重新推荐自治区科技进步奖的项目需提交以下说明材料

材料名称	上次推荐	本次推荐
推荐年度	2017	2018
项目名称	维吾尔族重大精神疾病的临床和分子生物学研究	维吾尔族重大精神疾病的临床和分子生物学研究
完成单位	新疆医科大学第一附属医院	新疆医科大学第一附属医院
完成人员	伊琦忠、安景荣·安治国、王江涛、罗晓、母代斌、徐斌、傅松年、胡红星、丽扎·满苏尔、陈霞、孙乐乐	伊琦忠、安景荣·安治国、王江涛、罗晓、母代斌、徐斌、傅松年、胡红星、丽扎·满苏尔、陈霞、孙乐乐
创新内容（创新点）	1、在全球范围内建立了首家维吾尔族重大精神疾病生物学信息样本库； 2、利用维吾尔族重大精神疾病生物学样本库的资源，在全球范围内首次进行了维吾尔族重大精神疾病病因及发病机制的关联性研究且有了比较重大的发现； 3、基础研究和临床紧密结合，将基础研究的成果转化为临床应用。促进了精准医学的发展（基因多态性与抗精神病药，抗抑郁药的精准医疗）。	1、项目研究成果为维吾尔族重大精神疾病的早发现、早诊断奠定了基础。 2、依托项目研究成果，搭建精神卫生安全质量控制平台转化成覆盖全疆的精神卫生医疗安全质量控制体系。
经济效益	无	无
社会效益	1、2013 年至 2017 年，开办自治区精神科医师和护士转岗培训班 11 期，培训学员达 218 人。 2、指导帮助喀什地区第一人民医院、新源县人民医院、博州人民医院、奇台县人民医院建立了精神科病房或门诊。培训的学员中已有多位在当地准备筹建精神科病房或门诊。 3、新疆维吾尔族精神分裂症新发生的拷贝数变异（de novo cnv）研究，国家自然科学基金（项目经费 49 万元）（81360209），2014.1—2017.12，主持，结题。 4、基于全外显子组测序探索新疆维吾尔族人群精神分裂症相关的 de novo 突变及其致病机理（项目经费 36 万元）（81660233），2017.1-2020.12，主持，在研。	1、推动了本地区的学科发展和建设（医院管理协会、质控中心、综合医院精神心理课的建设、标准、制度、人才培养）促进了区域精神卫生领域内转换医学、精准医学的发展。 2、举办 14 期继续教育培训班，提高了新疆基层精神卫生机构的医疗、教学、科研能力，培养了一批具有一定精神卫生科研能力的专业技术人员，促进了区域精神卫生事业的发展。 3、项目的实施促进了区域内精神卫生事业的发展，提高了自治区精神卫生的医疗质量和管理水平； 4、建立精神卫生医疗质量和管理体系，严格执行各项规章制度，搭建覆盖全疆的精神卫生安全质量控制平台。完善了维吾尔族重大精神疾病的临床路径的设置，并已在基层精神卫生机构临床全面展开。 5、成立自治区级精神卫生质量控制中

由 扫描全能王 扫描创建

重新推荐自治区科技进步奖的项目需提交以下说明材料

材料名称	上次推荐	本次推荐
推荐年度	2017	2018
项目名称	维吾尔族重大精神疾病的临床和分子生物学研究	维吾尔族重大精神疾病的临床和分子生物学研究
完成单位	新疆医科大学第一附属医院	新疆医科大学第一附属医院
完成人员	伊琦忠、安景荣·安治国、王江涛、罗晓、母代斌、徐斌、傅松年、胡红星、丽扎·满苏尔、陈霞、孙乐乐	伊琦忠、安景荣·安治国、王江涛、罗晓、母代斌、徐斌、傅松年、胡红星、丽扎·满苏尔、陈霞、孙乐乐
创新内容（创新点）	1、在全球范围内建立了首家维吾尔族重大精神疾病生物学信息样本库； 2、利用维吾尔族重大精神疾病生物学样本库的资源，在全球范围内首次进行了维吾尔族重大精神疾病病因及发病机制的关联性研究且有了比较重大的发现； 3、基础研究和临床紧密结合，将基础研究的成果转化为临床应用。促进了精准医学的发展（基因多态性与抗精神病药，抗抑郁药的精准医疗）。	1、项目研究成果为维吾尔族重大精神疾病的早发现、早诊断奠定了基础。 2、依托项目研究成果，搭建精神卫生安全质量控制平台转化成覆盖全疆的精神卫生医疗安全质量控制体系。
经济效益	无	无
社会效益	1、2013 年至 2017 年，开办自治区精神科医师和护士转岗培训班 11 期，培训学员达 218 人。 2、指导帮助喀什地区第一人民医院、新源县人民医院、博州人民医院、奇台县人民医院建立了精神科病房或门诊。培训的学员中已有多位在当地准备筹建精神科病房或门诊。 3、新疆维吾尔族精神分裂症新发生的拷贝数变异（de novo cnv）研究，国家自然科学基金（项目经费 49 万元）（81360209），2014.1—2017.12，主持，结题。 4、基于全外显子组测序探索新疆维吾尔族人群精神分裂症相关的 de novo 突变及其致病机理（项目经费 36 万元）（81660233），2017.1-2020.12，主持，在研。	1、推动了本地区的学科发展和建设（医院管理协会、质控中心、综合医院精神心理课的建设、标准、制度、人才培养）促进了区域精神卫生领域内转换医学、精准医学的发展。 2、举办 14 期继续教育培训班，提高了新疆基层精神卫生机构的医疗、教学、科研能力，培养了一批具有一定精神卫生科研能力的专业技术人员，促进了区域精神卫生事业的发展。 3、项目的实施促进了区域内精神卫生事业的发展，提高了自治区精神卫生的医疗质量和管理水平； 4、建立精神卫生医疗质量和管理体系，严格执行各项规章制度，搭建覆盖全疆的精神卫生安全质量控制平台。完善了维吾尔族重大精神疾病的临床路径的设置，并已在基层精神卫生机构临床全面展开。 5、成立自治区级精神卫生质量控制中

	5、汉密尔顿抑郁量表维吾尔语版的修订及其信效度研究，自治区自然科学基金（项目经费 5 元）（2014211C067），2014.1—2016.12，主要参与，结题。	心和医院管理的组织结构。促进了国家基本公共卫生服务项目的实施。在全疆各地区开展贯彻执行《精神卫生法》和《全国精神卫生工作规划（2015－2020 年）》宣传及指导基层精神卫生机构提高精神卫生服务能力并举行义诊、会诊、培训、科普讲座等公益活动。 6、为寻找维吾尔族重大精神疾病生物学标记物，以便对维吾尔族重大精神疾病的早发现、早诊断奠定了基础。为制定维吾尔族重大精神疾病的个体化、精准化诊断治疗方案奠定了基础，为新药开发，精准化治疗做准备。
发表论文题目、作者、年卷期	[1] Han S, An Z, Luo X, et al. Association between CMYA5, gene polymorphisms and risk of schizophrenia in Uygur population and a meta-analysis. Early Intervention in Psychiatry, 2015, 22(8):685-691.(韩书贤、安治国、罗晓、张丽丽、钟衔江、杜雯、伊琦忠、师咏勇) [2] Zhang L, Zhong X, An Z, et al. Association analysis of the GRM8 gene with schizophrenia in the Uygur Chinese population. Hereditas, 2015, 151(6):140-144.(张丽丽、钟衔江、安治国、韩书贤、罗晓、杜雯、师咏勇，伊琦忠) [3] Zhong X, Zhang L, Han S, et al. Case control study of association between the ANK3 rs10761482 polymorphism and schizophrenia in persons of Uyghur nationality living in Xinjiang China. Shanghai Archives of Psychiatry, 2014, 26(5):288-293(钟衔江、张丽丽、伊琦忠) [4] Raja Amjad Waheed Khan , Jianhua Chen , Shen J, et al. Common variants	[1] Zhiqiang L,Jianhua Chen,Qizhong Yi,YongyongShi et al. Genome-Wide association analysis identifies 30 new susceptibility loci for schizophrenia.Nature Genetics, 2017,doi:10.1038/ng.3973.

	in QPCT, gene confer risk of schizophrenia in the Han Chinese population. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics,015, 171B(2):237.)(Raja Amjad Waheed Khan，陈剑华，沈佳薇，李志强，王蒙，温组佳，宋治建，许一峰，师咏勇，伊琦忠，季卫东） [5] Wang Q, Wang Y, Ji W, et al. SNAP25 is associated with schizophrenia and major depressive disorder in the Han Chinese population. Journal of Clinical Psychiatry, 2015, 76(1):e76.（王庆忠，王燕玲，季卫东，贺宽军，李志强，陈剑华，温组佳，沈佳薇，季珏，师咏勇，伊琦忠，王永刚） [6] He K, An Z, Wang Q, et al. CACNA1C, schizophrenia and major depressive disorder in the Han Chinese population. British Journal of Psychiatry the Journal of Mental Science, 2014, 204(1):36.（贺宽军,安治国,王庆忠,李志强,陈剑华,季珏,贺林,伊琦忠,师咏勇） [7] Ji W,Li T, Pan Y,et al.CNTNAP2,is significantly associated with schizophrenia and major depression in the Han Chinese population. Psychiatry Research, 2013, 207(3):225-228.（季卫东，李涛，温组佳，安治国，赵倩，贺林，伊琦忠，师咏勇） [8] Xu W ,Liu Y, Chen J ,Guo Q ,Liu K .Genetic risk between the CACNA1I gene and schizophrenia in Chinese Uygur population. Hereditas,2017Jul	

	17,155.（许伟，陈剑华,温组佳,宋智建,伊琦忠,师咏勇） [9] 罗晓，伊琦忠，CMYA5 基因多态性与新疆维吾尔族精神分裂症的关联性研究，中国神经精神疾病杂志.2014,（12）：726-730. [10] 张丽丽,伊其忠,新疆维吾尔族人群中GRM7基因rs3749380多态性与精神分裂症的关联性研究；中华行为医学与脑科学杂志，2014,23（3）:203-206. [11] 韩书贤，伊琦忠,心肌病相关蛋白 5 基因 rs10043986 多态性与中国维吾尔族人群精神分裂症关联研究.中华行为医学与脑科学杂志,2015，24(1):27-30. [12] 张丽丽,伊其忠*.新疆维吾尔族抑郁症基因样本库的初步建立及应用,新疆医科大学学报,2014（10）:1375-1378.	
撰写专著	无	
获得的专利	无	精神卫生质量控制数据处理方法及装置（专利号：20180461013.7）; 病历质量控制系统（简称;MRQCS）V6.0（登记号：2017SR470817）
获得的自主知识产权	无	精神卫生质量控制数据处理方法及装置（专利号：20180461013.7）; 病历质量控制系统（简称;MRQCS）V6.0（登记号：2017SR470817）

应用推广单位名称	1、 新疆医科大学第一附属医院 2、 乌鲁木齐市安宁医院 3、 和田地区精神病医院 4、 喀什地区康宁医院 5、 伊犁地区康仁医院	1、博尔塔拉蒙古自治州人民医院 2、塔城地区人民医院 3、独山子人民医院

中华人民共和国国家知识产权局

100190

北京市海淀区中关村东路66号世纪科贸大厦C座12层1206
北京汇信合知识产权代理有限公司 吴甘棠(010-51282990)

发文日：

2018年05月16日

申请号或专利号：201810461013.7　　发文序号：2018051600340470

专利申请受理通知书

根据专利法第28条及其实施细则第38条、第39条的规定，申请人提出的专利申请已由国家知识产权局受理。现将确定的申请号、申请日、申请人和发明创造名称通知如下：

申请号：201810461013.7
申请日：2018年05月15日
申请人：伊琦忠, 纪悦
发明创造名称：精神卫生质量控制数据处理方法及装置

经核实，国家知识产权局确认收到文件如下：
实质审查请求书 每份页数:1页 文件份数:1份
权利要求书 每份页数:2页 文件份数:1份 权利要求项数：9项
说明书摘要 每份页数:1页 文件份数:1份
发明专利请求书 每份页数:4页 文件份数:1份
说明书 每份页数:10页 文件份数:1份
专利代理委托书 每份页数:2页 文件份数:1份
说明书附图 每份页数:3页 文件份数:1份

提示：

1. 申请人收到专利申请受理通知书之后，认为其记载的内容与申请人所提交的相应的内容不一致时，可以向国家知识产权局请求更正。
2. 申请人收到专利申请受理通知书之后，向国家知识产权局办理各种手续时，均应当准确、清晰地写明申请号。
3. 国家知识产权局收到向外国申请专利保密审查请求书后，依据专利法实施细则第9条予以审查。

审 查 员：自动受理　　审查部门：专利局初审及流程管理部

200101
2013.4

纸件申请，回函请寄：100088 北京市海淀区蓟门桥西土城路6号 国家知识产权局受理处收
电子申请，应当通过电子专利申请系统以电子文件形式提交相关文件。除另有规定外，以纸件等其他形式提交的文件视为未提交。

中华人民共和国国家版权局

计算机软件著作权登记证书

证书号：软著登字第2066101号

软 件 名 称：病历质量控制系统
[简称：MRQCS]
V6.0

著 作 权 人：纪悦;伊琦忠

开发完成日期：2017年05月10日

首次发表日期：2017年05月11日

权利取得方式：原始取得

权 利 范 围：全部权利

登 记 号：2017SR470817

根据《计算机软件保护条例》和《计算机软件著作权登记办法》的规定，经中国版权保护中心审核，对以上事项予以登记。

中华人民共和国国家版权局
计算机软件著作权
登记专用章

2017年08月25日

No. 01922528

中国科技核心期刊
中国学术期刊（光盘版）全文收录期刊
美国《乌利希期刊指南》收录期刊
美国《化学文摘》收录期刊
科学引文数据库（SCD）来源期刊

ISSN 1009-5551
CN 65-1204/R

新疆医科大学学报

شىنجاڭ تېببىي ئۇنىۋېرسىتېتى ئىلمىي ژۇرنىلى

JOURNAL OF XINJIANG MEDICAL UNIVERSITY

10
2014
第37卷 第10期
Vol.37 No.10
ISSN 1009-5551

新疆医科大学学报

XINJIANG YIKE DAXUE XUEBAO

月刊　1978年1月创刊　2014年37卷第10期　2014年10月出版

目　次

本期执行编委：张建龙 王云海 徐新娟 郑玉建 热娜·卡斯木
本期责任编辑：周 芳
本期英文编辑：尼格尔·买买提依明 杨 华 田桂玲 季 亮 朱洪斌
期刊基本参数：CN65-1204/R＊1978＊m＊A4＊128＊zh＊P＊￥10.00＊800＊47＊2014-10

新疆维吾尔族抑郁症基因样本库的初步建立及应用

经审数据库核实，自前数据库仅收录到第9期，该刊为第10期上发表

张丽丽，韩书贤，安治国，钟衔江，罗 晓，伊琦惠(Y05) 未来

（新疆医科大学第一附属医院心理医学中心，乌鲁木齐 830054）

摘要：目的 初步建立标准的新疆维吾尔族抑郁症DNA样品库，并进行TPH-2基因多态性的研究。**方法** 收集2010年1月－2013年1月于新疆医科大学第一附属医院精神科及新疆其他精神病院治疗的800例维吾尔族抑郁症患者的一般人口学资料、临床信息及DNA样本，DNA样品经质量控制后，保存于新疆医科大学第一附属医院重大疾病资源标本库，并建立与之一一对应的临床资料数据库，进行危险因素的评估。采用DNA直接测序法进行TPH-2基因多态性与抑郁症自杀倾向的关联性研究。**结果** (1)初步应用DNA样品库后发现rs7305115位点中基因型成功测定率为99.6%。(2)不同文化程度、职业的患者HAMD-17评分差异均有统计学意义（P均<0.05）；(3)基因型A/A、A/G、G/G的HAMD-17评分差异无统计学意义（$P>0.05$）；(4)无自杀倾向组和有自杀倾向组间rs7305115位点等位基因与基因型分布频率差异无统计学意义（$P>0.05$）。**结论** 初步建立了标准的新疆维吾尔族抑郁症DNA样品库，文化程度和职业可影响疾病的严重程度，未发现TPH-2基因与抑郁症自杀倾向存在明显关联。

关键词：抑郁症；DNA样本库；TPH-2基因；基因多态性

中图分类号：R749.4 **文献标识码：**A **文章编号：**1009-5551(2014)10-1375-04

doi：10.3969/j.issn.1009-5551.2014.10.035

The initial building and application of the DNA specimen database in Uygur Chinese population with depression

ZHANG Lili, HAN Shuxian, AN Zhiguo, ZHONG Xianjiang, LUO Xiao, YI Qizhong

(Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, China)

Abstract: Objective To built up the normative DNA specimen bank of Uyghur population with depression initially, and to analyze the polymorphism of TPH-2 gene. **Methods** 800 Uyghur patients with depression were collected by the demography, clinical data and DNA specimens from January, 2010 to January 2013. After quality control, DNA specimens were retained in the vital diseases repository of the first Affiliated Hospital to Xinjiang Medical University, then we set up the clinical data repository, and assessed the possible risk factors of the disense. According to direct DAN sequencing , we investigated the association between the polymorphism of TPH-2 gene and suicidal tendencies of depression. **Results** (1) The result of preliminary application shows that 99.6% of samples were genotyped successfully for rs7305115. (2) The significant differences of HAMD-17 scores were found between groups with different educational background or professions ($P<0.05$). (3) The scores of HAMD-17 had no differences statistically among different genotypes(A/A, A/G, G/G) (all $P>0.05$). (4) rs7305115 was not found to have genotypic or allelic association with suicidal tendencies between the cases with suicide intension and those without ($P>0.05$). **Conclusion** The normative DNA specimen database of Uyghur population with depression was built

基金项目：新疆维吾尔自治区自然科学基金(2010211A51)；国家自然科学基金(81160209)

作者简介：张丽丽(1987－)，女，在读硕士，研究方向：精神疾病遗传学。

通信作者：伊琦忠，男，主任医师，硕士生导师，研究方向：精神疾病遗传学，E-mail：qizhongyi@126.com。

up initially. Literacy level and social status were associated with the condition of the disease. But it failed to find the correlation between TPH-2 gene and depression.

Key words: depression; DNA specimen bank; TPH-2 gene; polymorphism

抑郁症是一种慢性复发性精神疾病，在所有精神障碍中抑郁症患病率最高。1993年，世界范围的流行病学调查显示，普通人群抑郁症的患病率为5.8%[1]，且有很高的自杀风险[2]。

研究显示，抑郁症是一种多基因遗传病，有家族聚集现象，从遗传学的角度阐述发病机制及风险因素，对于抑郁症的诊断及治疗意义重大[3-5]。目前国内多个研究机构建立了抑郁症基因数据库，并发现与抑郁症密切相关的88个基因[6-8]。新疆维吾尔族很少与汉族通婚，保有本民族的遗传特色，是基因研究中不可多得的资源[9]。抑郁症中的自杀行为一直为人们所关注[10-12]。有研究发现，5-HT合成限速酶色氨酸羟化酶2(TPH-2)与抑郁症自杀倾向密切相关[13-17]。Zhang等[18]提出，在抑郁症有自杀行为患者中，TPH-2基因位点rs7305115等位基因A与基因型A/A的频率要低于无自杀行为的患者。本研究旨在初步建立新疆维吾尔族抑郁症的基因样本库，并以rs7305115为例进行危险因素的评估。

1 资料与方法

1.1 研究对象 研究对象均来自世居新疆及无生物学亲缘关系的维吾尔族人群。入组前签署知情同意书，并通过新疆医科大学第一附属医院伦理委员会的审查。样本共计800例，男性354例，女性446例，平均年龄(40.85±0.43)岁，按职业分组，脑力劳动者350例，体力劳动者450例，按文化程度分组，高中文化程度以上313例，高中文化程度以下(包括高中)487例。入组标准：(1)2010年3月—2013年1月于新疆医科大学第一附属医院精神科及新疆其他精神病院治疗的维吾尔族抑郁症患者；(2)诊断，一般人口学资料及临床信息的收集由2名主治医师以上精神科医生完成，采用半定式临床检查(SCID)进行精神检查，符合《美国精神障碍诊断与统计手册》第4版(DSM-IV)抑郁发作的诊断标准；(3)按照一般划界分，汉密顿抑郁量表(HAMD-17)评分≥7分者视为有抑郁症状。排除标准：(1)中枢神经系统及脑器质性疾病患者；(2)严重躯体疾病，如肿瘤、内分泌、心血管疾病、肝肾功能损伤及由躯体疾病继发的抑郁；(3)其他精神疾病与精神发育迟滞患者；(4)有精神疾病阳性家族史者；(5)孕期、哺乳期及月经期女性。

1.2 方法

1.2.1 一般人口学资料及临床资料库的建立 收集一般人口学资料，包括姓名、出生年、性别、民族、文化程度、职业、联系方式等；临床资料包括住院号、初次发病年龄、本次诊断、HAMD前17项评分、本次出院评价、是否为双生儿、寄养子、近亲婚配、精神病互相婚配、一级亲族遗传史等信息。上述信息首先填入"精神疾病家系调查表"，然后应用Excel软件建立与上述信息一一对应的资料库。

1.2.2 DNA样本库的建立

1.2.2.1 采集外周血 采集研究对象晨起空腹肘静脉血各5 mL，2%EDTA抗凝处理。及时送往新疆医科大学第一附属医院重大疾病资源标本库。由专业人员控制质量，进行血清与血细胞的分离，统一编码(参照国际通用标准)。

1.2.2.2 基因组DNA的提取 每例样本各选取500 μL血细胞，采用天根血液基因组提取试剂盒(DP319)提取DNA。

1.2.2.3 基因组DNA的质量控制 用NanoDrop1000分光光度计对DNA样本浓度、纯度进行测定并予以记录，使纯度(OD_{260nm}/OD_{280nm})为1.6～1.8，浓度为200～500 ng/μL，不符合要求的重新提取。经质量控制后，将与Excel数据库信息一一对应的DNA样本存入我院的重大疾病资源标本库，-80℃长期保存，初步建立DNA样本库。

1.2.3 数据库及DNA样本库的初步应用

1.2.3.1 抑郁症危险因素评估 比较不同职业、文化程度各组间HAMD-17评分的差异，进行危险因素的评估。

1.2.3.2 TPH-2基因与抑郁症自杀倾向的研究 随机抽取DNA样品268例，按有无自杀倾向(有自杀观念或未遂)分组：自杀倾向组123例，无自杀倾向组145例，以rs7305115为例，进行关联性研究。具体方法如下：(1)利用primer 5.0设计引物，引物为：5′-ACCTGAGCCCACGAGACTTT -3′和5′-TCGAGCCAGAGCTGGAATAT-3′。(2)PCR扩增目的片段，反应体系为25 μL，包括模板DNA 50 ng、12.5 μL PCR Master Mix、正向及反向引物各0.5 μL、ddH_2O 6.5 μL。反应条件为：94℃ 5 min，94℃ 30 s，55℃ 30 s，72℃ 30 s，共35个循环，72℃ 5 min。(3)扩增产物经琼脂凝胶电泳检测后，由上海生物工程股份有限公司进行测序。

1.3 统计学处理 根据是否符合正态性及方差齐性检验，应用SPSS17.0软件对不同文化程度、不

间 HAMD-17 评分差异进行 t 检验或秩和检验；不同基因型组间 HAMD-17 评分差异进行单因方差分析。利用 SHEsis 软件(http://analysis.o-x.cn)进行 Hardy-Weinberg 遗传平衡度检验、间等位基因和基因型频率差异分析。

结果

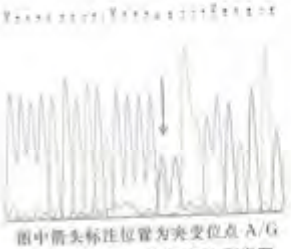
.1 DNA 样本库及临床数据库的初步建立 收集维吾尔族抑郁症患者基因组 DNA 共计 800 例，纯为 1.6～1.9，浓度为 200～500 ng/μL；对样本库行初步应用后发现，rs7305115 位点中基因型成功测定率为 99.6%，进一步证实了 DNA 样本质量可靠。且 DNA 样品与临床数据库信息严格对应，由本院重大疾病资源标本库专业人员保存，初步构建了 DNA 样品库和资料库。

.2 不同文化程度及职业患者 HAMD-17 评分的比较 高中以下(包括高中)组 HAMD-17 评分为(26.45±0.30)分，与高中以上组 HAMD-17 评分(25.28±0.37)分比较，差异有统计学意义($Z=3.04$，$P=0.042$)；体力劳动者组 HAMD-17 评分为(26.43±0.31)分，和脑力劳动者组 HAMD-17 评分(24.79±0.34)分比较，差异有统计学意义($Z=4.23$，$P<0.001$)。

.3 不同基因型间 HAMD-17 评分的差异性比较 对于 rs7305115 位点，剔除 1 例未成功测序的样本，发现 A/A、A/G、G/G 3 种不同基因型组间 HAMD-17 评分差异无统计学意义($P>0.05$)，见表 1，测序图见图 1。

表 1　不同基因型间 HAMD-17 评分的比较($\bar{x}\pm s$，分)

分组	n	HAMD-17	F 值	P 值
A/A	71	24.1±5.7		
A/G	126	25.4±6.3	1.43	0.24
G/G	70	25.9±7.1		



图中箭头标注位置为突变位点 A/G

图 1　rs7305115 位点 DNA 测序图

2.4 rs7305115 基因多态性与抑郁症自杀倾向的关联性 自杀倾向组与无自杀倾向组间性别、年龄均衡可比($P_{性别}=0.35$；$P_{年龄}=0.85$)，且基因型的分布均符合 Hardy-Weinberg 遗传平衡定律($P>0.05$)。两组间等位基因($P=0.20$，$OR=0.80$，95% CI 为 0.57～1.12)与基因型($P=0.45$)的分布频率差异均无统计学意义，见表 2。

表 2　rs7305115 基因多态性与抑郁症自杀倾向的关联性/例(%)

	等位基因		基因型			HWE	P 值
	A	G	A/A	A/G	G/G		
自杀倾向组	116(47.2)	130(52.8)	29(23.6)	58(47.2)	36(29.3)	0.55	0.45
无自杀倾向组	152(55.8)	120(44.2)	42(29.2)	68(47.2)	34(23.6)	0.53	

3　讨论

新疆维吾尔族和汉族存在遗传学和地理及社会文化背景等差异。本研究以 rs7305115 为例进行的研究与 Zhang 等[6]的研究结论不相一致，提示内地建立的汉族抑郁症基因库及寻找的基因位点不一定适合新疆维吾尔族抑郁症病人。因此，建立维吾尔族抑郁症样品 DNA 库和临床数据库，是研究维吾尔族抑郁症发病机制、诊断、个体治疗及新药研究的基础。

通过对基因库质量的控制发现，DNA 样本质量可靠，纯度为 1.6～1.9，浓度为 200～500 ng/μL，以 rs7305115 为例进行研究时，基因型成功测定率高达 99.6%，且符合 Hardy-Weinberg 遗传平衡定律，进一步说明 DNA 样品质量可靠，且有良好的群体代表性。通过对临床数据库相关风险因素的分析，发现低文化程度和体力劳动患者的病情严重，符合抑郁症发病的一般规律。

郝伟[7]研究提示 5-HT 相关基因可能参与抑郁症的发病机制。本研究通过初步应用该基因库，进行 TPH-2 基因多态性与抑郁症的关联性研究，结果显示 A/A、A/G、G/G 3 种不同基因型组间 HAMD-17 评分差异无统计学意义；有自杀倾向组与无自杀倾向组间等位基因与基因型的分布频率差异无统计学意义，未能找到 rs7305115 与抑郁症自杀倾向有关的证据，也未能找到影响疾病严重程度的危险基因或基因型。考虑一方面可能与样本量偏小有关；另一方面，可能是由于维吾尔族特有的遗传因素所致。

综上所述，本研究初步建立了规范化的维吾尔族抑郁症 DNA 样品库及临床资料库，并以 TPH-2 基因为例对该库初步应用，进行其与抑郁症的关联性研究，进一步证实了该遗传资源库的可靠、科学，但离建立大规模基因样本库仍有一定距离，目前对维吾尔族抑郁症的 DNA 样本收集工作仍在继续，争取为今后维吾尔族抑郁症研究提供一定规模的生物学资源。

参考文献：

[1] Patten SB. Recall bias and major depression lifetime prevalence [J]. Soc Psychiatry Epidemiol, 2003,38(6):290-296.

[2] 张国芳，侯钢. 五羟色胺与抑郁症自杀的分子遗传学[J]. 国外医学：精神病学分册，2003，30(2)：92-94.

[3] 段冬梅，雨雁，陈利平，等. 基因组学研究在抑郁症发病机制及诊断中的应用[J].中华中医药学刊，2009，27(5)：944-948.

[4] Verhoeven KJ, Macel M, Wolfe LM, et, al. Population admixture, biological invasions and the balance between local adaptation and inbreeding depression[J]. Proc Biol Sci, 2011, 278(1702):2-8.

[5] 王栋. 抑郁症相关基因与蛋白数据库的构建[D].西安：第四军医大学，2005:4-7.

[6] 王栋，梁蜀忠，孙金立，等.抑郁症相关基因数据库的构建[J].中国临床康复，2005，9(32)：30-31.

[7] 梁黎.中国少数民族基因库[N]. 中国民族，2009(Z1).

[8] Beghi M, Rosenbaum JF, Cerri C, et al. Risk factors for fatal and nonfatal repetition of suicide attempts: a literature review [J]. Neuropsychiatr Dis Treat, 2013, 9(1):1725-1736.

[9] Li JJ, Berk MS, Lee SS. Differential susceptibility in ... dinal models of gene-environment interaction for adolescent de... pression[J]. Dev Psychopathol, 2013, (4 Pt 1):991-...

[10] Walther DJ, Peter JU, Bashammakh S, et al. Synthesis of serotonin by a second tryptophan hydroxylase isoform[J]. Science, 2003, 299(5603):76.

[11] Sugden D.Comparison of circadian expression of tryptophan hydroxylase isoform mRNAs in the rat pineal gland ... real-time PCR [J]. J Neurochemistry, 2003, ... 1308-1311.

[12] Lazary J, Viczena V, Dome P, et al. Hopelessness, a potential endophenotpye for suicidal behavior, is influenced by TPH2 gene variants[J]. Pro Neuropsychopharmacol ... Psychiatry, 2012, 36(1):155-160.

[13] Musil R, Zill P, Seemüller F, et al. Genetics of emergent suicidality during antidepressive treatment--data from a naturalistic study on a large sample of inpatients with a major depressive episode[J]. Eur Neuropsychopharmacol, ... (7): 863-874.

[14] 张玉琦，徐文炜，袁国桢，等. TPH2 基因单核苷酸多态性与单相抑郁症及其症状表型的关联研究[J]. 现代预防医学，2011，38(10)，1904-1907.

[15] Zhang Y, Zhang C, Yuan G, et al. Effect of tryptophan hydroxylase-2 rs7305115 SNP on suicide attempts ... major depression[J]. Behav Brain Funct, 2010, ...

[16] 郝伟.精神病学[M].北京：人民卫生出版社，2008...

［收稿日期：...］

（本文编辑 ...）

（上接 1374 页）

3.3 影响汉族食管患者配偶焦虑抑郁状态得分的因素 本研究通过多因素分析，发现汉族食管患者配偶焦虑得分的影响因素包括对疾病的了解程度，收入。随着汉族患者配偶对疾病的认知水平增加，焦虑评分下降，原因可能是：(1)汉族食管癌患者的配偶对食管癌的了解程度不高且存在较多错误的认识，比如说癌症必死等错误理念，从而导致患者配偶在精神上存在较大的压力；(2)汉族食管癌患者配偶随着对疾病的认识加深，对治疗的信心也随之加强，从而减轻了其焦虑的心情。本研究显示，汉族食管癌患者配偶的焦虑状况还受收入的影响，其可能的机制与维、哈族食管癌患者配偶相似。

综上所述，维、汉、哈族食管癌患者配偶的焦虑状态不尽相同，但均随着治疗的进行而得到一定的改善，不同民族食管癌患者配偶焦虑状态的影响因素不同，应给予不同的支持方式。

参考文献：

[1] Darling GE. Quality of life in patients with esophageal cancer [J]. Thorac Surg Clin, 2013, 23(4):569-575.

[2] Jacobs M, Macefield RC, Elbers RG, et al. Meta-analysis shows clinically relevant and long-lasting ... health-related quality of life after esophageal ... [J]. Qual Life Res, 2014, 23(4):1155-1176.

[3] 任国军，苏纯音，薛永生，等. 食管癌患者生存质量... [J]. 中华护理杂志，2008，43(2)：151-153.

[4] 王宁燕，魏运红，汤晓霞，等. 食管癌放疗患者配偶... 质性研究[J]. 护理学杂志，2008，23(19)：59-60.

[5] Chang YL, Tsai YF, Wu YC, et al. Factors Re... ty of Life After Esophagectomy for Cancer Pa... [J]. Cancer Nurs, 2014, 37(1):4-13.

[6] 陈小燕.食管癌患者配偶心理状态的影响因素分析... 瘤，2011，20(11)：830-833.

[7] Remai D. Domains for global health education ... Perspect Med Educ, 2013, 2(5-6):356-357.

[8] 丁敏，陈小燕，朱一真，等. "知信行" 健康教育... 管癌配偶生活质量影响的研究[J].护理研究... 旬版，3388-3389.

[9] 祝敏英，周涛，杨秀木，等. 乳腺癌患者配偶... 查及相关因素分析[J]. 蚌埠医学院学报，... 1170-1172.

［收稿日期：...］

（本文编辑 ...）

中华医学会系列杂志

ISSN 1674-6554
CN 37-1468/R

中华行为医学与脑科学杂志®

ZHONGHUA XINGWEI YIXUE YU NAOKEXUE ZAZHI

2015年1月 第24卷 第1期

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

Volume 24 Number 1
January 2015

中華醫學會

CHINESE MEDICAL ASSOCIATION

ISSN 1674-6554

中华行为医学与脑科学杂志®

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

月刊　1992年6月创刊　第24卷　第1期　2015年1月20日出版

主　管
中华人民共和国国家卫生和计划生育委员会

主　办
中华医学会
100710，北京市东四西大街42号
济宁医学院
272067，山东省济宁市荷花路16号

编　辑
中华行为医学与脑科学杂志编辑委员会
272067，山东省济宁市荷花路16号
电话：(0537)3616263
传真：(0537)3616263-87
Email：zwyxkjbzz@163169.net
http://www.zwyx.cn
http://www.zwyx.cnjournals.org.cn
http://www.medline.org.cn

总编辑
白　波

编辑部主任
张作记

出　版
《中华医学杂志》社有限责任公司
100710，北京市东四西大街42号
电话（传真）：(010)85158180
Email：office@cmaph.org

广告经营许可证
3708004403021号

印　刷
济宁市火炬书刊印务中心

发　行
范围：公开
国内：济宁市报刊发行局
国外：中国国际图书贸易集团有限公司
（北京399信箱，100044）
代号 M3269

订　购
全国各地邮政局
邮发代号　24-115

邮　购
中华行为医学与脑科学杂志编辑部
272067，山东省济宁市荷花路16号
电话：(0537)3616263
传真：(0537)3616263-87
Email：zwyxkjbzz@163169.net
http://www.zwyx.cn
http://www.zwyx.org.cn

定　价
每期15.00元，全年180.00元

中国标准连续出版物号
ISSN 1674-6554
CN 37-1468/R

2015年版权归中华医学会所有

未经授权，不得转载、摘编本刊文章，不得使用本刊的版式设计

除非特别声明，本刊刊出的所有文章不代表中华医学会和本刊编委会的观点

本刊如有印装质量问题，请向本刊编辑部调换

目　次

基础研究

临床研究

卫生预防

本期责任编辑 冯学泉 英文编辑 张玫美 杨静 责任排版 张建设 英文审校 王福顺 朱滨

· 临床研究 ·

心肌病相关蛋白5基因rs10043986多态性与中国维吾尔族人群精神分裂症关联研究

[illegible]

【摘要】 目的 [illegible] 方法 [illegible] 结果 [illegible] 结论 [illegible]

【关键词】 [illegible]

Association analysis of the rs10043986 polymorphism of cardiomyopathy-associated 5 gene with schizophrenia in Uygur Chinese population

[illegible]

【Abstract】 **Objective** [illegible] **Methods** [illegible] **Results** [illegible] **Conclusion** [illegible]

【Key words】 [illegible]

[illegible]

[illegible]

境因素共同作用的结果，其遗传度为 80%[1]，因此探讨遗传因素与精神分裂症病因及发病机制对该病的诊断及个体化治疗具有重要意义。有研究发现，心肌病相关蛋白 5（cardiomyopathy-associated 5，CMYA5）基因编码的蛋白为 myospryn，属于 TRIM 蛋白家族，CMYA5 基因变异与杜氏营养不良、心肌病及高血压相关，又能引起精神症状，增加精神分裂症的患病风险[2-3]。Chen 等[4] 通过全基因组关联研究（genome wide association studies，GWAS）发现，CMYA5 基因 rs10043986 多态性可能增加精神分裂症的易感性，并对筛选结果在多个欧洲高加索人群的核心家系病例对照研究中得到了重复验证[4]。然而，近年来在以中国汉族人群和日本人群为对象的多项研究中均未发现 rs10043986 位点在亚洲人群中呈现多态性现象[5-10]。CMYA5 基因在不同种族人群中可能存在遗传异质性。中国新疆维吾尔族人群长期居住在中国西北地区，具有独特的生活环境和习俗且极少与其他民族通婚，适合进行遗传学研究。本研究对维吾尔族人群 CMYA5 基因 rs10043986 位点基因型进行分析，进一步探讨该位点是否也与中国维吾尔族人群精神分裂症存在关联。

对象与方法

一、对象

1.病例组：来自于新疆医科大学第一附属医院及新疆维吾尔自治区其他精神病专科医院住院的精神分裂症患者。每位患者由两名副高以上职称精神科医师根据《美国精神障碍诊断与统计手册第 4 版》（diagnostic and statistical manual of mental disorders-IV，DSM-IV）精神分裂症的诊断标准做出诊断，采用适于 DSM-IV的结构式临床访谈（structured clinical interview for DSM-IV，SCID）进行精神检查，用阳性与阴性症状量表（PANSS）[11] 评定患者病情的严重程度。排除标准：（1）患有严重躯体疾病或脑器质性疾病；（2）其它精神疾病及精神发育迟滞患者；（3）物质依赖与物质成瘾者；（4）有阳性精神疾病家族史者；（5）孕期、哺乳期及月经期女性。共入组 325 例，分为男性组（193 例）和女性组（132 例）；按首次发病年龄分为：青少年组（首次发病年龄<18 岁，63 例）和成年组（首次发病年龄≥18 岁，262 例）。研究对象的年龄范围：13～84 岁，平均年龄（38.5±12.9）岁；首次发病年龄：10～54 岁，平均年龄（24.6±8.5）岁。

2.对照组：均来自新疆医科大学第一附属医院体检中心的体检人员，身体健康，与患者组无血缘关系，经过 SCID 非病人版本的精神检查，无精神疾病既往史及家族史。共入组 183 例，分为男性组（107 例）和女性组（76 例）；按年龄分为：青少年组（年龄<18 岁，14 例）、成年组（年龄≥18 岁，169 例）。对照组年龄范围：16～68 岁，平均年龄（37.7±11.6）岁。患者组和对照组的性别（$X^2=0.04$，$P=0.84$）和年龄（$t=0.70$，$P=0.48$）分布差异均无统计学意义。

所有研究对象均来自世居于新疆维吾尔自治区、无生物学亲缘关系的维吾尔族人群。该研究通过新疆医科大学第一附属医院伦理委员会审查，向所有受试者详细解释本次研究目的，并由患者本人及其监护人签署知情同意书。

二、方法

1.标本收集与基因组 DNA 提取：采集所有研究对象的肘静脉血 2 ml，EDTA 抗凝，-20 ℃冰箱保存，采用血液基因组 DNA 提取系统（Tiangen Biotech CO. LTD，Beijing，China）提取 DNA，用 NanoDrop1000 分光光度计对 DNA 样本浓度、纯度进行测定并予以记录，使纯度（ODA280/A260）在 1.6～1.9 之间，浓度在 200～500 ng/μl 之间，不符合要求的重新提取。提取的 DNA 放置于-80 ℃保存备用。

2.单核苷酸多态性（SNP）的筛选：根据已报道的 CMYA5 基因多态性与精神分裂症关联性研究的成果[4]，选择与欧洲高加索人群精神分裂症有阳性关联的 CMYA5 基因外显子区非同义突变 SNP：rs10043986，最小等位基因频率为 0.097，该基因在中国汉族人群（HCB）和日本人群（JPT）基因组单体型图中无多态性（http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10043986）。本研究以中国维吾尔族人群为研究对象，对 rs10043986 位点基因型进行分析。

3.SNP 基因型检测：采用 TaqMan 荧光探针 SNP 基因分型技术对 CMYA5 基因 rs10043986 位点进行分型，所使用的荧光探针及 TaqMan® Genotyping Master Mix 均由美国应用生物系统公司（ABI）定制。PCR 反应在 GeneAmp PCR 9700 系统（Applied Biosystem）中进行。PCR 反应体系为 5 μl，包括模板 40 ng DNA，PCR 反应混合液（TaqMan® Genotyping Master Mix）2.5 μl 和 TaqMan 探针 0.035 μl。反应条件为：94 ℃ 15 min；（94 ℃ 20 s，60 ℃ 1 min）×55 个循环。PCR 反应产物利用 ABI 7900 DNA 检测系统（Applied Biosystem，Foster City，CA，USA）进行荧光信号读取，完成各待测样本基因型的确定。为保证基因分型的准确性及可靠性，本研究随机抽取 5%的样本两次进行基因分型，两次分型结果一致性为 100%。

4.统计分析：使用 SPSS 17.0 进行统计分析，计量资料采用 $\bar{x}\pm s$ 进行统计描述，运用 t 检验比较两组基因型患者 PANSS 量表得分；利用 SHEsis 软件平台（http://analysis.bio-x.cn）[12] 进行两组对象的 Hardy-Weinberg 遗传平衡度检验，组间等位基因和基因型分布频率差异、优势比（odds ratio，OR）及 95%可信区间（95% confidence interval，95% CI）分析。所有的统计检验均为双侧检验，以 $P<0.05$ 为差异有统计学意义。

结　果

一、Hardy-Weinberg 遗传平衡检验

病例组（$X^2=0.903$，$P=0.342$）和对照组（$X^2=$ [illegible]，$P=0.618$）基因分布频率均符合 Hardy-Weinberg 平衡。CMYA5 基因 rs10043986 位点的等位基因和基因型频率见表 1。

二、病例组与对照组 rs10043986 位点等位基因及基因型频率比较

病例组与对照组间等位基因（$X^2=9.038$，$P=$ [illegible]）和基因型（$X^2=9.417$，$P=0.009$）频率分布均差异有统计学意义。病例组携带 T 等位基因的频率高于对照组[$OR=0.398$，95% CI（0.215–0.740）]；按性别分层比较，发现男性病例组与男性对照组的等位基因和基因型分布均差异无统计学意义（均 $P>0.05$），女性病例组与女性对照组的等位基因（$X^2=11.812$，$P=0.001$）和基因型（$X^2=12.769$，$P=0.001$）频率分布均差异有统计学意义，女性病例组携带 T 等位基因频率高于女性对照组[$OR=0.117$，95% CI（0.027–0.499）]。见表 1。

三、首次发病年龄与 CMYA5 基因 rs10043986 多态性关联分析

青少年病例组与对照组间等位基因（$X^2=0.529$，$P=$ [illegible]）和基因型（$X^2=0.604$，$P=0.437$）分布均差异无统计学意义（$P>0.05$）；成年病例组和对照组等位基因（$X^2=8.219$，$P=0.004$）和基因型（$X^2=8.379$，$P=$ [illegible]）分布均差异有统计学意义（$P<0.05$），成年病例组携带 T 等位基因频率高于成年对照组[$OR=0.369$，95% CI（0.182–0.748）]。见表 2。

四、CMYA5 基因 rs10043986 多态性与精神分裂症患者 PANSS 量表评分关联分析

病例组中 C/C 基因型和 C/T 基因型患者在阳性量表分、阴性量表分、一般精神病理症状分及 PANSS 量表总分均差异无统计学意义（均 $P>0.05$）。见表 3。

讨　论

人类 CMYA5 基因又名 myospryn，位于 5q14.1 区域，含有 11kb 和 13 个外显子区，在心脏、骨骼肌和中枢神经系统中均有表达，但其功能仍不完全清楚[14]。CMYA5 与 Dysbindin 蛋白激酶 A 的调节亚基等存在直接相互作用，它们参与了溶酶体相关细胞器生物发生复合体作用和蛋白激酶 A 信号转导的细胞过程，这些生物学过程已被多项研究报道在精神分裂症中发挥着重要作用，这种交互作用提示 CMYA5 可能与精神分裂症有关[15-16]。目前有关 CMYA5 与精神分裂症的关系研究报道较少且阳性位点也不尽相同[6,10]，Chen 等[14] 通过 GWAS 和验证分析发现非同义突变位点 rs10043986 与欧洲高加索人群的精神分裂症存在关联性，但在中国汉族和日本人群中 rs10043986 未呈现多态性现象[7,10]。本研究首次以中国维吾尔族人群为对象，探讨 rs10043986 与精神分裂症的关联性。

本研究发现病例组中 rs10043986 的 T 等位基因频率增加，说明 rs10043986 与维吾尔族的精神分裂症可能相关，与欧洲高加索人群研究结果一致[6]，与中国汉族和日本人群研究结果不同[7,10]。既往研究认为，中国维吾尔族人群有其特殊的遗传特色[17]，这可能同新疆维吾尔族生活的地域环境处于古丝绸之路上，在漫长的人类历史中存在着多民族的相互交往有关。研究对象按性别分层后，发现在女性组中 rs10043986 与精神分裂症有关联，而在男性组中，两者无明显关联。这表明 rs10043986 与精神分裂症的关联可能存在性别差异，基因多态性与精神分裂症关系存在性别差异的在其它基因中也有类似发现[18]。

表 1　病例组与对照组等位基因及基因型频率分布比较[例(%)]

组别	n	等位基因频率		X^2 值	P 值	基因型频率			X^2 值	P 值
		C	T			C/C	C/T	T/T		
病例组	325	595(91.5)	55(8.5)	9.038	0.003	271(83.4)	53(16.3)	1(0.3)	9.417	0.009
对照组	183	353(96.4)	13(3.6)			170(92.9)	13(7.1)	0		
男性病例组	193	358(92.7)	28(7.3)	1.012	0.314	166(86.0)	26(13.5)	1(0.5)	1.231	0.54
男性对照组	107	203(94.9)	11(5.1)			96(89.7)	11(10.3)	0		
女性病例组	132	237(89.8)	27(10.2)	11.812	0.001	105(79.5)	27(20.5)	0	12.769	0.001
女性对照组	76	150(98.7)	2(1.3)			74(97.4)	2(2.6)	0		

注：病例组与对照组比较，经 X^2 检验 $P<0.05$，$OR=0.398$，95% CI（0.215–0.740）；男性病例组与男性对照组比较，经 X^2 检验 $P>0.05$[$OR=0.693$，95% CI（0.338–1.421）]；女性病例组与女性对照组比较，经 X^2 检验 $P<0.05$[$OR=0.117$，95% CI（0.027–0.499）]

表 2　青少年和成年病例组与对照组等位基因与基因频率的比较[例(%)]

组别	n	等位基因频率		X^2 值	P 值	基因型频率			X^2 值	P 值
		C	T			C/C	C/T	T/T		
青少年病例组	63	111(88.1)	15(11.9)	0.529	0.467	48(76.2)	15(23.8)	0	0.604	0.437
青少年对照组	14	26(92.9.)	2(7.1)			12(85.7)	2(14.3)	0		
成年病例组	262	484(92.4)	40(7.6)	8.219	0.004	223(85.1)	38(14.5)	1(0.4)	8.379	0.015
成年对照组	169	328(97.0)	10(3.0)			159(94.1)	10(5.9)	0		

注：青少年病例组和青少年对照组比较，经 X^2 检验 $P>0.05$[$OR=0.569$，95% CI（0.123–2.645）]；成年病例组和成年对照组比较，经 X^2 检验 $P<0.05$[$OR=0.369$，95% CI 为（0.182–0.748）]

表3 C/C基因型和C/T+TT型基因型两组患者PANSS量表评分比较(分，$\bar{x}\pm s$)

变量	n	阳性量表分	阴性量表分	一般精神病理量表分	总分
C/C型	77	18.38±6.97	21.88±7.13	37.07±9.22	34.69±18.73
C/T+TT型	51	19.85±7.20	21.42±6.76	35.58±8.89	32.78±16.82
t值		1.58	1.37	1.19	0.71
P值		0.12	0.08	0.24	0.48

般认为精神分裂症患者存在性别差异主要与雌激素有关，但这种差异的确切机制尚不明了[17]。根据以上结果提示，CMYA5基因是否可能与雌激素相互作用使其在女性精神分裂症患者中表现出特异性关联，为今后精神分裂症性别差异研究提供了线索。

在精神分裂症的中间表型中，发病年龄是修饰疾病外显率和表现程度的重要因素。Hare等[18]研究指出精神障碍发病年龄的遗传度约为33%，许多双生子和家系研究也证明精神分裂症发病年龄与遗传变异密切相关[19]。目前国内外关于CMYA5与精神分裂症的研究均未对患者首次发病年龄进行关联分析，本研究根据首次发病年龄将患者分为青少年组和成年组，发现在成年人群中rs10043986与精神分裂症密切关联，而在青少年中两者无显著关联，这在一定程度上提示rs10043986在青少年和成年中可能作用不同。环境因素、病程因素及治疗因素等是否会造成青少年和成年病人的这种差异，仍需进一步探讨。

有研究认为，基因多态性与精神分裂症临床症状严重程度存在相关性[20-21]，本研究未发现rs10043986与精神分裂症临床症状严重程度存在明显关联。这可能与精神分裂症遗传异质性有关，不同亚型的易感基因可能不同。本研究未能对患者进行临床分型，这可能会导致研究结果与真实情况存在偏倚，仍需在今后的研究中进一步验证。

综上所述，本研究结果提示rs10043986中T等位基因可能增加中国维吾尔族女性和成年人群精神分裂症的易感性，其多态性与精神分裂症临床症状严重程度可能无关。但本次研究仍存在诸多不足：(1)研究遗传变异可能和疾病分型相关[22]，不同亚型的精神分裂症可能存在不同的生物学机制，本研究未能进行不同亚型的相关分析；(2)所纳入研究人群样本量在患者组和对照组中未能严格匹配，青少年研究对象较少，可能使研究结果统计效能不足；(3)研究位点单一，样本量偏小，以及难以排出对照组以后发生精神分裂症的可能性等。要全面揭示CMYA5基因的功能，下一步需在加强精神分裂症亚型的标准化诊断的基础上，扩大样本量，并结合基因功能网络更深入地探讨CMYA5基因在精神分裂症病因和发病机制中的作用。

参 考 文 献

[1] 沈渔邨.精神病学[M].5版.北京:人民卫生出版社,2009:504.

[2] Andrea S,Bernal M,Alkomiet H.The impact of environmental factors in severe psychiatric disorders[J].Front Neurosci,2014,8(19):1-10.

[3] Sullivan PF,Kendler KS,Neale MC.Schizophrenia as a complex trait:evidence from a meta-analysis of twin studies[J].Arch Gen Psychiatry,2003,60(12):1187-1192.

[4] Benson MA,Tinsley CL,Blake DJ.Myospryn is a novel binding partner for dysbindin in muscle[J].J Biol Chem,2004,279(11):10450-10458.

[5] Morris DW,Murphy K,Kenny N,et al.Dysbindin(DTNBP1) and the biogenesis of lysosome-related organelles complex 1(BLOC-1):main and epistatic gene effects are potential contributors to schizophrenia susceptibility[J].Biol Psychiatry,2008,63(1):24-31.

[6] Chen X,Lee G,Maher B,et al.GWA study data mining and independent replication identify cardiomyopathy-associated 5(CMYA5) as a risk gene for schizophrenia[J].Mol Psychiatry,2010,16(11):1117-1129.

[7] Zhang R,Zhang H,Li M,et al.Genetic analysis of common variants in the CMYA5(cardiomyopathy-associated 5) gene with schizophrenia[J].Prog Neuropsychopharmacol Biol Psychiatry,2013,46(10):64-69.

[8] Li M,Luo X-j,Zhang X,et al.A common variant of the cardiomyopathy associated 5 gene(CMYA5) is associated with schizophrenia in Chinese population[J].Schizophr Res,2011,129(2-3):217-219.

[9] Furukawa H,Tochigi M,Otowa T,et al.An association analysis of the cardiomyopathy associated 5(CMYA5) gene with schizophrenia in a Japanese population[J].Psychiatr Genet,2013,23(4):179-180.

[10] Wang Q,He K,Chen J,et al.The CMYA5 gene confers risk for both schizophrenia and major depressive disorder in Han Chinese population[J].World J Biol Psychiatry,2014,15(7):553-560.

[11] 何庆玲,张明园.阳性和阴性症状量表的中国常模和因子分析[J].中国临床心理学杂志,2000,8(2):65-69.

[12] Shi Y,He L.SHEsis,a powerful software platform for analyses of linkage disequilibrium,haplotype construction,and genetic association at polymorphism loci[J].Cell Res,2005,15(2):97-98.

[13] Watanabe Y,Shibuya M,Someya T.The cardiomyopathy-associated 5(CMYA5) gene and risk of schizophrenia:Meta-analysis of rs3828611 and rs4704591 in East Asian populations[J].Asian J Psychiatry,2014,7(1):95-96.

[14] Tsoupri E,Capetanaki Y.Myospryn:a multifunctional desmin-associated protein[J].Histochem Cell Biol,2013,140(1):55-63.

[15] 张丽丽,邹其忠.新疆维吾尔族人群中GRM7基因rs3749380多态性与精神分裂症的关联性研究[J].中华行为医学与脑科学杂志,2014,23(3):203-206.

[16] 温晓凤,魏铁华,吴小立,等.ZNF804A rs1344706多态性与精神分裂症的关联分析[J].中国神经精神疾病杂志,2013,39(4):213-217.

[17] Min JA,Kim H,Pae CU,et al.Association of estrogen receptor genes and schizophrenia:a preliminary study[J].Prog Neuropsychopharmacol Biol Psychiatry,2012,36(1):1-4.

[18] Hare E,Glahn DC,Dassori A,et al.Heritability of age of onset of psychosis in schizophrenia[J].Am J Med Genet B Neuropsychiatr Genet,2010,153(1):298-302.

[19] Harrison PJ,Tunbridge EM.Catechol-O-methyltransferase(COMT):a gene contributing to sex differences in brain function,and to sexual dimorphism in the predisposition to psychiatric disorders[J].Neuropsychopharmacology,2008,33(13):3037-3045.

[20] 郭伟云,李文强,梁红星,等.偏执型精神分裂症患者精神分裂症断裂基因1单核苷酸多态性研究[J].中华行为医学与脑科学杂志,2012,21(4):337-339.

[21] 翟金国,李军,陈敏,等.脑神经营养因子G169A基因多态性与精神分裂症患者认知功能的关系[J].中华行为医学与脑科学杂志,2013,22(6):494-496.

[22] Bergen SE,O'Dushlaine CT,Lee PH,et al.Genetic modifiers and subtypes in schizophrenia:Investigations of age at onset,severity,sex and family history[J].Schizophr Res,2014,154(1-3):48-53.

(收稿日期:2014-05-25)
(本文编辑:冯学泉)

中华医学会系列杂志

ISSN 1674-6554
CN 37-1468/R

中华行为医学与脑科学杂志®

ZHONGHUA XINGWEI YIXUE YU NAOKEXUE ZAZHI

2014年3月 第23卷 第3期

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

Volume 23 Number 3
March 2014

中华医学会
CHINESE MEDICAL ASSOCIATION

ISSN 1674-6554
03
9 771674 655148

中华行为医学与脑科学杂志®

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

月刊　1992年6月创刊　第23卷　第3期　2014年3月20日出版

主　管
中华人民共和国国家卫生和计划生育委员会

主　办
中华医学会
100710，北京市东四西大街42号
济宁医学院
272067，山东省济宁市荷花路16号

编　辑
中华行为医学与脑科学杂志编辑委员会
272067，山东省济宁市荷花路16号
电话：(0537)3616263
传真：(0537)3616263-87
Email:zxyxlqk@163169.net
http://www.zxyx.cn
http://www.zxyx.org.cn
http://www.medline.org.cn

总编辑
杨志寅

编辑部主任
张作记

出　版
《中华医学杂志》社有限责任公司
100710，北京市东四西大街42号
电话(传真)：(010)85158180
Email:office@cma.org.cn

广告经营许可证
3708000403019号

印　刷
济宁市火炬书刊印务中心

发　行
范围：公开
国内：济宁市报刊发行局
国外：中国国际图书贸易集团有限公司
（北京399信箱，100044）
代号M5269

订　购
全国各地邮政局
邮发代号　24-115

邮　购
中华行为医学与脑科学杂志编辑部
272067，山东省济宁市荷花路16号
电话：(0537)3616263
传真：(0537)3616263-87
Email:zxyxlqk@163169.net
http://www.zxyx.cn
http://www.zxyx.org.cn

定　价
每期15.00元，全年180.00元

中国标准连续出版物号
ISSN 1674-6554
CN 37-1468/R

2014年版权归中华医学会所有

未经授权，不得转载、摘编本刊文章，不得使用本刊的版式设计

除非特别声明，本刊刊出的所有文章不代表中华医学会和本刊编委会的观点

本刊如有印装质量问题，请向本刊编辑部调换

目　次

临床研究

基础研究

· 临床研究 ·

新疆维吾尔族人群中 *GRM7* 基因 rs3749380 多态性与精神分裂症的关联性研究

张丽丽 伊其忠

【摘要】 **目的** 探讨 *GRM7* 基因多态性与中国维吾尔族人群中精神分裂症的关联。**方法** 利用 Taqman 探针分型技术对 360 例维吾尔族精神分裂症患者及 384 例维吾尔族非精神分裂症对照进行 GRM7 基因 rs3749380 位点基因分型，使用阳性和阴性症状量表对精神分裂症患者进行临床精神病性症状的评定，利用 SHEsis、SPSS 17.0 软件系统进行统计分析。**结果** 在病例组中，*GRM7* 基因 rs3749380 位点的 C 等位基因频率为 60.1%，T 等位基因频率为 39.9%，对照组中 C 等位基因频率为 58.6%，T 等位基因频率为 41.4%；病例组中 C/C 基因型频率为 38.6%，T/C 基因型频率为 43.1%，T/T 基因型频率为 18.3%，对照组中 C/C 基因型频率为 35.9%，T/C 基因型频率为 45.4%，T/T 基因型频率为 18.7%，两组间 rs3749380 位点基因型与等位基因频率差异无统计学意义（$P>0.05$）；青少年病例组与成年病例组间，青少年病例组与青少年对照组，成年病例组与成年对照组间，rs3749380 位点基因型与等位基因频率差异无统计学意义（$P>0.05$）；不同性别病例组与对照组间，rs3749380 位点基因型与等位基因频率差异无统计学意义（$P>0.05$）；各基因型与精神分裂症症状无明显相关性（$P>0.05$）。**结论** GRM7 基因 rs3749380 多态性和中国维吾尔族人群精神分裂症的发病无明显关联。

【关键词】 精神分裂症； GRM7 基因； 单核苷酸多态性； 关联性研究

Association of *GRM7* gene rs3749380 polymorphism with schizophrenia in Uygur Chinese populations

Zhang Lili, Yi Qizhong. Psychological Medicine Center, The First Affiliated Hospital to Xinjiang Medical University, Urumqi 830054, China

【**Abstract**】 **Objective** To investigate the association between *GRM7* gene polymorphism and schizophrenia in the Uygur Chinese population. **Methods** rs3749380 at the *GRM7* gene was selected for genotyping in a Uygur Chinese patients-control sample (case = 360, control = 384) by Taqman assays. The symptoms of schizophrenia were assessed by positive and negative syndrome scale (PANSS). SHEsis on line and SPSS 17.0 soft were used for calculating the data. **Results** For rs3749380 at *GRM7* gene, the C allele frequency was 60.1%, and T allele frequency was 39.9% in the patients. In the control, the C allele frequency was 58.6%, and T allele frequency was 41.4%. In the patients, the C/C genotype frequency was 38.6%, T/C genotype frequency was 43.1%, and T/T genotype frequency was 18.3%. In the controls, the C/C genotype frequency was 35.9%, T/C genotype frequency was 45.4%, and T/T genotype frequency was 18.7%. rs3749380 was not found to have genotypic or allelic association with schizophrenia ($P>0.05$). And genotypic or allelic association with schizophrenia were not found between adolescent and adult cases, adolescent cases and controls or adult cases and controls ($P>0.05$). The results showed rs3749380 genotypic or allelic was not association with schizophrenia in the different gender of the population ($P>0.05$). The genotypes were not significantly correlated with symptoms of schizophrenia ($P>0.05$). **Conclusion** There is no association of *GRM7* gene rs3749380 polymorphism with the incidence of schizophrenia in Uygur Chinese.

【**Key words**】 Schizophrenia; *GRM7* gene; Single nucleotide polymorphism; Association study

精神分裂症是一种患病率高，危害性大，具有反复发作倾向，病程较长的精神疾病，以精神活动和客观现实不协调及精神活动不协调为核心症状，造成社会功能损害。部分病人最终会导致精神衰退和精神残疾。流行病学调查发现，精神分裂症的患病率大约为 1%[1]。然而，其发病机制至今尚不明确。有研究表明，代谢型谷氨酸受体 7 亚型（metabotropic glutamate receptor 7, *GRM7*）基因作为精神分裂症的一个候选基因，有可能在精神分裂症的发生、发展过程中发挥了一定的作用。有研究明确指出 *GRM7* 基因多态性在精神分裂症的发病机制中意义重大[2-4]。如 Ohtsuki 等[4]的研究中，以日本人群为研究对象，选取了 2293 例精神分裂症患者及 2382 例非精神分裂症对照进行等位基因及基因型分析，在 *GRM7* 基因中的 25 个单核苷酸多态性（SNP）位点，只有 rs3749380（T/C）位点的等位基因分布频率在病例组与对照组间的差异有统计学意义（等位基因 $P=0.009$）。既往关于 *GRM7* 基因多态性与精神分裂症关联性的研究大多在印度和日本人群中进行，我国

[doi:10.3760/cma.j.issn.1674-6554.2014.03.004]

[基金项目：国家自然科学基金项目（81360209）；"973"计划子课题（2009CB522601）；"863"计划子课题（2008AA02A407）；自治区自然科学基金项目（2010211A51）]

[作者单位：830054 乌鲁木齐市，新疆医科大学第一附属医院心理医学中心（张丽丽）；伊其忠]

[通讯作者：伊其忠，Email：yqzhongyi@126.com]

目前此方面的报道极少，尤其是新疆维吾尔族人群的研究。为了进一步了解 *GRM7* 基因多态性是否与新疆维吾尔族精神分裂症存在关联，本研究以新疆维吾尔族人群为研究对象，选取 *GRM7* 基因中的位点 rs3749380(T/C)进行基因分型，比较病例组与对照组等位基因与基因型的分布差异。

对象与方法

一、对象

1.病例组：均来自新疆医科大学第一附属医院及新疆维吾尔自治区其他精神病专科医院的住院及门诊病人。诊断和临床资料的收集由两名副高以上职称精神科医师组成的小组完成。采用定式临床检查(SCID)进行精神检查，用阳性与阴性症状量表(PANSS)及简明精神病量表(BPRS)评定患者病情的严重程度，所有病人均符合《美国精神障碍诊断与统计手册第 4 版》(DSM-IV)精神分裂症的诊断标准。排除：(1)中枢神经系统及脑外伤患者。(2)其他精神疾病与精神发育迟滞患者。(3)物质依赖与物质成瘾者。(4)严重的躯体疾病患者，包括肿瘤，内分泌系统疾病，严重的心、脑血管疾病及肝肾功能损伤。(5)有阳性精神疾病家族史者。(6)孕期、哺乳期及月经期女性。共计 360 例，男性 213 例，女性 147 例，年龄范围：12~83 岁，平均(37±13)岁。按发病年龄分组：首次发病年龄≤18 岁患者为青少年病例组，共计 63 例；首次发病年龄>18 岁患者为成年病例组，共计 297 例。

2.对照组：均来自新疆医科大学第一附属医院体检中心的体检人员，身体健康，无遗传性精神疾病家族史，共计 384 例，男性 213 例，女性 171 例，年龄范围：18~80 岁，平均(38±14)岁。按年龄分组：年龄≤18 岁者为青少年对照组，共计 49 例；年龄>18 岁者为成年对照组，共计 335 例。

所有研究对象均来自世居于新疆维吾尔自治区，无生物学亲缘关系的维吾尔族人群。在实验进行前，对所有受试者进行本次研究目的详细解释，患者本人及第一监护人签署知情同意书，并通过新疆医科大学第一附属医院伦理委员会的审查。

二、方法

1.DNA 提取方法：取病例及对照的肘静脉血 2 ml，利用血液基因组 DNA 提取系统(Tiangen Biotech(Beijing) CO.LTD)提取外周血 DNA，用于基因分型。

2.单核苷酸多态性(SNP)的筛选：总结大量 *GRM7* 基因多态性与精神分裂症关联性研究的成果，我们选取候选 SNP：rs3749380，其位于 *GRM7* 基因的 1 号外显子区，最小等位基因频率>0.1。本次研究，以新疆维吾尔族人群为研究对象，对 rs3749380 进行基因分型的研究。

3.rs3749380 基因分型方法：本研究采用 Taqman 探针分型技术，所使用的基因分型探针及 TaqManUniversal PCR MasterMix 均由美国应用生物系统公司(ABI)定制。PCR 反应在 GeneAmp PCR 9700 系统(Applied Biosystem)中进行。PCR 反应体系 5 μl，包括：40 ng DNA，2.5 μl PCR 反应混合液(TaqManUniversal PCR MasterMix)和 0.035 μl 的 Taqman 探针。反应条件设定为：94 ℃ 1 min；94 ℃ 30 s，72 ℃ 30 s，30 个循环；4 ℃保温。反应产物利用 ABI 7900 DNA 检测系统(Applied Biosystem，Foster City，CA，USA)进行基因型的确定。其中有 98.7%对照及 98.6%病例的基因型被成功确定。为保证基因分型的准确性及可靠性，本研究从两组中随机选取 3%的样本再次进行基因分型，发现与之前的实验结果相一致。

因 Choi 等[5]在研究中指出 *GRM7* 基因作为精神分裂症的候选基因，在前额叶皮质中的表达是随着年龄发生变化的，在发育过程中如出现异常，可能会导致疾病的发生。因此，为了了解 *GRM7* 基因多态性是否与精神分裂症的首次发病年龄存在关联，本项研究将在新疆维吾尔族人群中分析 rs3749380 多态性是否与精神分裂症的首次发病年龄存在相关性。同时还将按性别进行分组，分别比较病例组与对照组中是否存在等位基因及基因型分布频率的差异。除此，有研究发现基因多态性可能与精神分裂症的临床症状存在明显关联，例如：PRODH 基因的 rs385440 与住院精神分裂症的"被动或谈漠社交"、"判断和自知力缺乏"症状严重程度相关联[6]；DISC1 基因的 rs821616 与 PANSS 阳性症状等存在关联[7]；BDNF 基因 G196A 多态性与精神分裂症阳性症状相关[8]。因此在本研究中，也将进行 rs3749380 多态性与疾病症状的关联性分析。

4.统计分析：利用 EXCEL 软件建立数据库，采用 SPSS17.0 软件对两组人群的年龄进行 *t* 检验，对性别进行 χ^2 检验，利用 SHEsis 软件平台(http://analysis.bio-x.cn)进行病例组与对照组的 Hardy-Weinberg 遗传平衡度检验、组间等位基因和基因型频率差异及 *OR* 值的分析。病例组 rs3749380 基因型与精神分裂症的症状做关联性分析，依据数据是否符合方差齐性，分别进行方差分析和秩和检验。以 $P<0.05$ 为差异有统计学意义。

结　果

一、病例组与对照组一般人口学资料分析

经 SPSS17.0 软件分析，两组受试者在年龄、性别上均差异无统计学意义(年龄：$P=0.086$，性别：$P=0.228$)。

二、Hardy-Weinberg 遗传平衡度检验

经 Hardy-Weinberg 遗传平衡度(HWE)检验，各组 *GRM7* 基因中位点 rs3749380 多态性基因型符合 Hardy-Weinberg 平衡(HWE)定律($P>0.05$)。

三、精神分裂症与 *GRM7* 基因 rs3749380 多态性的关联性研究结果

经 SHEsis 软件剔除未能成功测定基因型的病例及对照组各 5 例，数据处理后发现，病例组与对照组 *GRM7* 基因 rs3749380 多态性等位基因与基因型总体差上均差异无统计学意义（*P*>0.05）。见表 1。

表 1 病例组与对照组间等位基因与基因型频率的比较[例(%)]

组别	等位基因		OR	P值	基因型			P值
	T	C			C/C	T/C	T/T	
病例组	283(70.9)	427(80.1)	1.067	0.501	137(34.6)	155(43.1)	65(16.3)	0.743
对照组	314(71.4)	446(58.6)			136(35.9)	172(45.4)	71(18.7)	

注：*OR* 95% *CI* 为 0.866～1.314

四、精神分裂症首发年龄与 *GRM7* 基因 rs3749380 多态性的关联性

青少年、成年病例组间进行等位基因与基因型分布频率的比较，均差异无统计学意义（*P*>0.05）；青少年病例组、对照组，成年病例组、对照组间分别进行等位基因与基因型分布频率的比较，各组间均差异无统计学意义（*P*>0.05）。也就是说，本研究并未发现 *GRM7* 基因 rs3749380 多态性与新疆维吾尔族精神分裂症的首次发病年龄存在明显关联性。见表 2、3。

表 2 青少年、成年病例组间等位基因与基因频率的比较[例(%)]

组别	等位基因		OR	P值	基因型			P值
	T	C			C/C	T/C	T/T	
青少年病例组	53(42.7)	71(57.3)	1.159	0.438	20(9.0)	29(46.8)	12(19.4)	0.706
成年病例组	230(36.2)	356(63.8)			116(18.6)	124(42.3)	55(19.1)	

注：*OR* 95% *CI* 为 0.789～1.180

表 3 青少年病例组、成年病例组分别与青少年对照组、成年对照组等位基因与基因频率的比较[例(%)]

组别	等位基因		OR	P值	基因型			P值
	T	C			C/C	T/C	T/T	
青少年病例组	53(42.7)	71(57.3)	0.655	0.195	20(32.0)	29(46.8)	13(21.0)	0.362
青少年对照组	102(51.3)	96(48.7)			52(52.5)	38(38.4)	9(9.1)	
成年病例组	230(36.2)	406(63.8)	1.230	0.220	116(36.5)	124(39.0)	78(24.5)	0.013
成年对照组	282(42.6)	380(57.4)			114(34.5)	152(46.0)	64(19.4)	

注：青少年病例组与对照组 *OR* 95% *CI* 为 0.385～1.165；成年病例组与对照组 *OR* 95% *CI* 为 0.916～1.440

五、精神分裂症中不同性别组与 *GRM7* 基因 rs3749380 多态性的关联性研究结果

按性别分组后，男性病例组与对照组、女性病例组与对照组间，等位基因与基因型频率均差异无统计学意义（*P*>0.05）。这意味着，新疆维吾尔族精神分裂症患者的性别与 *GRM7* 基因的 rs3749380 多态性不存在明显关联。见表 4。

六、*GRM7* 基因 rs3749380 基因型与 PANSS 量表分的相关分析

经数据分析，只有 PANSS 量表总分符合方差齐性，对其进行方差分析，对阳性量表分、阴性量表分进行秩和检验后可知，均差异无统计学意义（*P*>0.05），即 *GRM7* 基因多态性与精神分裂症临床症状可能不存在明显相关性。见表 5。

表 4 不同性别间病例组与对照组等位基因与基因频率比较[例(%)]

组别	等位基因		OR	P值	基因型			P值
	T	C			C/C	T/C	T/T	
男性病例组	105(41.7)	147(58.3)	1.000	0.809	47(37.2)	53(42.1)	26(20.6)	0.759
男性对照组	170(42.6)	241(57.4)			72(34.3)	97(46.2)	41(19.5)	
女性病例组	153(39.2)	275(80.8)	1.021	0.857	58(40.3)	59(41.0)	27(18.8)	0.832
女性对照组	135(39.9)	205(60.1)			64(37.9)	75(44.4)	30(17.8)	

注：男性病例组与对照组 *OR* 95% *CI* 为 0.758～1.427；女性病例组与对照组 *OR* 95% *CI* 为 0.747～1.420

表 5 不同基因型患者 PANSS 评分比较（分，$\bar{x}\pm s$）

项目	rs3749380			P值
	C/C	T/C	T/T	
总分	73.5±13.5	73.7±14.5	74.9±15.2	0.812
阳性量表分	18.0±5.9	18.2±6.8	19.0±5.4	0.404
阴性量表分	23.8 ±6.7	23.2±7.9	23.6±5.9	0.647

讨 论

有研究证实，异常的谷氨酸传导途经可能与精神分裂症的发病存在相关关系。已有学者在基因及蛋白质水平对精神分裂症患者的谷氨酸受体进行了大量研究[9-11]。这些受体参与的生物过程在人类大脑发育中发挥重要作用[12-13]。例如，N-甲基-D-天门冬氨酸受体 3A 亚基（GRIN3A），可抑制树突的生长，在出生时高表达，在成年过程中呈逐渐下降趋势，而在精神分裂症患者中，发现整个成年期，GRIN3A 在前额叶皮质中一直处于高表达状态[14-15]；而氨甲基磷酸受体亚单位 GluR1（GRIA1）也被报道在精神分裂症患者的一生中都处于较高的表达水平[16]；G 蛋白耦联受体家族中的谷氨酸代谢型受体，则参与了神经干细胞/原始细胞的增殖、分化、存活等最基本的生命过程。在大脑发育的初始阶段有着至关重要的作用，Choi 等[5] 在研究中指出，很多精神分裂症候选基因在前额叶皮质中的表达是随着不同的年龄阶段而改变的，这些基因在发育的关键阶段出现表达异常都会导致精神分裂症的发病，其中包括谷氨酸代谢型受体中的 *GRM 7*、*GRM 5*。因此，推测编码谷氨酸受体的基因与精神分裂症的发病是密切相关的。通过总结近年来对谷氨酸受体基因与精神分裂症关联性的研究已发现，谷氨酸代谢型受体第Ⅲ家族基因在精神分裂症的发生、发展过程中发挥了重要作用[17-20]。有研究证实了 *GRM 4* 基因的单核苷酸多态性与精神分裂症密切相关[21-22]。Takaki 等[23] 也发现 *GRM 8* 基因中，SNP4-SNP5-SNP6（*P*=0.015）和 SNP5-SNP6-SNP7（*P*=0.0022）与日本人群中精神分裂症的发病密切关联。

本研究首次以中国维吾尔族人群作为研究对象，

探讨精神分裂症与谷氨酸代谢型受体 *GRM7* 基因多态性是否存在关联性。但通过对相关数据的分析，并未得到阳性结果。

本研究得到的结论与 Ohtsuki 等[4]的结论不一致，考虑可能有以下原因：(1)与所选研究人群相关，中国维吾尔族人群有本民族的特殊性，既有欧洲人群(属高加索人种)，又有亚洲人群(属蒙古利亚人种)的遗传特色。维吾尔族和汉族精神分裂症患者的临床表现因民族的社会文化背景和生活习俗的不同而有一定的差异。维吾尔族较少与汉族人群通婚的情况，因此保有本民族的遗传特色，并不严格服从亚洲人群或是欧洲人群的基因频率分布情况，因此，在维吾尔族人群与日本人群中得到不完全一致的结果可能是由于基因分布频率差异所致；(2)很多文献指出[27-30]，一些 SNPs 是和疾病的不同分型相关的，但是，现有的临床资料并没有给出精神分裂症具体的分型诊断，因此我们未能进行相关性的分析。(3)所选位点单一，且样本量较小，并不一定能反映各自群体的客观情况。本研究首次选择中国维吾尔族人群作为研究对象，对 *GRM7* 基因多态性与该人群中精神分裂症的相关性做了报道，尽管未能找到明显的证据证实二者之间的关联性，但仍需要改善试验中的局限性做进一步深入的研究。

参 考 文 献

[1] Barr CE, Mednick SA, Munk-Jorgensen P. Exposure to influenza epidemics during gestation and adult schizophrenia: a 40-year study[J]. Arch Gen Psychiatry, 1990, 47(9): 869-874.

[2] Gupta C, Schwab SG, Amir N, et al. A family-based association study of DNA sequence variants in GRM7 with schizophrenia in an Ashkenazi-Russian population[J]. Int J Neuropsychopharmacol, 2009, 12(9): 1283-1289.

[3] Shibata H, Tani A, Chikahara T, et al. Association study of polymorphisms in the group III metabotropic glutamate receptor genes, GRM4 and GRM7, with schizophrenia[J]. Psychiatry Res, 2009, 167(1-2): 88-96.

[4] Ohtsuki T, Koga M, Ishiguro H, et al. A polymorphism of the metabotropic glutamate receptor mGluR7 (GRM7) gene is associated with schizophrenia[J]. Schizophr Res, 2008, 101(1-3): 9-16.

[5] Choi KH, Zepp ME, Higgs BW, et al. Expression profiles of schizophrenia susceptibility genes during human prefrontal cortical development[J]. J Psychiatry Neurosci, 2009, 34(6): 450-458.

[6] 苏莉，韦波，陈强，等. 广西壮族、汉族精神分裂症与 PRODH 基因 rs385440 多态性的关联研究[J]. 中华行为医学与脑科学杂志, 2012, 21(1): 36-39.

[7] 郭伟云，李文强，张红星，等. 偏执型精神分裂症患者精神分裂症照裂基因 1 单核苷酸多态性研究[J]. 中华行为医学与脑科学杂志, 2012, 21(4): 337-339.

[8] 翟金国，李军，陈敏，等. 脑神经营养因子 G196A 基因多态性与精神分裂症患者认知功能的关系[J]. 中华行为医学与脑科学杂志, 2013, 22(6): 494-496.

[9] Stone JM, Morrison PD, Pilowsky LS. Glutamate and dopamine dysregulation in schizophrenia--a synthesis and selective review[J]. J Psychopharmacol, 2007, 21(4): 440-452.

[10] Tamminga CA. The neurobiology of cognition in schizophrenia[J]. J Clin Psychiatry, 2006, 67: 9-13.

[11] Coyle JT. The glutamatergic dysfunction hypothesis for schizophrenia[J]. Harv Rev Psychiatry, 1996, 3(5): 241-253.

[12] Catania MV, D'Antoni S, Bonaccorso CM, et al. Group I metabotropic glutamate receptors: A role in neurodevelopmental disorders?[J]. Mol Neurobiol, 2007, 35(3): 298-307.

[13] Mattson MP, Lee RE, Adams ME, et al. Interactions between entorhinal axons and target hippocampal neurons: a role for glutamate in the development of hippocampal circuitry[J]. Neuron, 1988, 1(9): 865-876.

[14] Contestabile A. Roles of NMDA receptor activity and nitric oxide production in brain development[J]. Brain Res Brain Res Rev, 2000, 32(2-3): 476-509.

[15] du Bois TM, Huang XF. Early brain development disruption from NMDA receptor hypofunction: relevance to schizophrenia[J]. Brain Res Rev, 2007, 53(2): 260-270.

[16] Sasaki YF, Rothe T, Premkumar LS, et al. Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons[J]. J Neurophysiol, 2002, 87(4): 2052-2063.

[17] Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches[J]. Neuron, 2004, 44(1): 5-21.

[18] Choi DW. Glutamate neurotoxicity and diseases of the nervous system[J]. Neuron, 1988, 1(8): 623-634.

[19] Mueller HT, Meador-Woodruff JH. NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder[J]. Schizophr Res, 2004, 71(2-3): 361-370.

[20] O'Connor JA, Hensler SE. Elevated GRIA1 mRNA expression in Layer II/III and V pyramidal cells of the DLPFC in schizophrenia[J]. Schizophr Res, 2007, 97(1-3): 277-288.

[21] Carter CJ. eIF2B and oligodendrocyte survival: where nature and nurture meet in bipolar disorder and schizophrenia?[J]. Schizophr Bull, 2007, 33(6): 1343-1353.

[22] Fallin MD, Lasseter VK, Avramopoulos D, et al. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios[J]. Am J Hum Genet, 2005, 77(6): 918-936.

[23] Fukuda H, Kikuchi R, Shibata H, et al. Positive associations of polymorphisms in the metabotropic glutamate receptor type 8 gene (GRM8) with schizophrenia[J]. Am J Med Genet B Neuropsychiatr Genet, 2004, 128B(1): 6-14.

[24] Makoff A, Pilling C, Harrington K, et al. Human metabotropic glutamate receptor type 7: molecular cloning and mRNA distribution in the CNS[J]. Brain Res Mol Brain Res, 1996, 40(1): 165-170.

[25] Kinoshita A, Shigemoto R, Ohishi H, et al. Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and mGluR7b, in the central nervous system of the adult rat and mouse: a light and electron microscopic study[J]. J Comp Neurol, 1998, 393(3): 332-352.

[26] Kosinski CM, Risso Bradley S, Conn PJ, et al. Localization of metabotropic glutamate receptor 7 mRNA and mGluR7a protein in the rat basal ganglia[J]. J Comp Neurol, 1999, 415(2): 266-284.

[27] Shigemoto R, Kulik A, Roberts JD, et al. Target-cell-specific concentration of a metabotropic glutamate receptor in the presynaptic active zone[J]. Nature, 1996, 381(6582): 523-525.

[28] Boudin H, Doan A, Xia J, et al. Presynaptic clustering of mGluR7a requires the PICK1 PDZ domain binding site[J]. Neuron, 2000, 28(2): 485-497.

[29] Rousseau F, Katsel P, Davis KL, et al. Molecular and genetic evidence for abnormalities in the nodes of Ranvier in schizophrenia. Arch Gen Psychiatry, 2012, 69(1): 7-15.

[30] Sanz E, Brunet A, Amengol L, et al. Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients[J]. J Psychiatr Res, 2010, 44(14): 971-978.

(收稿日期：2013-10-15)
(本文编辑：冯学泉)

中华医学会系列杂志

ISSN 1674-6554
CN 37-1468/R

中华行为医学与脑科学杂志®

ZHONGHUA XINGWEI YIXUE YU NAOKEXUE ZAZHI

2015年1月 第24卷 第1期

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

Volume 24 Number 1
January 2015

中華醫學會

CHINESE MEDICAL ASSOCIATION

ISSN 1674-6554

中华行为医学与脑科学杂志®

CHINESE JOURNAL OF BEHAVIORAL MEDICINE AND BRAIN SCIENCE

月刊　1992 年 6 月创刊　第 24 卷　第 1 期　2015 年 1 月 20 日出版

主　管
中华人民共和国国家卫生和计划生育委员会

主　办
中华医学会
100710，北京市东四西大街 42 号
济宁医学院
272067，山东省济宁市荷花路 16 号

编　辑
中华行为医学与脑科学杂志编辑委员会
272067，山东省济宁市荷花路 16 号
电话：(0537)3616263
传真：(0537)3616263-87
Email：zwxykdb@163.169.net
http://www.zwyx.cn
http://www.zwyx.org.cn
http://www.medline.org.cn

总编辑
白　波

编辑部主任
张作记

出　版
《中华医学杂志》社有限责任公司
100710，北京市东四西大街 42 号
电话（传真）：(010)85158180
Email：office@cma.org.cn

广告经营许可证
3708004003021 号

印　刷
济宁市火炬书刊印务中心

发　行
范围：公开
国内：济宁市报刊发行局
国外：中国国际图书贸易集团有限公司
（北京 399 信箱，100044）
代号 M5269

订　购
全国各地邮政局
邮发代号　24-115

邮　购
中华行为医学与脑科学杂志编辑部
272067，山东省济宁市荷花路 16 号
电话：(0537)3616263
传真：(0537)3616263-87
Email：zwxykdb@163.169.net
http://www.zwyx.cn
http://www.zwyx.org.cn

定　价
每期 15.00 元，全年 180.00 元

中国标准连续出版物号
ISSN 1674-6554
CN 37-1468/R

2015 年版权归中华医学会所有

未经授权，不得转载、摘编本刊文章，不得使用本刊的版式设计

除非特别声明，本刊刊出的所有文章不代表中华医学会和本刊编委会的观点

本刊如有印装质量问题，请向本刊编辑部调换

目　次

本期责任编辑 冯学泉 英文编辑 张玫美 杨静 责任排版 张建设 英文审校 王福顺 朱滨

· 临床研究 ·

心肌病相关蛋白5基因rs10043986多态性与中国维吾尔族人群精神分裂症关联研究

Association analysis of the rs10043986 polymorphism of cardiomyopathy-associated 5 gene with schizophrenia in Uygur Chinese population

境因素共同作用的结果，其遗传度为 80%[1]，因此探讨遗传因素与精神分裂症病因及发病机制对该病的诊断及个体化治疗具有重要意义。有研究发现，心肌病相关蛋白 5（cardiomyopathy-associated 5，CMYA5）基因编码的蛋白为 myospryn，属于 TRIM 蛋白家族，CMYA5 基因变异与杜氏营养不良、心肌病及高血压相关，又能引起精神症状，增加精神分裂症的患病风险[2-3]。Chen 等[4] 通过全基因组关联研究（genome wide association studies，GWAS）发现，CMYA5 基因 rs10043986 多态性可能增加精神分裂症的易感性，并对筛选结果在多个欧洲高加索人群的核心家系病例对照研究中得到了重复验证[4]。然而，近年来在以中国汉族人群和日本人群为对象的多项研究中均未发现 rs10043986 位点在亚洲人群中呈现多态性现象[5-10]。CMYA5 基因在不同种族人群中可能存在遗传异质性。中国新疆维吾尔族人群长期居住在中国西北地区，具有独特的生活环境和习俗且极少与其他民族通婚，适合进行遗传学研究。本研究对维吾尔族人群 CMYA5 基因 rs10043986 位点基因型进行分析，进一步探讨该位点是否也与中国维吾尔族人群精神分裂症存在关联。

对象与方法

一、对象

1.病例组：来自于新疆医科大学第一附属医院及新疆维吾尔自治区其他精神病专科医院住院的精神分裂症患者。每位患者由两名副高以上职称精神科医师根据《美国精神障碍诊断与统计手册第 4 版》（diagnostic and statistical manual of mental disorders-IV，DSM-IV）精神分裂症的诊断标准做出诊断，采用适于 DSM-IV的结构式临床访谈（structured clinical interview for DSM-IV，SCID）进行精神检查，用阳性与阴性症状量表（PANSS）[11] 评定患者病情的严重程度。排除标准：（1）患有严重躯体疾病或脑器质性疾病；（2）其它精神疾病及精神发育迟滞患者；（3）物质依赖与物质成瘾者；（4）有阳性精神疾病家族史者；（5）孕期、哺乳期及月经期女性。共入组 325 例，分为男性组（193 例）和女性组（132 例）；按首次发病年龄分为：青少年组（首次发病年龄<18 岁，63 例）和成年组（首次发病年龄≥18 岁，262 例）。研究对象的年龄范围：13～84 岁，平均年龄（38.5±12.9）岁；首次发病年龄：10～54 岁，平均年龄（24.6±8.5）岁。

2.对照组：均来自新疆医科大学第一附属医院体检中心的体检人员，身体健康，与患者组无血缘关系，经过 SCID 非病人版本的精神检查，无精神疾病既往史及家族史。共入组 183 例，分为男性组（107 例）和女性组（76 例）；按年龄分为：青少年组（年龄<18 岁，14 例）、成年组（年龄≥18 岁，169 例）。对照组年龄范围：16～68 岁，平均年龄（37.7±11.6）岁。患者组和对照组的性别（$X^2=0.04$，$P=0.84$）和年龄（$t=0.70$，$P=0.48$）分布差异均无统计学意义。

所有研究对象均来自世居于新疆维吾尔自治区、无生物学亲缘关系的维吾尔族人群。该研究通过新疆医科大学第一附属医院伦理委员会审查，向所有受试者详细解释本次研究目的，并由患者本人及其监护人签署知情同意书。

二、方法

1.标本收集与基因组 DNA 提取：采集所有研究对象的肘静脉血 2 ml，EDTA 抗凝，-20 ℃冰箱保存，采用血液基因组 DNA 提取系统（Tiangen Biotech CO. LTD，Beijing，China）提取 DNA，用 NanoDrop1000 分光光度计对 DNA 样本浓度、纯度进行测定并予以记录，使纯度（ODA280/A260）在 1.6～1.9 之间，浓度在 200～500 ng/μl 之间，不符合要求的重新提取。提取的 DNA 放置于-80 ℃保存备用。

2.单核苷酸多态性（SNP）的筛选：根据已报道的 CMYA5 基因多态性与精神分裂症关联性研究的成果[4]，选择与欧洲高加索人群精神分裂症有阳性关联的 CMYA5 基因外显子区非同义突变 SNP：rs10043986，最小等位基因频率为 0.097，该基因在中国汉族人群（HCB）和日本人群（JPT）基因组单体型图中无多态性（http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10043986）。本研究以中国维吾尔族人群为研究对象，对 rs10043986 位点基因型进行分析。

3.SNP 基因型检测：采用 TaqMan 荧光探针 SNP 基因分型技术对 CMYA5 基因 rs10043986 位点进行分型，所使用的荧光探针及 TaqMan® Genotyping Master Mix 均由美国应用生物系统公司（ABI）定制。PCR 反应在 GeneAmp PCR 9700 系统（Applied Biosystem）中进行。PCR 反应体系为 5 μl，包括模板 40 ng DNA，PCR 反应混合液（TaqMan® Genotyping Master Mix）2.5 μl 和 TaqMan 探针 0.035 μl。反应条件为：94 ℃ 15 min；（94 ℃ 20 s，60 ℃ 1 min）×55 个循环。PCR 反应产物利用 ABI 7900 DNA 检测系统（Applied Biosystem，Foster City，CA，USA）进行荧光信号读取，完成各待测样本基因型的确定。为保证基因分型的准确性及可靠性，本研究随机抽取 5%的样本两次进行基因分型，两次分型结果一致性为 100%。

4.统计分析：使用 SPSS 17.0 进行统计分析，计量资料采用 $\bar{x}\pm s$ 进行统计描述，运用 t 检验比较两组基因型患者 PANSS 量表得分；利用 SHEsis 软件平台（http://analysis.bio-x.cn）[12] 进行两组对象的 Hardy-Weinberg 遗传平衡度检验，组间等位基因和基因型分布频率差异、优势比（odds ratio，OR）及 95%可信区间（95% confidence interval，95% CI）分析。所有的统计检验均为双侧检验，以 $P<0.05$ 为差异有统计学意义。

结　果

一、Hardy-Weinberg 遗传平衡检验

病例组（$X^2=0.903$，$P=0.342$）和对照组（$X^2=$ [illegible]，$P=0.618$）基因分布频率均符合 Hardy-Weinberg 平衡。CMYA5 基因 rs10043986 位点的等位基因和基因型频率见表 1。

二、病例组与对照组 rs10043986 位点等位基因及基因型频率比较

病例组与对照组间等位基因（$X^2=9.038$，$P=$ [illegible]）和基因型（$X^2=9.417$，$P=0.009$）频率分布均差异有统计学意义。病例组携带 T 等位基因的频率高于对照组[$OR=0.398$，95% CI（0.215–0.740）]；按性别分层比较，发现男性病例组与男性对照组的等位基因和基因型分布均差异无统计学意义（均 $P>0.05$），女性病例组与女性对照组的等位基因（$X^2=11.812$，$P=0.001$）和基因型（$X^2=12.769$，$P=0.001$）频率分布均差异有统计学意义，女性病例组携带 T 等位基因频率高于女性对照组[$OR=0.117$，95% CI（0.027–0.499）]。见表 1。

三、首次发病年龄与 CMYA5 基因 rs10043986 多态性关联分析

青少年病例组与对照组间等位基因（$X^2=0.529$，$P=$ [illegible]）和基因型（$X^2=0.604$，$P=0.437$）分布均差异无统计学意义（$P>0.05$）；成年病例组和对照组等位基因（$X^2=8.219$，$P=0.004$）和基因型（$X^2=8.379$，$P=$ [illegible]）分布均差异有统计学意义（$P<0.05$），成年病例组携带 T 等位基因频率高于成年对照组[$OR=0.369$，95% CI（0.182–0.748）]。见表 2。

四、CMYA5 基因 rs10043986 多态性与精神分裂症患者 PANSS 量表评分关联分析

病例组中 C/C 基因型和 C/T 基因型患者在阳性量表分、阴性量表分、一般精神病理症状分及 PANSS 量表总分均差异无统计学意义（均 $P>0.05$）。见表 3。

讨　论

人类 CMYA5 基因又名 myospryn，位于 5q14.1 区域，含有 11kb 和 13 个外显子区，在心脏、骨骼肌和中枢神经系统中均有表达，但其功能仍不完全清楚[14]。CMYA5 与 Dysbindin 蛋白激酶 A 的调节亚基等存在直接相互作用，它们参与了溶酶体相关细胞器生物发生复合体作用和蛋白激酶 A 信号转导的细胞过程，这些生物学过程已被多项研究报道在精神分裂症中发挥着重要作用，这种交互作用提示 CMYA5 可能与精神分裂症有关[15-16]。目前有关 CMYA5 与精神分裂症的关系研究报道较少且阳性位点也不尽相同[6,10]，Chen 等[14] 通过 GWAS 和验证分析发现非同义突变位点 rs10043986 与欧洲高加索人群的精神分裂症存在关联性，但在中国汉族和日本人群中 rs10043986 未呈现多态性现象[7,10]。本研究首次以中国维吾尔族人群为对象，探讨 rs10043986 与精神分裂症的关联性。

本研究发现病例组中 rs10043986 的 T 等位基因频率增加，说明 rs10043986 与维吾尔族的精神分裂症可能相关，与欧洲高加索人群研究结果一致[6]，与中国汉族和日本人群研究结果不同[7,10]。既往研究认为，中国维吾尔族人群有其特殊的遗传特色[17]，这可能同新疆维吾尔族生活的地域环境处于古丝绸之路上，在漫长的人类历史中存在着多民族的相互交往有关。研究对象按性别分层后，发现在女性组中 rs10043986 与精神分裂症有关联，而在男性组中，两者无明显关联。这表明 rs10043986 与精神分裂症的关联可能存在性别差异，基因多态性与精神分裂症关系存在性别差异的在其它基因中也有类似发现[18]。

表 1　病例组与对照组等位基因及基因型频率分布比较[例(%)]

组别	n	等位基因频率		X^2 值	P 值	基因型频率			X^2 值	P 值
		C	T			C/C	C/T	T/T		
病例组	325	595(91.5)	55(8.5)	9.038	0.003	271(83.4)	53(16.3)	1(0.3)	9.417	0.009
对照组	183	353(96.4)	13(3.6)			170(92.9)	13(7.1)	0		
男性病例组	193	358(92.7)	28(7.3)	1.012	0.314	166(86.0)	26(13.5)	1(0.5)	1.231	0.54
男性对照组	107	203(94.9)	11(5.1)			96(89.7)	11(10.3)	0		
女性病例组	132	237(89.8)	27(10.2)	11.812	0.001	105(79.5)	27(20.5)	0	12.769	0.001
女性对照组	76	150(98.7)	2(1.3)			74(97.4)	2(2.6)	0		

注：病例组与对照组比较，经 X^2 检验 $P<0.05$，$OR=0.398$，95% CI（0.215–0.740）；男性病例组与男性对照组比较，经 X^2 检验 $P>0.05$[$OR=0.693$，95% CI（0.338–1.421）]；女性病例组与女性对照组比较，经 X^2 检验 $P<0.05$[$OR=0.117$，95% CI（0.027–0.499）]

表 2　青少年和成年病例组与对照组等位基因与基因频率的比较[例(%)]

组别	n	等位基因频率		X^2 值	P 值	基因型频率			X^2 值	P 值
		C	T			C/C	C/T	T/T		
青少年病例组	63	111(88.1)	15(11.9)	0.529	0.467	48(76.2)	15(23.8)	0	0.604	0.437
青少年对照组	14	26(92.9.)	2(7.1)			12(85.7)	2(14.3)	0		
成年病例组	262	484(92.4)	40(7.6)	8.219	0.004	223(85.1)	38(14.5)	1(0.4)	8.379	0.015
成年对照组	169	328(97.0)	10(3.0)			159(94.1)	10(5.9)	0		

注：青少年病例组和青少年对照组比较，经 X^2 检验 $P>0.05$[$OR=0.569$，95% CI（0.123–2.645）]；成年病例组和成年对照组比较，经 X^2 检验 $P<0.05$[$OR=0.369$，95% CI 为（0.182–0.748）]

表3　C/C基因型和C/T+TT型基因型两组患者PANSS量表评分比较(分，$\bar{x}\pm s$)

组别	n	阳性量表分	阴性量表分	一般精神病理量表分	总分
C/C型	77	18.18±6.97	23.38±7.13	37.07±9.22	94.66±19.73
C/T+TT型	51	19.93±7.20	21.93±6.76	35.58±8.09	82.70±16.82
t值		1.38	1.87	1.19	0.71
P值		0.17	0.06	0.24	0.48

般认为精神分裂症患者存在性别差异主要与雌激素有关，但这种差异的确切机制尚不明了[17]。根据以上结果提示，CMYA5基因是否可能与雌激素相互作用使其在女性精神分裂症患者中表现出特异性关联，为今后精神分裂症性别差异研究提供了线索。

在精神分裂症的中间表型中，发病年龄是修饰疾病外显率和表现程度的重要因素。Hare等[18]研究指出精神障碍发病年龄的遗传度约为33%，许多双生子和家系研究也证明精神分裂症发病年龄与遗传变异密切相关[19]。目前国内外关于CMYA5与精神分裂症的研究均未对患者首次发病年龄进行关联分析，本研究根据首次发病年龄将患者分为青少年组和成年组，发现在成年人群中rs10043986与精神分裂症密切关联，而在青少年中两者无显著关联，这在一定程度上提示rs10043986在青少年和成年中可能作用不同。环境因素、病程因素及治疗因素等是否会造成青少年和成年病人的这种差异，仍需进一步探讨。

有研究认为，基因多态性与精神分裂症临床症状严重程度存在相关性[20-21]，本研究未发现rs10043986与精神分裂症临床症状严重程度存在明显关联。这可能与精神分裂症遗传异质性有关，不同亚型的易感基因可能不同。本研究未能对患者进行临床分型，这可能会导致研究结果与真实情况存在偏倚，仍需在今后的研究中进一步论证。

综上所述，本研究结果提示rs10043986中T等位基因可能增加中国维吾尔族女性和成年人群精神分裂症的易感性，其多态性与精神分裂症临床症状严重程度可能无关。但本次研究仍存在诸多不足：(1)研究遗传变异可能和疾病分型相关[22]，不同亚型的精神分裂症可能存在不同的生物学机制，本研究未能进行不同亚型的相关分析；(2)所纳入研究人群样本量在患者组和对照组中未能严格匹配，青少年研究对象较少，可能使研究结果统计效能不足；(3)研究位点单一，样本量偏小，以及难以排出对照组以后发生精神分裂症的可能性等。要全面揭示CMYA5基因的功能，下一步需在加强精神分裂症亚型的标准化诊断的基础上，扩大样本量，并结合基因功能网络更深入地探讨CMYA5基因在精神分裂症病因和发病机制中的作用。

参考文献

[1] 沈渔邨. 精神病学[M]. 5版. 北京：人民卫生出版社，2009：504.

[2] Andrea S, Berrouï M, Alkomet H. The impact of environmental factors in severe psychiatric disorders[J]. Front Neurosci, 2014, 8(19): 1-10.

[3] Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies[J]. Arch Gen Psychiatry, 2003, 60(12): 1187-1192.

[4] Benson MA, Tinsley CL, Blake DJ. Myospryn is a novel binding partner for dysbindin in muscle[J]. J Biol Chem, 2004, 279(11): 10450-10458.

[5] Morris DW, Murphy K, Kenny N, et al. Dysbindin (DTNBP1) and the biogenesis of lysosome-related organelles complex 1 (BLOC-1): main and epistatic gene effects are potential contributors to schizophrenia susceptibility[J]. Biol Psychiatry, 2008, 63(1): 24-31.

[6] Chen X, Lee G, Maher B, et al. GWA study data mining and independent replication identify cardiomyopathy-associated 5 (CMYA5) as a risk gene for schizophrenia[J]. Mol Psychiatry, 2010, 16(11): 1117-1129.

[7] Zhang R, Zhang H, Li M, et al. Genetic analysis of common variants in the CMYA5 (cardiomyopathy-associated 5) gene with schizophrenia[J]. Prog Neuropsychopharmacol Biol Psychiatry, 2013, 46(10): 64-69.

[8] Li M, Luo X-j, Zhang X, et al. A common variant of the cardiomyopathy associated 5 gene (CMYA5) is associated with schizophrenia in Chinese population[J]. Schizophr Res, 2011, 129(2-3): 217-219.

[9] Furukawa H, Tochigi M, Otowa T, et al. An association analysis of the cardiomyopathy associated 5 (CMYA5) gene with schizophrenia in a Japanese population[J]. Psychiatr Genet, 2013, 23(4): 179-180.

[10] Wang Q, He K, Chen J, et al. The CMYA5 gene confers risk for both schizophrenia and major depressive disorder in Han Chinese population[J]. World J Biol Psychiatry, 2014, 15(7): 553-560.

[11] [illegible][J]. 中国临床心理学杂志, 2000, 8(2): 65-69.

[12] Shi Y, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci[J]. Cell Res, 2005, 15(2): 97-98.

[13] Watanabe Y, Shibuya M, Someya T. The cardiomyopathy-associated 5 (CMYA5) gene and risk of schizophrenia: Meta-analysis of rs3828611 and rs4704591 in East Asian populations[J]. Asian J Psychiatry, 2014, 7(1): 95-96.

[14] Tsoupri E, Capetanaki Y. Myospryn: a multifunctional desmin-associated protein[J]. Histochem Cell Biol, 2013, 140(1): 55-63.

[15] 张俐丽，[illegible]. 新疆维吾尔族人群中GRM7基因rs3749380多态性与精神分裂症的关联性研究[J]. 中华行为医学与脑科学杂志, 2014, 23(3): 203-206.

[16] [illegible]. ZNF804A rs1344706多态性与精神分裂症的关联分析[J]. 中国神经精神疾病杂志, 2013, 39(4): 213-217.

[17] Min JA, Kim H, Pae CU, et al. Association of estrogen receptor genes and schizophrenia: a preliminary study[J]. Prog Neuropsychopharmacol Biol Psychiatry, 2012, 36(1): 1-4.

[18] Hare E, Glahn DC, Dassori A, et al. Heritability of age of onset of psychosis in schizophrenia[J]. Am J Med Genet B Neuropsychiatr Genet, 2010, 153(1): 298-302.

[19] Harrison PJ, Tunbridge EM. Catechol-O-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders[J]. Neuropsychopharmacology, 2008, 33(13): 3037-3045.

[20] 郭伟云，李文强，[illegible]，等. 偏执型精神分裂症患者精神分裂症断裂基因1单核苷酸多态性研究[J]. 中华行为医学与脑科学杂志, 2012, 21(4): 337-339.

[21] [illegible]，等. 脑神经营养因子G196A基因多态性与精神分裂症患者认知功能的关系[J]. 中华行为医学与脑科学杂志, 2013, 22(6): 494-496.

[22] Bergen SE, O'Dushlaine CT, Lee PH, et al. Genetic modifiers and subtypes in schizophrenia: Investigations of age at onset, severity, sex and family history[J]. Schizophr Res, 2014, 154(1-3): 48-53.

(收稿日期：2014-05-25)
(本文编辑：冯学泉)

Early Intervention IN PSYCHIATRY
First Impact Factor released in June 2010 and now listed in MEDLINE!
Early Intervention in Psychiatry 2015; ••: ••–••
doi:10.1111/eip.12276

Original Article

Association between *CMYA5* gene polymorphisms and risk of schizophrenia in Uygur population and a meta-analysis

Shuxian Han, Zhiguo An, Xiao Luo, Lili Zhang, Xianjiang Zhong, Wen Du, Qizhong Yi and Yongyong Shi

Abstract

Aim: Previous evidence has found that some single nucleotide polymorphisms (SNPs) in cardiomyopathy-associated 5 gene (*CMYA5*) were associated with schizophrenia in the Caucasian and Chinese Han populations. In this study, we aimed to investigate the relationship between *CMYA5* gene polymorphisms and schizophrenia in Chinese Uygur population and perform a meta-analysis to synthetically analyse the association of *CMYA5* gene polymorphisms with schizophrenia in Asian populations.

Method: We retrospectively analysed 985 schizophrenia cases and 1123 healthy controls in Chinese Uygur population. Four SNPs (*rs259127*, *rs3828611*, *rs4704591* and *rs6883197*) of *CMYA5* were genotyped using TaqMan SNP genotyping assay. Meta-analysis was conducted across Asian studies by Review Manager 5.2.

Results: Results showed no significant difference in either allelic or genotypic frequency in four SNPs of the *CMYA5* gene between cases and controls ($P > 0.05$). However, the age of onset and the PANSS positive-factor subscale score were significantly lower in schizophrenia patients with the A/A genotype of *rs6883197* than those with A/G and G/G genotypes ($P < 0.05$). In addition, the meta-analysis showed the significant association of *rs3828611* with risk of schizophrenia ($P = 0.03$, OR = 0.92, 95% CI: 0.91–0.99).

Conclusions: Our results support the association between *CMYA5 rs6883197* and schizophrenia in Chinese Uygur population. Meta-analysis demonstrated that rs3828611 was significantly associated with schizophrenia in Asian population. Genetic heterogeneity among populations may be the main reason of results conflict between studies. In conclusion, association between *CMYA5* gene polymorphisms and schizophrenia was confirmed in Asian population.

Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China

Corresponding author: Professor Qizhong Yi or Yongyong Shi, Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, 137 Liyushan South Road, Urumqi 830054, China. Email: qizhongyi@126.com

Received 15 January 2015; accepted 17 August 2015

Key words: *CMYA5,* polymorphism, schizophrenia, Uygur Chinese population.

INTRODUCTION

Schizophrenia is a chronic and severe mental disorder affecting patients' professional and social life.[1,2] The disease is characterized by heterogeneous psychotic features including positive symptoms (hallucination, delusions and disorganized behaviour), negative symptoms (affective blunting, apathy and social withdrawal) and cognitive dysfunctions (attention, learning and memory impairments).[3] Schizophrenia has a lifetime prevalence of approximately 1% in general population worldwide, and the high heritability estimated at around 80% was reported in a family study and a meta-analysis of multiple twin studies.[4,5] Hitherto, a great number of candidate gene studies and genome-wide association studies have identified many candidate genes which might confer risk for schizophrenia, such as cardiomyopathy-associated 5 gene (*CMYA5*).

© 2015 Wiley Publishing Asia Pty Ltd

CMYA5 protein was originally identified as a binding partner for dysbindin,[6] which has been found to play a role in the aetiology of schizophrenia.[7–9] Recently, a meta-analysis reported three single nucleotide polymorphisms (SNPs) (*rs3828611*, *rs4704591* and *rs10043986*) within *CMYA5* gene located on chromosome 5q14.1 being implicated in schizophrenia.[7] Subsequently, Li *et al*. carried out a case–control study in three independent Chinese Han populations; they found that *rs3828611* was positively associated with schizophrenia, and confirmed that *rs10043986* was not polymorphic in their samples.[10] However, this association was not found in a Japanese population and a Chinese Han population from Shanxi province.[11,12] However, nominally significant association of *rs7714250* and *rs13158477* with schizophrenia was found by Zhang *et al*.[12] In addition, Wang *et al*. showed significant association between two SNPs (*rs6883197* and *rs259127*) and schizophrenia in a Chinese Han population from Shanghai city.[13] Taken together, although increasing evidence indicated that *CMYA5* gene is a potential susceptibility gene for schizophrenia, genetic heterogeneity may exist across populations which have been found in the association between other genes and other diseases.[9,14,15] Thus, we performed this study to further investigate the association between *CMYA5* gene polymorphism and schizophrenia.

The ethnic intermarriages were rare in Uygur population and they had a relatively stable area of residence,[16] which makes it suitable for genetic studies. In the present study, we attempted to evaluate the association of *CMYA5* gene polymorphisms with schizophrenia in the Chinese Uygur population. In addition, given inconsistent results were existing in previous studies, we also performed a systematic meta-analysis of *rs3828611* and *rs4704591* in allele frequency by combining our finding with the results of previous studies across Asian population.

METHODS

Subjects

We performed a case–control study with 985 schizophrenia patients (612 male and 373 female, average age of 37.38 ± 11.90, average age of onset 25.32 ± 7.97) and 1123 normal controls (585 male and 538 female, average age of 42.60 ± 13.04). The cases were recruited from the inpatients and outpatients with schizophrenia in First Affiliated Hospital of Xinjiang Medical University. Patients were interviewed by two independent experienced psychiatrists and were diagnosed strictly according to DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition).[17] The exclusion criteria were: (i) history of serious somatic illness (neurological disease, endocrine disorders or autoimmune disease); (ii) comorbidity patients with other mental disorders; (iii) psychoactive substance abuse or addiction; and (iv) pregnancy or postpartum period. Controls were drawn from the healthy Uygur Chinese population in Health Examination Center of First Affiliated Hospital of Xinjiang Medical University. They have no current or lifetime psychiatric history according to SCID,[18] as well as no first-degree relative with history of psychosis.

The study protocols and process were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. It was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical symptom assessment

The Positive and Negative Syndrome Scale (PANSS)[19] was used for the severity of the patients' clinical symptoms assessment by two independent experienced psychiatrists who had attended a training in the use of the PANSS. After training, repeated assessments showed that the inter-observer correlation coefficient was greater than 0.84 for the scores of PANSS. The clinical symptoms (measured by the PANSS) were the mental status before psychiatric drug treatment in this study. The PANSS categorizes the symptoms into five-factor subscales: positive, negative, disorganized, excited and depressed. The item scores of PANSS are summed to determine the total score and scores of five-factor subscales.[20] Age of onset was assessed from medical records and/or interviews. The onset was determined according to the Interview for the Retrospective Assessment of the Onset of Schizophrenia.[21]

SNP selection and genotyping of SNPs

We have totally genotyped four SNPs (*rs259127*, *rs3828611*, *rs4704591* and *rs6883197*) of *CMYA5* gene, which were selected based on public database (National Center for Biotechnology Information, dbSNP, http://www.ncbi.nlm.nih.gov/SNP/) and data from previous studies.[11,13,22,23] The information of four SNPs was shown in Table 1.

Genomic DNA was extracted from Peripheral blood samples using Tiangen DNA isolation kit according to the manufacturer's protocol. Genotyping was conducted using the TaqMan SNP

© 2015 Wiley Publishing Asia Pty Ltd

genotyping assay on the ABI 7900 DNA detection system. All probes were designed and synthesized by Applied Biosystems. According to the guidelines, the 5 μL mixture consisting of 2.5 μL of TaqMan Universal PCR Master Mix reagent, 0.035 μL of Taqman assay and 40 ng of DNA was used for PCR amplification by the GeneAmp PCR 9700 System with the following protocol: initial denaturation at 94°C for 15 minutes, followed by 45 cycles of 94°C for 20 seconds and 60°C for 1 minute. The genotyping success rate ranged from 94.2% (*rs259127*) to 95.7% (*rs4704591*). For quality control, the experimenters were completely blind to the case or control status and 10% of the samples were genotyped again to test the reliability and stability of results.

Statistical analysis

All continuous variables were expressed as mean ± standard deviation and compared using one-way ANOVA. Differences in categorical variables were analysed using the chi-squared test. Deviation from the Hardy–Weinberg equilibrium (HWE), allele and genotype frequencies and odds ratio (OR) of individual SNPs, pairwise linkage disequilibrium, and haplotype analysis were performed by SHEsis software (http://analysis.bio-x.cn).[24] All tests were two tailed and statistical significance was set at the threshold of 0.05. Bonferroni correction was applied for each test to adjust for multiple testing.

Meta-analysis

Recent studies were searched for meta-analysis. The studies with Asian populations were included in this study. The pooled ORs and their 95% confidence intervals (CIs) were calculated using fixed-effect (Mantel–Haenszel) model. When there was no heterogeneity among studies, random-effect (inverse-variance) model was applied. The percentage of variability across studies attributable to heterogeneity beyond chance was estimated using the I^2 statistic. Differences in pooled OR were compared using a Z test. Review Manager, version 5.2, was used for data synthesis and statistical analysis.

RESULTS

Association of *CMYA5* with schizophrenia in Chinese Uygur population

As shown in Table 2, the genotype distributions of four SNPs were all in HWE. Moreover, the allele and genotype distributions of four SNPs were similar between case and control groups ($P > 0.05$).

In addition, we also examined the relationship between the genotype and clinical variables in

TABLE 1. The information of four SNPs in the *CMYA5* gene region

SNP ID	*rs6883197*	*rs3828611*	*rs259127*	*rs4704591*
Position	78991373	79034662	79058642	79103471
Functional	Intron	Exon/UTR3	Intron	Intergenic/unknown
Polymorphism	A/G	C/G	A/T	C/G

SNP, single nucleotide polymorphism.

TABLE 2. Genotype and allele frequencies of polymorphisms of *CMYA5* gene in schizophrenia patients and controls

SNP ID	Genotype frequency (%)			*P*	Allele frequency (%)		*P*	OR (95% CI)	HWE *P*-value
rs6883197	A/A	A/G	G/G		A	G			
Case	350 (36.3)	454 (47.1)	160 (16.6)	0.697	1154 (59.9)	774 (40.1)	0.393	0.947 (0.836–1.073)	0.534
Control	415 (37.8)	513 (46.7)	170 (15.5)		1343 (61.2)	853 (38.8)			0.582
rs3828611	C/C	C/G	G/G		C	G			
Case	523 (53.9)	380 (39.2)	67 (6.9)	0.833	1426 (73.5)	514 (26.5)	0.963	1.003 (0.873–1.154)	0.857
Control	575 (54.3)	404 (38.2)	79 (7.5)		1554 (73.4)	562 (26.6)			0.491
rs259127	A/A	A/T	T/T		A	T			
Case	484 (50.2)	388 (40.2)	93 (9.6)	0.349	1346 (69.7)	584 (30.3)	0.206	0.917 (0.801–1.049)	0.239
Control	572(52.0)	441(40.1)	87(7.9)		1585 (72.0)	615 (28.0)			0.876
rs4704591	C/C	C/G	G/G		C	G			
Case	64(6.6)	415(42.7)	493(50.7)	0.094	543 (27.9)	1401 (72.1)	0.582	1.039(0.906–1.192)	0.059
Control	87(8.0)	416(38.3)	583(53.7)		590 (27.2)	1582 (72.8)			0.292

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds radio; SNP, single nucleotide polymorphism.

© 2015 Wiley Publishing Asia Pty Ltd

schizophrenia (Table 3). We found that the polymorphisms of *rs6883197* could significantly affect the age of onset ($P = 0.006$, Bonferroni corrected $P = 0.018$) and PANSS positive-factor subscale score ($P = 0.001$, Bonferroni corrected $P = 0.003$), whereas *rs6883197* showed nominal significant association with PANSS excited-factor subscale score ($P = 0.040$, Bonferroni corrected $P = 0.120$). The age of onset of G/G genotype carriers was significantly lower than that of A/A genotype carriers ($P = 0.004$, Bonferroni corrected $P = 0.012$). However, there was no significant difference between age of onset of G/G and A/G genotype carriers after Bonferroni corrected ($P = 0.050$, Bonferroni corrected $P = 0.150$). Besides, the positive-factor subscale score of PANSS in the patients with G/G genotype was significantly lower than that in the patients with A/G ($P = 0.002$, Bonferroni corrected $P = 0.006$) and A/A genotypes ($P = 0.003$, Bonferroni corrected $P = 0.009$).

Meta-analysis of *rs3828611* and *rs4704591* in Asian population

A total of five studies were included for meta-analysis of *rs3828611* and *rs4704591*. There were 8053 patients and 8126 healthy controls. The characteristics of these studies were described in Table 4.

In the analysis, there was no evidence of significant heterogeneity among studies (*rs3828611*: $P = 0.26$, $I^2 = 24\%$; *rs4704591*: $P = 0.52$, $I^2 = 0\%$). So, the fixed-effects model was used to pool the data. The pooled estimate showed that schizophrenia was significantly associated with the allele frequency of *rs3828611* ($P = 0.03$, pooled OR = 0.92, 95% CI: 0.91–0.99) but not significantly associated with the allele frequency of *rs4704591* ($P = 0.97$, pooled OR = 1.00, 95% CI: 0.95–1.06). A detailed reproduction of these results is shown in Table 4 and Figure 1.

DISCUSSION

The human *CMYA5* gene is located on the long arm of chromosome 5 at the 5q14.1. Evidence that comes from previous studies has shown the association between *CMYA5* gene polymorphism and schizophrenia. However, genetic heterogeneity in different populations should not be ignored. Therefore, we have investigated this association in Chinese Uygur population. The results showed that the polymorphisms of *CMYA5 rs6883197* were significantly associated with the age of onset and positive-factor subscale score of PANSS in Chinese Uygur schizophrenia patients, suggesting there was some

TABLE 3. Demographic and clinical characteristics in schizophrenia of SNPs in *CMYA5* gene

SNP ID	Genotype	Sex (M/F)	Age of onset	PANSS score					
				Positive	Negative	Disorganized	Excited	Depressed	Total
rs6883197	A/A	217/133	26.02 ± 8.61	10.95 ± 4.37	28.32 ± 8.51	15.59 ± 5.39	9.41 ± 4.75	12.19 ± 3.88	76.47 ± 18.44
	A/G	282/172	25.32 ± 7.83	10.97 ± 4.49	27.69 ± 8.39	15.37 ± 4.96	9.38 ± 4.50	11.91 ± 3.81	75.30 ± 18.41
	G/G	102/58	23.61 ± 6.63	9.58 ± 4.31	27.87 ± 8.02	15.49 ± 5.27	8.40 ± 4.00	11.38 ± 3.63	72.71 ± 18.06
	P	0.922	0.006	0.001	0.568	0.827	0.040	0.080	0.100
rs3828611	C/C	331/192	25.21 ± 8.05	10.89 ± 4.45	28.10 ± 8.18	15.35 ± 5.10	9.40 ± 4.58	11.92 ± 3.85	75.64 ± 18.04
	C/G	230/150	25.68 ± 7.85	10.66 ± 4.38	27.82 ± 8.67	15.62 ± 5.26	9.14 ± 4.44	11.99 ± 3.82	75.24 ± 19.07
	G/G	43/24	24.22 ± 7.79	9.91 ± 4.54	26.94 ± 8.31	14.73 ± 5.13	8.55 ± 4.28	11.63 ± 3.65	71.76 ± 17.13
	P	0.661	0.344	0.212	0.549	0.395	0.301	0.770	0.267
rs259127	A/A	306/178	25.18 ± 8.45	10.57 ± 4.44	27.56 ± 8.04	15.21 ± 5.01	9.10 ± 4.42	11.81 ± 3.76	74.22 ± 17.83
	A/T	238/150	25.18 ± 7.46	10.94 ± 4.43	28.60 ± 8.26	15.87 ± 5.13	9.53 ± 4.68	12.09 ± 3.78	77.03 ± 18.54
	T/T	51/42	26.59 ± 7.64	10.65 ± 4.55	27.21 ± 10.19	14.84 ± 4.77	9.14 ± 4.01	11.74 ± 3.99	73.17 ± 20.39
	P	0.309	0.269	0.473	0.130	0.087	0.343	0.503	0.053
rs4704591	C/C	43/21	23.72 ± 6.82	11.68 ± 4.65	27.98 ± 8.43	15.44 ± 4.77	9.75 ± 5.28	11.34 ± 3.26	76.00 ± 18.22
	C/G	249/166	25.43 ± 8.24	10.86 ± 4.28	27.86 ± 8.44	15.31 ± 5.07	9.15 ± 4.43	12.07 ± 3.80	75.25 ± 18.23
	G/G	314/179	25.40 ± 7.90	10.49 ± 4.52	27.89 ± 8.38	15.51 ± 5.32	9.24 ± 4.46	11.86 ± 3.89	74.96 ± 18.68
	P	0.369	0.261	0.161	0.993	0.852	0.613	0.326	0.904

M/F, male/female; PANSS, Positive and Negative Syndrome Scale; SNP, single nucleotide polymorphism.

© 2015 Wiley Publishing Asia Pty Ltd

TABLE 4. Meta-analysis of *rs4704591* and *rs3828611* with schizophrenia in Asian populations

Author, year	Sample area	Diagnosis	Sample size (cases/controls)	Investigated SNPs	Minor allele frequency (cases, controls)	*P*-value (allele)	OR (95% CI)
Current study	Chinese Uygur	DSM-IV	985/1123	Rs4704591	0.279, 0.272	0.58	1.04 (0.91–1.19)
				Rs3828611	0.265, 0.266	0.96	1.00 (0.88–1.15)
Li *et al.*, 2011	Chinese Han	ICD-10/DSM-IV	2797/2808	Rs4704591	0.228, 0.223	0.53	0.97 (0.89–1.06)
				Rs3828611	0.408, 0.435	<0.01	0.89 (0.83–0.96)
Zhang *et al.*, 2013	Chinese Han	DSM-IV	467/503	Rs4704591	0.213, 0.239	0.18	0.86 (0.70–1.07)
				Rs3828611	0.455, 0.435	0.34	0.92 (0.77–1.10)
Furukawa *et al.*, 2013	Japanese	ICD-10 and DSM-IV	2474/2457	Rs4704591	0.166, 0.161	0.51	1.04 (0.94–1.16)
				Rs3828611	0.486, 0.487	0.93	1.00 (0.93–1.09)
Wang *et al.*, 2014	Chinese Han	DSM-IV	1330/1235	Rs4704591	0.234, 0.229	0.67	1.03(0.90–1.17)
				Rs3828611	0.445, 0.434	0.42	0.96(0.86–1.07)

CI, confidence interval; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; ICD-10, Tenth Revision of the International Classification of Diseases; OR, odds radio; SNP, single nucleotide polymorphism.

relationship between *CMYA5 rs6883197* and schizophrenia in Chinese Uygur population. However, we failed to confirm statistically significant association between schizophrenia and other three SNPs (*rs3828611*, *rs259127* and *rs4704591*). In addition, we also performed a meta-analysis combining previous data and our results. The meta-analysis supports the existence of a significant association between allele frequencies of *rs3828611* and schizophrenia in Asian population. Thus, *CMYA5* gene polymorphism was associated with schizophrenia.

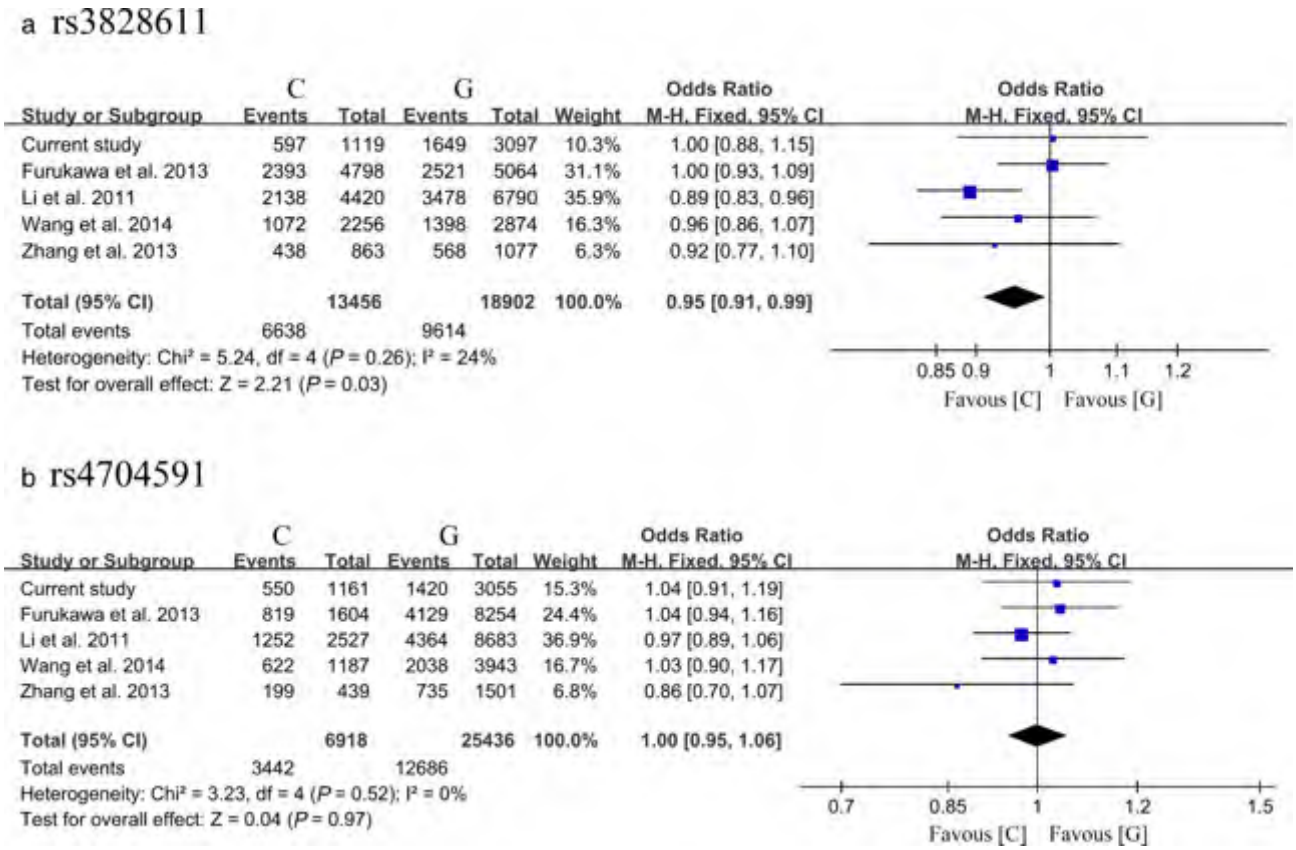
The CMYA5 protein, also known as myospryn, was a binding partner of dysbindin which is an essential component of BLOC-1 (biogenesis of lysosome-related organelles complex 1) processes.[25,26] The dysbindin gene has been confirmed as a schizophrenia susceptibility gene in the previous studies.[25–27] Moreover, the cellular processes of BLOC-1 have been suggested to be involved in schizophrenia pathogenesis.[28] Thus, polymorphisms of *CMYA5* gene and schizophrenia may be correlated through cellular processes of BLOC-1 and dysbindin may play key roles in this mechanism.

Additionally, there were some differences between the results of this study and the previous studies. Although some previous studies also concluded that *CMYA5* gene polymorphism is associated with schizophrenia,[10–13] the significant associated SNPs in these studies were different from these studies. These results contradict each other, and may be due to ethnic background, as the frequency distribution of *CMYA5* polymorphism may vary significantly in various populations. For example, it has been reported that G allele frequency of *rs3828611* in Chinese Han schizophrenia patients from Shanxi province is about 43.5%, whereas the G allele frequency was 26.5% in Chinese Uygur schizophrenia patients in this study. In addition to inter-ethnic differences, several other factors may also contribute to the divergent association results, such as subtypes in schizophrenia, population stratification and cultures.

Several limitations should be noted in our study. Firstly, we did not fully investigate all the SNPs in *CMYA5* gene which were reported in previous studies. Further investigations concerning the other SNPs would be performed in the future. Secondly, the variants in this gene may be associated with subtypes in schizophrenia. It is incapable to get a conclusion because of insufficient clinical information. Thirdly, the relatively small sample size may be responsible for the negative association results obtained in this study. Thus, further studies with larger sample size are essential to verify the results of this study.

© 2015 Wiley Publishing Asia Pty Ltd

FIGURE 1. Forest plots for association of schizophrenia with allele frequency of *rs3828611* (a) and *rs4704591* (b).



In conclusion, we have demonstrated that polymorphism of *CMYA5 rs6883197* was associated with schizophrenia in Chinese Uygur populations simultaneously likely to be relevant to patients' age of onset and positive-factor subscale score. Whether our genetic association on specific clinical symptoms can apply to other ethnic groups still needs to be confirmed. In addition, association between allele frequencies of *rs3828611* and schizophrenia in Asian population was supported by the meta-analysis. In summary, these results give evidence for the association between *CMYA5* gene polymorphisms and schizophrenia in Asian population.

ACKNOWLEDGEMENTS

We are grateful to all the individuals who have participated for their contribution to our research. This work was supported by the Xinjiang Uygur Autonomous Region Science and Technology Funds (2010211A51), the Natural Science Foundation of China (81360209, 81130022, 81272302, 31000553), the National 863 Project (2012AA02A515), the sub-topic of the National 973 Project (2007CB512301) and the sub-topic of the National 863 Project (2006AA02A407).

REFERENCES

1. Bakanidze G, Roinishvili M, Chkonia E et al. Association of the Nicotinic Receptor alpha7 Subunit Gene (CHRNA7) with Schizophrenia and Visual Backward Masking. *Front Psychiatry* 2013; **4**: 133.
2. Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012. *Mol Psychiatry* 2012; **17**(12): 1228–38.
3. Zhang C, Li Z, Shao Y et al. Association study of tryptophan hydroxylase-2 gene in schizophrenia and its clinical features in Chinese Han population. *J Mol Neurosci* 2011; **43**(3): 406–11.
4. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003; **60**: 1187–92.
5. Wang KS, Liu X, Arana TB et al. Genetic association analysis of ITGB3 polymorphisms with age at onset of schizophrenia. *J Mol Neurosci* 2013; **51**(2): 446–53.
6. Tsoupri E, Capetanaki Y. Myospryn: a multifunctional desmin-associated protein. *Histochem Cell Biol* 2013; **140**: 55–63.
7. Chen X, Lee G, Maher BS et al. GWA study data mining and independent replication identify cardiomyopathy-associated 5 (CMYA5) as a risk gene for schizophrenia. *Mol Psychiatry* 2010; **16**(11): 1117–29.

© 2015 Wiley Publishing Asia Pty Ltd

8. Papaleo F, Weinberger DR. Dysbindin and schizophrenia: it's dopamine and glutamate all over again. *Biol Psychiatry* 2011; **69**: 2–4.
9. Watanabe Y, Shibuya M, Someya T. The cardiomyopathy-associated 5 (CMYA5) gene and risk of schizophrenia: meta-analysis of rs3828611 and rs4704591 in East Asian populations. *Asian J Psychiatr* 2014; **7**: 95–6.
10. Li M, Luo XJ, Zhang X et al. A common variant of the cardiomyopathy associated 5 gene (CMYA5) is associated with schizophrenia in Chinese population. *Schizophr Res* 2011; **129**(2–3): 217–9.
11. Furukawa M, Tochigi M, Otowa T et al. An association analysis of the cardiomyopathy-associated 5 (CMYA5) gene with schizophrenia in a Japanese population. *Psychiatr Genet* 2013; **23**(4): 179–80.
12. Zhang R, Zhang H, Li M et al. Genetic analysis of common variants in the CMYA5 (cardiomyopathy-associated 5) gene with schizophrenia. *Prog Neuropsychopharmacology Biol psychiatry* 2013; **46**: 64–9.
13. Wang Q, He K, Li Z et al. The CMYA5 gene confers risk for both schizophrenia and major depressive disorder in the Han Chinese population. *World J Biol Psychiatry* 2014;**15**(7): 553–60.
14. Menzel O, Bekkeheien R, Reymond A et al. Knobloch syndrome: novel mutations in COL18A1, evidence for genetic heterogeneity, and a functionally impaired polymorphism in endostatin. *Hum Mutat* 2004; **23**: 77–84.
15. Arnott ID, Nimmo ER, Drummond HE et al. NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe. *Genes and immunity.* 2004; **5**(5): 417–25.
16. Wang J, Wen J, Si-Ma-Yi MH et al. Association of ADAM33 gene polymorphisms with asthma in the Uygur population of China. *Biomed Rep* 2014; **1**(3): 447–53.
17. Hasin D, Hatzenbuehler ML, Keyes K, Oqburn E. Substance use disorders: Diagnostic and Statistical Manual of Mental Disorders, (DSM-IV) and International Classification of Diseases, (ICD-10). *Addiction* 2006; **101**(s1): 59–75.
18. So E, Kam I, Leung CM, Chung D, Liu Z, Fong S. The Chinese-bilingual SCID-I/P project: stage 1-reliability for mood disorders and schizophrenia. *Hong Kong J Psychiatry* 2003; **13**: 7–18.
19. Wallwork RS, Fortgang R, Hashimoto R, Weinberger DR, Dickinson D. Searching for a consensus five-factor model of the positive and negative syndrome Scale for schizophrenia. *Schizophr Res* 2012; **137**: 246–50.
20. Leutwyler H, Hubbard E, Jeste D, Miller B, Vinogradov S. Association between schizophrenia symptoms and neurocognition on mobility in older adults with schizophrenia. *Aging Ment Health* 2014; **18**(8): 1006–12.
21. Häfner H, Riecher R, Hambrecht M et al. IRAOS: an instrument for the assessment of onset and early course of schizophrenia. *Schizophr Res* 1992;**6**: 209–23.
22. Morris DW, Murphy K, Kenny N et al. Dysbindin(DTNBP1) and the biogenesis of lysosome-related organelles complex1 (BLOC-1): main and epistatic gene effects are potential contributors to schizophrenia susceptibility. *Biol. Psychiatry* 2008; **63**: 24–31.
23. Lee G. Schizophrenia Candidate Genes Study. 2009. http://scholarscompass.vcu.edu.etd.
24. Yong Y, Lin H. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005; **15**: 97–8.
25. Mullin AP, Gokhale A, Larimore J, Faundez V. Cell biology of the BLOC-1 complex subunit dysbindin, a schizophrenia susceptibility gene. *Mol Neurobiol* 2011; **44**: 53–64.
26. Gokhale A, Larimore J, Werner E et al. Quantitative proteomic and genetic analyses of the schizophrenia susceptibility factor dysbindin identify novel roles of the biogenesis of lysosome-related organelles complex 1. *J Neurosci* 2012; **32**: 3697–711.
27. Kendler KS. Schizophrenia genetics and dysbindin: a corner turned?. *Am J Psychiatry.* 2004; **161**(9): 1533–6. http://dx.doi.org/10.1176/appi.ajp.161.9.1533
28. Morris DW, Murphy K, Kenny N et al. Dysbindin (DTNBP1) and the biogenesis of lysosome-related organelles complex 1 (BLOC-1): main and epistatic gene effects are potential contributors to schizophrenia susceptibility. *Biol Psychiatry* 2008; **63**(1): 24–31.

© 2015 Wiley Publishing Asia Pty Ltd

Hereditas 151: 140–144 (2014)

Association analysis of the *GRM8* gene with schizophrenia in the Uygur Chinese population

LILI ZHANG[1], XIANJIANG ZHONG[1], ZHIGUO AN[1], SHUXIAN HAN[1], XIAO LUO[1], YONGYONG SHI[2] and QIZHONG YI[1]

[1]*Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi, PR China*
[2]*Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai JiaoTong University, Shanghai, PR China*

Zhang, L., Zhong, X., An, Z., Han, S., Luo, X., Shi, Y. and Yi, Q. 2014. Association analysis of the *GRM8* gene with schizophrenia in the Uygur Chinese population. – *Hereditas 151*: 140–144. Lund, Sweden. eISSN 1601-5223. Received 22 January 2014. Accepted 25 July 2014.

GRM8 is a schizophrenia candidate gene that is also thought to be involved in the glutamate pathway, which is very important in the pathogenesis of schizophrenia. In this study, we aim to investigate the association between *GRM8* and schizophrenia in the Uygur Chinese population.

Rs2237748 and rs2299472, located in the *GRM8* gene, were selected for genotyping in a set of Uygur Chinese case-control samples, which included 723 cases and 561 controls, using TaqMan assays and capillary sequencing. The statistical analysis was carried out using the online software program SHEsis, and a meta-analysis was carried out to identify other relevant studies using Review Manager 5. We found that the rs2299472 genotype was significantly associated with schizophrenia ($P = 0.015$, $P = 0.030$, after Bonferroni correction). The frequency of the CC genotype was higher in the schizophrenic patients ($P = 0.008$), and the frequency of the AC genotype was lower ($P = 0.008$). Furthermore, the meta-analysis incorporating the previous and current studies also showed that rs2299472 is associated with schizophrenia. This study indicates that the *GRM8* gene may play an important role in the pathogenesis of schizophrenia.

Lili Zhang, Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830054, PR China. E-mail: zhanglili6372@gmail.com

Schizophrenia is a severe mental disorder with a high recurrence rate and a prolonged course. The cardinal symptoms of the disease are delusion, hallucination and disturbance of thought, which may lead to a regression from society and mental disability in some patients. Even now, it is still not possible to reach a consistent conclusion regarding its pathologies. However, family studies indicate that genetic factors play a very important role (Farmer and McGuffin 1988).

In recent years, glutamate receptors and other relevant genes have been studied in populations with schizophrenia (Coyle 1996; Meador-Woodruff and Healy 2000; Tamminga 2006; Stone et al. 2007). For instance, the *NMDA, AMPA*/kainate and glutamate receptors were found to be positively associated with schizophrenia. *NMDA* receptor subunit 3A, which can suppress dendritic spine formation (Choi 1998; Sasaki et al. 2002; Malenka and Bear 2004), appears to be maintained at high levels throughout adulthood in the prefrontal cortexes of individuals with schizophrenia (Mueller and Meador-Woodruff 2004). Schizophrenics also have high expression levels of the *AMPA* receptor subunit *GluR1* throughout life (O'Connor and Hemby 2007). Likewise, the glutamate genes, such as GRIA1, GRIK1, etc., are closely related to schizophrenia. Any disruption in these genes during the critical period of development may predispose an individual to schizophrenia (Choi et al. 2009). Therefore, there are several indications that abnormal glutamate receptors and genes may participate in the pathophysiology of schizophrenia.

After conducting systematic studies on the associations between the glutamate receptor genes and schizophrenia, we found that the group III metabotropic glutamate receptor, composed of the *mGluR4, 6, 7* and *8* genes, has important significance for the pathophysiology of schizophrenia, such as the proper function of the *GRM4* and *GRM7* genes (Fallin et al. 2005; Carter 2007; Ohtsuki et al. 2008; Ganda et al. 2009; Shibata et al. 2009).

Among the group III metabotropic glutamate receptors, *mGluR8*, which is encoded by the *GRM8* gene, localizes to the presynaptic grid of glutamate synapses and is thought to control presynaptic glutamate release (Shigemoto et al. 1997). We inferred that the polymorphism of the *GRM8* gene may cause the dysfunction of *mGluR8* and is thereby involved in the pathogenesis of psychotic disorders. A study by Hiromi Takaki and colleagues proved this viewpoint. In their study, they tested the association of schizophrenia with *GRM8*

© 2015 The Authors. This is an Open Access article. DOI: 10.1111/hrd2.00045

using 22 single nucleotide polymorphisms (SNPs) in 100 case-control pairs of Japanese subjects. Significant associations were detected for the combinations of rs2237797-rs1361963-rs2283094 and rs1361963-rs 2283094-rs 2402851. However, for the single polymorphism sites, only rs2237748 and rs2299472 were observed to have significant associations with schizophrenia. However, none of the SNPs showed any associations after Bonferroni correction (Takaki et al. 2004).

Based on the study by Takaki et al. (2004), we concluded that the glutamate receptors and their encoding genes, especially the group III metabotropic glutamate receptor genes, show significant associations with schizophrenia. The small sample size of 100 case-control pairs or the type I errors in the study by Takaki et al. may have led to the failure to find significant associations between schizophrenia and rs2237748 and rs2299472. Therefore, in our study, we selected 1284 subjects (723 cases and 561 controls) of Uygur Chinese origin and genotyped the SNPs rs2237748 (C/T) and rs2299472 (A/C), which are located in *GRM8,* to revalidate whether *GRM8* is involved in the etiology of schizophrenia in the Uygur Chinese population.

MATERIAL AND METHODS

Subjects

All of the samples were of Uygur Chinese origin, including 723 cases (445 men and 278 women with a mean age of 36.9 ± 12.3 years) and 561 controls (303 men and 258 women with a mean age of 45.1 ± 13.5 years). At the same time, all of the participants were informed of the purpose of our study in detail and provided written consent for the genetic analysis, which was approved by the Xinjiang Medical University Ethics Committee. All of the subjects were biologically unrelated and belong to the local Xinjiang population. All of the subjects were assessed via the guidelines provided in the DSM-IV by at least two independent senior psychiatrists. The cases included both out-patients and in-patients with schizophrenia, and the controls were drawn from the healthy population and from patients with other disorders. To make the inclusive criteria more reasonable, we also evaluated all of the patients' conditions via the PANSS and BPRS rating scales. The exclusion criteria included the following: 1) central nervous system disorders and brain injury or disease, 2) mental retardation, 3) alcohol and drug dependence, 4) cancer, 5) endocrine system disorder, 6) family history of psychiatric disorders and 7) pregnancy.

SNP selection and genotyping

Using the SNP database (<www.ncbi.nlm.nih.gov/snp/>), we chose rs2237748 (C/T) and rs2299472 (A/C), which are located in *GRM8*, for genotyping. Their minor allele frequencies were both higher than 0.1 in the Han Chinese population, and the average intervals of the two SNPs were 58.4 kb.

The genomic DNA was extracted from the peripheral blood using the Tiangen RelaxGene Blood System (DP319,Tiangen Biotech (Beijing) Co. Ltd.).

Rs2237748 was genotyped using the TaqMan assay on an ABI 7900 DNA Detection System (Applied Biosystems, Foster City, CA, USA). The PCR reaction was performed on a GeneAmp PCR 9700 System (Applied Biosystems). For each reaction, 40 ng of DNA was used in a 5-µl volume containing 2.5 µl of TaqMan Universal PCR Master Mix reagent and 0.035 µl of TaqMan probe. The PCR cycling parameters were 95°C for 10 min followed by 50 cycles at 95°C for 15 s and 58°C for 1 min. All of the genotypes of the cases and controls were blind called to guarantee quality control during the genotyping process. The genotyping call rates ranged from 88.1% (controls) to 97.2% (cases) for rs2237748.

We genotyped the rs2299472 polymorphism using capillary sequencing. The sequence of the forward primer was 5′-CCAAGACTCCCTCTTTTTGC-3′, and the sequence of the reverse primer was 5′-TATGGGAGACCAGGAGATGG-3′. The product size was 210 bp. The PCR reaction system (5-µl volume) contained 2.5 µl of 2 × Taq PCR Master Mix, 0.1 µl of forward primer, 0.1 µl of reverse primer, 1.3 µl of diH_2O and 20 ng of DNA. We adopted the PCR cycling parameters that are described in detail in Takaki et al. (2004). After finishing the SAP, BDT and purification, the products which were analyzed using an ABI Prism 3730XL Genetic Analyzer by the ShangHai JIE LI BIOLOGY Company (<http://genebioseq.com>). The genotyping call rates ranged from 95.2% (cases) to 95.4% (controls) for rs2299472.

Statistical analyses

The odds ratios, allele and genotype frequencies and Hardy–Weinberg equilibriums for the cases and controls were calculated using the online software program SHEsis (<http://analysis.bio-x.cn>) (Li et al. 2009).

Meta-analysis

Using the keywords '*GRM8*', '*schizophrenia*' and 'polymorphism' in the PubMed database, we only found the study by Takaki et al. (2004), which was included in the meta-analysis. We combined and analyzed the data from the previous and current studies using the software program Review Manager 5. A fixed-effect model was applied when there was no heterogeneity, otherwise a random effect model was employed.

Table 1. *Results of the case-control analyses for rs2237748 and rs2299472.*

SNP ID	Allele (frequency)		Odds ratio	95% CI	P*	Genotype (frequency)			HWE	P**
rs2237748										
	C	T				C/C	C/T	T/T		
case	748 (0.532)	658 (0.468)	0.966	0.82~1.14	0.682	203 (0.289)	342 (0.486)	158 (0.225)	0.542	0.798
control	534 (0.540)	454 (0.460)				151 (0.306)	232 (0.470)	111 (0.225)	0.225	
rs2299472										
	A	C				A/A	A/C	C/C		
case	574 (0.417)	802 (0.583)	0.873	0.74~1.03	0.099	132 (0.192)	310 (0.451)	246 (0.358)	0.054	0.015
control	482 (0.450)	588 (0.550)				100 (0.187)	282 (0.527)	153 (0.286)	0.135	

P* are for the allele test.
P** are for the genotype test.

RESULTS

Case-control study

The SNPs on all of the samples were genotyped, and we did not find any deviation from Hardy–Weinberg equilibrium for any of the SNPs. Rs2237748 was not found to have any allelic or genotypic association with schizophrenia. However, rs2299472 showed a genotypic significance with schizophrenia after Bonferroni correction (P** = 0.015; P = 0.030, after Bonferroni correction). The results are shown in Table 1. The frequency of the CC genotype was higher in the patients than in the controls ($\chi^2 = 7.015$, P = 0.008), and the frequency of the AC genotype was lower in the patients than in the controls ($\chi^2 = 7.015$, P = 0.008). The AA genotype was not found to be associated with schizophrenia ($\chi^2 = 0.048$, P = 0.827).

Meta-analysis

There was no evidence of heterogeneity between the two studies for rs2299472; therefore, a fixed-effect model was employed. The OR and 95% CI values were calculated separately for the 'CC' versus 'AC + AA', 'AA' versus 'AC + CC' or 'AC' versus 'AA + CC' models. The CC and AC genotypes were found to be related to schizophrenia (CC/AC + AA: OR = 1.48, 95% CI = 1.18~1.16, P = 0.0006; AC/AA + CC: OR = 0.72, 95% CI = 0.58~0.89, P = 0.002). The AA genotype was not found to be significantly associated with schizophrenia (AA/AC + CC: OR = 0.96, 95% CI = 0.73~1.26, P = 0.78). The statistical results of the meta-analysis for rs2299472 are shown in Table 2 and Fig. 1.

DISCUSSION

In the present study, we investigated the associations of the *GRM8* polymorphisms and schizophrenia in 723 cases and 561 controls of Uygur Chinese origin. We tested the rs2237748 and rs2299472 polymorphisms in our samples. Rs2299472 continued to show a genotypic association with schizophrenia following Bonferroni correction, and we detected that the CC and AC genotypes of rs2299472 were associated with schizophrenia in the Uygur Chinese population (CC: $\chi^2 = 7.015$, P = 0.008; AC: $\chi^2 = 7.015$, P = 0.008). In addition, the meta-analysis also demonstrated that the two genotypes were associated with schizophrenia. Our data showed that a higher frequency of CC or a lower frequency of AC may increase the risk of schizophrenia in the Uygur Chinese population.

The protein products encoded by the *GRM8* gene participate in the glutamate pathway. This pathway has

Table 2. *Results of the meta-analysis for rs2299472.*

SNP ID	Studies	CC		AC + AA		Odds ratio	95% *CI*	*P*(*Z*)	*P*(*Q*)
		case	control	case	control				
rs2299472	Takaki et al.	50	32	50	68	1.48	1.18~1.86	0.0006	0.18
	present study	246	153	442	382				
		AC		AA + CC					
	Takaki et al.	39	50	61	50	0.72	0.58~0.89	0.002	0.65
	present study	310	282	378	253				
		AA		AC + CC					
	Takaki et al.	11	18	89	82	0.96	0.73~1.26	0.78	0.17
	present study	132	100	556	435				

The odds ratio (95% *CI*) is calculated with respect to CC versus AC + AA, AC versus AA + CC, and AA versus AC + CC. *P*(*Z*) is for the overall effect. *P*(*Q*) is for heterogeneity.

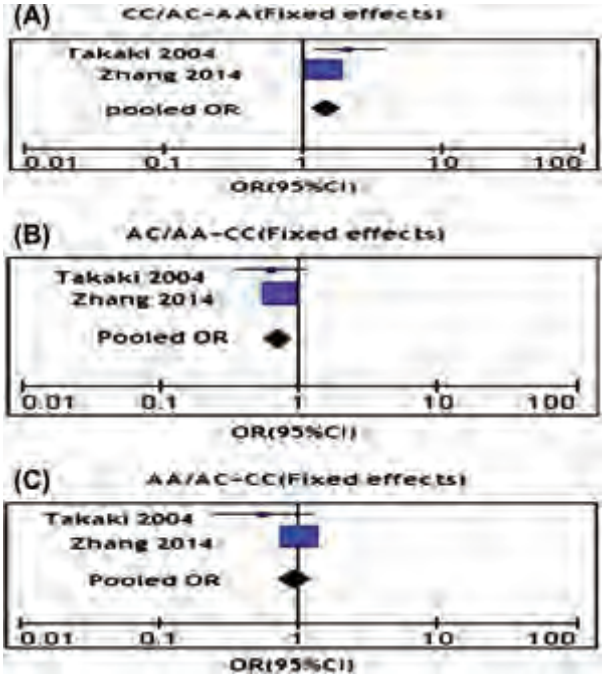


Fig. 1. Forest plots for schizophrenia associated with rs2299472. A: CC versus AC + AA model, B: AC versus AA + CC model, C: AA versus AC + CC model.

been considered to play important roles in neural plasticity, neural development and neurodegeneration (Okamoto et al. 1998). The protein products are also involved in cognitive functions (Shimizu et al. 2000) and are candidates for several nervous system diseases and psychiatric disorders, such as autistic spectrum disorders, depression and schizophrenia. In 2004, Takaki et al. reported that at least one susceptibility locus for schizophrenia is located within the *GRM8* region in a Japanese population. They chose 22 SNPs for genotyping, and the single nucleotide polymorphisms rs2237748 and rs2299472 showed significant associations with schizophrenia (rs2237748: P = 0.0279 for the allele, P = 0.0124 for the genotype; rs2299472: P = 0.0302 for the allele, P = 0.0127 for the genotype), but neither SNP was found to be associated with the disease following Bonferroni correction (Takaki et al. 2004).

There are several possible explanations for the discrepancy between the previous study and our results. First, it may be partially due to the differences in the allele frequencies of each population. When tracing the origins, the Uygur population contains both European and Asian blood. The data from the dbSNP bank indicate that the C allele frequency is 75% in European and 57.1% in JPT + CHB for rs2237748. The A allele frequency is 48.3% in JPT + CHB for rs2299472. The allele frequencies of rs2237748 and rs2299472 are different between other ethnic populations and the Uygur (Table 1). Therefore, the conclusions from the Uygur Chinese population cannot be completely consistent with that of the Japanese population. In addition, the sample size in the previous study consisted of only 100 case-control subjects, which may have led to the negative result.

Although we found that the rs2299472 genotypes are significantly associated with schizophrenia, there were still some limitations to our study. For instance, we could not obtain the clinical data on the schizophrenic subtypes or the drug treatments that were used, which have been proven to be associated with the gene polymorphism. In addition, to strengthen the results of the study, we must study additional SNPs located in *GRM8* and validate the results in different ethnic groups. However, we still lack evidence of functional experiments to support our findings.

In this study, our results supported that *GRM8* may play an important role in the pathogenesis of schizophrenia in the Uygur Chinese population. Further genetic studies in independent sample sets and in addition to functional experiments are suggested for the future.

Acknowledgments – We are deeply grateful to all of the participants and mental health workers that took part in this project. This work was supported by the Natural Science Foundation of China (to YYS, grant no. 81130022, 81272302, 31000553; to QY, grant no. 81360209); the National 863 project (to YYS, grant no. 2012AA02A515); the sub-topic of the National 973 project (to QY, grant no. 2007CB512301); the sub-topic of the National 863 project (to QY, grant no. 2006AA02A407); and the Xinjiang Uygur Autonomous Region Science and Technology Funds (to QY, grant no. 2010211A51). The relationship between the two institutions is cooperative. There are no conflicts of interest.

REFERENCES

Carter, C. J. 2007. eIF2B and oligodendrocyte survival: where nature and nurture meet in bipolar disorder and schizophrenia? – Schizophrenia Bull. 33: 1343–1353.

Choi, D. W. 1998. Glutamate neurotoxicity and diseases of the nervous system. – Neuron 1: 624–634.

Choi, K. H., Zepp, M. E., Higgs, B. W. et al. 2009. Expression profiles of schizophrenia susceptibility genes during human prefrontal cortical development. – J. Psychiatry Neurosci. 34: 450–458.

Coyle, J. T. 1996. The glutamatergic dysfunction hypothesis for schizophrenia. – Harv. Rev. Psychiatry 3: 241–253.

Fallin, M. D., Lasseter, V. K., Avramopoulos, D. et al. 2005. Bipolar I disorder and schizophrenia: a 440-single-nucleotide polymorphism screen of 64 candidate genes among Ashkenazi Jewish case-parent trios. – Am. J. Hum. Genet. 77: 918–936.

Farmer, A. E. and McGuffin, P. 1988. The pathogenesis and management of schizophrenia. – Drugs 35: 177–185.

Ganda, C., Schwab, S. G., Amir, N. et al. 2009. A family-based association study of DNA sequence variants in GRM7 with schizophrenia in an Indonesian population. – Int. J. Neuropsychopharmacol. 12: 1283–1289.

Li, Z. Q., Lin, H., Shi, Y. Y. et al. 2009. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (< http://analysis.bio-x.cn>). – Cell Res. 19: 519–523.

Malenka, R. C. and Bear, M. F. 2004. LTP and LTD: an embarrassment of riches. – Neuron 44: 5–21.

Meador-Woodruff, J. H. and Healy, D. J. 2000. Glutamate receptor expression in schizophrenic brain. – Brain Res. Brain Res. Rev. 31: 288–294.

Mueller, H. T. and Meador-Woodruff, J. H. 2004. NR3A NMDA receptor subunit mRNA expression in schizophrenia, depression and bipolar disorder. – Schizophrenia Res. 71: 361–370.

O'Connor, J. A. and Hemby, S. E. 2007. Elevated GRIA1 mRNA expression in Layer II/III and V pyramidal cells of the DLPFC in schizophrenia. – Schizophrenia Res. 97: 277–288.

Ohtsuki, T., Koga, M., Ishiguro, H. et al. 2008. A polymorphism of the metabotropic glutamate receptor mGluR7 (GRM7) gene is associated with schizophrenia. – Schizophrenia Res. 101: 9–16.

Okamoto, T., Sekiyama, N., Otsu, M. et al. 1998. Expession and purification of the extracellular ligand binding region of metabotropic glutamate receptor subtype1. – Biol. Chem. 273: 13089–13096.

Sasaki, Y. F., Rothe, T., Premkumar, L. S. et al. 2002. Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. – J. Neurophysiol. 87: 2052–2063.

Shibata, H., Tani, A., Chikuhara, T. et al. 2009. Association study of polymorphisms in the group III metabotropic glutamate receptor genes, GRM4 and GRM7, with schizophrenia. – Psychiatry Res. 167: 88–96.

Shigemoto, R., Kinoshita, A., Wada, E. et al. 1997. Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. – J. Neurosci. 17: 7503–7522.

Shimizu, E., Tang, Y. P., Rampon, C. et al. 2000. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. – Science 290: 1170–1174.

Stone, J. M., Morrison, P. D. and Pilowsky, L. S. 2007. Glutamate and dopamine dysregulation in schizophrenia – a synthesis and selective review. – J. Psychopharmacol. 21: 440–452.

Tamminga, C. A. 2006. The neurobiology of cognition in schizophrenia. – J. Clin. Psychiatry 67: 9–13.

Takaki, H., Kikuta, R., Shibata, H. et al. 2004. Positive associations of polymorphisms in the metabotropic glutamate receptor type 8 gene (GRM8) with schizophrenia. – Am. J. Med. Genet. B Neuropsychiatr. Genet. 128B: 6–14.

Shanghai Archives of Psychiatry, 2014, Vol. 26, No. 5

•Original article•

Case control study of association between the *ANK3* rs10761482 polymorphism and schizophrenia in persons of Uyghur nationality living in Xinjiang China

Xianjiang ZHONG [1,2], Lili ZHANG [1,3], Shuxian HAN[1], Xiao LUO[1], Zhiguo AN[1], Qizhong YI[1]*

Background: The rs10761482 polymorphism of the *ANK3* gene has been associated with the occurrence of schizophrenia.

Aim: Assess the relationship between the *ANK3* gene and schizophrenia in individuals of Uyghurian descent.

Methods: A total of 630 patients with schizophrenia and 535 healthy controls of Uyghur descent were genotyped for the *ANK3* gene rs10761482 locus using Taqman probe technology. SHEsis and SPSS17.0 software were used for data analysis.

Results: There were no significant differences in the genotype or allele frequencies between the case group and control group. Within the case group there was no relationship between gender or age of onset of schizophrenia and the genotype or allele frequencies. Separate analyses among men and among women also failed to identify significant differences in the allele and genotype frequencies between cases and controls or between patients with adolescent-onset schizophrenia and those with adult-onset schizophrenia

Conclusions: Our findings do not support previous reports about the relationship of the *ANK3* gene and schizophrenia. In the Uyghur nationality group recruited for this study there was no significant association between the *ANK3* gene rs10761482 polymorphism and schizophrenia. If these results are replicated in further studies, then the focus should change to understanding *why* this widely acknowledged association does not exist in this particular ethnic group.

Keywords: schizophrenia; *ANK3* gene; rs10761482 polymorphism, association studies, Uyghur nationality, China

[*Shanghai Arch Psychiatry*. 2014; **26**(5): 288-293. Epub 2014 Sept 29. doi: http://dx.doi.org/10.11919/j.issn.1002-0829.214033]

1. Background

Although linkage studies suggested that genetic factors play a major role in the onset and course of schizophrenia, the exact mechanisms remain unclear.[1] One gene of particular interest is *ANK3*, located on chromosome 10q21. It acts on the early development of the Ranviers nodes of axons in the central and peripheral nervous systems[2] and, thus, plays a key role in the regulation and differentiation of the nervous system.[3] When the *ANK3* gene is knocked out in mice, the mice show abnormal hypothalamic - pituitary - adrenal axis (HPA) functioning.[4] Ankyrin G (ANKG), which is encoded by the *ANK3* gene, is crucial for the stability of the neuronal membrane[5,6] and facilitates the connection between axons and ribbon synapses.[7] ANKG also regulates ion channels involved in the release of neurotransmitters;[8] abnormal expression of ANKG may induce abnormal activity in glutamate receptors (GluRs).[9] Many studies have demonstrated that abnormal release of brain neurotransmitters in different neural pathways is

[1] First Affiliated Hospital of Xinjiang Medical University, Xinjiang Uyghur Autonomous Region, China
[2] First People's Hospital of Xiantao City, Hubei Province, China
[3] Sixth People's Hospital of Hebei Province, Hebei Province, China
*correspondence: qizhongyi@126.com

A full-text Chinese translation of this article will be available at www.shanghaiarchivesofpsychiatry.org on November 25, 2014.

involved in the pathogenesis of schizophrenia. Thus, HPA axis dysfunction and abnormal glutamate activity – both of which are influenced by *ANK3* – are key targets in the search for the genetic causes of schizophrenia.

Several studies suggest that *ANK3* is important in the pathogenesis of schizophrenia. Athanasiu and colleagues conducted a genome-wide association study (GWAS) in 2663 European individuals with schizophrenia and 13,780 controls and reported that the *ANK3* gene rs10761482 polymorphism was associated with schizophrenia.[10] Yuan and colleagues found the same results in a sample of Han Chinese.[11] Another study reported that the *ANK3* was also related to the age of onset of schizophrenia.[12] In this study, we aim to assess the relationship of the *ANK3* rs10761482 polymorphism with schizophrenia among individuals of Uyghur decent (one of China's large ethnic minority groups) living in the Xinjiang region of western China and determine whether or not the polymorphism is different in persons with schizophrenia who have an early versus late age of onset.

2. Methods

2.1 Sample

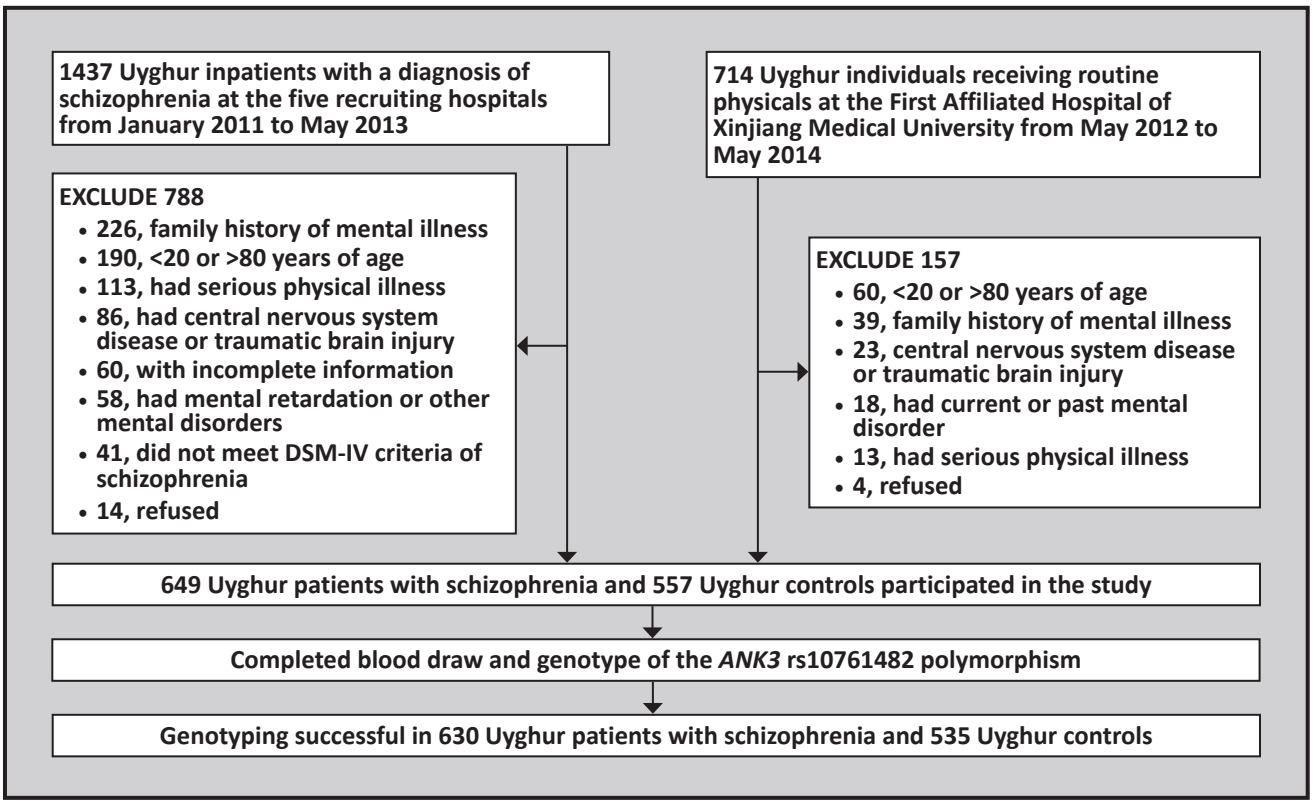
Figure 1 shows the enrollment process for the study. All the participants were natives to Xinjiang of the Uyghur nationality and had no biological connections with each other. Two senior psychiatrists conducted the diagnosis and collected the clinical data in the patient group. We provided a detailed explanation to all participants about the purpose of this study and obtained informed consent signed by the subjects or their guardians. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

2.1.1 Case group

A total of 649 Uyghur inpatients diagnosed with schizophrenia were recruited from six hospitals in the Xinjiang Uyghur Autonomous Region of China between January 2011 and May 2013: the Department of Psychology of the First Affiliated Hospital of Xinjiang Medical University, the Urumqi Peace Hospital, the Hotan Mental Health Hospital, the Kashgar First People's Hospital, the Yili Mental Health Hospital, and the Aksu Prefecture Kangning Hospital. Inclusion criteria were: (a) 20 to 80 years of age; (b) diagnosis of schizophrenia based on the criteria specified in the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV) as assessed by two trained psychiatrists using the Chinese version of the Structured Clinical Interview for DSM-IV (SCID)[13]; (c) no mental retardation or other mental disorders; (d) no family history of mental illnesses; (e) no central nervous system diseases or traumatic brain injury; and (f) no serious physical illnesses, such as cancer, endocrine disease, severe cardiovascular disease, or liver or kidney dysfunction.

The 649 patients in the case group included 389 (60%) males and 260 (40%) females. Their mean (sd) age was 44.4 (12.1) years. Among them, 134 (75 males and 59 females) had the first onset of illness when they

Figure 1. Identification of study participants



were 18 years of age or younger (the 'adolescent-onset' group); the remaining 515 (314 males and 201 females) had the first onset after 18 years of age (the 'adult-onset' group).

2.1.2 Control group

Individuals of the Uyghur minority who participated in a routine physical examination at the First Affiliated Hospital of Xinjiang Medical University from May 2012 to May 2014 were potential control subjects. They had to meet the same inclusion criteria as the case group (above) except that they had no present or prior history of mental disorders. Potential participants were administered the Composite International Diagnostic Interview (CIDI) by psychology research fellows to identify those with current or past mental illnesses. A total of 557 control subjects participated in the study, including 303 (54%) males and 254 (46%) females. Their mean age was 45.1 (15.50) years.

There was a borderline excess of males in the case group compared to the control group (60% vs. 54%; χ^2=3.76, p=0.052) but there was no significant difference in the mean age of the two groups (t=0.82, p=0.414).

2.2 Genotype assessment

Two milliliters of venous blood was drawn from each participant. Blood samples were prepared for genotyping using the DNA extraction system provided by the Tiangen Biotech Company in Beijing.

Based on findings from previous GWAS studies, we selected the loci rs10761482 of the *ANK3* gene for genotyping. According to the PubMed nucleic acid database, the rs10761482 (C/T) is in the first intron of the *ANK3* gene in Asian and European populations and the allele frequencies were greater than 0.1.

Taqman probe-based polymerase chain reaction (PCR) was used for genotyping. PCR was conducted using the GeneAmp PCR 9700 system (Applied Biosystem). Each well of the 384-well plate contains 5μl of PCR reaction material: 40ng DNA, 2.5μl PCR reaction mixture, and 0.035μl Taqman probes. Forty five cycles of amplification were performed using the following settings: 95°C for 10 minutes, 92°C for 15 seconds, and 60°C for 1 min. The product was genotyped using the ABI 7900 DNA detection system (Applied Biosystem, Foster City, CA, USA). In the patient group 97.1% (630/649) of the samples were successfully genotyped; in the control group 96.1% (535/557) were successfully genotyped. In order to ensure accuracy and reliability, we randomly selected 3% of the samples from each of the two groups to genotype again; the same results were obtained from all of these re-tested samples.

2.3 Statistical Analysis

Microsoft Excel was used to construct the database. SPSS17.0 software was used for analysis. Two-sample t-test was used to compare the age of onset and χ^2 test was used to compare the male-female ratios. In addition, the SHEsis software (http://analysis.bio-x.cn) was used to test if the data were in accordance with the Hardy-Weinberg equilibrium (HWE) and to analyze differences in the allele frequencies and genotypes between the two groups. Due to the borderline difference in the male-female ratios between the two groups, we also conducted a stratified analysis by gender.

3. Results

3.1 Comparisons of the allele and genotype frequencies between the case group and control group

The distribution of the rs10761482 polymorphisms met the Hardy-Weinberg equilibrium (HWE) requirements in all four subgroups of subjects considered in this analysis: male cases (p=0.059), male controls (p=0.116), female cases (p=0.956), and female controls (p=0.855). No statistically significant differences were found in the rs10761482 allele and genotype frequencies between the 630 cases and 535 controls who were successfully genotyped. As shown in Table 1, similar results were found when the analysis was stratified by gender; neither the allele frequency nor the genotype frequency were significantly different between male cases and controls or between female cases and controls.

Separate comparison of the results of the 381 males with schizophrenia who were successfully genotyped and the 249 females with schizophrenia who were successfully genotyped found no significant difference by gender in the allele frequency (OR=1.01, CI=0.77~1.33, p=0.942) or in the genotype frequency (χ^2=1.52, p=0.468).

3.2 Association between the onset age and the *ANK3* gene rs10761482 polymorphism

As shown in Table 2, there were no differences in either the genotype or the allele frequencies of the *ANK3* rs10761482 polymorphism between the 131 individuals with adolescent-onset schizophrenia and the 499 individuals with adult-onset schizophrenia who successfully completed the genotyping. Stratified analysis revealed similar results by gender: the frequencies of alleles and genotypes between male adolescent-onset and adult-onset patients with schizophrenia were not significantly different, and the frequencies of alleles and genotypes between female adolescent-onset and adult-onset patients were also not significantly different.

Separate analyses comparing the 131 adolescent-onset patients' results to those of all 535 controls found no difference in allele frequency (OR=0.97, CI=0.59~1.45, p=0.871) or in genotype frequency (χ^2=0.06, p=0.971). A similar comparison of the 499 adult-onset patients' results to those of all controls found no difference in allele frequency (OR=1.01, CI=0.82~1.28, p=0.907) or in genotype frequency (χ^2=0.02, p=0.992). These comparisons remained statistically insignificant after stratifying by gender (results provided on request).

Table 1. Comparisons of allele and genotype frequencies of the *ANK3* gene rs10761482 polymorphism between persons of Uyghur nationality with and without schizophrenia

rs10761482	Allele					Genotype				
	C n (%)	T n (%)	odds ratio	95%CI	*p*	C/C n (%)	C/T n (%)	T/T n (%)	χ^2	*p*
BOTH GENDERS										
cases (n=630)	983 (78.0)	277 (22.0)	1.00	[0.83~1.22]	0.967	377 (59.8)	229 (36.3)	24 (3.8)	0.01	0.995
controls (n=535)	834 (77.9)	236 (22.1)				320 (59.8)	194 (36.3)	21 (3.9)		
MALES										
cases (n=381)	595 (78.1)	167 (21.9)	0.98	[0.75~1.27]	0.855	226 (59.3)	143 (37.5)	12 (3.1)	0.04	0.981
controls (n=293)	460 (78.5)	126 (21.5)				176 (60.1)	108 (36.9)	9 (3.1)		
FEMALES										
cases (n=249)	388 (77.9)	110 (22.1)	1.04	[0.77~1.40]	0.810	151 (60.6)	86 (34.5)	12 (4.8)	0.07	0.967
controls (n=242)	374 (77.3)	110 (22.7)				144 (59.5)	86 (35.5)	12 (5.0)		

CI, confidence interval

Table 2. Comparisons of allele and genotype frequencies of the *ANK3* gene rs10761482 polymorphism between persons of Uyghur nationality with adolescent-onset schizophrenia (ADL) and persons of Uyghur nationality with adult-onset schizophrenia (ADU)

rs10761482	Allele					Genotype				
	C n (%)	T n (%)	odds ratio	95%CI	*p*	C/C n (%)	C/T n (%)	T/T n (%)	χ^2	*p*
BOTH GENDERS										
ADL group (n=131)	203 (77.5)	59 (22.5)	0.96	[0.69~1.33]	0.814	77 (58.8)	49 (37.4)	5 (3.8)	0.08	0.960
ADU group (n=499)	780 (78.2)	218 (21.8)				300 (60.1)	180 (36.1)	19 (3.8)		
MALES										
ADL group (n=74)	113 (76.4)	35 (23.6)	0.88	[0.80~1.35]	0.570	43 (58.1)	27 (36.5)	4 (5.4)	1.53	0.465
ADU group (n=307)	482 (78.5)	132 (21.5)				183 (59.6)	116 (37.8)	8 (2.6)		
FEMALES										
ADL group (n=57)	90 (78.9)	24 (21.1)	1.08	[0.65~1.80]	0.761	34 (59.6)	22 (38.6)	1 (1.8)	1.81	0.405
ADU group (n=192)	298 (77.6)	86 (22.4)				117 (60.9)	64 (33.3)	11 (5.7)		

CI, confidence interval

4. Discussion

4.1 Main findings

This study is the first to explore the role of the *ANK3* gene in schizophrenia in a relatively large sample of individuals of Uyghur descent. We found no statistically significant differences between cases and controls in either the allele frequencies or the genotypes of one of the *ANK3* polymorphisms that is most frequently associated with schizophrenia. We also found no link between the *ANK3* rs10761482 polymorphism and the age of onset of schizophrenia. Other studies[14] have also failed to replicate findings about the role of *ANK3* in schizophrenia, but the weight of the evidence – including a meta-analysis[11] confirming the role of allele C of the *ANK3* rs10761482 polymorphism and of allele T of the rs10994336 locus – still supports the hypothesized role of *ANK3* in the pathogenesis of schizophrenia. Several studies have identified mechanisms via which *ANK3* could be involved; for example, allele C of the *ANK3* rs9804190 polymorphism is associated with the down-regulation of mRNA expression in schizophrenia.[15] Nevertheless, our negative findings and those of other investigators suggest that other factors may play a role in moderating the effects of *ANK3*.

4.2 Limitations

Several factors may have contributed to our null findings. (a) The study sample was limited to individuals of the Uyghur minority group in China, a fairly homogenous group (with limited out-group marriage) that has both European and Asian genetic characteristics. Despite the similar C allele frequency of the locus rs10761482 in our sample (78%) to that reported for the European and Asian populations (74.8% and 75%, respectively, based on the PubMed database), the Uyghur group may have unique genetic characteristics that alter the relationship between *ANK3* and schizophrenia. (b) In limiting our sample to individuals with schizophrenia who do not have a family history of mental illness, we may have decreased the relative importance of genetic factors in the group of patients considered in the analysis. (c) We did not assess several other *ANK3* loci that may be related to the pathogenesis of schizophrenia including rs10761482, rs9804190, and rs10994336.[11,15] (d) Some studies suggest that genetic polymorphisms are associated with specific clinical subtypes of schizophrenia;[16,17] other than considering age of onset, we did not subclassify our results by other potentially important factors. (e) The sample size was too small to justify stratifying results by multiple variables.

4.3 Implications

Replication of widely accepted genetic findings about psychiatric illnesses in different, relatively homogenous ethnic groups may help identify factors that moderate the role of these presumed 'universal' genetic factors. Our failure to replicate findings of a relationship between the *ANK3* gene rs10761482 polymorphism and schizophrenia in a relatively large sample of patients with schizophrenia from the Uyghur minority group in western China is an example of the potential promise of such studies. If these results are replicated in further studies, then the focus should change to understanding *why* this widely acknowledged association does not exist in this particular ethnic group. Such follow-up studies would have considerable value in highlighting the genetic environment in which *ANK3* does or does not exert a role in the pathogenesis of schizophrenia.

Acknowledgements

The authors thank all the investigators for their assistance in this project, and the Bio-X Institute of Shanghai Jiao Tong University for providing technical support.

Conflict of Interest

The authors report no conflicts of interests related to this study.

Funding

This work was supported by the Natural Science Foundation of China (project name: 'de novo CNV in schizophrenia in persons of Uyghur nationality in Xinjiang', no. 81360209) and the Xinjiang Uyghur Autonomous Region Science and Technology Fund (project name:'Establishing a genetic database for depression in persons of Uyghur nationality in Xinjiang', no. 2010211A51).

Ethical approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

Informed consent

All participants or their guardians provided written informed consent to participate in the study.

中国新疆维吾尔族人群中 *ANK3* 基因 rs10761482 多态性与精神分裂症关联性的病例对照研究

钟衔江，张丽丽，韩书贤，罗晓，安治国，伊琦忠

背景：ANK3 基因 rs10761482 多态性已被发现与精神分裂症的发生相关联。

目的：评估新疆维吾尔族人群 ANK3 基因和精神分裂症之间的关联。

方法：使用 Taqman 探针技术对 630 例新疆维吾尔族精神分裂症患者和 535 名新疆维吾尔族健康人群进行 *ANK3* 基因 rs10761482 位点的基因分型。 采用 SHEsis 和 SPSS17.0 软件进行数据分析。

结果：病例组和对照组之间的基因型和等位基因频率无显著差异。在病例组，性别或精神分裂症发病年龄与基因型或等位基因频率之间没有显著关联。将男性和女性单独分析，病例组与对照组之间的等位基因和基因型频率均未发现显著差异，青春期发病与成年后发病的精神分裂症患者之间的等位基因和基因型频率也无显著差异。

结论：我们的研究结果不支持以往 ANK3 基因与精神分裂症有关联的报告。本研究招募的维吾尔族人群中，*ANK3* 基因 rs10761482 多态性与精神分裂症之间没有显著关联。如果这些结果在进一步的研究中得到证实，那么研究重点将转而了解为什么在这个特定的族群中不存在上述已经被广泛认可的关联。

关键词：精神分裂症，*ANK3* 基因，rs10761482 多态性，关联研究，维吾尔族，中国

本文全文中文版从 2014 年 11 月 25 日起在 www.shanghaiarchivesofpsychaitry.org 可供免费阅览下载

References

1. Farmer AE, McGuffin P. The pathogenesis and management of schizophrenia. *Drugs*. 1988; **35**: 177-185
2. Kapfhamer D, Miller DE, Lambert S, Bennett V, Glover TW, Burmeister M. Chromosomal localization of the ankyrinG gene (ANK3/Ank3) to human 10q21 and mouse 10. *Genomics.* 1995; **27**(1): 189-191. doi: http://dx.doi.org/10.1006/geno.1995.1023
3. Hansell NK, Medland SE, Ferreira MAR, Geffen GM, Zhu G, Montgomery GW, et al. Linkage analyses of event-related potential slow wave phenotypes recorded in a working memory task. *Behav Genet.* 2006; **36**(1): 29-44. doi: http://dx.doi.org/10.1007/s10519-005-9002-2
4. Sobotzik JM, Sie JM, Politi C, Del Turco D, Bennett V, Deller T, et al. AnkyrinG is required to maintain axo-dendritic polarity in vivo. *Proc Natl Acad Sci USA.* 2009; **104**(41): 17564-17569. doi: http://dx.doi.org/10.1073/pnas.0909267106
5. Bennett V. Ankyrins: adaptors between diverse plasma membrane proteins and the cytoplasm. *J Biol Chem.* 1992; **267**(13): 8703-8706
6. Bennett V, Otto E, Kunimoto M, Kordeli E, Lambert S. Diversity of ankyrins in the brain. *Biochem Soc Trans*. 1991; **19**(4): 1034-1039
7. Hedstrom KL, Ogawa Y, Rasband MN. AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. *Cell Biol*. 2008; **183**(4): 635-640. doi: http://dx.doi.org/10.1083/jcb.200806112
8. Kosaka T, Komada M, Kosaka K. Sodium channel cluster, betaIV-spectrin and ankyrinG positive ''hot spots'' on dendritic segments of parvalbumin-containing neurons and some other neurons in the mouse and rat main olfactory bulbs. *Neurosci Res.* 2008; **62**(3): 176-186. doi: http://dx.doi.org/10.1016/j.neures.2008.08.002
9. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, et al. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell*. 2012; **149**(3): 525-537. doi: http://dx.doi.org/10.1016/j.cell.2012.03.028
10. Athanasiu, L, Mattingsdal M, Kähler AK, Brown A, Gustafsson O, Agartz I, et al. Gene variants associated with schizophrenia in a Norwegian genome-wide study are replicated in a large European cohort. *J Psychiatr Res.* 2012; **44**(12): 748-753. doi: http://dx.doi.org/10.1016/j.jpsychires.2010.02.002
11. Yuan A, Yi Z, Wang Q, Sun J, Li Z, Du Y, et al. ANK3 as a risk gene for schizophrenia: new data in Han Chinese and meta analysis. *Am J Med Genet B Neuropsychiatr Genet*. 2012; **159**(8): 997-1005. doi: http://dx.doi.org/10.1002/ajmg.b.32112
12. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, et al. Sequencing choromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell*. 2012; **149**(3): 525-537. Epub 2012 Apr 19. doi: http://dx.doi.org/10.1016/j.cell.2012.03.028
13. Phillips MR, Liu XH. *Adapted Chinese version of Structured Clinical Interview for DSM-IV-TR Axis I Disorders*, *Research Version, Patient Edition (SCID-I/P)* by Michael B. First, Robert L. Spitzer, Miriam Gibbon, and Janet B.W. Williams. Shanghai: Suicide Research and Prevention Center, Shanghai Mental Health Center; 2011
14. Gella A, Segura M, Durany N, Pfuhlmann B, Stöber G, Gawlik M. Is Ankyrin a genetic risk factor for psychiatric phenotypes? *BMC Psychiatry*. 2011; **11**(103): 1-5. doi: http://dx.doi.org/10.1186/1471-244X-11-103
15. Roussos P, Katsel P, Davis KL, Bitsios P, Giakoumaki SG, Jogia J, et al. Molecular and genetic evidence for abnormalities in the nodes of Ranvier in schizophrenia. *Arch Gen Psychiatry*. 2012; **69**(1): 7-15. doi: http://dx.doi.org/10.1001/archgenpsychiatry.2011.110
16. Su L, Wei B, Chen Q, Feng Q, Pan R, Zhou Y, et al. [Association study of PRODH gene variant rs385440 with schizophrenia in Zhuang and Han nationality of Guangxi]. *Zhonghua Xing Wei Yi Xue Yu Nao Ke Xue Za Zhi*. 2012; **21**(1): 36-39. Chinese
17. Guo W, Li W, Zhang H, Hao W. [Association study of disrupted-in-schizophrenia-1 gene single nucleotide polymorphism with schizophrenia in Han Chinese population]. *Zhonghua Xing Wei Yi Xue Yu Nao Ke Xue Za Zhi*. 2012; **21**(4): 337-339. Chinese

(received: 2014-03-20; accepted: 2014-07-01)

Xianjiang Zhong obtained his Master's degree from Xinjiang Medical University in June 2014. He has been a resident physician in the Mental Health Department of the First People's Hospital in Xiantao City, Hubei Province since July 2014. His research interest is in the molecular genetics of schizophrenia.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Common Variants in *QPCT* Gene Confer Risk of Schizophrenia in the Han Chinese Population

Raja Amjad Waheed Khan,[1,2#] Jianhua Chen,[1,3#] Jiawei Shen,[1] Zhiqiang Li,[1] Meng Wang,[1] Zujia Wen,[1] Zhijian Song,[1] Wenjin Li,[1] Yifeng Xu,[3] Yongyong Shi,[1,4,5]* Qizhong Yi,[6]* and Weidong Ji[4,5]*

[1]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, Shanghai, People's Republic of China

[2]University of Azad Jammu and Kashmir, Department of Chemistry, Muzaffarabad 13100, Pakistan

[3]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China

[4]Shanghai Changning Mental Health Center, Shanghai, People's Republic of China

[5]Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai, People's Republic of China

[6]Department of Psychiatry, First Teaching Hospital of Xinjiang Medical University, Urumqi, People's Republic of China

Manuscript Received: 3 June 2015; Manuscript Accepted: 14 October 2015

Schizophrenia (SCZ) is a common and severe mental disorder, its etiology has not been elucidated completely. In one previous genome-wide association study (GWAS) of SCZ in the Caucasian population, the *QPCT* has been reported as susceptible gene for SCZ. The *QPCT* gene encodes Glutaminyl cyclase (QC), an enzyme which is involved in the post translational modification by converting N-terminal glutamate of protein to pyroglutamate, which is resistant to protease degradation, more hydrophobic, and prone to aggregation and neurotoxic. To further investigate the role of this gene in the pathogenesis of schizophrenia in the Han Chinese population, we conducted this study in 1,248 (Mean age ± S.D, 36.44 years ± 9.0) SCZ cases, 1,248 (Mean age ± S.D, 30.62 years ± 11.35) healthy control samples for a case control study. We genotyped six SNPs in this study, including one positive SNP of the previous study, using the Sequenom MassARRAY platform. We found that rs2373000 was significantly associated with SCZ before correction [rs2373000: *P* allele = 0.016, $\chi^2 = 5.784$, OR [95%CI] = 0.861 [0.762–0.972], *P* genotype = 0.018, $\chi^2 = 0.069$]. After permutation correction for multiple testing, rs2373000 [rs2373000: *P* Allele corrected = 0.063, *P* genotype corrected = 0.069] showed marginal association with SCZ. Additionally, one pathogenic haplotype (TGT) containing rs2373000 was also significantly associated with SCZ. Our results are consistent with the findings of previous study and the genetic risk of *QPCT* gene for SCZ also exists in the Han Chinese population. © 2015 Wiley Periodicals, Inc.

Key words: schizophrenia; *QPCT* gene; genetic association; SNP; the Han Chinese

How to Cite this Article:
Khan RAW, Chen J, Shen J, Li Z, Wang M, Wen Z, Song Z, Li W, Xu Y, Shi Y, Yi Q, Ji W. 2016. Common Variants in *QPCT* Gene Confer Risk of Schizophrenia in the Han Chinese Population.
Am J Med Genet Part B 171B:237–242.

INTRODUCTION

Schizophrenia (SCZ) is a common and severe psychiatric disorder. The population prevalence of SCZ is 1% and heritability is 70–85 %

[#]Raja Amjad Waheed Khan and Jianhua Chen contributed equally to this work.

Conflict of interest: None.
Grant sponsor: 973 Program; Grant number: 2015CB559100; Grant sponsor: National 863; Grant numbers: 2012AA02A515, 2012AA021802; Grant sponsor: Natural Science Foundation of China; Grant numbers: 31325014, 81130022, 81272302, 81121001, 81171271; Grant sponsor: Shanghai Key Laboratory of Psychotic Disorders; Grant number: 13dz2260500; Grant sponsor: Shanghai Health and Family Planning Commission; Grant number: 20134Y082; Grant sponsor: Shanghai Municipal Education Commission and Shanghai Education Development Foundation; Grant number: 12SG17; Grant sponsor: Shanghai Subject Chief Scientist; Grant number: 15XD1502200; Grant sponsor: Shanghai Jiao Tong University Liberal Arts and Sciences Cross-Disciplinary Project; Grant number: 13JCRZ02.
*Correspondence to:
Yongyong Shi, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), and the Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China.
Email: shiyongyong@gmail.com
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 22 October 2015
DOI 10.1002/ajmg.b.32397

© 2015 Wiley Periodicals, Inc.

[Burmeister et al., 2008]. It is also evident from family, adoption, and twin studies that heritability in the development of SCZ is 64% [Paul et al., 2009]. Disability-adjusted life years (DALYs) are 7.4% for Schizophrenia [Whiteford et al., 2013]. The exact etiology of SCZ is unknown, however based on the family, adoption, and twin studies, we may conclude that genetic factors play an important role in the development of this disease [Craddock, 2005]. Nowadays, molecular approaches like candidate gene association study and GWAS (genome wide association study) have proposed various susceptible genes which play an important role in the pathogenesis of SCZ. Meanwhile, *QPCT* gene has been reported as a new locus for SCZ in Caucasian by GWAS plus meta-analysis and replication study [Ripke et al., 2013].

QPCT gene is located on chromosome 2 at co-ordinate 37571753-37600465, spanning about 28.71–Kbp of DNA and contains seven exons (NM_012413) (http://genome.ucsc.edu/cgi-bin/hgGateway). This gene encodes Glutaminyl cyclase (QC), an enzyme which shows high level of expression in brain and other peripheral tissues. This enzyme catalyzes the formation of pyroglutamate (pGlu) at the N terminus of the various peptides and proteins [Sykes et al., 1999; Hartlage-Rübsamen et al., 2009]. The QC is localized in the Golgi complex, endoplasmic reticulum, and secretary granules where it is supposed to play an important role in maturation of different proteins [Cynis et al., 2008a; Hartlage-Rübsamen et al., 2009].

Glutaninyl cyclase (QC) enzyme has also been reported to involve in post translational modification of amyloid- β peptide by converting N terminal glutamate of this peptide at position 3 or 11 into pyroglutamic acid, which is resistant to protease degradation, more hydrophobic and prone to aggregation which is neurotoxic [Cynis et al., 2008b; Kimpe et al., 2012].

Currently, it is known that unsettled Ca^{2+} homeostasis not only enhances mRNA level of QC but also its enzyme activity. The QC promoter is considered to contain binding sites for calcium dependent transcription factors such as c-*fos* and *c-jun*. These two transcription factors are proposed to be induced by the same Ca^{2+} related stimuli as QC, and their upregulation precedes QC expression. This mutual up regulation of enzyme and these factors is not observed in non-neuronal cell. Upregulation of QC selectively in neuronal cells via Ca^{2+} dependent transcription factors is the major consequence of unsettled Ca^{2+} homeostasis [Kimpe et al., 2012].

Previous GWAS of schizophrenia in the Caucasian with meta-analysis and replication has shown significant association of single nucleotide polymorphism (SNP), rs2373000 ($P = 6.78 \times 10^{-9}$) with SCZ [Ripke et al., 2013]. In our study, a case control analysis was designed to further investigate whether common variants in this gene are associated with schizophrenia in the Han Chinese population. Total six SNPs including one positive SNP of previous study covering this gene were included for genotyping by using Sequenom MassARRAY platform.

MATERIALS AND METHODS

Samples/Subjects

Totally 1,248 unrelated SCZ patients (845 males and 403 females) and 1,248 healthy controls (672 males and 576 females) were included in this study. The mean ages of SCZ patients and healthy controls were 36.44 years (±9.0) and 30.62 years (±11.35), respectively.

All the patients were out-patients or sometimes stable in-patients, Shanghai in origin and living in Shanghai China. Patients were interviewed by two independent psychiatrists and diagnosed strictly according to DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) based on SCID-1 (structured Clinical Interview for DSM-IV Axis 1 Disorders). Normal controls were selected from general public of the Han Chinese Population in Shanghai. The control subjects were also interviewed by two independent psychiatrists according to DSM-IV criteria based on (SCID-1) [American Psychiatric Association, 1994].

In written information from volunteers regarding their medical histories with detailed questions about psychosis, and other complex disease was obtained. A face to face interview was conducted from volunteers before blood collection and physical examinations such as height, weight, blood pressure, etc. were also carried out.

Informed consent was obtained from all the subjects and the study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). This study was reviewed and approved by the local ethical committee.

SNP Selection and Genotyping

Genomic DNA was extracted from peripheral blood samples using Quick Gene DNA whole blood kit L (FUJIFILM) protocol. Five tag SNPs (rs6708524, rs3770752, rs4384764, rs3770748, rs6708310) were selected using haploview software version 4.2, with pair-wise tagging $r^2 \geq 0.6$ and minor allele frequency (MAF) ≥ 0.05 [Barrett et al., 2005] and human genome browser of the University of California-Santa Cruz (UCSC) (http//genome.ucsc.edu/). In addition to this, one positive SNP of previous study (rs2373000) was also included in this study. Detail of six SNPS is given in the Table I. Relative position of SNPs obtained from Vector NTI (www.invitrogen.com/VectorNTI) is shown in (Fig. 1). The coverage of tag SNPs for *QPCT* gene was calculated by using Haploview, which was 100% [Barrett et al., 2005]. All the selected SNPs were

TABLE I. The Information of Six SNPs in *QPCT* Gene Region

SNP ID	rs6708524	rs3770752	rs4384764	rs2373000	rs3770748	rs6708310
Position	Chr. 2:37572687	Chr. 2:37576136	Chr. 2:37590284	Chr. 2:37592628	Chr. 2:37595525	Chr. 2:37600427
Functional	Intron	Intron	Intron	Intron	Intron	3-UTR
Polymorphism	A/G	C/T	A/G	C/T	C/T	A/G

genotyped using the Sequenom MassARRAY matrix—assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry platform (Sequenom, San Diego, CA) using DNA isolated from peripheral leucocytes of blood samples.

MassARRAY design software package (v4.0) was used to design the specific SNP filtering. The quality of PCR amplified fragments and extension primer specificity was ascertained prior to running the reaction. Shrimp Alkaline phosphate phosphatase was utilized for the dephosphorylation of residual nucleotides prior to iPLEX Gold reaction. Following a single-base extension, reaction products were desalted with spectro clean resin (Sequenom), and 10 nl was spotted onto the SpectroCHIP using the MassARRAY Nanodispenser.

The MassARRAY Analyzer Compact MALDI-TOF mass spectrometer was used to determine product mass. In order to clarify uncertain genotype calls, a manual review was done. An assay with a call rate of less than 80% within the same Spectro Chip was considered to have failed. The overall call rate in our assay was more than 97%.

Statistical Analysis

Allele and genotype frequencies, haplotype analysis, Hardy-Weinberg equilibrium (HWE) analysis, 1,000 permutation tests, and association tests for schizophrenia were performed by using online SHEsis software (http://shesisplus.bio-x.cn/SHEsis.html) [SHI and HE, 2005; Li et al., 2009]. This is a user friendly software platform which is equipped with a series of highly efficient analytic tools designed for association studies.

The Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) was used to calculate case-control genetic power [Purcell et al., 2003]. The statistical significance was assumed at the threshold of 0.05. A total 1,000 permutation were carried out to correct the *P* values for multiple testing. The χ^2 test was used to evaluate Hardy–Weinberg equilibrium. Differences in schizophrenia cases and controls were also evaluated by using χ^2 test. The D'value between markers was used to represent the linkage disequilibrium. The only those haplotypes with an estimated frequency >3% were included for analysis.

RESULTS

Single Site Analysis

The *P* values of Hardy–Weinberg equilibrium (HWE) for one SNP exceeded from 0.05 in healthy controls. In the following studies, we excluded SNP (rs6708524) due to its failure in Hardy–Weinberg equilibrium tests ($P < 0.05$).

The allele and genotype frequencies of five SNPs in the patient samples and normal controls were shown in Table II. For SCZ, rs2373000 showed significant association before correction [rs2373000: *P* allele = 0.016, $\chi^2 = 5.784$, OR (95%CI) = 0.861 (0.762–0.972), *P* genotype = 0.018, $\chi^2 = 8.001$]. After 1,000 permutation correction, rs2373000 showed marginal association with SCZ [rs2373000: *P* corrected allele = 0.063, *P* corrected genotype = 0.069] (Table II). It is important to note that the same risk allele (T) for corresponding positive SNP in previous [Ripke et al., 2013] as well as in our study is same which further supports our results (Table II).

In summary, after 1,000-permutation correction, rs2373000 marginally associated with SCZ [rs2373000: *P* corrected allele x2009;= 0.063, *P* corrected genotype = 0.069] (Table II). Some of the selected SNPs in the present study are in relatively strong linkage disequilibrium. Although the *P*-value after correction did not reach the global significant, this is the potential casual SNP because the global significant threshold is too stringent. However, our results are consistent with results of previous study and same risk allele is shared between these two studies. So, we successfully replicated rs2373000 in the Han Chinese population (Table II).

Linkage Disequilibrium

Adjacent SNPs with pairwise D' $\geq$ 0.90 were classified in the same block. Therefore, two haplotype blocks were identified in SCZ. Haplotype blocks with the following sequence were analyzed in SCZ: rs3770752-rs4384764-rs2373000 (16.49 Kb) in block 1 and rs3770748-rs6708310 (4.90 Kb) in block 2 as shown in Figure 2.

Haplotype Analysis

Haplotype analysis results are shown in (Table III). One haplotype (TGT: $P = 4.50 \times 10^{-4}$, *P* corrected = 0.001) for the block rs3770752- rs4384764-rs2373000 showed significant association with SCZ. Notably, this haplotype contains corresponding positive SNP which was significantly associated in previous as well as in our study. Interestingly, positive haplotype containing risk allele (T) for corresponding positive SNP in our study and previous study is same (Table III).

DISCUSSION

Schizophrenia is a severe psychiatric disorder. The complete etiology of this disorder is still unknown. The genetic factors play an important role in the pathogenesis of this disorder [Craddock, 2005]. Recently, Schizophrenia working group of the psychiatric genomic consortium (PGC) provided new insights regarding SCZ. In this study, schizophrenia related associations were profoundly enriched at enhancers active in brain rather than other tissues [Consortium, 2014].

Glutaminyl cyclase (QC) and its isoenzyme (iso *QC*) have been reported to modify N-terminal of CCL2, a chemokine which is associated with tumor progression in several cancer types. Expression of *QPCT* gene has been reported to correlate with mRNA level of substrate CCL2 in NF-kB dependent pathway [Kehlen et al., 2012]. Previous GWAS of schizophrenia in Caucasian, meta-analysis, and replication has shown significant association of rs2373000 ($P = 6.78 \times 10^{-9}$) in *QPCT* gene with SCZ [Ripke et al., 2013].

In our study, after permutation correction, rs2373000 [rs2373000: *P* corrected allele = 0.063, *P* corrected genotype = 0.069] showed marginal association with SCZ in the Han Chinese population. In previous study, minor allele (T) was more prevalent in schizophrenia cases than controls in Caucasian population. Similarly, in our study major allele (T) was also more frequently observed in schizophrenia cases than controls. The minor allele frequency in the Han Chinese population is (MAF = 0.31), whereas in the Caucasian population it is (MAF = 0.44). In the Caucasian

TABLE II. Allelic and Genotypic Frequency in Cases and Controls of SCZ and Normal Controls Against Five SNPs in *QPCT* Gene

SNP	Alleles		OR [95%CI]	χ^2	*P*-Value	Permutation *P*	Genotypes			χ^2	*P*-Value	Permutation *P*
rs3770752	T	C					T/T	T/C	C/C			
SCZ	2218 (0.895)	258(0.104)	0.859 [0.718–1.027]	2.754	0.096	0.335	999 (0.806)	220 (0.177)	19 (0.015)	3.022	0.22	0.618
Control	2091(0.88)	283(0.119)					924 (0.778)	243 (0.204)	20 (0.016)			
rs4384764	G	A					G/G	G/A	A/A			
SCZ	1461 (0.591)	1007 (0.408)	1.004 [0.896–1.126]	0.006	0.935	1	431 (0.349)	599 (0.485)	204 (0.165)	0.034	0.982	1
Control	1414 (0.593)	970(0.406)					416 (0.348)	582 (0.488)	194 (0.162)			
rs2373000	T	C					T/T	T/C	C/C			
SCZ	1786 (0.719)	696(0.28)	0.861 [0.762–0.972]	5.784	**0.016**	**0.063**	651 (0.524)	484 (0.39)	106 (0.085)	8.001	**0.018**	**0.069**
Control	1717 (0.688)	777(0.311)					584 (0.468)	549 (0.44)	114 (0.091)			
rs3770748	T	C					C/C	T/C	T/T			
SCZ	1250 (0.507)	1214 (0.492)	0.982 [0.877–1.099]	0.095	0.756	0.997	299 (0.242)	616 (0.5)	317 (0.257)	0.096	0.953	1
Control	1219 (0.511)	1163 (0.488)					284 (0.238)	595 (0.499)	312 (0.261)			
rs6708310	A	G					A/A	G/G	G/A			
SCZ	1389 (0.575)	1023 (0.424)	0.953 [0.85–1.069]	0.648	0.42	0.857	405 (0.335)	222 (0.184)	579 (0.48)	0.663	0.717	0.993
Control	1325 (0.564)	1023 (0.435)					377 (0.321)	226 (0.192)	571 (0.486)			

Bold digits represent $P < 0.05$.
SCZ, schizophrenia; CI, confidence interval; OR, odd ratio; χ^2, chi square test.

FIG. 1. Relative position of selected SNPs inside *QPCT* gene. This structure was designed using Vector NTI (Invitrogen, Carlsbad, CA, USA). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

population, the minor allele is (T) whereas in the Han Chinese population it is major allele. Notably, in both studies (T) allele emerged as the risk allele which hints that this marker may influence the activity of *QPCT* gene in schizophrenia.

Genetic power was calculated for rs2373000 in both allelic (1df) and genotypic (2df) models. For allele, power was 0.55 whereas for genotype it was merely 0.47. The genetic power calculation software is available at (http://pngu.mgh.harvard.edu/~purcell/gpc/)[Purcell et al., 2003]. The genetic power analysis for aforementioned SNP indicates that we might find significant association of this SNP with SCZ if we increase the sample size while the genetic power is more

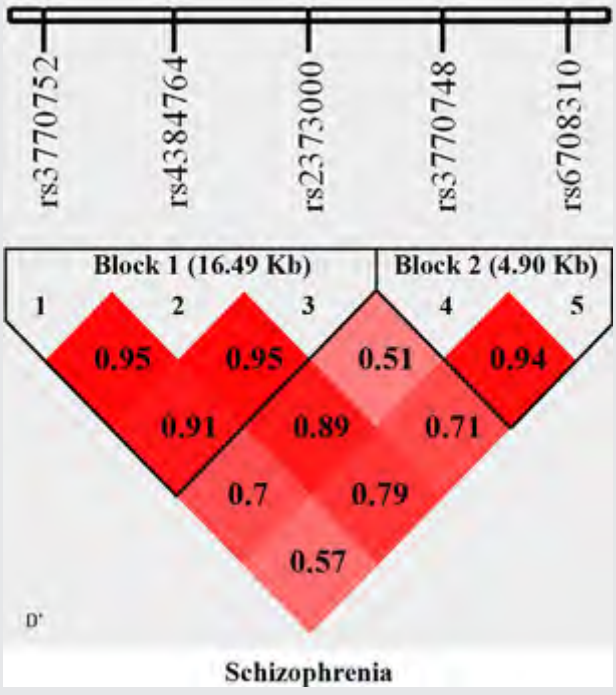


FIG. 2. Linkage disequilibrium among 5 SNPs in Schizophrenia cases obtained from SHEsis platform. The number inside each square of the plot corresponds to the D' value between the corresponding markers. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmgb].

TABLE III. Haplotye Analysis for *QPCT* Gene in Schizophrenia

SNPs blocks with D'≥0.90	Haplotype	Case (freq)	Control (freq)	χ^2	OR [95%CI]	*P*-value	Corrected *P*
rs3770752- rs4384764- rs2373000	TGT	769 (0.312)	657 (0.277)	12.314	1.246 [1.102–1.409]	**4.50e-04**	**0.001**
	TGC	438 (0.177)	470 (0.198)	1.378	0.917 [0.794–1.059]	0.24	
	TAT	990 (0.401)	946 (0.399)	1.633	1.077 [0.961–1.207]	0.201	
	CGC	242 (0.098)	264 (0.111)	1.064	0.907 [0.755–1.091]	0.302	
rs3770748- rs6708310	CA	1160 (0.482)	1115 (0.475)	1.635	1.075 [0.962–1.202]	0.2	
	TA	227 (0.094)	209 (0.089)	0.814	1.094 [0.899–1.332]	0.366	
	TG	996 (0.413)	990 (0.421)	0.03	1.01 [0.901–1.131]	0.862	

Haplotypes with frequency <0.03 in cases or controls were not included.
Bold letters represent significant *P* value <0.05.

than 0.8 in future studies. Comparing our study with previous one [Ripke et al., 2013], which has comparatively very large sample size. This is the first study, where we replicated the SNP (rs2373000) in the Han Chinese population.

Furthermore, one haplotype (TGT) for block rs3770752-rs4384764-rs2373000 containing corresponding positive SNP in our study was also significantly associated with SCZ [P corrected $= 0.001$], further validating this study.

Previous study conducted in the Chinese population, rs3770748 located in the intron of *QPCT* was found significantly associated with bone mineral density (BMD) in pre-menopausal ($P = 0.002$) and post-menopausal ($P = 0.023$) women [Huang and Kung, 2007]. Another study conducted in Japanese population has highlighted *QPCT* as the essential modifier of pituitary hormone, and single nucleotide polymorphism (SNP) variation inside this gene was attributed to BMD among postmenopausal women [Ezura et al., 2004]. In a multistage GWAS in the Caucasian, SNP (rs3770745) ($P = 2.71 \times 10^{-10}$) was successfully associated with Chronic Lymphocytic Leukemia [Berndt et al., 2013].

In summary, based on our results and potential involvement of this gene in different disorders, we may conclude that *QPCT* is an important gene which might regulate a key pathway toward pathogenesis of schizophrenia and other disorders. This is the first study which revealed that genetic risk exists in *QPCT* gene for Schizophrenia in the Han Chinese population. Comparing our study with previous one, previous study has comparatively very large sample size which is the limitation of our study. Further studies are needed to validate the association of this SNP with large sample size in the Chinese as well as other populations. It is also required to study the potential role of this gene in the correlated pathways.

ACKNOWLEDGMENTS

We thank and acknowledge all the participants of this study. This work was supported by the 973 Program (2015CB559100), the National 863 projects (2012AA02A515, 2012AA021802), the Natural Science Foundation of China (31325014, 81130022, 81272302, 81121001, 81171271), Shanghai Key Laboratory of Psychotic Disorders (13dz2260500), the Youth Research Project of Shanghai Health and Family Planning Commission (20134Y082), "Shu Guang" project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17), Program of Shanghai Subject Chief Scientist (15XD1502200), and the Shanghai Jiao Tong University Liberal Arts and Sciences Cross-Disciplinary Project (13JCRZ02).

REFERENCES

American Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders (DSM-IV). American Psychiatric Press, Washington DC.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics 21(2):263–265.

Berndt S, Skibola C, Joseph VC, Nicola JN, Alexandra W, Zhaoming C, Wendy M, Alain W, Sophia SK, Rachel SL, et al. 2013. Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. Nat Genet 45(8):868–876.

Burmeister M, McInnis MG, Zöllner S. 2008. Psychiatric genetics: Progress amid controversy. Nat Rev Genet 9(7):527–540.

Craddock N. 2005. The genetics of schizophrenia and bipolar disorder: Dissecting psychosis. J Med Genet 42(3):193–204.

Cynis H, Rahfeld J-U, Stephan A, Kehlen A, Koch B, Wermann M, Demuth H-U, Schilling S. 2008a. Isolation of an isoenzyme of human glutaminyl cyclase: Retention in the golgi complex suggests involvement in the protein maturation machinery. J Mol Biol 379(5):966–980.

Cynis H, Scheel E, Saido TC, Schilling S, Demuth H-U. 2008b. Amyloidogenic processing of amyloid precursor protein: Evidence of a pivotal role of glutaminyl cyclase in generation of pyroglutamate-modified amyloid-β. Biochemistry 47:7405–7413.

Ezura Y, Kajita M, Ishida R, Yoshida S, Yoshida H, Suzuki T, Hosoi T, Inoue S, Shiraki M, Orimo H, et al. 2004. Association of multiple nucleotide variations in the pituitary glutaminyl cyclase gene (QPCT) with low radial BMD in adult women. J Bone Miner Res 19(8):1296–1301.

Hartlage-Rübsamen M, Staffa K, Waniek A, Wermann M, Hoffmann T, Cynis H, Schilling S, Demuth HU, Roßner S. 2009. Developmental expression and subcellular localization of glutaminyl cyclase in mouse brain. Int J Dev Neurosci 27(8):825–835.

Huang Q-Y, Kung AWC. 2007. The association of common polymorphisms in the QPCT gene with bone mineral density in the Chinese population. J Hum Genet 52:757–762.

Kehlen A, Haegele M, Menge K, Gans K, Immel UD, Hoang-Vu C, Klonisch T, Demuth HU. 2012. Role of glutaminyl cyclases in thyroid carcinomas. Endocr Relat Cancer 20(1):79–90.

Kimpe LD, Bennis A, Zwart R, Haastert ESv, Hoozemans JJM, Scheper W. 2012. Disturbed Ca2+ homeostasis increases glutaminyl cyclase expression; connecting two early pathogenic events in alzheimer's disease in vitro. PLoS ONE 7(9):1–8.

Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L, Shi Y. 2009. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: Update of the SHEsis (http://analysis.bio-x.cn). Cell Res 19(4):519–523.

Paul L, Benjamin HY, Camilla B, Yudi P, Tyrone DC, Patrick FS, Christina MH. 2009. Common genetic determinants of schizophrenia and bipolar disorder in Swedish families: A population-based study. Lancet 373:234–239.

Purcell S, Cherny S, Sham PC. 2003. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 19(1):149–150.

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kähler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, et al. 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45(10):1150–1159.

Schizophrenia Working Group of the Psychiatric Genomics Consortium. 2014. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511:421–427.

SHI YY, HE L. 2005. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15(2):97–98.

Sykes PA, Watson SJ, Temple Jr S, Bateman Jr RC. 1999. Evidence for tissue-specic forms of glutaminyl cyclase. FEBS Lett 455:159–161.

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, et al. 2013. Global burden of disease attributable to mental and substance use disorders: Findings from the global burden of disease study 2010. Lancet 382(9904):1575–1586.

ORIGINAL RESEARCH

SNAP25 Is Associated With Schizophrenia and Major Depressive Disorder in the Han Chinese Population

Qingzhong Wang, PhD; Yanlin Wang, PhD; Weidong Ji, PhD; Guoquan Zhou, PhD; Kuanjun He, PhD; Zhiqiang Li, PhD; Jianhua Chen, PhD; Wenjin Li, PhD; Zujia Wen, PhD; Jiawei Shen, PhD; Yu Qiang, MS; Jue Ji, BS; Yujiong Wang, PhD; Yongyong Shi, PhD; Qizhong Yi, MD, PhD; and Yonggang Wang, PhD

ABSTRACT

Objective: Synaptosomal-associated protein of 25 kDa (*SNAP25*) is a member of the soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) protein complex, which plays essential roles in the modulation of different voltage-gated calcium channels and neurotransmitter release. Many previous studies have reported the *SNAP25* gene to be significantly associated with attention-deficit/hyperactivity disorder (ADHD). Recently, shared genetic variants have been demonstrated in 5 major psychiatric disorders, including schizophrenia, major depressive disorder, bipolar disorder, autism spectrum disorders, and ADHD. However, no compelling, convincing evidence has suggested an association between *SNAP25* and schizophrenia or major depressive disorder. Thus, we investigated the association between *SNAP25* and both schizophrenia and major depressive disorder in the Han Chinese population.

Method: We performed a large-scale case-control study to test the association between *SNAP25* and 2 major mental disorders, schizophrenia (*DSM-IV* criteria) and major depressive disorder (*DSM-IV* criteria), in the Han Chinese population. Seven single-nucleotide polymorphisms (SNPs) were genotyped in 1,330 schizophrenia patients, 1,045 major depressive disorder patients, and 1,520 healthy controls of Han Chinese origin.

Results: Two SNPs, rs3787283 and rs3746544, were found to be associated with both schizophrenia (rs3746544, adjusted $P = .00257$) and major depressive disorder (rs3746544, adjusted $P = .0485$; rs3787283, adjusted $P = .00387$) in this study. The AG haplotype consisting of rs3787283 and rs3746544 was also significantly associated with both schizophrenia and major depressive disorder (schizophrenia: adjusted $P = .0126$; major depressive disorder: adjusted $P = .000580$). Additionally, we carried out a meta-analysis of the current data and published association results and further confirmed the association between rs3746544 and schizophrenia ($P_{meta} = .002$, $OR_{meta} = 1.213$ [95% CI, 1.077–1.367]).

Conclusions: Our results indicated that SNPs in *SNAP25* represented a common risk factor of both schizophrenia and major depressive disorder in the Han Chinese population.

J Clin Psychiatry 2015;76(1):e76–e82
© Copyright 2015 Physicians Postgraduate Press, Inc.

Submitted: *December 20, 2013; accepted April 11, 2014 (doi:10.4088/JCP.13m08962).*
Corresponding author: *Yongyong Shi, PhD, Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi; Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200127, PR China (shiyongyong@gmail.com).*

Mental disorders are caused by a combination of biological, psychological, and environmental factors.[1] These mental diseases have been recognized as leading causes of morbidity, and they require extensive long-term medical and social care.[2] Schizophrenia and major depressive disorder are 2 of the most common mental disorders. Schizophrenia is a severe mental disorder with a lifetime risk of approximately 1% and is characterized by hallucinations, delusions, and cognitive deficits, with a heritability estimated at up to 80%.[3] Major depressive disorder includes a distinct change in mood and is characterized by sadness or irritability and is accompanied by at least several psychophysiological changes.[4] Previous studies comparing the concordance rates of major depressive disorder between monozygotic and dizygotic twins have suggested a heritability of approximately 37%.[5]

Recent applications of genome-wide association studies (GWAS) and next generation sequencing have discovered rare copy number variants and common single-nucleotide polymorphisms (SNPs) that are associated with the risk of psychiatric disorders.[6] Furthermore, these studies have shown an overlap between the genetic variant that is susceptible to different diseases.[7] To examine the shared genetic etiology, Lee et al[8] collected GWAS data from the Psychiatric Genomics Consortium and applied univariate and bivariate methods to 5 disorders—schizophrenia, bipolar disorder, major depressive disorder, autism spectrum disorders, and attention-deficit/hyperactivity disorder (ADHD). These researchers reported that the values of SNP-based heritability are reasonably robust and were significantly greater than 0 for all 5 disorders. They also found that the genetic risk variants were substantially shared between schizophrenia and bipolar disorder (high genetic mean ± standard error correlation, 0.68 ± 0.04), bipolar disorder and major depressive disorder (moderate genetic correlation, 0.43 ± 0.06), schizophrenia and major depressive disorder (moderate genetic correlation, 0.47 ± 0.06), and ADHD and major depressive disorder (moderate genetic correlation, 0.32 ± 0.07); and they found a low genetic correlation exists between schizophrenia and autism spectrum disorders (0.68 ± 0.04).[8] In *The Lancet*,[9] the Psychiatric Genomics Consortium performed a meta-analysis of the GWAS data for 33,332 cases and 27,888 controls, which were distributed among the 5 major psychiatric disorders in Psychiatric Genomics Consortium (major depressive disorder, bipolar disorder, schizophrenia, autism spectrum disorders, and ADHD). In that study, they reported SNPs at 4 loci in regions on chromosomes 3p21 and 10q24 and SNPs in 2 L-type

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Clinical Points

- Schizophrenia and major depressive disorder share clinical features and genetic risks factors.
- Synaptosomal-associated protein of 25 kDa (*SNAP25*) might contribute to biological pathogenic factors that are pivotal to the identification of suitable treatments.

voltage-gated calcium-channel subunits, *CACNA1C* and *CACNB2*, which exceeded the threshold of genome-wide significance ($P < 5 \times 10^{-8}$). Their results provided evidence that genetic variation in calcium channel signaling can increase the risk of these 5 neuropsychiatric disorders.[9]

SNAP25 is an integral part of the SNARE complex, which enables synaptic vesicle exocytosis in conjunction with syntaxin and synaptobrevin.[10] Multiple studies have revealed that the level of *SNAP25* mRNA or protein expression was altered in specific brain regions of patients with schizophrenia, which provides supportive evidence for the potential importance of *SNAP25* and synaptic-mediated signal pathway in the etiology of schizophrenia.[11–15] Until recently, several groups have analyzed the association between *SNAP25* and schizophrenia. For example, Carroll et al[16] investigated 38 tagging SNPs that span the *SNAP25* locus in UK schizophrenic cases. They found that several independent SNPs showed nominal significance, and rs3787283 was the most significant SNP associated with schizophrenia ($P = .006$, OR = 1.25). They also found that the strongest associated SNP of schizophrenia in *SNAP25* was also associated with ADHD with the opposite risk allele.[16] In addition, Fanous et al[17] genotyped 18 haplotype-tagging SNPs within the *SNAP25* gene in a sample of 270 Irish high-density families and performed an association study in an independent sample set with 657 cases and 411 controls. They observed robust association in both single marker and haplotype-based analyses between *SNAP25* and schizophrenia in an Irish family.[17] Moreover, a Japanese research group[18] conducted a 2-stage genetic association analysis of *SNAP25* with schizophrenia. In the first-stage screening, they detected only 1 SNP (rs12626080) and a haplotype (rs363014 and rs12626080) in *SNAP25*, with nominal significance in 377 cases and 377 controls. However, they could not replicate these nominally significant SNPs and haplotypes in the second-stage analysis. Taken together, these association studies provided clues of *SNAP25*'s important role in the etiology of schizophrenia; however, due to an insufficient sample size, there were no compelling reports of a positive association between *SNAP25* and schizophrenia and major depressive disorder.

To investigate whether *SNAP25* is associated with mental diseases in the Han Chinese population, we genotyped 7 SNPs (rs363039, rs363050, rs362549, rs362998, rs363006, rs3787283, rs3746544) within *SNAP25* in 1,330 schizophrenia patients, 1,045 major depressive disorder patients, and 1,520 healthy controls of Han Chinese origin.

METHOD

Subjects

Our sample sets consisted of 1,330 schizophrenia patients (805 male and 525 female; mean ± SD age = 36.4 ± 8.96 years), 1,045 major depression patients (729 male and 316 female; mean ± SD age = 34.4 ± 12.1 years), and 1,520 healthy controls (774 male and 746 female; mean ± SD age = 30.6 ± 11.4 years). All subjects were of Han Chinese origin. The patients were interviewed by 2 independent psychiatrists and diagnosed strictly according to the *Diagnostic and Statistical Manual of Mental Disorders,* Fourth Edition (*DSM-IV*). Healthy controls were randomly selected from the Han Chinese general population, and they resided in the same area as the patient group. All volunteers were interviewed by 2 psychiatrists to rule out control subjects with a family history of mental illness. We also obtained written informed consent from all participants. Our study was reviewed and approved by the local ethics committee of Human Genetics Resources.

Selection and Genotyping of SNPs

We used the University of California-Santa Cruz (UCSC) human genome browser (http://genome.ucsc.edu/) and Haploview 4.1 software[19] to select tag SNPs within the *SNAP25* gene in the Hapmap Han Chinese in Beijing, China + Japanese in Tokyo, Japan (CHB + JPT) population (Release 21). Single nucleotide polymorphisms with a reported minor allele frequency below 0.03 were not considered in the analysis. In addition, 7 tag SNPs were selected for genotyping. These tag SNPs can capture 93% of common SNPs information with $r^2 > 0.5$ (analyzed using tagger server online software, http://www.broadinstitute.org/mpg/tagger/server.html). Among the 7 SNPs, rs3746544 and rs3787283 were reported to be positive by Carroll et al.[16] The other 5 SNPs were randomly chosen according to different blocks. The structure of the *SNAP25* gene and the location of the 7 selected tag SNPs are shown in Figure 1. All SNPs were genotyped using TaqMan SNP Genotyping Assays on Fluidigm EP1 platform. All probes were designed and synthesized by Applied Biosystems (Foster City, California). All 7 SNPs had a genotype call rate > 93%.

Sample Preparation

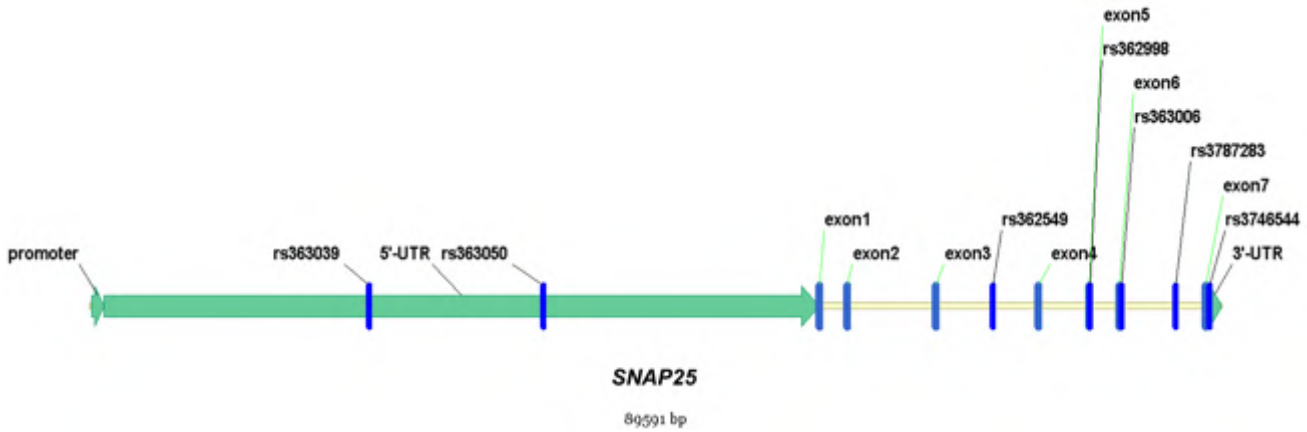
Genomic DNA was extracted from peripheral blood using the Quick Gene DNA whole blood kit (Fugifilm, Tokyo, Japan). Isolated DNA from each sample was used for SNP genotyping with TaqMan chemistry (Applied Biosystems, Foster City, California) and Fluidigm dynamic array chips (Fluidigm Corporation; San Francisco, California).

Statistical Analysis

We calculated Hardy-Weinberg equilibrium, allelic association, genotypic association, and odds ratio (OR) using SHEsis software (http://analysis.bio-x.cn).[20,21] Hardy-Weinberg equilibrium was determined using the χ^2 test for goodness of fit. Linkage disequilibrium estimation and haplotype analyses were performed on Haploview 4.1.[19]

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Figure 1. Distribution of the 7 SNPs in the *SNAP25* Gene



Abbreviations: *SNAP25* = synaptosomal-associated protein of 25kDa, SNP = single-nucleotide polymorphism, UTR = untranslated region.

We corrected the *P* values of genotypes and alleles using Bonferroni correction in which the *P* values obtained were multiplied by the number of SNPs, and Bonferroni correction for haplotype analysis was multiplied by the haplotype numbers. The global *P* value of haplotype analysis was calculated using the omnibus χ^2 test. All tests were 2-tailed and statistical significance was established at $P < .05$.

Meta-Analysis

For the meta-analysis, we searched PubMed using keywords *rs3746544* or *MnlI (rs3746544)* and *schizophrenia*. All the case-control studies between rs3746544 and schizophrenia, up to February 2014, were included in the analysis. A χ^2-based *Q* statistic test was performed to assess the heterogeneity. If the result of the heterogeneity test was $P > .05$, the fixed-effects model (Mantel-Haenszel methods) was used to pool ORs; otherwise, the random-effects model was chosen. The significance of the pooled ORs was determined by the *Z* test. Meta-analytic procedures were carried out using Comprehensive Meta-Analysis v.2.0 (Biostat Inc; Englewood, New Jersey; http://www.meta-analysis.com/index.php).

RESULTS

Genotype distributions of all SNPs were in Hardy-Weinberg equilibrium in healthy controls. The allele and genotype frequencies of 7 SNPs in all samples are listed in Table 1. The results of haplotype analysis are shown in Table 2. Pairwise linkage disequilibrium analysis of the 7 SNPs is shown in Figure 2. In addition, we identified 2 haplotype blocks, where 1 block contained 3 SNPs (rs362549 and rs362998 and rs363006) and another block contained 2 SNPs (rs3787283 and rs3746544) (Figure 2).

For schizophrenia, we found that rs3746544 was significantly associated with schizophrenia in the χ^2 test (allele: $\chi^2_1 = 12.705$, $P = .000368$, OR = 1.277 [95% CI, 1.160–1.460]; genotype: $\chi^2_2 = 15.126$, $P = .000525$). After Bonferroni correction, rs3746544 was still significant in the allele and genotype distribution ($P_{allele} = .00257$, $P_{genotype} = .00368$). Haplotype analysis revealed that the *P* value for the A-G haplotype consisting of rs3746544 and rs3787283 was .0284 and the corrected global *P* value was .0126.

For major depressive disorder, we found that rs3787283 and rs3746544 were significant in both allele and genotype distribution in the χ^2 test (rs3787283: [allele] $\chi^2_1 = 11.943$, $P = .000553$; [genotype] $\chi^2_2 = 11.796$, $P = .002764$, OR = 0.8017 [95% CI, 0.71–0.91]; rs3746544: [allele] $\chi^2_1 = 7.295$, $P = .00694$; [genotype] $\chi^2_2 = 8.476$, $P = .0145$, OR = 1.213 [95% CI, 1.054–1.396]). After Bonferroni correction, the allele of rs3787283 and rs3746544 still remained significant (rs3787283, allele: $P_{corrected} = .00387$; rs3746544, allele: $P_{corrected} = .0485$). Haplotype analysis revealed that rs3746544 and rs3787283 were significant between major depressive disorder patients and healthy controls (global $P = .000580$, after correction) (Table 2).

Including our current data and published results, we analyzed 5,293 subjects in total in a meta-analysis of rs3746544, including 2,400 schizophrenia cases and 2,893 healthy controls. In the homogeneity analysis, no significant heterogeneity was detected among 5 individual sample groups (rs3746544: $\chi^2_4 = 3.270$, $P = .514$). The meta-analysis (*Z* score test) of the combined samples was assessed with a fixed-effect model. Combining all samples in the meta-analysis, we found that rs3746544 showed significant association with schizophrenia ($Z = 3.171$, $P = .002$, OR = 1.213; Table 3). The forest plot of the meta-analysis is presented in Figure 3.

DISCUSSION

Many studies have demonstrated that alterations of the *SNAP25* gene structure, expression, and function can directly contribute to neuropsychiatric and neurologic disorders, including ADHD, epilepsy, autism spectrum disorder, schizophrenia.[22] The *coloboma* mutant mouse model contains a neutron-irradiation–induced gene region deletion located on mouse chromosome 2, which includes *SNAP25*.[23] The *coloboma* mutant mice displayed spontaneous hyperactivity, inattention, impulsivity, the symptoms of which are parallel to ADHD.[23] Interestingly, the normal phenotype was restored using transgenic *SNAP25* in *coloboma* mutant mice.[24] The number of linkage studies on

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Table 1. Allele and Genotype Association Analysis of 7 SNPs in *SNAP25*

SNP ID	Allele Frequency		OR (95% CI)	Allelic *P* Value[a]	Genotype Frequency			Genotypic *P* Value[a]	H-W *P* Value	Call Rates
rs363039	A	G			AA	AG	GG			
Schizophrenia	0.510	0.490	0.922 (0.817 – 0.039)	.601	0.265	0.489	0.246	.280	.487	0.949
MDD	0.538	0.462	1.030 (0.90 – 1.167)	.638	0.281	0.514	0.205	.824	.288	0.951
Control	0.530	0.470			0.277	0.507	0.216		.582	0.941
rs363050	A	G			AA	AG	GG			
Schizophrenia	0.307	0.693	1.040 (0.925 – 1.170)	.510	0.102	0.410	0.488	.802	.230	0.939
MDD	0.291	0.709	0.964 (0.843 – 1.101)	.586	0.086	0.410	0.504	.761	.863	0.938
Control	0.293	0.707			0.095	0.407	0.498		.262	0.976
rs362549	A	G			AA	AG	GG			
Schizophrenia	0.567	0.433	0.932 (0.829 – 1.048)	.241	0.327	0.481	0.193	.473	.465	0.962
MDD	0.571	0.429	0.949 (0.839 – 1.072)	.401	0.320	0.503	0.178	.545	.414	0.954
Control	0.584	0.416			0.342	0.483	0.174		.863	0.976
rs362998	A	G			AA	AG	GG			
Schizophrenia	0.207	0.793	1.017 (0.876 – 1.180)	.823	0.044	0.328	0.629	.724	.916	0.952
MDD	0.210	0.790	1.036 (0.887 – 1.210)	.655	0.047	0.327	0.626	.805	.646	0.961
Control	0.205	0.795			0.048	0.313	0.639		.050	0.962
rs363006	A	G			AA	AG	GG			
Schizophrenia	0.157	0.843	1.093 (0.929 – 1.286)	.280	0.031	0.328	0.629	.724	.118	0.953
MDD	0.146	0.854	1.004 (0.846 – 1.194)	.955	0.026	0.313	0.639	.805	.289	0.938
Control	0.145	0.855			0.025	0.313	0.639		.265	0.972
rs3787283	A	G			AA	AG	GG			
Schizophrenia	0.419	0.581	0.999 (0.887 – 1.126)	.999	0.176	0.486	0.338	.963	.963	0.939
MDD	0.366	0.634	0.802 (0.70 – 0.909)	**.00387**[b]	0.140	0.452	0.408	**.0196**[b]	.415	0.942
Control	0.419	0.581			0.179	0.481	0.341		.669	0.947
rs3746544	G	T			GG	GT	TT			
Schizophrenia	0.281	0.719	1.277 (1.160 – 1.460)	**.00257**[b]	0.068	0.427	0.505	**.00368**[b]	.0569	0.954
MDD	0.272	0.728	1.226 (1.065 – 1.410)	**.0485**[b]	0.066	0.411	0.524	**.0144**	.213	0.953
Control	0.235	0.765			0.056	0.357	0.587		.994	0.965

[a]Significant *P* values (< .05) are in boldface.
[b]Corrected *P* value derived using the Bonferroni correction.
Abbreviations: H-W = Hardy-Weinberg equilibrium, MDD = major depressive disorder, OR = odds ratio, *SNAP25* = synaptosomal-associated protein of 25kDa, SNP = single-nucleotide polymorphism.

Table 2. Haplotype Association Results for *SNAP25* in Case-Control Samples

		Schizophrenia				MDD			
Haplotype	Samples	*P* (case frequency)	*P* (control frequency)	*P* Value[a]	Global *P*	*P* (case frequency)	*P* (control frequency)	*P* Value[a]	Global *P*
rs362549-	A-G-A	.153	.139	.235	.025	.138	.139	.886	
rs362998-	A-G-G	.419	.446	**.0490**		.435	.446	.361	.775
rs363006	G-A-G	.202	.193	.544		.204	.193	.481	
	G-G-G	.219	.297	.452		.217	.209	.589	
rs3787283-	A-G	.235	.201	**.0284**[b]	**.0126**[b]	.206	.201	.694	
rs3746544	A-T	.194	.218	.053		.166	.218	**.0012**[b]	
	G-G	.044	.032	**.0329**		.063	.032	**.0016**[b]	**.000580**[b]
	G-T	.526	.548	.139		.565	.548	.285	

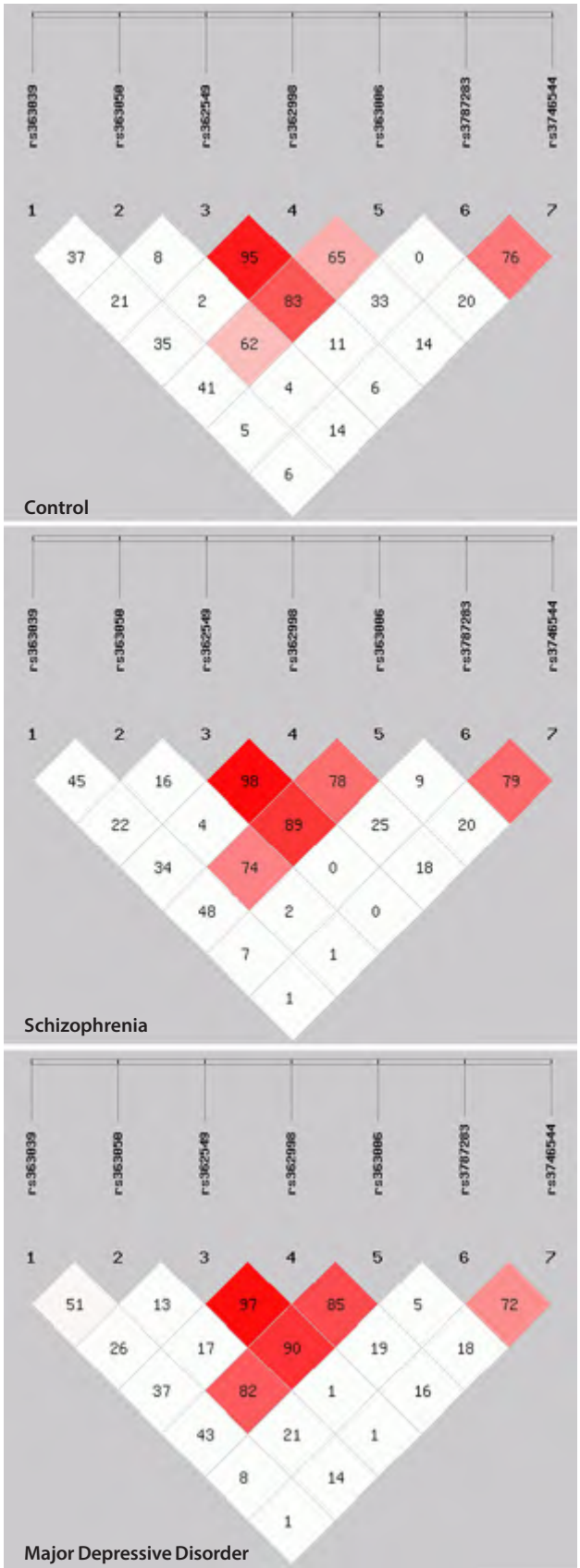
[a]Significant *P* values (< .05) are in boldface.
[b]Corrected *P* value derived using Bonferroni correction.
Abbreviation: MDD = major depressive disorder, *SNAP25* = synaptosomal-associated protein of 25kDa.

polymorphisms in the *SNAP25* gene locus have revealed its linkage with ADHD.[25–27,28] Recent GWAS have confirmed the association of *SNAP25* with ADHD.[29] However, there has been no additional evidence supporting the association between *SNAP25* and schizophrenia until now.

In this study, the main finding was a significant association between rs3746544 within the *SNAP25* gene and schizophrenia and major depressive disorder in a Chinese Han population. The data suggested that the *SNAP25* gene might be involved in schizophrenia and major depressive disorder susceptibilities, and the data also supported previous reports that schizophrenia and major depressive disorder may overlap in pathogenesis.[30] Carroll et al[16] have reported that rs3746544 showed a nominal association significance with schizophrenia in a UK population via a mutation screening and genotyping ($P = .004$, OR = 1.2).[16] Feng et al[27] reported a significant association between the rs3746544 SNP as well as the haplotype transmission of 2 polymorphic loci and ADHD in 97 nuclear families. Kim and colleagues[31] investigated further polymorphisms and confirmed the role of *SNAP25* in the inheritance of ADHD. Interestingly, they also showed that this association was stronger in a subgroup of ADHD patients who suffered from comorbid major depressive disorder.[31] In addition, Etain et al[32] observed the association of a common variant located in the *SNAP25* promoter region with early-onset bipolar

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Figure 2. Linkage Disequilibrium Among the 7 SNPs[a]



[a]The linkage disequilibrium structure (D′ value) between marker pairs is indicated by the shaded matrices. The figure was generated using Haploview 4.1.

Abbreviation: SNP = single-nucleotide polymorphism.

disorder, but not with a late-onset subgroup. Taken together, these data indicated that the risk loci within *SNAP25* region might contribute to the genetics risk shared by different psychiatric disorders.

In this study, we genotyped a large sample set, which ensured that our data would be reliable. Our data demonstrated that only rs3746544 was significantly associated with schizophrenia ($P = .00257$, after correction). The meta-analysis P value for rs3746544, based on combining the results from this study and published results, was .002, suggesting robust association of rs3746544 with schizophrenia, further supporting the hypothesis that *SNAP25* might be potential susceptibility genes for schizophrenia. We reported that rs3746544, which is located in the 3′ untranslated region (3′UTR) of the *SNAP25* gene, affected the binding of microRNAs (mirSNPs). mirSNPs are a novel class of functional SNPs, which are located either in the gene of the microRNA or in the target mRNA.[33] mirSNPs can alter the interaction between a microRNA and large genes to modulate homeostatic protein levels, resulting in phenotypical changes, such as diseases.[34] To predict rs3746544 polymorphism effects on microRNA binding splicing, we employed mirSNPs finder software (http://www.bioguo.org/miRNASNP/), which enabled the investigation of the predicted target gain and loss due to SNPs in microRNA seed regions or in target mRNA 3′UTRs.[35] We found that hsa-mir-3617 and *SNAP25* produced miRNA/SNP target duplexes if the rs3746544 allele was T (Figure 4). Previous studies have demonstrated a decrease of *SNAP25* expression in the hippocampus of schizophrenia patients.[12] We speculated that the aberrant expression in schizophrenia may be caused by hsa-mir-3617 silencing of *SNAP25* expression via the generation of microRNA binding sites. However, further functional studies are required to authenticate the role of hsa-mir-3617 and the T allele of rs3746544 in schizophrenia.

We also found that rs3787283 was significantly associated with major depressive disorder ($P = .00387$, after correction). Kim et al[31] observed that rs3787283 was most significantly associated with ADHD via family-based association test analysis ($P = .002$). They also observed that ADHD with comorbid major depressive disorder may enhance the association observed with *SNAP25* among subphenotypes of ADHD.[31] However, to the best of our knowledge, no previous study has investigated the potential involvement of major depressive disorder susceptibility. Thus, we speculated that potential functional mutations near this marker should be explored and then tested for their association with major depressive disorder.

In the analysis of haplotypes, significant associations with schizophrenia and major depressive disorder were also found in haplotypes of rs3787283-rs3746544, which covered the large part of the tested region. According to the study, the haplotype linkage disequilibrium test has a higher power and is more robust than the corresponding single-marker linkage disequilibrium tests; our results suggested that the rs3787283-rs3746544 might encompass the susceptibility

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Table 3. Meta-Analysis of 5 Population-Based Association Studies Between Schizophrenia and rs3746544

Study, Year	Population	Cases, n	Controls, n	A-Allele Frequencies Cases	A-Allele Frequencies Controls	*P* Value[a]	OR (95% CI)
Musil et al,[38] 2008	Caucasian	162	312	0.360	0.390	.483	0.869 (0.586–1.287)
Golimbet et al,[39] 2010	Caucasian	66	136	0.378	0.324	.438	1.275 (0.690–2.355)
Lochman et al,[40] 2013	Czech	192	213	0.360	0.330	.515	1.146 (0.760–1.728)
Carroll et al,[16] 2009	UK	650	712	0.370	0.320	.050	1.251 (1.000–1.565)
Current study	Chinese	1,330	1,520	0.281	0.235	.005	1.274 (1.077–1.508)
Total		2,400	2,893	0.320	0.284	.002	1.213 (1.077–1.367)

[a]Heterogeneity analysis: $\chi^2_4 = 3.270$, $P = .514$, $I^2 = 0.000$.
Abbreviation: OR = odds ratio.

Figure 3. Forest Plot of Meta-Analysis for rs3746544 [G] (risk allele)

Study	Odds Ratio	Upper Limit	Lower Limit	*Z* Value	*P* Value	Relative Weight
Musil et al,[38] 2008	0.869	1.287	0.586	−0.702	.483	9.19
Golimbet et al,[39] 2010	1.275	2.355	0.690	0.776	.438	3.78
Lochman et al,[40] 2013	1.146	1.728	0.760	0.650	.515	8.44
Carroll et al,[16] 2009	1.251	1.565	1.000	1.960	.050	28.40
Current study	1.274	1.508	1.077	2.823	.005	50.19
Total	1.213	1.367	1.077	3.171	.002	

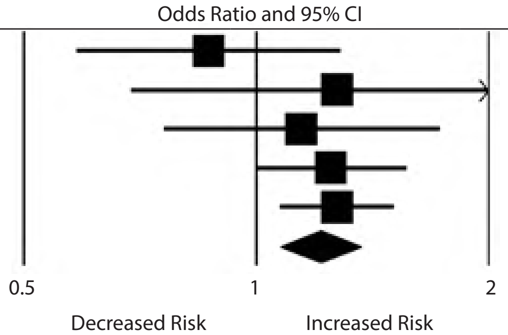


Figure 4. The Allele of rs3746544 Gain miRNA/SNP Target Duplexes[a]

SNP in gene 3'UTR	miRNA	SNP location and Target site on UTR	Energy change (kcal/mol)	miRNA/SNP-target duplexes	Effect by SNP on 3'UTR
SNAP25; rs3746544 [illegible]	hsa-miR-3617	239 222-215	Wild: 0.00 SNP: -20.60	[illegible]	gain

[a]hsa-mir-3617 and *SNAP25* produce miRNA/SNP target duplexes if the rs3746544 allele is T.
Abbreviations: miRNA = microRNA, *SNAP25* = synaptosomal-associated protein of 25kDa, SNP = single-nucleotide polymorphism, UTR = untranslated region.

variants for schizophrenia and major depressive disorder.[36,37] This result was consistent with a report by Carroll et al,[16] in which they found strong linkage disequilibrium between rs3787283 and rs3746544 within *SNAP25*, which was associated with schizophrenia in the United Kingdom.

Taken together, our results indicate that SNPs and haplotype within the *SNAP25* gene were significantly associated with schizophrenia and major depressive disorder in the Han Chinese population. Further studies using a larger sample size are suggested to validate our findings.

Author affiliations: Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi (Drs Q. Wang, Shi, and Yi); Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education) (Drs Q. Wang, He, Z. Li, Chen, W. Li, Wen, Shen, and Shi and Mss Qiang and Ji); Institute of Social Cognitive and Behavioral Sciences (Drs Q. Wang, He, Z. Li, Chen, Shen, Shi, and Yonggang Wang); Prenatal Diagnosis Center, International Peace Maternity and Child Health Hospital (Dr Yanlin Wang); Institute of Neuropsychiatric Science and Systems Biological Medicine (Dr Shi); and Department of Neurology, Renji Hospital, School of Medicine (Dr Yonggang Wang), Shanghai Jiao Tong University; Key Laboratory of Molecular Medicine, Ministry of Education, Department of Biochemistry and Molecular Biology, Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, (Dr Yanlin Wang); Shanghai Changning Mental Health Center (Drs Ji and Shi); Shanghai Changning Beixinjing Street Community Health Service Center (Dr Zhou); Shanghai Institute of Mental Health (Dr Chen), Shanghai; and Key Laboratory of Ministry of Education for Conservation and Utilization of Special eBiological Resources in the Western, and College of Life Science, Ningxia University, Yinchuan, Ningxia (Dr Yuijong Wang), P.R. China.

Author contributions: Drs Q. Wang, Yanlin Wang, Ji, and Zhou contributed equally to this work. The 3 primary investigators for this study, Drs Shi (shiyongyoung@gmail.com), Yi (13079911689@126.com), and Yonggang Wang (w100yg@163.com), all contributed equally and should be considered as authors to have responsibility for this article. Actual contributions of each author are listed as follows—study concept and design: Drs Shi, Yi, and Q. Wang; analysis and interpretation of data: Drs Ji, Zhou, Z. Li, Wen, Shen, and Qiang and Ms Ji; drafting of the manuscript: Drs Q. Wang, Yanlin Wang, and Ji; critical revision of the manuscript: Drs Shi, Yi, and Yonggang Wang; statistical analysis: Drs Yanlin Wang, He, Chen, W. Li, and Yujiong Wang; obtainment of funding: Drs Shi and Yi; and study supervision: Dr Shi.

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

Potential conflicts of interest: The authors declare no competing financial interests.
Funding/support: This work was supported by the Natural Science Foundation of China (31325014, 81130022, 81272302, 31000553, 81121001), National 863 project (2012AA02A515), the Shanghai Jiao Tong University Liberal Arts and Sciences Cross-Disciplinary Project (13JCRZ02), 973 Program (2010CB529600), Shanghai Changning Health Bureau program (20104Y06001), Program for Changjiang Scholars and Innovative Research Team in University (IRT1025), Foundation for the Author of National Excellent Doctoral Dissertation of China (201026), Shanghai Rising-Star Program Shanghai Science and Technology Development Funds (12QA1401900), and "Shu Guang" project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17).
Role of sponsors: The funding organizations had no role in the design or conduct of the study; the collection, management, analysis or interpretation of the data; or the preparation, review, or approval of the manuscript.
Acknowledgments: The authors thank all of the patients and healthy individuals who participated in the study.

REFERENCES

1. Ghaemi SN. Paradigms of psychiatry: eclecticism and its discontents. *Curr Opin Psychiatry*. 2006;19(6):619–624.
2. Craddock N, O'Donovan MC, Owen MJ. The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *J Med Genet*. 2005;42(3):193–204.
3. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry*. 2003;60(12):1187–1192.
4. Spitzer RL. *DSM-IV-TR Case Book*. Arlington, VA: American Psychiatric Publishing; 2002.
5. Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry*. 2000;157(10):1552–1562.
6. Gershon ES, Alliey-Rodriguez N, Liu C. After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. *Am J Psychiatry*. 2011;168(3):253–256.
7. Frazer KA, Murray SS, Schork NJ, et al. Human genetic variation and its contribution to complex traits. *Nat Rev Genet*. 2009;10(4):241–251.
8. Lee SH, Ripke S, Neale BM, et al; International Inflammatory Bowel Disease Genetics Consortium (IIBDGC). Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat Genet*. 2013;45(9):984–994.
9. Smoller JW, Craddock N, Kendler K, et al; Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet*. 2013;381(9875):1371–1379.
10. Wang Y, Tang BL. SNAREs in neurons—beyond synaptic vesicle exocytosis [review]. *Mol Membr Biol*. 2006;23(5):377–384.
11. Thompson PM, Sower AC, Perrone-Bizzozero NI. Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. *Biol Psychiatry*. 1998;43(4):239–243.
12. Thompson PM, Egbufoama S, Vawter MP. SNAP-25 reduction in the hippocampus of patients with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2003;27(3):411–417.
13. Young CE, Arima K, Xie J, et al. SNAP-25 deficit and hippocampal connectivity in schizophrenia. *Cereb Cortex*. 1998;8(3):261–268.
14. Karson CN, Mrak RE, Schluterman KO, et al. Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: a possible neurochemical basis for 'hypofrontality.' *Mol Psychiatry*. 1999;4(1):39–45.
15. Fatemi SH, Earle JA, Stary JM, et al. Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia. *Neuroreport*. 2001;12(15):3257–3262.
16. Carroll LS, Kendall K, O'Donovan MC, et al. Evidence that putative ADHD low risk alleles at SNAP25 may increase the risk of schizophrenia. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(7):893–899.
17. Fanous AH, Zhao Z, van den Oord EJ, et al. Association study of SNAP25 and schizophrenia in Irish family and case-control samples. *Am J Med Genet B Neuropsychiatr Genet*. 2010;153B(2):663–674.
18. Kawashima K, Kishi T, Ikeda M, et al. No association between tagging SNPs of SNARE complex genes (STX1A, VAMP2 and SNAP25) and schizophrenia in a Japanese population. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(7):1327–1331.
19. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005;21(2):263–265.
20. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*. 2005;15(2):97–98.
21. Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res*. 2009;19(4):519–523.
22. Corradini I, Verderio C, Sala M, et al. SNAP-25 in neuropsychiatric disorders. *Ann N Y Acad Sci*. 2009;1152(1):93–99.
23. Hess EJ, Jinnah HA, Kozak CA, et al. Spontaneous locomotor hyperactivity in a mouse mutant with a deletion including the Snap gene on chromosome 2. *J Neurosci*. 1992;12(7):2865–2874.
24. Steffensen SC, Henriksen SJ, Wilson MC. Transgenic rescue of SNAP-25 restores dopamine-modulated synaptic transmission in the coloboma mutant. *Brain Res*. 1999;847(2):186–195.
25. Brophy K, Hawi Z, Kirley A, et al. Synaptosomal-associated protein 25 (SNAP-25) and attention deficit hyperactivity disorder (ADHD): evidence of linkage and association in the Irish population. *Mol Psychiatry*. 2002;7(8):913–917.
26. Mill J, Curran S, Kent L, et al. Association study of a SNAP-25 microsatellite and attention deficit hyperactivity disorder. *Am J Med Genet*. 2002;114(3):269–271.
27. Feng Y, Crosbie J, Wigg K, et al. The SNAP25 gene as a susceptibility gene contributing to attention-deficit hyperactivity disorder. *Mol Psychiatry*. 2005;10(11):998–1005, 973.
28. Brookes K, Xu X, Chen W, et al. The analysis of 51 genes in *DSM-IV* combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. *Mol Psychiatry*. 2006;11(10):934–953.
29. Franke B, Neale BM, Faraone SV. Genome-wide association studies in ADHD. *Hum Genet*. 2009;126(1):13–50.
30. Green EK, Grozeva D, Jones I, et al; Wellcome Trust Case Control Consortium. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry*. 2010;15(10):1016–1022.
31. Kim JW, Biederman J, Arbeitman L, et al. Investigation of variation in SNAP-25 and ADHD and relationship to co-morbid major depressive disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(6):781–790.
32. Etain B, Dumaine A, Mathieu F, et al. A SNAP25 promoter variant is associated with early-onset bipolar disorder and a high expression level in brain. *Mol Psychiatry*. 2010;15(7):748–755.
33. Mishra PJ, Mishra PJ, Banerjee D, et al. MiRSNPs or MiR-polymorphisms, new players in microRNA mediated regulation of the cell: introducing microRNA pharmacogenomics. *Cell Cycle*. 2008;7(7):853–858.
34. Saunders MA, Liang H, Li W-H. Human polymorphism at microRNAs and microRNA target sites. *Proc Natl Acad Sci U S A*. 2007;104(9):3300–3305.
35. Gong J, Tong Y, Zhang HM, et al. Genome-wide identification of SNPs in microRNA genes and the SNP effects on microRNA target binding and biogenesis. *Hum Mutat*. 2012;33(1):254–263.
36. Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet*. 1999;65(4):1161–1169.
37. Akey J, Jin L, Xiong M. Haplotypes vs single marker linkage disequilibrium tests: what do we gain? *Eur J Hum Genet*. 2001;9(4):291–300.
38. Musil R, Spellmann I, Riedel M, et al. SNAP-25 gene polymorphisms and weight gain in schizophrenic patients. *J Psychiatr Res*. 2008;42(12):963–970.
39. Golimbet VE, Alfimova MV, Gritsenko IK, et al. Association between a synaptosomal protein (SNAP-25) gene polymorphism and verbal memory and attention in patients with endogenous psychoses and mentally healthy subjects. *Neurosci Behav Physiol*. 2010;40(4):461–465.
40. Lochman J, Balcar VJ, Sťastný F, et al. Preliminary evidence for association between schizophrenia and polymorphisms in the regulatory Regions of the ADRA2A, DRD3 and SNAP-25 Genes. *Psychiatry Res*. 2013;205(1-2):7–12.

© 2015 COPYRIGHT PHYSICIANS POSTGRADUATE PRESS, INC. NOT FOR DISTRIBUTION, DISPLAY, OR COMMERCIAL PURPOSES.

CACNA1C, schizophrenia and major depressive disorder in the Han Chinese population

Kuanjun He, Zhiguo An, Qingzhong Wang, Tao Li, Zhiqiang Li, Jianhua Chen, Wenjin Li, Ti Wang, Jue Ji, Guoyin Feng, He Lin, Qizhong Yi and Yongyong Shi

BJP 2014, 204:36-39.
Access the most recent version at DOI: 10.1192/bjp.bp.113.126979

Supplementary Material Supplementary material can be found at: http://bjp.rcpsych.org/content/suppl/2013/11/14/bjp.bp.113.126979.DC1.html

References This article cites 42 articles, 7 of which you can access for free at: http://bjp.rcpsych.org/content/204/1/36#BIBL

Reprints/ permissions To obtain reprints or permission to reproduce material from this paper, please write to **permissions@rcpsych.ac.uk**

You can respond to this article at **http://bjp.rcpsych.org/letters/submit/bjprcpsych;204/1/36**

Downloaded from http://bjp.rcpsych.org/ on September 4, 2014
Published by The Royal College of Psychiatrists

To subscribe to *The British Journal of Psychiatry* go to:
http://bjp.rcpsych.org/site/subscriptions/

BJPsych | The British Journal of Psychiatry (2014) 204, 36–39. doi: 10.1192/bjp.bp.113.126979

CACNA1C, schizophrenia and major depressive disorder in the Han Chinese population

Kuanjun He,* Zhiguo An,* Qingzhong Wang, Tao Li, Zhiqiang Li, Jianhua Chen, Wenjin Li, Ti Wang, Jue Ji, Guoyin Feng, He Lin, Qizhong Yi and Yongyong Shi

Background
Common psychiatric disorders are highly heritable, indicating that genetic factors play an important role in their aetiology. The *CACNA1C* gene, which codes for subunit alpha-1C of the Cav1.2 voltage-dependent L-type calcium channel, has been consistently found to be the shared risk gene for several kinds of mental disorder.

Aims
To investigate whether *CACNA1C* is a susceptibility gene for schizophrenia and major depressive disorder in the Han Chinese population.

Method
We carried out a case–control study of 1235 patients with schizophrenia, 1045 with major depressive disorder and 1235 healthy controls. A tag single nucleotide polymorphism (SNP) rs1006737 along with another 10 tag SNPs in the *CACNA1C* gene were genotyped in all samples.

Results
We found that rs1006737 was associated with both schizophrenia ($P_{allele} = 0.0014$, $P_{genotype} = 0.006$, odds ratio (OR) = 1.384, 95% CI 1.134–1.690) and major depressive disorder ($P_{allele} = 0.0007$, $P_{genotype} = 0.003$, OR = 1.425, 95% CI 1.160–1.752).

Conclusions
Our findings support *CACNA1C* being a risk gene for both schizophrenia and major depressive disorder in the Han Chinese population.

Declaration of interest
None.

As leading causes of morbidity that require significant long-term medical and social care, mental disorders have increasingly attracted attention in recent years. Schizophrenia, major depressive disorder and bipolar disorder are the three major disorders. Numerous family, twin and adoption studies confirmed that genetic factors play an important role in these mental disorders.[1] Some of the underlying genetic risk factors for these three disorders have been identified in genetic association studies. Although the findings from these studies have often been inconsistent and have not been validated in different populations, some encouraging results have emerged recently. The *CACNA1C* gene has been found to be a risk gene for several major psychiatric disorders and has been validated in different populations.[2–9] This gene is located at 12p13.3, spanning an approximately 645 kb genomic region, consists of 56 exons and encodes the alpha-1C subunit of the L-type voltage-dependent calcium channel Cav1.2. Cav1.2 couples transient increase of membrane permeability for calcium-causing cell-membrane depolarisation, leading to activated intracellular gene transcription and plays an important role in dendritic development, neuronal survival, synaptic plasticity, memory formation, learning and behaviour.[10–13] The *CACNA1C* gene is widely expressed in the cardiovascular system and the entire nervous system, especially hippocampus and thalamus of brain.[14,15] Genome-wide association studies (GWASs) have detected the single nucleotide polymorphism (SNP) rs1006737 in intron of the *CACNA1C* gene as a shared risk factor for schizophrenia, bipolar disorder and major depressive disorder in the White population.[4,16] However, few genetic study of the *CACNA1C* gene have been carried out in the Han Chinese population. To investigate whether *CACNA1C* is associated with schizophrenia and major depressive disorder in the Han Chinese population, we genotyped rs1006737 along with ten other SNPs (online Table DS1) in 1235 people with schizophrenia, 1045 with major depressive disorder and 1235 controls of Han Chinese origin.

*These authors contributed equally to the work.

Method

Participants

Our sample set consists of 1235 people with schizophrenia (805 males and 430 females), 1045 participants with major depressive disorder (729 males and 316 females) and 1235 normal controls (665 males and 570 females) recruited from the Han Chinese population. All of the participants in our study were unrelated, living in Shanghai, China, and were of Shanghai origin. Patients were out-patients or in-patients whose condition was stable and were interviewed by two independent psychiatrists and diagnosed strictly according to DSM-IV criteria.[17] All participants gave informed consent, the details of which had been reviewed and approved by the local ethical committee. Controls were randomly selected from the general population in Shanghai.

The mean age of individuals in the schizophrenia group was 36.4 years (s.d. = 9.0). All of the participants with schizophrenia had paranoid schizophrenia and no lifetime history of an episode of mania or depression. The mean age of individuals in the major depressive disorder group was 34.4 years (s.d. = 12.1). They were carefully selected on the basis that all of them had experienced at least two distinct major depressive disorder episodes and displayed no signs of bipolar disorder during the 2-year period after the onset of depression. The mean age of individuals in the control group was 30.6 years (s.d. = 11.4). All controls were randomly selected from the general public of the Han Chinese population. Volunteers who replied to a written invitation completed an evaluation of their medical history, with supplementary questions about psychosis and other major complex diseases. Before collecting their blood, a face-to-face interview was conducted that included a physical examination (height, weight, blood pressure, etc.).

Genotyping

Genomic DNA was prepared from peripheral blood samples of participants using the QuickGene DNA whole blood kit L protocol. The tag SNP selection was performed using haploview software, with pair-wise tagging, $r^2 \geqslant 0.5$ and minor allele frequency (MAF) $\geqslant 0.05$.[18,19] Details of all 11 SNPs genotyped in the *CACNA1C* gene can be found in Table DS1. All SNPs were genotyped using TaqMan SNP Genotyping Assays on a Fludigm EP1 platform. All probes were designed and synthesised by Life Technology. The criteria for excluding poorly performing samples and SNPs were determined by the genotype calls of each sample with a call-rate better than 96.5% (online Tables DS2 and DS3).

Statistical analysis

All the parameter calculations, including allele and genotype frequencies, and Hardy–Weinberg equilibrium analysis were carried out online using a SHEsis platform on Windows 7 (http://analysis2. bio-x.cn/myAnalysis.php).[20,21] All tests were two-tailed and statistical significance was assumed at the threshold of 0.05.

Population-stratification analysis

To avoid the false-positive association caused by potential population stratification, we performed the stratification analysis on STRUCTURE software on Windows 7 (version 2.3.4, http://pritch.bsd.uchicago. edu/structure.html).[22–25] We used genotype data from 79 random SNPs to undertake population-stratification analysis on the third-stage sample set. We obtained data about these 79 SNPs from 522 HapMap samples, 174 samples from Utah residents with ancestry from northern and western Europe in the United States (CEU), 209 samples from Yoruba in Ibadan, Nigeria (YRI), and 139 samples from Han Chinese in Beijing (CHB) (HapMap public release 28 at http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap28_B36/).[26] The software assumes that there were K populations (K is the number of assumed populations) in the data-set and then tries to find the distinct populations using the genotype data. Taking into consideration the immigration and geographical genetic isolation and the fact that the samples of the two disorders were both recruited from the same population, we applied the admixture model and correlated-frequencies model, with a burn-in length of 10 000 and MCMC (Markov chain Monte Carlo) repeats of 10 000. To make sure the results were consistent, we ran the program several times at each K from two to seven.

Results

We found that rs1006737 was positively associated with both schizophrenia (rs1006737: $P_{allele} = 0.0014$, $P_{genotype} = 0.006$, odds ratio (OR) = 1.384, 95% CI 1.134–1.690) and major depressive disorder (rs1006737: $P_{allele} = 0.0007$, $P_{genotype} = 0.003$, OR = 1.425, 95% CI 1.160–1.752) (online Tables DS4 and DS5). Moreover, after Bonferroni multiple tests correction, rs1006737 was still significantly associated with both disorders (online Tables DS4 and DS5).

Online Fig. DS1 shows the results of population-stratification analysis, which is the triangle chart of $K = 3$. Each angle represents a possible independent ancestry and the different coloured dots represent the individuals in assumed population components. When $K = 3$, the results described by the triangle chart are best. The combined population of CEU, CHB and YRI displayed a clear stratified pattern (Fig. DS1a). Our samples of the two disorders and controls distributed evenly in the triangle, which indicates that there was no obvious significant stratification in the population (Fig. DS1b). When K ranged from two to seven, the results were consistent with each other. Taken together, we can therefore conclude that our positive results before correction were unlikely to have been caused by population stratification. (See online supplement DS1 for additional results.)

Discussion

Findings relating to the *CACNA1C* gene

Schizophrenia, major depressive disorder and bipolar disorder are three severe mental disorders. They not only affect individuals and their families but also challenge society and health services worldwide. A high degree of heritability has indicated that genetic factors play an important role in their aetiologies. The *CACNA1C* gene was reported to have a nominal association with bipolar disorder in a family-based association study.[27] Combined with data from the Wellcome Trust Case–Control Consortium GWAS,[28] Sklar *et al* reported that rs1006737, within the *CACNA1C* gene, was associated with bipolar disorder ($P = 1 \times 10^{-4}$) in a GWAS of 1461 people with bipolar disorder and 2008 controls.[9] A subsequent study with a larger sample size confirmed that rs1006737, in *CACNA1C*, was associated with bipolar disorder ($P = 7.0 \times 10^{-8}$, OR = 1.181) in a White population.[2] The *CACNA1C* gene has also been found to be associated with other psychiatric disorders. Genetic studies have discovered that the genotype of *CACNA1C* was also associated with schizophrenia[5,29] and major depressive disorder.[7,30] Green *et al* reported in a GWAS that the risk SNP (rs1006737) for bipolar disorder was also a conferred risk for schizophrenia and early recurrent major depressive disorder.[3] A meta-analysis of two separate GWASs of people with bipolar disorder and major depressive disorder revealed that rs1006737 reached genome-wide significance after combining bipolar with unipolar mood disorders.[4] The consistent findings in genetic studies indicates that the *CACNA1C* gene belongs to a class of shared susceptibility factors for major psychiatric disorders and has likely played an important role in the pathogenesis of them.

Nevertheless, the mechanisms underlying how genetic changes in *CACNA1C* modify risk for developing psychiatric disorders are still unclear. This gene encodes the alpha-1C subunit of the L-type voltage-dependent calcium channel Cav1.2. Calcium channels are involved in various aspects of neuronal development and in the establishment of maintenance of connectivity during development and throughout adulthood.[31] Previous studies of people with bipolar disorder have consistently reported elevated basal and stimulated intracellular calcium levels in peripheral blood cells.[32,33] The evidences suggested that calcium signalling may play a role in bipolar disorder. Cav1.2 participates in the proper function of numerous neurological circuits in the hippocampus, amygdala and mesolimbic reward and motivation systems.[34,35] These are strongly implicated in psychiatric disease pathophysiology.

The *CACNA1C* gene has also been found to be expressed in many tissues in the human body across various developmental stages.[36] And, it is widely expressed in the brain, especially hippocampus and thalamus.[15] The relevance of *CACNA1C* to bipolar disorder was supported by the observed downregulation of messenger (m)RNA transcripts in mouse brain in response to lithium.[37] Notably, a missense mutation in *CACNA1C* can cause Timothy syndrome, which is characterised by multi-organ dysfunction such as cardiac arrhythmias and cognitive abnormalities.[38] The heterozygous knockout adult mice of *CACNA1C* were found expressing decreased Cav1.2 protein levels and L-type calcium channel current, and protecting against depression-like phenotypes.[39] A larger effect-size study clearly

indicated that genetic variant at SNP rs1006737 was associated with changes in brain structure and function in normal controls.[40] Genetic variation at rs1006737 may be involved in modulating gene expression, whereas the risk allele of rs1006737 is associated with increased expression levels of *CACNA1C*.[29] Three studies found the rs1006737 risk allele was associated with increased brain grey matter, either total grey matter volume or in specific brain regions in healthy controls. [41–43]

In our study, the main finding was a significant association between the *CACNA1C* gene and both schizophrenia and major depressive disorder in the Han Chinese population. We have confirmed that rs1006737 in *CACNA1C* was significantly associated with schizophrenia ($P_{allele} = 0.0014$, $P_{genotype} = 0.006$, OR = 1.384, MAF = 0.101) and major depressive disorder ($P_{allele} = 0.0007$, $P_{genotype} = 0.003$, OR = 1.425, MAF = 0.103) in a case–control study with a large sample size in the Han Chinese population. Even when Bonferroni correction (it is considered the most conservative method) was used, we still found that rs1006737 was associated with schizophrenia ($P_{allele} = 0.028$) and major depressive disorder ($P_{allele} = 0.014$). At the same time, we found another SNP (rs2239015) was significantly associated with schizophrenia (after Bonferroni correction: $P_{allele} = 0.006$, $P_{genotype} = 0.030$, OR = 1.249, MAF = 0.355) (Table DS4).

Nyegaard *et al* reported that rs1006737 was associated with schizophrenia ($P = 0.015$, OR = 1.16, MAF = 0.361) in a case–control study of 976 people with schizophrenia and 1489 healthy controls of European origin.[5] Green *et al* have reported that rs1006737 is associated with major depressive disorder ($P = 0.013$, OR = 1.15, MAF = 0.363) in recurrent major depressive disorder ($n = 1196$) and UK non-psychiatric comparison groups ($n = 15\,316$).[3] Comparing our results with theirs, the MAFs for the SNPs were quite different but the ORs are all in the same direction (for schizophrenia, OR: 1.384 *v.* 1.16, MAF: 0.101 *v.* 0.361; for major depressive disorder, OR 1.425 *v.* 1.15, MAF 0.103 *v.* 0.363).

Avoiding the potential influence of population stratification in our samples was important. We therefore used the software STRUCTURE 2.3.4 and data from 79 additional random SNPs dispersed on different chromosomes to analyse the potential population stratification in our samples. We did not detect any population stratification, and therefore, the results could not be affected by this confounding factor.

Future directions for research

Given rs1006737 and rs2239015 were both located in introns of *CACNA1C*, they were not expected to directly interfere with properties relating to the structure and function of Cav1.2. We know that intronic variation is likely to be involved in regulating gene expression and that extensive alternative splicing exists in the *CACNA1C* transcripts. It may potentially generate thousands of splice variants. Now, it is important to confirm the consequences of changes in splice variation of *CACNA1C* on neurophysiology. If we can find relevant answers, it might be very helpful for deciding novel therapeutic targets and approaches. In addition, it is also necessary to test its association with bipolar disorder in a large sample of individuals of Han Chinese origin in the future.

Funding

This work was supported by the Natural Science Foundation of China (81130022, 81272302, 31000553, 81121001), the National 863 project (2012AA02A515), the 973 Program (2010CB529600), Program for Changjiang Scholars and Innovative Research Team in University (IRT1025), the Foundation for the Author of National Excellent Doctoral Dissertation of China (201026), Shanghai Rising-Star Program (12QA1401900) and 'Shu Guang' project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17).

Acknowledgements

We are grateful to all patients and healthy controls participating in this study, as well as to the psychiatrists for their help in the recruitment and identification of patients with schizophrenia and major depressive disorder.

Kuanjun He, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai and College of Life Science, Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia; **Zhiguo An**, MS, Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi; **Qingzhong Wang**, PhD, **Tao Li**, PhD, **Zhiqiang Li**, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai; **Jianhua Chen**, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai and Schizophrenia Program, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai; **Wenjin Li**, PhD, **Ti Wang**, PhD, **Jue Ji**, BM, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai and College of Life Science, Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia; **Guoyin Feng**, BM, Schizophrenia Program, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai; **He Lin**, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai; **Qizhong Yi**, MD, PhD, Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi; **Yongyong Shi**, PhD, Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai, Shanghai Changning Mental Health Center, Shanghai and Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai, People's Republic of China

Correspondence: Yongyong Shi or Qizhong Yi or Lin He, Bio-X Institutes, Shanghai Jiao Tong University, The Central Little White House, 1954 Huashan Road, Shanghai 200030 or Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi 830002, People's Republic of China. Tel./fax: +86 2162822491. Email: shiyongyong@gmail.com (Y. Shi) or 13079911689@126.com (Q. Yi) or helinhelin@gmail.com (L. He).

First received 27 Jan 2013, final revision 10 May 2013, accepted 5 Jun 2013

References

1 Craddock N, O'Donovan MC, Owen MJ. The genetics of schizophrenia and bipolar disorder: dissecting psychosis. *J Med Genet* 2005; **42**: 193–204.

2 Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, et al. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008; **40**: 1056–8.

3 Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, et al. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry* 2010; **15**: 1016–22.

4 Liu Y, Blackwood DH, Caesar S, de Geus EJ, Farmer A, Ferreira MA, et al. Meta-analysis of genome-wide association data of bipolar disorder and major depressive disorder. *Mol Psychiatry* 2011; **16**: 2–4.

5 Nyegaard M, Demontis D, Foldager L, Hedemand A, Flint TJ, Sorensen KM, et al. CACNA1C (rs1006737) is associated with schizophrenia. *Mol Psychiatry* 2010; **15**: 119–21.

6 Ripke S, Sanders A, Kendler K, Levinson D, Sklar P, Holmans P, et al. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet* 2011; **43**: 969–76.

7 Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA, et al. Genome-wide association study of recurrent early-onset major depressive disorder. *Mol Psychiatry* 2011; **16**: 193–201.

8 Sklar P, Ripke S, Scott LJ, Andreassen OA, Cichon S, Craddock N, et al. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. *Nat Genet* 2011; **43**: 977–83.

9 Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, et al. Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008; **13**: 558–69.

10 Moosmang S, Haider N, Klugbauer N, Adelsberger H, Langwieser N, Muller J, et al. Role of hippocampal Cav1.2 Ca^{2+} channels in NMDA receptor-independent synaptic plasticity and spatial memory. *J Neurosci* 2005; **25**: 9883–92.

11 Shibasaki M, Kurokawa K, Ohkuma S. Upregulation of L-type Ca(v)1 channels in the development of psychological dependence. *Synapse* 2010; **64**: 440–4.

12 White JA, McKinney BC, John MC, Powers PA, Kamp TJ, Murphy GG. Conditional forebrain deletion of the L-type calcium channel Ca V 1.2 disrupts remote spatial memories in mice. *Learn Mem* 2008; **15**: 1–5.

13 Woodside BL, Borroni AM, Hammonds MD, Teyler TJ. NMDA receptors and voltage-dependent calcium channels mediate different aspects of acquisition and retention of a spatial memory task. *Neurobiol Learn Mem* 2004; **81**: 105–14.

14 Narayanan D, Xi Q, Pfeffer LM, Jaggar JH. Mitochondria control functional CaV1.2 expression in smooth muscle cells of cerebral arteries. *Circ Res* 2010; **107**: 631–41.

15 Striessnig J, Koschak A, Sinnegger-Brauns MJ, Hetzenauer A, Nguyen NK, Busquet P, et al. Role of voltage-gated L-type Ca^{2+} channel isoforms for brain function. *Biochem Soc Trans* 2006; **34**: 903–9.

16 Curtis D, Vine AE, McQuillin A, Bass NJ, Pereira A, Kandaswamy R, et al. Case-case genome-wide association analysis shows markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes. *Psychiatr Genet* 2011; **21**: 1–4.

17 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (4th edn) (DSM-IV)*. APA, 1994.

18 de Bakker PI, Burtt NP, Graham RR, Guiducci C, Yelensky R, Drake JA, et al. Transferability of tag SNPs in genetic association studies in multiple populations. *Nat Genet* 2006; **38**: 1298–303.

19 de Bakker PI, Yelensky R, Pe'Er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005; **37**: 1217–23.

20 Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res* 2009; **19**: 519–23.

21 Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005; **15**: 97–8.

22 Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol Ecol Notes* 2007; **7**: 574–8.

23 Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 2003; **164**: 1567–87.

24 Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000; **155**: 945–59.

25 Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with the assistance of sample group information. *Mol Ecol Resour* 2009; **9**: 1322–32.

26 International HapMap Consortium. The International HapMap Project. *Nature* 2003; **426**: 789–96.

27 Sklar P, Gabriel SB, McInnis MG, Bennett P, Lim YM, Tsan G, et al. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol Psychiatry* 2002; **7**: 579–93.

28 Burton P, Clayton D, Cardon L, Craddock N, Deloukas P, Duncanson A, et al. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661–78.

29 Bigos KL, Mattay VS, Callicott JH, Straub RE, Vakkalanka R, Kolachana B, et al. Genetic variation in CACNA1C affects brain circuitries related to mental illness. *Arch Gen Psychiatry* 2010; **67**: 939–45.

30 Casamassima F, Huang J, Fava M, Sachs GS, Smoller JW, Cassano GB, et al. Phenotypic effects of a bipolar liability gene among individuals with major depressive disorder. *Am J Med Genet B Neuropsychiatr Genet* 2010; **153B**: 303–9.

31 Spitzer NC. Electrical activity in early neuronal development. *Nature* 2006; **444**: 707–12.

32 Emamghoreishi M, Schlichter L, Li PP, Parikh S, Sen J, Kamble A, et al. High intracellular calcium concentrations in transformed lymphoblasts from subjects with bipolar I disorder. *Am J Psychiatry* 1997; **154**: 976–82.

33 Perova T, Wasserman MJ, Li PP, Warsh JJ. Hyperactive intracellular calcium dynamics in B lymphoblasts from patients with bipolar I disorder. *Int J Neuropsychopharmacol* 2008; **11**: 185–96.

34 Leitch B, Szostek A, Lin R, Shevtsova O. Subcellular distribution of L-type calcium channel subtypes in rat hippocampal neurons. *Neuroscience* 2009; **164**: 641–57.

35 Nestler EJ, Carlezon WJ. The mesolimbic dopamine reward circuit in depression. *Biol Psychiatry* 2006; **59**: 1151–9.

36 Pang L, Koren G, Wang Z, Nattel S. Tissue-specific expression of two human Ca(v)1.2 isoforms under the control of distinct 5′ flanking regulatory elements. *Febs Lett* 2003; **546**: 349–54.

37 McQuillin A, Rizig M, Gurling HM. A microarray gene expression study of the molecular pharmacology of lithium carbonate on mouse brain mRNA to understand the neurobiology of mood stabilization and treatment of bipolar affective disorder. *Pharmacogenet Genomics* 2007; **17**: 605–17.

38 Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 2004; **119**: 19–31.

39 Dao DT, Mahon PB, Cai X, Kovacsics CE, Blackwell RA, Arad M, et al. Mood disorder susceptibility gene CACNA1C modifies mood-related behaviors in mice and interacts with sex to influence behavior in mice and diagnosis in humans. *Biol Psychiatry* 2010; **68**: 801–10.

40 Rose EJ, Donohoe G. Brain vs behavior: an effect size comparison of neuroimaging and cognitive studies of genetic risk for schizophrenia. *Schizophr Bull* 2013; **39**: 518–26.

41 Kempton MJ, Ruberto G, Vassos E, Tatarelli R, Girardi P, Collier D, et al. Effects of the CACNA1C risk allele for bipolar disorder on cerebral gray matter volume in healthy individuals. *Am J Psychiatry* 2009; **166**: 1413–4.

42 Perrier E, Pompei F, Ruberto G, Vassos E, Collier D, Frangou S. Initial evidence for the role of CACNA1C on subcortical brain morphology in patients with bipolar disorder. *Eur Psychiatry* 2011; **26**: 135–7.

43 Wang F, McIntosh AM, He Y, Gelernter J, Blumberg HP. The association of genetic variation in CACNA1C with structure and function of a frontotemporal system. *Bipolar Disord* 2011; **13**: 696–700.

EXTRA CONTENT ONLINE

Psychiatry Research 207 (2013) 225–228

Contents lists available at SciVerse ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

CNTNAP2 is significantly associated with schizophrenia and major depression in the Han Chinese population

Weidong Ji [a,b,c,1], Tao Li [b,c,1], Yaosheng Pan [a,b,c,1], Hua Tao [a,b,c,1], Kang Ju [a,b,c,1], Zujia Wen [b,c,1], Yingchun Fu [a,b,c,1], Zhiguo An [e], Qian Zhao [c], Ti Wang [c], Lin He [a,b,c], Guoyin Feng [d], Qizhong Yi [e], Yongyong Shi [a,b,c,*]

[a] *Shanghai Changning Mental Health Center, 299 XieHe Road, Shanghai 200042, P.R. China*
[b] *Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai 200042, P.R. China*
[c] *Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200030, P.R. China*
[d] *Shanghai Institute of Mental Health, Shanghai 200030, P.R. China*
[e] *Department of Psychiatry, the First Teaching Hospital of Xinjiang Medical University, Urumqi 830002, P.R. China*

ARTICLE INFO

Article history:
Received 2 April 2012
Received in revised form
3 September 2012
Accepted 15 September 2012

Keywords:
Bipolar disorder
Schizophrenia
Major depression
Single nucleotide polymorphism (SNP)
CNTNAP2

ABSTRACT

CNTNAP2, located on 7q35–36.1, encodes a single-pass transmembrane protein mediating cell–cell interactions in the nervous system. *CNTNAP2* has been suggested to play an important role in mental diseases such as autism and language disorder. However, we still do not know whether it also confers risk to major psychiatric disorders such as schizophrenia, major depression and bipolar disorder. We analysed single nucleotide polymorphisms (SNPs) previously reported to be associated with autism or language impairment in 1135 schizophrenia patients, 1135 unrelated major depression patients, 1135 unrelated bipolar disorder patients and 1135 unrelated normal controls recruited from the Han Chinese population. We found that the genotypes of rs17236239 were significantly associated with schizophrenia and the alleles of rs2710102 and rs2710117 were significantly associated with major depression. According to the location of significant signals, our study indicated that exon 13–15 of *CNTNAP2* may play important roles in both schizophrenia and major depression in the Han Chinese population.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Schizophrenia, major depression and bipolar disorder are major psychiatric diseases exhibiting relatively high heritability. Schizophrenia is a severe mental illness affecting about 1% of the world population, major depression is a severe disorder with a prevalence of 3–5% for males and 8–10% for females within a year-long period in North America (Kessler et al., 2005; Murphy et al., 2000), and bipolar disorder is estimated to afflict about 1 out of every 45 adults in the USA (Murphy et al., 2000). Although the pathologies of the above diseases are still unclear, the genetic factor is known to be an important component in their aetiologies (Arias et al., 2002). Notably, some hypotheses of the mechanisms of those diseases are similar, for example, the monoamine hypothesis (Belmaker and Agam, 2008; Glatt et al., 2003; Serretti and Mandelli, 2008).

Terracciano and associates reported that Contactin-associated protein-like 2 (*CNTNAP2*) was significantly associated with openness by a genome-wide association study (Terracciano et al., 2010). Using ChIP and association Vernes and associates found that CNTNAP2 plays a role downstream of FoxP2, and is involved in the causation of impaired language disorder (Vernes et al., 2008). Another two research groups reported that the gene *CNTNAP2* was also significantly associated with autism (Alarcón et al., 2008; Bakkaloglu et al., 2008). Moreover, the internal and overlapping deletions in the *CNTNAP2* gene could increase the risk of schizophrenia (International Schizophrenia Consortium et al., 2008; Vernes et al., 2008). All of the above reports indicated that CNTNAP2 might play an important role in personality and mental state. Notably, Burbach and van der Zwaagurbach concluded that different mental disorders could share some genetic factors (Burbach and van der Zwaag, 2009). This viewpoint triggered us to check whether CNTNAP2 was involved in the pathogenesis of other psychiatric diseases.

CNTNAP2 is an extraordinarily large gene spanning 2.5 Mb on chromosome 7q35–36.1. It encodes a single-pass transmembrane protein that mediates cell–cell interactions in the nervous system (Oiso et al., 2009). Further, CNTNAP2 is associated with potassium channels and other cell-adhesion molecules, just like CNTN2 is, to

* Correspondence to: Bio-X Insititutes, Shanghai Jiao Tong University, the Little White Building, 55 Guangyuan Xi Road, Shanghai 200030, P.R. China or Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai 200042, P.R. China. or Shanghai Changning Mental Health Center, 299 XieHe Road, Shanghai 200042, P.R. China. Tel./fax: +86 21 62822491.
E-mail address: shiyongyong@gmail.com (Y. Shi).
[1] These authors contributed equally to this work.

0165-1781/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.psychres.2012.09.024

Table 1
Association tests of 13 SNPs in the *CNTNAP2* gene within schizophrenia, major depression, bipolar disorder patients and normal controls.

SNP ID		Allele frequency	Odds ratio[a]	95% CI	P value[b]	Genotype frequency			*P* value[c]
rs7794745		A				AA	AT	TT	
	Schizophrenia	1388(0.671)	1.086	0.96～1.23	0.203	489(0.473)	410(0.396)	136(0.131)	0.2111
	Major depression	1455(0.676)	1.114	0.98～1.26	0.0930	489(0.454)	477(0.443)	110(0.102)	0.0913
	Bipolar disorder	1396(0.629)	0.906	0.80～1.02	0.115	433(0.390)	530(0.478)	146(0.132)	0.0632
	Control	1449(0.652)				485(1.437)	479(0.431)	147(0.132)	
rs851715		C				CC	CT	TT	
	Schizophrenia	941(0.454)	1.131	1.00～1.28	0.045	218(0.210)	505(0.487)	314(0.303)	0.0747
	Major depression	991(0.453)	1.130	1.00～0.27	0.045	229(0.210)	533(0.488)	331(0.303)	0.0762
	Bipolar disorder	893(0.404)	0.925	0.82～1.04	0.201	184(0.167)	525(0.476)	395(0.358)	0.273
	Control	939(0.423)				191(0.172)	557(0.502)	361(0.326)	
rs2538976		C				CC	CT	TT	
	Schizophrenia	796(0.384)	0.893	0.79～1.01	0.0718	161(0.155)	474(0.457)	402(0.388)	0.143
	Major depression	825(0.382)	0.888	0.79～1.00	0.0548	149(0.138)	527(0.488)	403(0.373)	0.115
	Bipolar disorder	944(0.425)	1.061	0.94～1.20	0.334	189(0.170)	566(0.510)	355(0.320)	0.395
	Control	894(0.411)				183(0.168)	528(0.485)	377(0.347)	
rs6464774		C				CC	CT	TT	
	Schizophrenia	1361(0.656)	1.140	1.01～1.29	0.040	463(0.446)	435(0.419)	140(0.135)	0.106
	Major depression	1413(0.655)	1.136	1.00～1.29	0.0437	477(0.442)	459(0.425)	143(0.133)	0.133
	Bipolar disorder	1356(0.609)	0.933	0.83～1.05	0.265	402(0.361)	552(0.496)	159(0.143)	0.0741
	Control	1381(0.625)				443(0.401)	495(0.448)	166(0.150)	
rs986062		A				AA	AG	GG	
	Schizophrenia	708(0.344)	0.898	0.79～1.02	0.0936	139(0.135)	430(0.418)	459(0.446)	0.170
	Major depression	741(0.340)	0.881	0.78～1.00	0.0441	123(0.113)	495(0.454)	472(0.433)	0.0764
	Bipolar disorder	827(0.383)	1.061	0.94～1.20	0.344	145(0.134)	537(0.497)	398(0.369)	0.0828
	Control	814(0.369)				159(0.144)	496(0450)	448(0.406)	
rs10952659		C				CC	CG	GG	
	Schizophrenia	520(0.254)	0.894	0.78～1.02	0.106	78(0.076)	364(0.356)	580(0.568)	0.265
	Major depression	541(0.250)	0.874	0.76～1.00	0.0505	64(0.059)	413(0.382)	604(0.559)	0.0547
	Bipolar disorder	588(0.266)	0.949	0.83～1.08	0.444	74(0.067)	440(0.398)	591(0.535)	0.245
	Control	609(0.276)				94(0.085)	421(0.382)	587(0.533)	
rs2710102		A				AA	AG	GG	
	Schizophrenia	968(0.467)	1.158	1.03～1.31	0.0172				
	231(0.223)	506(0.488)	300(0.289)	0.0306					
	Major depression	1016(0.475)	1.198	1.06～1.35	0.00308	232(0.217)	552(0.516)	285(0.267)	0.0108
	Bipolar disorder	946(0.434)	1.013	0.90～1.14	0.836	208(0.191)	530(0.486)	353(0.324)	0.632
	Control	947(0.430)				196(0.178)	555(0.505)	349(0.317)	
rs2710117		A				AA	AT	TT	
	Schizophrenia	1138(0.554)	0.849	0.75～0.96	0.00855	319(0.311)	500(0.487)	208(0.203)	0.0320
	Major depression	1177(0.549)	0.834	0.74～0.94	0.00309	317(0.296)	543(0.507)	211(0.197)	0.00973
	Bipolar disorder	1295(0.592)	0.991	0.88～1.12	0.887	380(0.347)	535(0.489)	179(0.164)	0.913
	Control	1302(0.594)				388(0.354)	526(0.480)	182(0.166)	
rs10246256		C				CC	CT	TT	
	Schizophrenia	905(0.443)	1.124	0.99～1.27	0.0606	209(0.205)	487(0.477)	326(0.319)	0.0497
	Major depression	953(0.442)	1.118	0.99～1.26	0.0678	215(0.199)	523(0.485)	341(0.316)	0.0908
	Bipolar disorder	847(0.390)	0.903	0.80～1.02	0.0964	174(0.160)	499(0.459)	414(0.381)	0.0688
	control	913(0.414)				180(0.163)	553(0.502)	369(0.335)	
rs17236239		A				AA	AG	GG	
	Schizophrenia	1674(0.825)	1.0790	0.92～1.26	0.344	703(0.693)	268(0.264)	44(0.043)	0.00264
	Major depression	1764(0.813)	0.997	0.85～1.16	0.969	711(0.655)	342(0.315)	32(0.029)	0.842
	Bipolar disorder	1738(0.793)	0.878	0.75～1.02	0.0890	695(0.634)	348(0.318)	53(0.048)	0.0204
	Control	1765(0.813)				708(0.653)	349(0.322)	28(0.026)	

		A			AA	AG	GG		
rs1922892									
	Schizophrenia	1104(0.534)	0.880	0.78～0.99	0.0384				
0	302(0.292)	500(0.484)	232(0.224)	0.0480					
	Major depression	1137(0.522)	0.840	0.75～0.95	0.00399	289(0.265)	559(0.513)	241(0.221)	0.0121
	Bipolar disorder	1268(0.572)	1.026	0.91～1.16	0.673	366(0.330)	536(0.483)	207(0.187)	0.555
	Control	1245(0.565)				344(0.312)	557(0.506)	200(0.182)	
rs2538991									
	Schizophrenia	957(0.467)	1.132	1.00～1.28	0.0437	233(0.228)	491(0.479)	300(0.293)	0.0469
	Major depression	1028(0.471)	1.148	1.02～1.29	0.0229	229(0.210)	570(0.522)	293(0.268)	0.0602
	Bipolar disorder	939(0.426)	0.957	0.85～1.08	0.468	206(0.187)	527(0.478)	370(0.335)	0.402
	Control	962(0.436)				203(0.184)	556(0.505)	343(0.311)	
rs759178									
	Schizophrenia	900(0.471)	1.089		0.172	214(0.224)	472(0.494)	269(0.282)0.0676	0.0676
	Major depression	1044(0.492)	1.182		0.00631	249(0.234)	572(0.532)	306(0.284)	0.0112
	Bipolar disorder	954(0.435)	0.941		0.320	215(0.196)	524(0.478)	358(0.326)	0.0357
	Control	968				198(0.184)	572(0.532)	306(0.284)	
rs4431523									
	Schizophrenia	305(0.151)	0.827	0.70～0.97	0.0234	42(0.042)	221(0.218)	749(0.740)	0.000824
	Major depression	368(0.170)	0.955	0.82～1.12	0.567	32(0.030)	304(0.281)	746(0.689)	0.849
	Bipolar disorder	427(0.196)	1.135	0.97～1.32	0.103	51(0.047)	325(0.298)	714(0.655)	0.169
	Control	384(0.177)				35(0.032)	314(0.289)	738(0.679)	

[a] Odds ratio provided by SHEsis.
[b] *P* value using Haploview 4.0RC1.
[c] Pearson's *P* value provided by SHEsis.

help create functional subdomains on myelinated axons (Ye et al., 2008). In this study, we investigated the association between *CNTNAP2* and schizophrenia, major depression and bipolar disorder. In addition, we focussed on those single nucleotide polymorphisms (SNPs) previously reported to be associated with autism or impaired language disorder. Ten SNPs (rs851715, rs10246256, rs2710102, rs759178, rs1922892, rs2538991, rs17236239, rs2538976, and rs2710117 and rs4431523) were found to be significantly associated with nonsense-word repetition, which is a robust endophenotype of specific language impairment (Vernes et al., 2008). Further, rs7794745, rs986062, rs6464774, rs10952659 and rs2710102 were also validated to be significantly associated with autism (Alarcón et al., 2008; Bakkaloglu et al., 2008). Therefore, we genotyped these common SNPs for further analysis in the Han Chinese population.

2. Methods and Materials

2.1. Subjects

This sample set consisted of 1135 unrelated schizophrenia cases (630 males and 505 females), 1135 unrelated major depressive disorder cases (483 males and 652 females), 1135 unrelated bipolar disorder cases (618 males and 517 females) and 1135 normal controls (369 males and 766 females) recruited from the Chinese Han population.

All cases were selected from inpatients of mental health centres. The mean onset age of schizophrenia cases was 35.2 years ($\pm$7.2). The mean onset age of major depressive disorder cases was 35.0 years ($\pm$11.6). The mean onset age of bipolar disorder cases was 36.6 years ($\pm$13.8). All patients were given a standardised interview and diagnosed independently by two psychiatrists strictly according to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria.

The mean age of the controls was 58.7 years ($\pm$9.9). All controls were recruited among volunteers randomly selected from the Shanghai local population with both their parents being local Shanghai residents, and without family history of psychiatric disorders. Considering the high mean ages, those control samples should have a lower incidence of schizophrenia and major depression. Practice lists were screened for potentially suitable volunteers by exclusion of subjects with major mental illness or with first-degree relatives with major mental illness.

All participants gave written informed consent after the nature of the study had been fully explained. The study was reviewed and approved by the local Ethical Committee of Human Genetics Resources.

2.2. Genotyping

We genotyped all samples using TaqMan assays on the ABI 7900 DNA detection system (Applied Biosystems, Foster City, CA, USA). The standard 5 μl reaction was performed on the GeneAmp PCR 9700 System (Applied Biosystems) using TaqMan® Universal PCR Master Mix reagent kits according to the manufacturer's guidelines.

2.3. Statistical analysis

We calculated Hardy–Weinberg equilibrium, allelic association, genotypic association and odds ratio (OR) on the SHEsis software platform (http://analysis.bio-x.cn) (Shi and He, 2005; Li et al., 2009). The statistical significance threshold of adjusted *P* values in the association study was set at 0.05. Further, we used Bonferroni's multiple tests correction to adjust our results. In addition, we conducted a logistic regression analysis of disease traits using gender and age as covariates on the PLINK platform (http://pngu.mgh.harvard.edu/~purcell/plink/download.shtml).

3. Results

Among those genotyped 14 SNPs, rs759178 was discarded from further analysis due to its deviation from Hardy–Weinberg equilibrium in the control group. According to Table 1, rs851715, rs6464774, rs2710102, rs2710117, rs1922892, rs2538991, rs10246256 and rs17236239 were found to have allelic or genotypic association with schizophrenia before multiple tests' correction. However, after Bonferroni's correction, we only found that

rs17236239 (unadjusted $P=0.0026$, and $P=0.0343$ after correction) remained significant in the genotypic analysis.

Rs851715, rs6464774, rs986062, rs2710102, rs2710117, rs1922892, rs17236239 and rs2538991 showed allelic or genotypic significance with major depression before correction. We found that rs2710102 was still significant after Bonferroni correction (unadjusted $P=0.00308$; OR=1.20, 95% confidence interval (CI)=(1.06–1.35); and $P=0.04$ after correction) and rs2710117 (unadjusted $P=0.00309$; OR=0.83, 95% CI=(0.74–0.94); and $P=0.0402$ after correction).

In addition, we also carried out a logistic regression analysis using gender as a covariate for these positive loci. We found that the *P*-value adjusted for gender of rs17236239 was 0.002238, the *P*-value of rs2710102 was 0.004858 and the *P*-value was 0.007569 for rs2710117. All three SNPs were still significant. These results indicated that the associations between *CNTNAP2* and schizophrenia and major depression were not influenced by gender.

No SNP was associated with bipolar disorder after Bonferroni correction.

Detailed information about the association study is provided in Table 1.

4. Discussion

We found that the SNP rs17236239 was associated with schizophrenia, and rs2710102 and rs2710117 were significantly associated with major depression after Bonferroni correction for multiple comparisons. Further, in schizophrenia, rs2710102 showed a notably allelic association before correction (unadjusted $P=0.00855$). We also found that the significance of these SNPs was not influenced by gender effects.

All three SNPs are located in the exon13–15 region of the *CNTNAP2* gene. Vernes and associates concluded that the region of exon13–15 was a very possible interacting target of FoxP2, due to the fact that CNTNAP2's protein sequence coded by exon13–15 is just the right binding site of FoxP2, which regulates expression of *CNTNAP2* directly (Vernes et al., 2008). Therefore, the *FoxP2–CNTNAP2* pathway may play an important role in pathogenesis of psychiatric disorders. However, further analysis of this pathway and regulatory network is needed for a better understanding of neurogenetic mechanisms involved in psychiatric disorders.

Although our sample size was relatively large and we analysed a considerable sample size and achieved association signals of common variants, we still lacked further evidence of functional experiments to support our findings and even demonstrate the role of CNTNAP2 or related pathways in the pathogenesis of mental disorder.

In summary, we found a genetic association between common variants within the *CNTNAP2* gene and schizophrenia and major depression. Further studies in independent sample sets are suggested to validate our findings. In addition, we also provided evidence that different psychiatric disorders could share genetic risk factors in their aetiologies. However, the potential role of CNTNAP2 and the FoxP2–CNTNAP2 pathway in the pathogenesis of schizophrenia and major depression needs to be clarified by further functional studies.

Acknowledgements

We warmly thank all patients and healthy individuals who participated in our study. This work was supported by the Natural Science Foundation of China (81130022, 81272302, 31000553 and 81121001), the National 863 project (2012AA02A515), the 973 Program (2010CB529600), Program for Changjiang Scholars and Innovative Research Team in University (IRT1025), the Foundation for the Author of National Excellent Doctoral Dissertation of China (201026), the Program for New Century Excellent Talents in University (NCET-09-0550), Shanghai Science and Technology Development Funds (12QA1401900) and the Shanghai Changning Health Bureau program (20094Y06001).

References

Alarcón, M., Abrahams, B.S., Stone, J.L., Duvall, J.A., Perederiy, J.V., Bomar, J.M., Sebat, J., Wigler, M., Martin, C.L., Ledbetter, D.H., Nelson, S.F., Cantor, R.M., Geschwind, D.H., 2008. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. The American Journal of Human Genetics 82, 150–159.

Arias, B., Rosa, A., Fananas, L., 2002. Human genetic variation and mental disorders. Neurotoxicity Research 4, 523–530.

Bakkaloglu, B., O'Roak, B.J., Louvi, A., Gupta, A.R., Abelson, J.F., Morgan, T.M., Chawarska, K., Klin, A., Ercan-Sencicek, A.G., Stillman, A.A., Tanriover, G., Abrahams, B.S., Duvall, J.A., Robbins, E.M., Geschwind, D.H., Biederer, T., Gunel, M., Lifton, R.P., State, M.W., 2008. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. The American Journal of Human Genetics 82, 165–173.

Belmaker, R.H., Agam, G., 2008. Major depressive disorder. New England Journal of Medicine 358, 55–68.

Burbach, J.P., van der Zwaag, B., 2009. Contact in the genetics of autism and schizophrenia. Trends in Neurosciences 32, 69–72.

Glatt, S.J., Faraone, S.V., Tsuang, M.T., 2003. Meta-analysis identifies an association between the dopamine D2 receptor gene and schizophrenia. Molecular Psychiatry 8, 911–915.

International Schizophrenia Consortium, et al., 2008. Rare chromosomal deletions and duplications increase risk of schizophrenia. Nature 455, 237–241.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., Walters, E.E., 2005. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry 62, 617–627.

Li, Z., Zhang, Z., He, Z., Tang, W., Li, T., Zeng, Z., He, L., Shi, Y., 2009. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Research 19, 519–523.

Murphy, J.M., Laird, N.M., Monson, R.R., Sobol, A.M., Leighton, A.H., 2000. A 40-year perspective on the prevalence of depression: the Stirling County Study. Archives of General Psychiatry 57, 209–315.

Oiso, S., Takeda, Y., Futagawa, T., Miura, T., Kuchiiwa, S., Nishida, K., Ikeda, R., Kariyazono, H., Watanabe, K., Yamada, K., 2009. Contactin-associated protein (Caspr) 2 interacts with carboxypeptidase E in the CNS. Journal of Neurochemistry 109, 158–167.

Serretti, A., Mandelli, L., 2008. The genetics of bipolar disorder: genome 'hot regions,' genes, new potential candidates and future directions. Molecular Psychiatry 13, 742–771.

Shi, Y.Y., He, L., 2005. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Research 15, 97–98.

Terracciano, A., Sanna, S., Uda, M., Deiana, B., Usala, G., Busonero, F., Maschio, A., Scally, M., Patriciu, N., Chen, W.M., Distel, M.A., Slagboom, E.P., Boomsma, D.I., Villafuerte, S., Sliwerska, E., Burmeister, M., Amin, N., Janssens, A.C., van Duijn, C.M., Schlessinger, D., Abecasis, G.R., Costa Jr., P.T., 2010. Genome-wide association scan for five major dimensions of personality. Molecular Psychiatry 15, 647–656.

Vernes, S.C., Newbury, D.F., Abrahams, B.S., Winchester, L., Nicod, J., Groszer, M., Alarcon, M., Oliver, P.L., Davies, K.E., Geschwind, D.H., Monaco, A.P., Fisher, S.E., 2008. A functional genetic link between distinct developmental language disorders. New England Journal of Medicine 359, 2337–2345.

Ye, H., Tan, Y.L., Ponniah, S., Takeda, Y., Wang, S.Q., Schachner, M., Watanabe, K., Pallen, C.J., Xiao, Z.C., 2008. Neural recognition molecules CHL1 and NB-3 regulate apical dendrite orientation in the neocortex via PTPa. The EMBO Journal 27, 188–200.

Xu et al. Hereditas (2018) 155:5
DOI 10.1186/s41065-017-0037-1

Hereditas

RESEARCH Open Access

Genetic risk between the *CACNA1I* gene and schizophrenia in Chinese Uygur population

Wei Xu[1,2†], Yahui Liu[2†], Jianhua Chen[2,3†], Qingli Guo[2], Ke Liu[2], Zujia Wen[2], Zhaowei Zhou[2], Zhijian Song[2], Juan Zhou[2], Lin He[1,2*], Qizhong Yi[4*] and Yongyong Shi[1,2*]

Abstract

Background: Schizophrenia (SCZ) is a common mental disorder with high heritability, and genetic factors play a major role in the pathogenesis. Recent researches indicated that the *CACNA1I* involved in calcium channels probably affect the potential pathogenesis of SCZ.

Results: In this study, we attempted to investigate whether the *CACNA1I* gene contributes the risk to SCZ in the Uighur Chinese population, and performed a case-control study involving 985 patient samples and 1218 normal controls to analyze nine SNPs within the *CACNA1I* gene. Among these sites, six SNPs were significantly associated with SCZ in the allele distribution: rs132575 (adjusted P_{allele} = 0.039, OR = 1.159), rs713860 (adjusted P_{allele} = 0.039, OR = 0.792), rs738168 (adjusted P_{allele} = 0.039, OR = 0.785), rs136805 (adjusted P_{allele} = 0.014, OR = 1.212), rs5757760 (adjusted P_{allele} = 0.042, OR = 0.873) and rs5750871 (adjusted P_{allele} = 0.039, OR = 0.859). In addition, two SNPs turned to be risk factors for SCZ not only in the allele distribution, but also in the genotype distribution: rs132575 (adjusted $P_{genotype}$ = 0.037) and rs136805 (adjusted $P_{genotype}$ = 0.037).

Conclusions: Overall, the present study provided evidence that significant association exists between the *CACNA1I* gene and SCZ in the Uighur Chinese population, subsequent validation of functional analysis and genetic association studies are needed to further extend this study.

Keywords: Schizophrenia, *CACNA1I* gene, Case-control study, Uighur Chinese

Background

Schizophrenia (SCZ) is one of enigmatic, complex psychotic mental disease that characterized by abnormalities in the perception or expression of reality, causing a substantial burden on patients and public expenditure [1, 2]. The lifetime prevalence of SCZ is generally estimated to be 1%, and genetic risks account for up to 80% occurrences [3]. This chronic disorder poses series of typical manifestations resembling auditory hallucinations, delusions, and behavioral dysfunction [4, 5]. A lot of crucial developments in neuropathology, epidemiology, and medications are emerged, triggering better identification of etiology and effective therapeutics. Analysis of the genetic epidemiologic in family, twin, and adoption, the conclusion suggest that hereditary loci for which linkage to the SCZ play a critical role in the development of the disease [6].

With the deepening research of gene detection and disease mechanism, *CACNA1I* (calcium voltage-gated channel subunit alpha1 I) has been identified as a candidate gene for SCZ. Recently, a primary GWAS conducted by the Psychiatric Genomics Consortium-Schizophrenia Workgroup (PGC-SCZ) has made encouraging progress in identifying genetic susceptibility loci, and the *CACNA1I* gene is reported as a new locus for SCZ in Caucasian [7]. *CACNA1I* is located at 22p13.1, spanning about 118 kb genomic region, and consists of 38 exons. This gene encodes Cav3.3

* Correspondence: helin@sjtu.edu.cn; qizhongyi@126.com; shiyongyong@gmail.com
†Equal contributors
[1]Department of biology, School of Life Science, Anhui Medical University, 81 meishan road, Hefei, Anhui 230031, China
[4]Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, 137 Liyushan South Road, Urumqi, Xinjiang 830054, China
Full list of author information is available at the end of the article

BioMed Central

© The Author(s). 2017 **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

isoform that contains a pore-forming alpha subunit, and the coding product of *CACNA1I* is a member of low-threshold (T-type) Ca^{2+} channels [8, 9]. The *CACNA1I* gene is abundantly expressed in the thalamic reticular nucleus, and delineates the distinctive physiological properties of neuronal firing [10, 11]. There are three subtypes of low threshold voltage-activated T-type Ca^{2+} channels have been implicated and designated α_{1G} (Cav3.1), α_{1H} (Cav3.2) and α_{1I} (Cav3.3) by previous reports, which endow typical kinetic features and involve in different signatures of T-currents, respectively [12]. In view of the exploration of the thalamic reticular and relay neurons activities, increasing results point to Cav3.1 and Cav3.2 channels represent short burst firing and small conductance, while Cav3.3 leads to slower activation and inactivation [13, 14].

The normal physiological activities of human beings need to be maintained through the action potential discharge of specific ion channels. Ion exchange is responsible for the level of intracellular Ca^{2+}, carry out a series of electrical, chemical, and physical function [15]. Evidence demonstrates that *CACNA1I* mRNA is ubiquitously expressed in brain regions, and Cav3.3 channel provoked by small membrane depolarization can elicit spontaneous discharge. The channel encoded by *CACNA1I* plays a central role in the thalamic spindle generator [16], alongside reduced sleep spindles associate with SCZ [17]. Abnormalities of sleep spindles and disturbances in thalamic neurons, are found in people with schizophrenia. It is noteworthy that the encode proteins has been reported can meet the druggable target of SCZ [18]. Moreover, T-type calcium channels have been shown to be a crucial cause of insomnia and neuropathic pain [19]. There is evidence that a single copy of Chr22:39665939G > A *CACNA1I* triggers calcium channel disorder and is associated with the pathogenesis of SCZ [20]. These profound findings have prompted us to open up promising research idea that *CACNA1I* might regulates signaling pathways in SCZ.

Uygur is one of the minority nationalities in China, and mainly distributes in Xinjiang Province. The region located in the northwest border area of China, and the hinterland of the Eurasian continent. As a part of the ancient Silk Road, the mutual migration between the countries, the typical diets, and the different lifestyles play the important role in shaping the genetic structure [21, 22]. The Uygur populations therefore are results of admixture of Han Chinese and Western Europe [23], and also is the highlight of the current study.

To date, there have been no studies that *CACNA1I* SNPs association with SCZ in the Uygur Chinese population reported, so it is the first study which performed *CACNA1I* in the Uygur Chinese population. A total of nine SNPs were selected in *CACNA1I*, including eight tag SNPs which were examined to provide a good coverage of this region, and one positive SNP which identified from a genome-wide association study was selected [24].

Methods

Subjects

In total, 985 unrelated patients with SCZ (612 males and 373 females), and 1218 control individuals (629 males and 589 females) were enrolled from Xinjiang Province. The mean age of SCZ cases was 39.45 years (±12.12), and normal controls was 43.07 years (±13.14). The data was illustrated as Table 1.

All eligible subjects selected were the native population of Xinjiang province. Clinical diagnosis were carried out in strict accordance with DSM-IV criteria (Diagnostic and Statistical Manual of Mental Disorders, the fourth edition) based on SCID-I (Structured Clinical Interview for DSM-IV Axis I Disorders) by interviewed with two independent psychiatrists. The healthy controls were randomly selected from the general Uighur population. All participants signed informed consent. This study obtained the consent of the local ethnic ethics, and undertaken the support of its support.

Genomic assay

According to QuickGene DNA whole blood kit L (FUJIFILM), genomic DNA was isolated from the peripheral blood of the subjects. Eight tag SNPs (rs132567, rs738168, rs713860, rs11705208, rs132575, rs136805, rs5757760, rs5750871) are obtained through Haploview software version 4.2, with pair-wise r^2 threshold ≥0.5 and minor allele frequency ≥ 0.05 [25]. Besides, we put a positive site of the previous research (rs9607658) into the experiment. The specific information of these 9 SNPs is listed in Table 2, while, the nine SNPs in the relative position of *CACNA1I* gene is also shown in Fig. 1. All samples were subjected to genotyping by the Sequenom MassARRAY matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry platform (Sequenom Inc., San Diego, CA).

Statistical analysis

Powerful SHEsis software provides a set of processing parameters for maximum benefit, including allele and genotype frequencies, Hardy-Weinberg equilibrium, association tests and haplotype analysis (http://shesisplus.bio-x.cn/SHEsis.html) [26, 27]. This is a comprehensive platform for

Table 1 Demographic detail of sample set

	Patients with schizophrenia		Healthy controls	
Total sample(N)	985		1218	
	Male	Female	Male	Female
	612	373	629	589
Mean age ± SD	39.45 ± 12.12		43.07 ± 13.14	

Table 2 The information of 9 SNPs in *CACNA1I* gene

SNP ID	rs9607658	rs132567	rs132575	rs713860	rs738168	rs136805	rs11705208	rs5757760	rs5750871
Position	39,561,735	39,577,521	39,586,716	39,612,821	39,615,692	39,622,207	39,646,048	39,648,397	39,673,444
Function	intron	intron	intron	intron	intron	intron	intron	intron	intron
Polymorphism	C/T	A/G	C/T	C/T	A/G	C/T	C/T	C/T	A/G

processing association study, and perform expectation maximization algorithm in haplotype reconstruction and frequency estimation. Allele and genotype frequencies refer to the percentage of allele and genotype in a population, and show the diversity and abundance of the gene in a population. FDR correction is a conservative method to explain multiple comparisons. All outputted tests were two-tailed, the *P* value standard of the statistical significance were set to be less than 0.05.

Results

Single site analysis

The genotype *P* values of the 9 SNPs in Hard–Weinberg equilibrium test (HWE) were all larger than 0.05 in both patients and healthy controls. So they all did not deviate from Hard–Weinberg equilibrium, and demonstrated the genetic properties of this sample population remained relatively stable. Call rates of all loci exceeded 99% in all samples. Detailed information is referenced in Table 3.

In Table 4, all the allele and genotype *P* values for the 9 SNPs in the patient samples and normal controls are shown. rs132575 and rs136805 were significant in both allele and genotype distributions [rs132575: adjusted P_{allele} = 0.039, adjusted $P_{genotype}$ = 0.037; rs136805: adjusted P_{allele} = 0.014, adjusted $P_{genotype}$ = 0.037]. In addition, rs713860, rs738168, rs5757760 and rs5750871 were significantly associated with SCZ in the allele distributions [rs713860: adjusted P_{allele} = 0.039, OR[95% CI] = 0.792[0.652–0.963]; rs738168: adjusted P_{allele} = 0.039, OR[95% CI] = 0.785[0.651–0.947]; rs5757760: adjusted P_{allele} = 0.042, OR[95% CI] = 0.873 [0.773–0.985]; rs5750871: adjusted P_{allele} = 0.039, OR[95% CI] = 0.859 [0.76–0.97]]. It is notable that rs738168 showed genotypic significance with SCZ before FDR correction [$P_{genotype}$ = 0.03, $P_{genotype}$ = 0.084 after FDR correction].

According to the gender of the subjects, the two sample sets were obtained separately. Detailed analysis results are illustrated in Tables 5 and 6. For male samples, there are seven ninths of the genes significantly associated with SCZ. rs132575, rs136805, rs5757760 and rs5750871 showed association towards SCZ in both allele and genotype distributions, meanwhile, rs9607658, rs713860, rs738168 revealed stronger positive results in the allele distributions. Interestingly, there was no significant association between *CACNA1I* and SCZ in the female sample, all the *P* values of 9 SNPs were greater than 0.05.

Linkage disequilibrium

The pairwise linkage disequilibrium (LD) values among the all investigated SNPs were subjected to calculate in all subjects. A total of 4 haplotype blocks of *CACNA1I* (rs132575-rs713860, rs713860-rs738168, rs713860-rs11705208, rs11705208-rs5750871) were identified when SNPs with D′ > 0.95 were classified in the same block, as presented in Fig. 2.

Haplotype analysis

There were two haplotypes (A-T: adjusted *P* = 0.038, OR [95% CI] = 0.804 [0.661–0.977]; G-C: adjusted *P* = 0.025, OR[95% CI] = 1.175 [1.035–1.334]) in the block rs132575-rs713860, which were significantly associated with SCZ, haplotype A-T proved to be a protective factor, and haplotype G-C showed it was risk factor. In the block rs713860-rs738168, haplotype C-C and T-A demonstrated protective factor and risk factor of SCZ, respectively (C-C: adjusted *P* = 0.007, OR [95% CI] =1.299 [1.079–1.564]; T-A: adjusted *P* = 0.023, OR[95% CI] = 0.797 [0.656–0.969]). In the block rs11705208-rs5750871, one haplotype C-G presented protective factor of SCZ (adjusted *P* = 0.038, OR [95% CI] =0.873 [0.773–0.986]), another haplotype, C-A, was risk factor after data analysis (adjusted *P* = 0.015, OR [95%

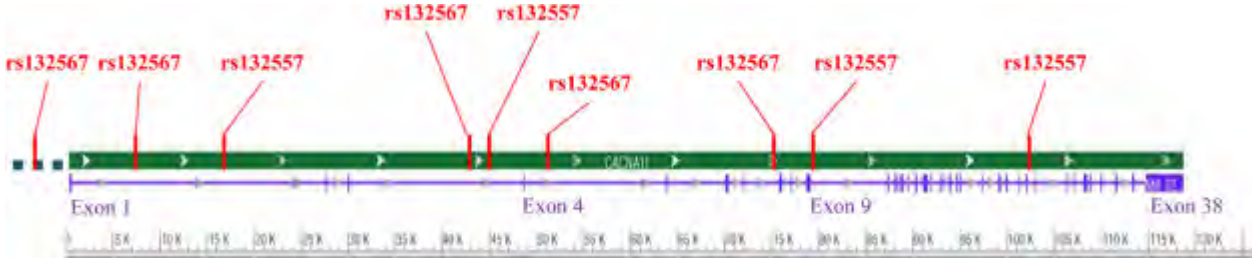


Fig. 1 Relative positions in gene *CACNA1I* of nine SNPs

Table 3 The call rate (%) and HWE test of 9 SNPs in SCZ patients and control

SNP ID	rs9607658		rs132567		rs132575		rs713860		rs738168		rs136805		rs11705208		rs5757760		rs5750871	
	case	control	case	control	case	control	case	control	case	control	case	control	case	control	case	control	case	control
call rate%	0.996		0.997		0.996		0.996		0.997		0.991		0.997		0.992		0.992	
HWE-P	0.904	0.654	0.549	0.222	0.231	0.483	0.515	0.929	0.729	0.955	0.973	0.837	0.658	0.999	0.999	0.989	0.58	0.78

CI] = 1.174 [1.042–1.322]). The result of haplotype analysis is suggested in Table 7.

Discussion

SCZ is a genetically complex neuropsychiatric disorder, but the specific etiology of this disease is still vague. SCZ is highly heritable, and the genes that contribute to the disorder play an important role [28]. In this context, we have attempted to confirm an association of *CACNA1I* variants with SCZ. We discovered nine variation sites within the *CACNA1I* locus, as well as one previously studied by Aiden Corvin et al. [24]. This is first study which replicated genetic susceptibility of *CACNA1I* gene in the Uygur Chinese population.

We found nominally association between several SNPs of *CACNA1I* and SCZ. There are four SNPs, rs713860, rs738168, rs5757760 and rs5750871 identified to be associated with SCZ in the allele distributions. In addition, both rs132575 and rs136805 were found to be significantly associated in allelic and

Table 4 Allele and genotype frequencies of 9 Loci in SCZ

SNP ID	Alleles		OR [95% CI]	*P*-value	P-FDR	Genotypes			*P*-value	P-FDR
rs9607658	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	618(0.314)	1350(0.685)	1.101 [0.968 ~ 1.253]	0.142	0.178	94(0.095)	430(0.436)	460(0.467)	0.218	0.245
Control	711(0.293)	1711(0.706)				111(0.091)	489(0.403)	611(0.504)		
rs132567	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	1073(0.545)	895(0.454)	0.917 [0.814 ~ 1.034]	0.159	0.178	284(0.288)	505(0.513)	195(0.198)	0.054	0.084
Control	1271(0.523)	1155(0.476)				348(0.286)	575(0.474)	290(0.239)		
rs132575	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	1295(0.658)	673(0.341)	1.159 [1.021 ~ 1.316]	*0.021*	*0.039*	414(0.42)	467(0.474)	103(0.104)	*0.008*	*0.037*
Control	1674(0.69)	750(0.309)				587(0.484)	500(0.412)	125(0.103)		
rs713860	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	1779(0.904)	187(0.095)	0.792 [0.652 ~ 0.963]	*0.019*	*0.039*	808(0.821)	163(0.165)	12(0.012)	0.056	0.084
Control	2142(0.882)	284(0.117)				947(0.78)	248(0.204)	18(0.014)		
rs738168	C(freq)	A(freq)				C/C(freq)	C/A(freq)	A/A(freq)		
Case	1763(0.895)	205(0.104)	0.785 [0.651 ~ 0.947]	*0.011*	*0.039*	792(0.804)	179(0.181)	13(0.013)	*0.03*	0.084
Control	2115(0.871)	313(0.128)				920(0.757)	275(0.226)	19(0.015)		
rs136805	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	1001(0.509)	965(0.49)	1.212 [1.075 ~ 1.365]	*0.001*	*0.014*	253(0.257)	495(0.503)	235(0.239)	*0.006*	*0.037*
Control	1339(0.556)	1065(0.443)				378(0.314)	583(0.485)	241(0.2)		
rs11705208	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	1775(0.901)	193(0.098)	1.039 [0.849 ~ 1.27]	0.708	0.708	803(0.816)	169(0.171)	12(0.012)	0.771	0.771
Control	2198(0.905)	230(0.094)				995(0.819)	208(0.171)	11(0.009)		
rs5757760	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
case	766(0.389)	1200(0.61)	0.873 [0.773 ~ 0.985]	*0.028*	*0.042*	149(0.151)	468(0.476)	366(0.372)	0.09	0.116
control	1017(0.422)	1391(0.577)				216(0.179)	585(0.485)	403(0.334)		
rs5750871	G(freq)	A(freq)				G/G(freq)	G/A(freq)	A/A(freq)		
case	748(0.38)	1218(0.619)	0.859 [0.76 ~ 0.97]	*0.014*	*0.039*	150(0.152)	448(0.455)	385(0.391)	0.051	0.084
control	1003(0.416)	1403(0.583)				215(0.178)	573(0.476)	415(0.344)		

Italics represent *P*-values < 0.05

Table 5 SNP analysis in men

SNP ID	Alleles		OR [95% CI]	*P*-value	P-FDR	Genotypes			*P*-value	P-FDR
rs9607658	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	394(0.322)	828(0.677)	1.218 [1.025 ~ 1.447]	*0.024*	*0.031*	59(0.096)	276(0.451)	276(0.451)	0.065	0.084
Control	350(0.28)	896(0.719)				49(0.078)	252(0.404)	322(0.516)		
rs132567	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	674(0.551)	548(0.448)	0.892 [0.761 ~ 1.044]	0.156	0.176	176(0.288)	322(0.527)	113(0.184)	0.167	0.188
Control	655(0.523)	597(0.476)				172(0.274)	311(0.496)	143(0.228)		
rs132575	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	790(0.646)	432(0.353)	1.266 [1.07 ~ 1.498]	*0.005*	*0.013*	247(0.404)	296(0.484)	68(0.111)	*0.008*	*0.02*
Control	873(0.698)	377(0.301)				307(0.491)	259(0.414)	59(0.094)		
rs713860	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	1111(0.91)	109(0.089)	0.731 [0.563 ~ 0.95]	*0.018*	*0.028*	507(0.831)	97(0.159)	6(0.009)	0.053	*0.079*
Control	1104(0.881)	148(0.118)				486(0.776)	132(0.21)	8(0.012)		
rs738168	C(freq)	A(freq)				C/C(freq)	C/A(freq)	A/A(freq)		
Case	1101(0.9)	121(0.099)	0.724 [0.564 ~ 0.928]	*0.01*	*0.019*	497(0.813)	107(0.175)	7(0.011)	*0.028*	0.051
Control	1087(0.868)	165(0.131)				470(0.75)	147(0.234)	9(0.014)		
rs136805	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	600(0.49)	622(0.509)	1.292 [1.102 ~ 1.513]	*0.001*	*0.013*	149(0.243)	302(0.494)	160(0.261)	*0.007*	*0.02*
Control	688(0.554)	552(0.445)				194(0.312)	300(0.483)	126(0.203)		
rs11705208	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
Case	1099(0.899)	123(0.1)	1 [0.769 ~ 1.299]	0.998	0.998	498(0.815)	103(0.168)	10(0.016)	0.315	0.315
Control	1126(0.899)	126(0.1)				505(0.806)	116(0.185)	5(0.007)		
rs5757760	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
case	464(0.379)	758(0.62)	0.79 [0.673 ~ 0.929]	*0.004*	*0.013*	86(0.14)	292(0.477)	233(0.381)	*0.004*	*0.02*
control	541(0.436)	699(0.563)				103(0.166)	335(0.54)	182(0.293)		
rs5750871	G(freq)	A(freq)				G/G(freq)	G/A(freq)	A/A(freq)		
case	454(0.371)	768(0.628)	0.794 [0.675 ~ 0.933]	*0.005*	*0.013*	92(0.15)	270(0.441)	249(0.407)	*0.009*	*0.02*
control	530(0.426)	712(0.573)				110(0.177)	310(0.499)	201(0.323)		

Italics represent *P*-values < 0.05

genotype analysis. Before FDR correction, rs738168 was associated with schizophrenia in the genotype distribution. Most of the investigated SNPs were positive in our subjects.

rs9607658 was reported as a risk factor for SCZ in population of Ireland in a genome–wide association study (GWAS) by Aiden Corvin et al. (combined $P = 3.3 \times 10^{-5}$, OR[95% CI] = 1.21[1.10–1.33]) [24]. However, rs9607658 did not confer susceptibility in the present study (adjusted $P = 0.142$, OR[95% CI] = 1.101[0.968–1.253]). This is likely to be caused by racial differences between Uygur and Ireland populations, and the existence of genetic heterogeneity can lead to such a result. A study on Uygur genetic characteristics suggest Uygur population from northern and southern Xinjiang Province share different proportions of ancestors from the European and Han population, so they are the results of admixture the anthropological features of the East and West [29, 30]. The minor allele frequency (MAF = T) in the Han Chinese population is 0.03, whereas in the Ireland population it is 0.54. The results of these two different populations are profound discrepancy, and Uighur population as mixture of the European and Han population also produce certain differences in MAF. Besides, the accuracy of the result is related to the sample size, and the small sample size in this study is used as a limitation for the significant analysis.

In addition, the result has been adopted segregation analysis of sex as a research strategy. We found that male had more susceptibility loci for SCZ, but all the SNPs were negative in the female group. This may be due to a difference in the prevalence and symptoms of psychiatric disorders from a gender standpoint. Previous literature also shows that the existence of significant gender differences in animal models of mental illness [31]. Compared with women SCZ patients, men with

Table 6 SNP analysis in women

SNP ID	Alleles		OR [95% CI]	*P*-value	P-FDR	Genotypes			*P*-value	P-FDR
rs9607658	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	224(0.3)	522(0.699)	0.968 [0.793 ~ 1.182]	0.755	0.907	35(0.093)	154(0.412)	184(0.493)	0.835	0.835
Control	361(0.306)	815(0.693)				62(0.105)	237(0.403)	289(0.491)		
rs132567	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	399(0.534)	347(0.465)	0.96 [0.798 ~ 1.153]	0.664	0.907	108(0.289)	183(0.49)	82(0.219)	0.407	0.808
Control	616(0.524)	558(0.475)				176(0.299)	264(0.449)	147(0.25)		
rs132575	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
Case	505(0.676)	241(0.323)	1.024 [0.841 ~ 1.247]	0.806	0.907	167(0.447)	171(0.458)	35(0.093)	0.302	0.808
Control	801(0.682)	373(0.317)				280(0.477)	241(0.41)	66(0.112)		
rs713860	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	78(0.104)	668(0.895)	0.891 [0.663 ~ 1.196]	0.443	0.907	6(0.016)	66(0.176)	301(0.806)	0.718	0808
Control	136(0.115)	1038(0.884)				10(0.017)	116(0.197)	461(0.785)		
rs738168	A(freq)	C(freq)				A/A(freq)	A/C(freq)	C/C(freq)		
Case	84(0.112)	662(0.887)	0.881 [0.662 ~ 1.172]	0.384	0.907	497(0.813)	72(0.193)	295(0.79)	0.646	0808
Control	148(0.125)	1028(0.874)				470(0.75)	128(0.217)	450(0.765)		
rs136805	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	343(0.461)	401(0.538)	1.085 [0.902 ~ 1.305]	0.384	0.907	75(0.201)	193(0.518)	104(0.279)	0.47	0808
Control	513(0.44)	651(0.559)				115(0.197)	283(0.486)	184(0.316)		
rs11705208	T(freq)	C(freq)				T/T(freq)	T/C(freq)	C/C(freq)		
Case	70(0.093)	676(0.906)	1.067 [0.776 ~ 1.466]	0.687	0.907	2(0.005)	66(0.176)	305(0.817)	0.523	0808
Control	104(0.088)	1072(0.911)				6(0.01)	92(0.156)	490(0.833)		
rs5757760	C(freq)	T(freq)				C/C(freq)	C/T(freq)	T/T(freq)		
case	442(0.594)	302(0.405)	0.993 [0.823 ~ 1.197]	0.943	0.943	133(0.357)	176(0.473)	63(0.169)	0.363	0808
control	692(0.592)	476(0.407)				221(0.378)	250(0.428)	113(0.193)		
rs5750871	A(freq)	G(freq)				A/A(freq)	A/G(freq)	G/G(freq)		
case	450(0.604)	294(0.395)	0.954 [0.79 ~ 1.151]	0.626	0.907	136(0.365)	178(0.478)	58(0.155)	0.563	0808
control	691(0.593)	473(0.406)				214(0.367)	263(0.451)	105(0.18)		

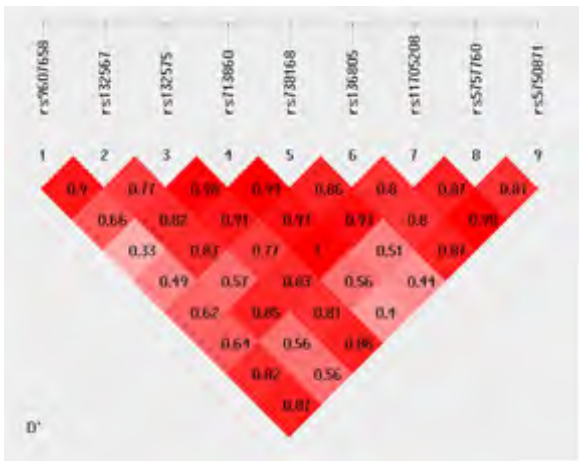


Fig. 2 Linkage disequilibrium among 5 SNPs of the *CACNA1I* gene

SCZ have a high rate of mortality (death, suicide) and earlier onset in the study of gender differences by Mao-Sheng Ran et al. [32]. For the present study, a total of 373 women in the patient sample, 589 women were recruited in the control group. Sample size is a critical factor in gender analysis, thus, there is a need for a larger sample to validate the association between gender and SCZ.

Although these nine SNPs are located in the intron region of *CACNA1I* gene, and they are not directly involved in the biological functions and characteristics of T-type calcium channel, intronic variations may provide some auxiliary cis-acting elements for gene expression regulation, which plays a role in modifying gene transcription efficiency. The protein encoded by *CACNA1I* is widely expressed in the nucleus reticularis thalami, different splice variants can affect the normal discharge of neurons [33]. Besides, we evaluated the protein interaction of *CACNA1I* gene by the version

Table 7 Haplotype Analysis for *CACNA1I* Gene in SCZ

Blocks with D' > 0.95	Haplotype	Case(freq)	Control(freq)	Chi ^{2}	OR [95% CI]	*P*-value	P-FDR
rs132575-rs713860	A-C	1107(0.563)	1393(0.575)	0.435	0.96 [0.851 ~ 1.082]	0.52	*0.509*
	A-T	187(0.095)	281(0.116)	4.788	0.804 [0.661 ~ 0.977]	*0.03*	*0.038*
	G-C	672(0.341)	745(0.307)	6.216	1.175 [1.035 ~ 1.334]	*0.013*	*0.025*
rs713860-rs738168	C-C	1762(0.896)	2112(0.87)	7.714	1.299 [1.079 ~ 1.564]	*0.006*	*0.007*
	T-A	187(0.095)	283(0.116)	5.161	0.797 [0.656 ~ 0.969]	*0.023*	*0.023*
rs713860-rs11705208	C-C	1588(0.807)	1913(0.788)	2.883	1.136 [0.98 ~ 1.317]	0.091	*0.134*
	C-T	191(0.097)	229(0.094)	0.109	1.034 [0.845 ~ 1.266]	0.757	*0.757*
	T-C	187(0.095)	284(0.117)	5.352	0.794 [0.653 ~ 0.965]	*0.021*	0.062
rs11705208-rs5750871	C-G	745(0.379)	1000(0.417)	4.761	0.873 [0.773 ~ 0.986]	*0.03*	*0.038*
	T-A	191(0.097)	225(0.093)	0.268	1.055 [0.861 ~ 1.292]	0.604	*0.604*
	C-A	1027(0.522)	1172(0.488)	7.041	1.174 [1.042 ~ 1.322]	*0.008*	*0.015*

Italics represent *P*-values < 0.05

10.0 of STRING [34], the result showed the *CACNB2* gene involved in SCZ interacts with *CACNA1I* gene, Whether different splice variants or protein-protein interactions, they may confers risk for SCZ.

The *CACNA1I* gene encodes the alpha-1 subunit of the T-type voltage-gated calcium channel Cav3.3, presenting series of function of calcium ion channel that are involved in the neural development and synapse formation [35]. Gene related to Ca^{2+} signaling, such as *CACNA1I* that encode VGCC subunits is associated with schizophrenia and other psychiatric disorders [36]. Evidence suggested that this gene is significantly associated with psychiatric disorders such as autism spectrum disorders. rs5750860, located in *CACNA1I*, has been reported to be associated with autism spectrum disorders by using existing genome-wide association study (GWAS) data and imputation methods [37] . Previous study indicated *CACNA1I* plays a crucial role in spindle activity by participating in the synchronous oscillation of thalamic cortical neurons, and expected to serve as a novel treatment biomarker associated with impaired cognition for individuals with SCZ by treating spindle deficits [17]. The release of neurotransmitters involved in the pathological process of SCZ, and simultaneously there is the research indicated that the *CACNA1I* gene triggers synaptic plasticity in reticular thalamic neurons. Presynaptic neurotransmitter release and postsynaptic receptor signal transduction play an important role in the transmission of information in the brain [38].

Conclusion

For this study, our efforts on mental illness represent a promising beginning. This is the first time that genetic factors of the *CACNA1I* gene have been verified to be associated with SCZ in the Uygur Chinese population. Obviously, *CACNA1I* plays a key role in the pathogenesis of SCZ. However, the present study remains a major bottleneck in the validation of larger samples, and a larger sample size could be better demonstrate the role of the *CACNA1I* gene in the etiology of schizophrenia. In addition, the Uighur Chinese population has been verified in the present study, and genetic association of other ethnic groups are suggested. Further functional studies of the *CACNA1I* gene are encouraged to conduct, especially in other ethnic groups. All the analysis will facilitate new therapeutic route for SCZ and may provide new insight into the pathogenesis of psychiatric illnesses.

Abbreviations

CACNA1I: Calcium voltage-gated channel subunit alpha1 I; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, the fourth edition; GWAS: Genome wide association study; HWE: Hardy–Weinberg equilibrium test; LD: Linkage disequilibrium; OR, odds ratio; MALDI-TOF: Matrix-assisted laser desorption ionization-time of flight; PGC-SCZ: Psychiatric Genomics Consortium -Schizophrenia Workgroup; SCID-I: Structured Clinical Interview for DSM-IV Axis I Disorders; SCZ: Schizophrenia

Funding

We are deeply grateful to all the participants in the study. And we appreciate psychiatrists working on this project as well as the normal controls and patients. This work is supported by the 973 Program (2015CB559100), the 863 project (2012AA02A515), the Natural Science Foundation of China (31,325,014, 81,130,022, 81,272,302, 81,421,061), the National High Technology Research and Development Program of China (2012AA021802), the Program of Shanghai Academic Research Leader (15XD1502200), National Program for Support of Top-Notch Young Professionals, Shanghai Key Laboratory of Psychotic Disorders (13dz2260500), "Shu Guang" project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17).

Availability of data and materials

Not applicable.

Authors' contributions

Author Wei Xu, Yahui Liu and Jianhua Chen co-deigned this study, wrote the protocol, carried on all experiments and managed the literature searches and analyses. Juan Zhou and Zujia Wen conducted the sample collection and verification. Qingli Guo and Zhijian Song undertook the statistical analysis. Ke Liu and Zhaowei Zhou were responsible for platform coordination and management. Author Wei Xu wrote the first draft of the manuscript,

while Yongyong Shi, Qizhong Yi and Lin He supervised the whole research process. All authors contributed to and have approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
The study was scrutinized and approved by the local ethical committee with all informed consent being accessible to subjects.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
[1]Department of biology, School of Life Science, Anhui Medical University, 81 meishan road, Hefei, Anhui 230031, China. [2]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education) and the Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China. [3]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, People's Republic of China. [4]Psychological Medicine Center, The First Affiliated Hospital of Xinjiang Medical University, 137 Liyushan South Road, Urumqi, Xinjiang 830054, China.

Received: 25 April 2017 Accepted: 7 June 2017
Published online: 17 July 2017

References

1. Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA psychiatry. 2015;72(4):334–41.
2. van Os J, Kapur S. Schizophrenia. Lancet (London, England). 2009;374(9690):635–45.
3. Burmeister M, McInnis MG, Zöllner S. Psychiatric genetics: progress amid controversy. Nat Rev Genet. 2008;9(7):527–40.
4. Freedman R. Schizophrenia. N Engl J Med. 2003;349(18):1738–49.
5. Javitt DC, Spencer KM, Thaker GK, Winterer G, Hajos M. Neurophysiological biomarkers for drug development in schizophrenia. Nat Rev Drug Discov. 2008;7(1):68–83.
6. Craddock N, O'Donovan MC, Owen MJ. The genetics of schizophrenia and bipolar disorder: dissecting psychosis. J Med Genet. 2005;42(3):193–204.
7. Consortium SWGotPG. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511(7510):421–7.
8. Perez-Reyes E. Molecular characterization of T-type calcium channels. Cell Calcium. 2006;40(2):89–96.
9. Monteil A, Chausson P, Boutourlinsky K, Mezghrani A, Huc-Brandt S, Blesneac I, et al. Inhibition of Cav3.2 T-type calcium channels by its intracellular I-II loop. J Biol Chem. 2015;290(26):16168–76.
10. Lee SE, Lee J, Latchoumane C, Lee B, Oh SJ, Saud ZA, et al. Rebound burst firing in the reticular thalamus is not essential for pharmacological absence seizures in mice. Proc Natl Acad Sci U S A. 2014;111(32):11828–33.
11. Shin HS. T-type Ca2+ channels and absence epilepsy. Cell Calcium. 2006;40(2):191–6.
12. Wang CY, Lai MD, Phan NN, Sun Z, Lin YC. Meta-analysis of public microarray datasets reveals voltage-gated calcium Gene signatures in clinical cancer patients. PLoS One. 2015;10(7):e0125766.
13. Hildebrand ME, David LS, Hamid J, Mulatz K, Garcia E, Zamponi GW, et al. Selective inhibition of Cav3.3 T-type calcium channels by Galphaq/11-coupled muscarinic acetylcholine receptors. J Biol Chem. 2007;282(29):21043–55.
14. Cataldi M, Lariccia V, Marzaioli V, Cavaccini A, Curia G, Viggiano D, et al. Zn(2+) slows down ca(V)3.3 gating kinetics: implications for thalamocortical activity. J Neurophysiol. 2007;98(4):2274–84.
15. Sun H, Varela D, Chartier D, Ruben PC, Nattel S, Zamponi GW, et al. Differential interactions of Na+ channel toxins with T-type Ca2+ channels. J Gen Physiol. 2008;132(1):101–13.
16. Astori S, Wimmer RD, Prosser HM, Corti C, Corsi M, Liaudet N, et al. The ca(V)3.3 calcium channel is the major sleep spindle pacemaker in thalamus. Proc Natl Acad Sci U S A. 2011;108(33):13823–8.
17. Manoach DS, Pan JQ, Purcell SM, Stickgold R. Reduced sleep spindles in schizophrenia: a treatable Endophenotype that links risk genes to impaired cognition? Biol Psychiatry. 2016;80(8):599–608.
18. Lencz T, Malhotra AK. Targeting the schizophrenia genome: a fast track strategy from GWAS to clinic. Mol Psychiatry. 2015;20(7):820–6.
19. Yue J, Liu L, Liu Z, Shu B, Zhang Y. Upregulation of T-type Ca2+ channels in primary sensory neurons in spinal nerve injury. Spine. 2013;38(6):463–70.
20. Andrade A, Hope J, Allen A, Yorgan V, Lipscombe D, Pan JQ. A rare schizophrenia risk variant of CACNA1I disrupts CaV3.3 channel activity. Sci Rep. 2016;6:34233.
21. Yao YG, Kong QP, Wang CY, Zhu CL, Zhang YP. Different matrilineal contributions to genetic structure of ethnic groups in the silk road region in china. Mol Biol Evol. 2004;21(12):2265–80.
22. Luo M, Zhou X, Ji H, Ma W, Liu G, Dai D, et al. Population difference in the associations of KLOTH promoter Methylation with mild cognitive impairment in Xinjiang Uygur and Han populations. PLoS One. 2015;10(7):e0132156.
23. Comas D, Calafell F, Mateu E, Perez-Lezaun A, Bosch E, Martinez-Arias R, et al. Trading genes along the silk road: mtDNA sequences and the origin of central Asian populations. Am J Hum Genet. 1998;63(6):1824–38.
24. Control ISGCatWTC. 2 C: genome-wide association study implicates HLA-C*01:02 as a risk factor at the major histocompatibility complex locus in schizophrenia. Biol Psychiatry. 2012;72(8):620–8.
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263–5.
26. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis. Cell Res. 2009;19(4):519–23. http://Analysis.Bio-x.Cn
27. Shen J, Li Z, Chen J, Song Z, Zhou Z, Shi Y. SHEsisPlus, a toolset for genetic studies on polyploid species. Sci Rep. 2016;6:24095.
28. Cariaga-Martinez A, Saiz-Ruiz J, Alelu-Paz R. From linkage studies to Epigenetics: what we know and what we need to know in the neurobiology of schizophrenia. Front Neurosci. 2016;10:202.
29. Xu S, Huang W, Qian J, Jin L. Analysis of genomic admixture in Uyghur and its implication in mapping strategy. Am J Hum Genet. 2008;82(4):883–94.
30. Shan M, Wang X, Sun G, Ma B, Yao X, Ainy A, et al. A retrospective study of the clinical differences of Uygur breast cancer patients compared to Han breast cancer patients in the Xinjiang region of China. Int J Clin Exp Med. 2014;7(10):3482–90.
31. Kokras N, Dalla C. Sex differences in animal models of psychiatric disorders. Br J Pharmacol. 2014;171(20):4595–619.
32. Ran MS, Mao WJ, Chan CL, Chen EY, Conwell Y. Gender differences in outcomes in people with schizophrenia in rural China: 14-year follow-up study. Br J Psychiatry. 2015;206(4):283–8.
33. Murbartian J, Arias JM, Perez-Reyes E. Functional impact of alternative splicing of human T-type Cav3.3 calcium channels. J Neurophysiol. 2004;92(6):3399–407.
34. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015;43(Database issue):D447–52.
35. Yunker AMR, Sharp AH, Sundarraj S, Ranganathan V, Copeland TD, McEnery MW. Immunological characterization of T-type voltage-dependent calcium channel CaV3.1 (alpha1G) and CaV3.3 (alpha1I) isoforms reveal differences in their localization, expression, and neural development. Neuroscience. 2003;117(2):321–35.
36. Nakao A, Miki T, Shoji H, Nishi M, Takeshima H, Miyakawa T, et al. Comprehensive behavioral analysis of voltage-gated calcium channel beta-anchoring and -regulatory protein knockout mice. Front Behav Neurosci. 2015;9:141.
37. Lu AT, Dai X, Martinez-Agosto JA, Cantor RM. Support for calcium channel gene defects in autism spectrum disorders. Molecular autism. 2012;3(1):18.
38. Astori S, Luthi A. Synaptic plasticity at intrathalamic connections via CaV3.3 T-type Ca2+ channels and GluN2B-containing NMDA receptors. J Neurosci. 2013;33(2):624–30.

Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia

Zhiqiang Li[1–4,22], Jianhua Chen[2,5,22], Hao Yu[6–8], Lin He[2,4], Yifeng Xu[5], Dai Zhang[6,7,9], Qizhong Yi[10], Changgui Li[11], Xingwang Li[2], Jiawei Shen[2], Zhijian Song[2], Weidong Ji[4,12], Meng Wang[2], Juan Zhou[2], Boyu Chen[2], Yahui Liu[2], Jiqiang Wang[2], Peng Wang[13], Ping Yang[13], Qingzhong Wang[2], Guoyin Feng[5], Benxiu Liu[14], Wensheng Sun[14], Baojie Li[2], Guang He[2], Weidong Li[2], Chunling Wan[2], Qi Xu[15], Wenjin Li[2], Zujia Wen[2], Ke Liu[2], Fang Huang[2], Jue Ji[2], Stephan Ripke[16–18], Weihua Yue[6,7], Patrick F Sullivan[19,20], Michael C O'Donovan[21] & Yongyong Shi[1–4,10,12]

We conducted a genome-wide association study (GWAS) with replication in 36,180 Chinese individuals and performed further transancestry meta-analyses with data from the Psychiatry Genomics Consortium (PGC2). Approximately 95% of the genome-wide significant (GWS) index alleles (or their proxies) from the PGC2 study were overrepresented in Chinese schizophrenia cases, including ~50% that achieved nominal significance and ~75% that continued to be GWS in the transancestry analysis. The Chinese-only analysis identified seven GWS loci; three of these also were GWS in the transancestry analyses, which identified 109 GWS loci, thus yielding a total of 113 GWS loci (30 novel) in at least one of these analyses. We observed improvements in the fine-mapping resolution at many susceptibility loci. Our results provide several lines of evidence supporting candidate genes at many loci and highlight some pathways for further research. Together, our findings provide novel insight into the genetic architecture and biological etiology of schizophrenia.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Schizophrenia (MIM181500) is a chronic, severe and disabling brain disorder that affects approximately 1% of the worldwide population and imposes an enormous burden on society[1,2]. It is a highly heritable psychiatric disorder (with an estimated heritability of 70–85%[3]) with a substantial polygenic component including thousands of common alleles with small effects that contribute to disease risk[4]. Approximately 33–50% of the genetic risk of schizophrenia has been captured by common alleles in GWAS[5]. The evidence to date suggests that many risk alleles for common schizophrenia-associated genetic loci may be shared across ancestry groups, but others may be population specific because of differing causal variants or linkage disequilibrium (LD) patterns in populations of different ancestries[6]. Previous GWAS have identified more than 110 schizophrenia-associated loci and have substantially advanced understanding of this condition[5,7–13]. In particular, the most recent and largest schizophrenia GWAS (from the Schizophrenia Working group of the Psychiatric Genomics Consortium, PGC2) which, with discovery and extension, included a total of 36,989 schizophrenia cases and 113,075 controls, has identified 128 independent genome-wide significant associations spanning 108 loci[7].

However, a large proportion of the genetic factors underlying schizophrenia remain unknown. Most of the heritability of schizophrenia is not yet attributable to specific loci; only 3.5% of the liability can be explained by GWS loci[7]. Moreover, to date, most GWAS participants with schizophrenia are of European descent. Thus, although the PGC2 report includes samples from East Asia, the proportions are small (approximately 5% and 3% for cases and controls, respectively). Large-scale GWAS including individuals of non-European descent

[1]Affiliated Hospital of Qingdao University and Biomedical Sciences Institute of Qingdao University (Qingdao Branch of SJTU Bio-X Institutes), Qingdao University, Qingdao, China. [2]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, Shanghai, China. [3]Institute of Social Cognitive and Behavioral Sciences, Shanghai Jiao Tong University, Shanghai, China. [4]Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai, China. [5]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [6]Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, China. [7]Institute of Mental Health, Sixth Hospital, Peking University, Beijing, China. [8]Department of Psychiatry, Jining Medical University, Jining, China. [9]Peking-Tsinghua Joint Center for Life Sciences/PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing, China. [10]Department of Psychiatry, First Teaching Hospital of Xinjiang Medical University, Urumqi, China. [11]Shandong Provincial Key Laboratory of Metabolic Disease and Metabolic Disease Institute of Qingdao University, Qingdao, China. [12]Changning Mental Health Center, Shanghai, China. [13]Wuhu Fourth People's Hospital, Wuhu, China. [14]Longquan Mountain Hospital of Guangxi Province, Liuzhou, China. [15]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [16]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, USA. [17]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. [18]Department of Psychiatry and Psychotherapy, Charité-Universitätsmedizin, Berlin, Germany. [19]Departments of Genetics and Psychiatry, University of North Carolina, Chapel Hill, North Carolina, USA. [20]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [21]MRC Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, Cardiff, UK. [22]These authors contributed equally to this work. Correspondence should be addressed to Y.S. (shiyongyong@gmail.com).

Received 17 April; accepted 19 September; published online 9 October 2017; doi:10.1038/ng.3973

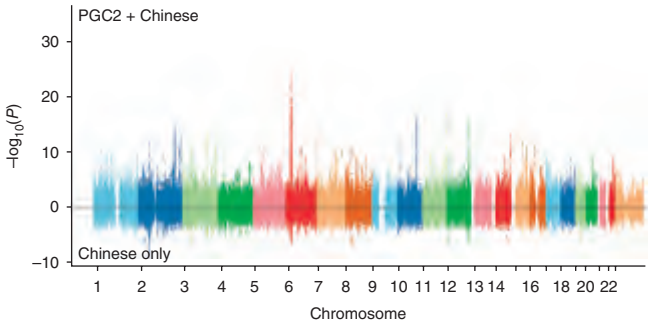


Figure 1 Comparison of Manhattan plots for the Chinese and transancestry analyses. Manhattan plot of results from the Chinese-only (7,699 schizophrenia cases and 18,327 controls) and PGC2-plus-Chinese transancestry (43,175 cases and 65,166 controls) analyses. $-\log_{10}P$ values for PGC2 plus Chinese transancestry analyses and $\log_{10}P$ values for Chinese-only analyses are shown.

are essential for extending understanding of the genetic architecture of schizophrenia in the human population as a whole, for testing the generalizability of the results from European populations regarding this global disorder[6] and for identifying population-specific risk factors, should they exist.

To identify additional schizophrenia susceptibility loci and to gain a better understanding of the genes and biological pathways implicated in schizophrenia, we performed a GWAS including 7,699 cases and 18,327 controls of Chinese ancestry, as well as a transancestry GWAS meta-analysis with PGC2 (43,175 cases and 65,166 controls in total). The candidate loci found in each analysis were then studied in an independent replication sample of 4,384 schizophrenia cases and 5,770 controls of Chinese ancestry.

RESULTS

Results of GWAS screening in the Chinese population

We first conducted a GWAS for schizophrenia in the Chinese population (in comparison to the discovery phase of our prior GWAS report[10], the number of cases was doubled, and the number of controls was tripled). After systematic quality control (QC) analysis and imputation to the 1000 Genomes Project data (Online Methods), we assessed the associations of 5,107,227 genetic variants in 7,699 schizophrenia cases and 18,327 controls (**Supplementary Table 1**). The primary GWAS comprised three samples that were genotyped on different platforms: 4,175 cases and 10,470 controls genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0 (SNP6.0); 2,472 cases and 5,928 controls genotyped with the Affymetrix Axiom Genome-Wide CHB1 Array Plate (CHB1); and 1,052 cases and 1,929 controls genotyped with CHB1 or the Illumina 1M Array (1M). Principal component analysis (PCA) was used to assess population substructure (**Supplementary Fig. 1** and Online Methods). For each subset, association testing was conducted with logistic regression including ancestry principal components (PCs) as covariates to adjust for population stratification. The results were combined with inverse-variance-weighted meta-analysis (based on a fixed-effects model). The genomic inflation factor (λ_{GC}) was 1.22, and the $\lambda_{1,000}$ (a scaled value to 1,000 cases and 1,000 controls) was 1.02. We conducted LD-score regression analysis[14] to distinguish the relative contributions of confounding bias and polygenicity. The LD-score regression intercept was 1.07 (s.e. = 0.01), and the slope was greater than zero, thus suggesting that most of the increase in the mean χ^2 statistic was from polygenic architecture rather than population stratification, in agreement with the previously documented polygenic nature of schizophrenia inheritance[7,15]. However, given this modest elevation in the intercept, we further corrected the meta-analysis statistics for residual test-statistic inflation[14,16] (Online Methods). Quantile–quantile and Manhattan plots are shown in **Supplementary Figures 2** and **3**. In this analysis (**Fig. 1**), we observed 66 GWS variants in a region previously reported to be associated with schizophrenia (2p16.1)[11,12].

The proportion of variance in susceptibility to schizophrenia explained by genome-wide SNP genotypes for individuals of Han Chinese ancestry (Online Methods) was estimated to be 31.5% (s.e. = 1.9%), assuming a population risk of 0.01. This result was similar to the corresponding estimate for European samples (33%)[5], thus providing further evidence of the highly polygenic nature of schizophrenia beyond that in previous studies[7,15].

Results of the Chinese and PGC2 genome-wide meta-analysis

We performed a meta-analysis of the Chinese GWAS samples (7,699 schizophrenia cases and 18,327 controls) (denoted Chinese GWAS) and PGC2 GWAS samples (35,476 schizophrenia cases and 46,839 controls) to explore the effects of power and heterogeneity. A total of 4,303,606 genetic variants were common to the two data sets and were retained in the combined analysis. For combining the data, we used a fixed-effects model, but for variants with pronounced heterogeneity (I^2 >75%)[17], we used a random-effects model to allow for the possibility that the presence of heterogeneity might result in test-statistic inflation. In our final result, the λ_{GC} was 1.50, and the $\lambda_{1,000}$ was less than 1.01. The deviation of the observed statistics from the null hypothesis was less than that expected under a polygenic model for schizophrenia[7,18]. Quantile–quantile and Manhattan plots are shown in **Supplementary Figures 4** and **5**. In the combined analysis, we detected 5,618 SNPs surpassing the threshold for GWS for association with schizophrenia. These SNPs mapped to 104 physically distinct associated regions, as defined by clumping the variants by using r^2 >0.1 and merging the LD-independent variants within 250 kb (**Fig. 1** and **Supplementary Table 2**).

Results of the combined analysis with replication samples

We then obtained association results from an independent Chinese cohort of 4,384 schizophrenia cases and 5,770 controls[19] (**Supplementary Table 1**) for LD-independent SNPs with $P < 1 \times 10^{-5}$ in the Chinese-only GWAS meta-analysis or with $P < 5 \times 10^{-7}$ in the Chinese and PGC2 GWAS meta-analysis (Online Methods).

The combined analysis of the Chinese GWAS and replication samples resulted in a data set of 12,083 cases and 24,097 controls. Seven loci were GWS for association with schizophrenia in the meta-analysis of individuals of Chinese ancestry. Of those loci, three have been previously reported to be associated with schizophrenia (**Supplementary Fig. 6**), and the other four are novel: rs2073499 at 3p21.31 (odds ratio (OR) = 0.899, fixed-effects meta-analysis $P = 2.61 \times 10^{-8}$), rs7757969 at 6q21 (OR = 1.110, $P = 4.82 \times 10^{-8}$), rs4479915 at 6q27 (OR = 0.876, $P = 4.82 \times 10^{-9}$) and rs11534004 at 7q31.1 (OR = 0.890, $P = 1.71 \times 10^{-8}$) (**Fig. 2**). Four additional loci were significant at $P < 1 \times 10^{-5}$ in the Chinese GWAS meta-analysis and showed nominal evidence of replication (P <0.05) but were not GWS in the combined analysis. Results for all tested SNPs are presented in **Supplementary Table 3**.

The combined results of the transancestry meta-analysis (43,175 schizophrenia cases and 65,166 controls) and replication samples (4,384 schizophrenia cases and 5,770 controls) identified a total of 109 GWS loci (**Supplementary Table 4** and **Supplementary Data 1**). Of the 109 loci, 83 had previously been reported, and 26 loci were novel.

Together, the above results identified 124 SNPs that were GWS and were associated with schizophrenia. The SNPs mapped to 113

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

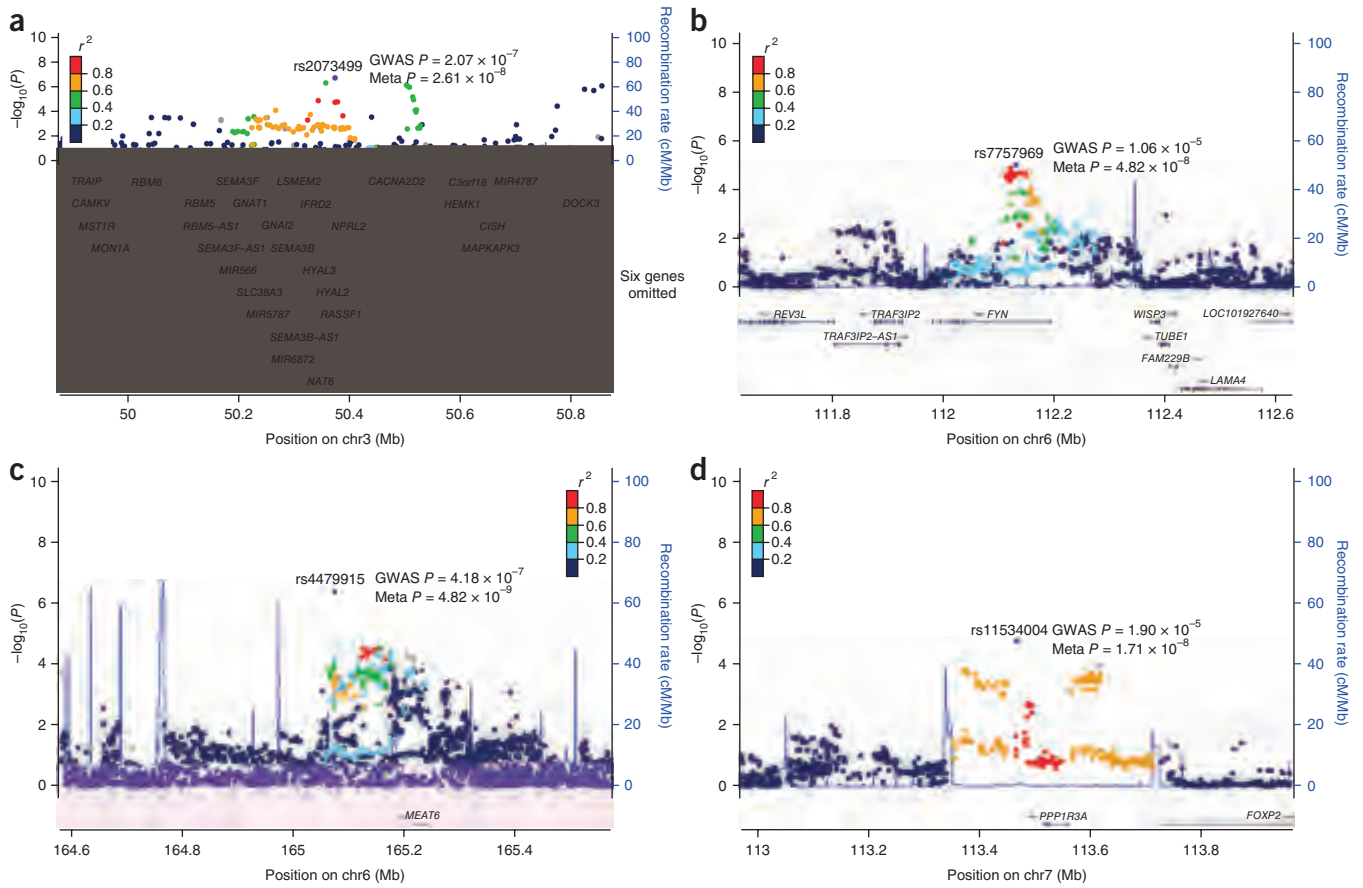


Figure 2 Regional plots for novel GWS loci in Chinese people. (**a**) rs2073499 at 3p21.31. (**b**) rs7757969 at 6q21. (**c**) rs4479915 at 6q27. (**d**) rs11534004 at 7q31.1. Meta, meta-analysis; chr, chromosome. $-\log_{10}P$ values are shown for SNPs for the region 500 kb on either side of the marker SNPs. The index SNP is shown in purple, and the r^2 values of the other SNPs are indicated by color. The r^2 values were established on the basis of 1000 Genomes data (November 2014). *P* values for the GWAS stage are shown with circles, and *P* values for the meta-analysis combining all data sets are shown with text. The genes within the relevant regions are annotated and shown as arrows.

physically distinct loci: four loci were GWS only in the Chinese-only analysis, 106 were GWS only in the transancestry analysis, and three were present in both analyses (**Supplementary Table 5**). Of the 113 associated loci, 30 have not been previously reported (**Table 1**), four of which were GWS in the Chinese sample but not the transancestry analysis. In addition, at three of the previously reported loci, the GWS SNPs in the present study were in low LD with the previously identified GWS SNPs (r^2 <0.1 in both the European and Chinese populations), thus possibly suggesting independent signals in these regions (**Supplementary Table 5**).

Similarities and differences across ancestries

Of the 108 loci (128 index SNPs) identified in the PGC2 report[7], we were able to investigate 103 loci (117 index SNPs or their proxies) that were in common between PGC2 and Chinese data sets (**Supplementary Table 6**). Of these, the PGC2-associated risk alleles were overrepresented in Chinese cases at 109 SNPs (from 98 loci), and at 58 SNPs (from 56 loci) this overrepresentation achieved nominal significance (*P* <0.05). In transancestry meta-analyses, 85 SNPs at 78 loci continued to be GWS. It is known that the random-effects model might be overly conservative[20], and therefore on an exploratory basis, we performed a fixed-effects model meta-analysis for all these SNPs regardless of the existence of heterogeneity. Under the fixed-effects model, an additional eight SNPs (93 in total) at eight loci (86 loci in total) were GWS in the combined analysis. However, the results for the GWS SNPs indicated by fixed-effects meta-analysis and with evidence of heterogeneity should be interpreted with caution. Nevertheless, this finding suggested that the schizophrenia susceptibility loci identified in European samples were applicable to the Chinese sample. Moreover, the transancestry meta-analyses also confirmed two GWS loci (8p12 and 7q11.22) identified in our previous reports[10,21].

Regarding the seven GWS index SNPs analyzed in the Chinese-only analysis in this study, three replicated at *P* <0.05 in the PGC2 data set but showed significant heterogeneity (Higgins and Thompson I^2 index >75%) across populations and were not GWS in transancestry meta-analyses. In addition, one of the index SNPs (rs78681500) was absent in the PGC2 data set, owing to its rarity (minor allele frequency (MAF)<1%). Of the 117 GWS index SNPs identified in the transancestry analysis, all showed the same direction of effect across ancestries, and the I^2 was less than 75%.

We next assessed the genome-wide congruence of risk alleles across the PGC2 and Chinese GWAS data sets for LD-clumped independent SNPs[22]. For the schizophrenia-associated SNPs ($P \leq 0.0001$) identified in the Chinese data set, we observed a highly significant excess of directional concordance in the PGC2 data set (67.7%, binomial test $P = 3.06 \times 10^{-7}$). For the SNPs demonstrating weaker evidence of an association with schizophrenia ($0.0001 < P \leq 0.05$), we also observed an excess of consistency in the direction of effect. In contrast, for the

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Table 1 Novel schizophrenia GWS loci and notable genes

Chromosome	SNP	Position	*P* value	Notable gene(s)[a]
2	rs999494	73157395	2.40×10^{-10}	*EMX1* (N, D)
2	rs62152284	104984387	5.86×10^{-9}	*LOC100287010* (N)
2	rs6430491	134840967	9.55×10^{-10}	*MIR3679* (N)
3	rs10510653	32058559	2.54×10^{-8}	*GPD1L* (Q), *ZNF860* (N)
3	rs2073499	50374293	2.61×10^{-8}	*HYAL3* (Q), *RASSF1* (N)
4	rs11722779	103827488	3.40×10^{-8}	*BDH2* (Q), *CENPE* (Q), *CISD2* (Q), *KRT8P46* (Q), *LRRC37A15P* (Q), *NHEDC1* (N), *SLC9B1* (Q)
5	rs10940346	49806042	1.11×10^{-8}	*EMB* (N, Q)
5	rs2247870	90151589	2.54×10^{-8}	*ADGRV1* (N, M, D)
5	rs2764766	127213625	1.94×10^{-8}	*LINC01184* (N)
6	rs6903570	64866857	2.70×10^{-8}	*EYS* (N), *PHF3* (D), *PTP4A1* (D)
6	rs160593	105466332	7.69×10^{-9}	*HACE1* (Q), *LIN28B* (N, Q)
6	rs7757969	112132032	4.82×10^{-8}	*FYN* (N, Q)
6	rs4479915	165075601	4.82×10^{-9}	*C6ORF118* (N)
7	rs323167	78336677	4.47×10^{-8}	*MAGI2* (N, D)
7	rs11534004	113467444	1.71×10^{-8}	*PPP1R3A* (N, M)
8	rs17687067	17036201	3.39×10^{-12}	*MTMR7* (Q), *VPS37A* (Q), *ZDHHC2* (N, D, Q)
8	rs73219805	26272768	1.94×10^{-11}	*BNIP3L* (N, D), *PPP2R2A* (D), *SDAD1P1* (Q)
10	rs111364339	64857872	5.37×10^{-9}	*JMJD1C* (D), *NRBF2* (N)
12	rs28607014	117708611	1.75×10^{-8}	*NOS1* (N)
14	rs10148671	29469373	4.46×10^{-8}	*LINC01551* (N)
14	rs2383377	33257914	2.36×10^{-8}	*AKAP6* (N, D), *NPAS3* (D)
14	rs8012642	84669481	4.66×10^{-8}	*FLRT2* (N)
15	rs783540	83254708	3.05×10^{-8}	*AP3B2* (D, Q), *CPEB1* (N, Q)
15	rs758129	89900887	2.87×10^{-8}	*MIR9-3* (N), *POLG* (D), *RLBP1* (Q)
16	rs6500596	4470027	5.24×10^{-9}	*CDIP1* (Q), *CORO7* (N, D, Q), *DNAJA3* (M, Q), *NMRAL1* (Q, S)
16	rs8058130	64371163	4.77×10^{-8}	*CDH11* (N)
17	rs56007784	1290950	1.16×10^{-9}	*YWHAE* (N)
17	rs72843506	19946287	3.73×10^{-8}	*AKAP10* (D), *CCDC144CP* (Q), *SPECC1* (N, D, Q), *USP32P3* (Q)
17	rs35065479	55736735	2.31×10^{-8}	*TSPOAP1-AS1* (Q), *MSI2* (N)
18	rs56775891	77575613	1.85×10^{-8}	*KCNG2* (N, Q, S)
18	rs28735056	77622879	4.60×10^{-10}	*KCNG2* (N)

Genomic position is based on the UCSC hg19/NCBI build 37. [a]Notable genes are indicated as follows: gene nearest to the index SNP (N); schizophrenia-associated variant in strong LD ($r^2 \geq 0.8$) with a missense variant in the indicated gene (M); gene prioritized by DEPICT (D); gene with mRNA levels in *cis* genetic linkage with the index SNPs (Q); and gene prioritized by SMR analysis (S).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

SNPs with no evidence of association ($P > 0.5$), there was no enrichment in coincident risk alleles across ancestry groups (**Supplementary Table 7**). We repeated this analysis by identifying the schizophrenia risk alleles at SNPs in the PGC2 data set and assessing concordance in the direction of the effect in the Chinese data set, and we found a very similar pattern (**Supplementary Table 7**). We concluded that there was a significant excess in directional concordance across ancestry groups for the SNPs with evidence of a schizophrenia association.

Potential biological mechanisms of the associated loci

To determine the likely causal genes of the schizophrenia-associated genetic loci, we considered each of the following to represent evidence supporting a gene's causality within a locus (Online Methods): (i) being the gene nearest the index SNP[23]; (ii) containing a missense mutation and being in high LD ($r^2 > 0.8$) with the GWS SNPs[23]; (iii) showing prioritization with DEPICT[24]; (iv) being *cis*-acting expression quantitative trait loci (*cis*-eQTL) genes for the index SNPs[23,25–28]; or (v) showing prioritization in summary-data-based Mendelian randomization (SMR) analysis[29]. Using these criteria, we prioritized 247 genes from the schizophrenia risk loci and found that 85 had more than one line of supporting evidence (defined as 'prioritized candidate genes') (**Supplementary Table 8**). We first focused on those genes in the newly identified loci (**Table 1**). As expected, some of those genes were plausibly biologically relevant. The index SNP rs2247870 (NP_115495.3, p.Val5876Ile) at 5q14.3 (GWS locus no. 37) is a missense variant in *ADGRV1* (also known as *GPR98*), which encodes a member of the G-protein-coupled-receptor superfamily and is expressed in the central nervous system. Multiple lines of evidence suggest that G-protein-coupled receptors play critical roles in major psychiatric disorders (including schizophrenia) and their treatment[30]. A variant in *GPR98* has been found to be associated with the response to antipsychotic treatment[31]. *FYN* (GWS locus no. 49) encodes a membrane-associated tyrosine kinase. FYN plays a critical role in neuronal apoptosis and is involved in brain development and synaptic transmission[32,33]. Lower expression of FYN protein has been observed in the platelets of schizophrenic patients compared with controls[34]. The results from whole-blood eQTL analysis[27] indicated that the schizophrenia risk allele identified in this study (rs7757969[C]) was correlated with a lower expression of *FYN* ($P = 1.71 \times 10^{-7}$, with a false discovery rate <0.05 and in the credible interval covered by the 99% credible set), in agreement with previous findings. The estimate (b_{XY}) for the effect of gene expression on schizophrenia risk under the SMR analysis was −0.70 ($P_{SMR} = 7.55 \times 10^{-4}$). *MAGI2* (GWS locus no. 54) encodes a synaptic scaffolding molecule that is essential for the development and maintenance of synapses[35]. Synaptic dysfunction has been suggested to play an important role in schizophrenia[36]. Common variants in *MAGI2* have been found to be associated with cognitive impairment in people with schizophrenia[37]. Although it is currently difficult to pinpoint a causal gene that is responsible for a given locus, the prioritized genes may be considered as favorable candidates for further research to unravel the plausible biological mechanisms underlying the associations.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Improved fine-mapping resolution at the associated loci

We sought to refine the localization of likely functional variants in the schizophrenia-associated loci by using a previously published approach[38,39]. We derived Bayesian credibility sets in different data sets and evaluated the evidence for improved fine-mapping resolution through transancestry meta-analysis. For the 99% credible SNP sets, the transancestry data set produced the smallest spanned regions for ~80% (n = 88) of the tested loci (**Supplementary Table 9**), including 11 loci with a spanned region less than 30 kb. At 53 of the 88 loci, the number of genes that overlapped with the transancestry interval defined by a credible set was two or fewer. Of those overlapped genes mapping to the credible intervals in the 53 loci, 49.1% was were in the list of prioritized candidate genes, whereas the proportion was 12.4% and 5.1% for the analysis of PGC2 and Chinese data, respectively.

We also conducted fine-mapping analysis with PAINTOR by leveraging the functional annotation data and LD information in multi-ancestry cohorts[40–42]. We integrated the primary functional categories (coding, UTR, promoter, DNase-hypersensitivity site, intronic and intergenic) proposed by Gusev *et al.*[41]. A total of 62 variants achieved a posterior probability of >0.80 in at least one of the single-population and transancestry analyses (**Supplementary Table 10**). Of them, 38 variants had a higher posterior probability in the transethnic analysis than in the single-population analyses, including an additional 16 variants that achieved a transancestry posterior probability of >0.80 but had a posterior probability <0.80 in the single-population analyses. Eleven (68.8%) of these 16 variants had at least one hit in the selected eQTL studies in HaploReg v4.1 (ref. 23 and **Supplementary Table 11**). For example, at GWS locus no. 80, rs12541 with a posterior probability of 0.926 (**Supplementary Fig. 7a**) is in the UTR region of *ESAM* and correlated with its expression in whole blood ($P = 3.62 \times 10^{-8}$ and in the 99% credible-set interval)[27]. A further example is GWS locus no. 103, rs3814883, which had a posterior probability of 0.911 (**Supplementary Fig. 7b**) and is a synonymous variant of *TAOK2* and also an eQTL SNP for several genes in different tissues[23] (**Supplementary Table 12**). It might also be correlated with the expression of *SEZ6L2* in the brain cerebellum and frontal cortex ($P = 2.37 \times 10^{-8}$ and 5.03×10^{-8}, respectively)[26]. TAOK2 has been found to affect basal-dendrite development in cortical neurons[43]. SEZ6L2 has been found to be a Cathepsin D transport receptor involved in neurite outgrowth[44]. To further explore the regulatory nature in the context of the cell-type-specific epigenome, we also integrated the reference epigenomes of seven highlighted marks for 127 human tissues and cell types produced by the Roadmap Epigenomics Project[45] (Online Methods). Of the top 100 enriched cell-type-specific epigenomic annotations for schizophrenia associations in the current and PGC2 analyses[7], over 40 were related to the brain and nervous system (**Supplementary Table 13**). In the further PAINTOR fine-mapping analyses with the top 100 epigenomic annotations, many SNPs had higher posterior probabilities, some of which increased to a value >0.80, thus indicating potential biologically relevant cell types for these associations (**Supplementary Table 14**). For example, rs6670165, a candidate causal SNP at GWS locus no. 7, mapped to enhancers and promoters active in several brain regions. The identification of these SNPs suggested an important benefit of the transancestry fine-mapping signal in functional annotation data. However, 14 variants had a posterior probability >0.80 in the single-population analyses, which decreased to <0.8 in the transancestry analysis (**Supplementary Table 10**).

Biological pathways and gene sets

To identify pathways and gene sets in the transancestry meta-analysis, we performed an enrichment analysis with MAGMA[46]. We identified

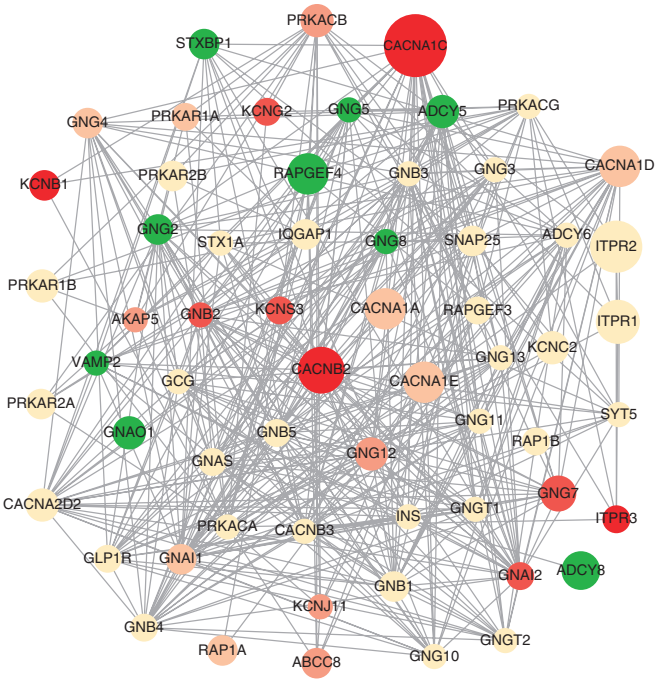


Figure 3 Interaction network of the schizophrenia-associated pathway 'glucagon-like peptide-1 regulates insulin secretion'. The network shows functional interactions for the genes in the pathway 'glucagon-like peptide-1 regulates insulin secretion' from the Reactome database. Each node represents a gene, and each edge represents a functional interaction. The node size corresponds to the gene size. The node color corresponds to the significance of the gene on the basis of the MAGMA analysis, and the green-to-red gradient corresponds to nonsignificance to high significance.

one gene set, 'regulation of insulin secretion by glucagon-like peptide 1' (from the Reactome database) that was significantly enriched (MAGMA competitive $P = 5.14 \times 10^{-7}$; **Fig. 3**). The MAGMA pathway analysis also highlighted several other pathways. Two of the previously highlighted schizophrenia-associated pathways, 'postsynaptic density'[47] and 'voltage-gated calcium channel complex'[7], also ranked highly in our analysis, with P values of 9.01×10^{-4} and 1.32×10^{-3}, respectively (**Supplementary Table 15**).

Polygenic risk-score profiling

Polygenic scoring analyses have been proposed to predict the case–control status in a target data set, on the basis of the results from a training GWAS[4]. To assess the overlap between the common-variant signal in the European and Chinese populations and to provide estimates of the proportions of variance additionally explained by the Chinese sample, we conducted a polygenic scoring analysis. We randomly selected approximately 1,000 schizophrenia cases and 1,000 controls from the Chinese sample as the target sample and used four training data sets: (i) the PGC2 European-only data set (EUR49); (ii) the full PGC2 data set; (iii) the Chinese sample, excluding the target sample; and (iv) the Chinese plus PGC2 combined data set (**Fig. 4**). The risk-profile SNPs (P thresholds (P_T) = 5×10^{-8}, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1) from the European-only data set alone explained approximately 1.11% to 2.34% of the variance in the case–control status of the Chinese sample on the liability scale[48] (assuming a population risk of 0.01). When the Asian samples were included, the PGC2 data set explained approximately 1.52% to 3.51% of the variance. The Chinese data set alone explained approxi-

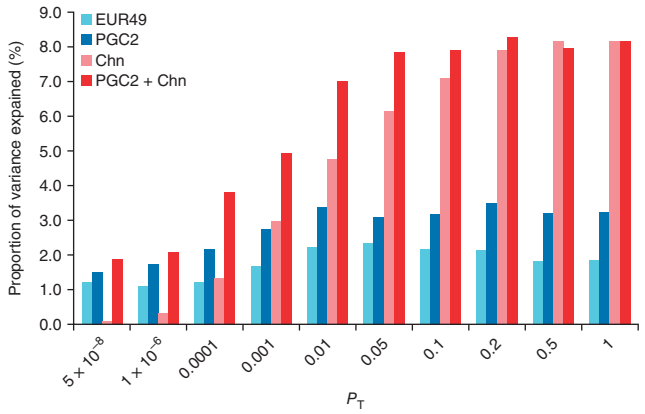


Figure 4 Polygenic risk-score profiling analysis. Polygenic risk-score profiling analysis using approximately 1,000 randomly selected schizophrenia cases and 1,000 controls from the Chinese sample as a target and deriving risk alleles from three training data sets: the PGC2 European-only (EUR49) data set (light blue); the full PGC2 data set (blue); the Chinese (Chn) sample excluding the target sample (light red); and the Chinese and PGC2 data sets combined (red). The *x* axis shows ten *P*-value thresholds ($P_T = 5 \times 10^{-8}$, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1). The *y* axis is the estimate of the proportion of variance explained on the liability scale, which is converted from Nagelkerke's pseudo R^2 (computed by comparison of a full model including covariates and polygenic risk scores to a reduced model including covariates only).

mately 0.10% to 8.15% of the variance. In almost all situations, the combined data set (PGC2 plus Chinese) explained larger proportions of the variance (approximately 1.89% to 8.28%). For $P_T = 5 \times 10^{-8}$, the proportion of the explained variance increased from 1.20% (EUR49), 1.52% (PGC2) and 0.10% (Chinese) to 1.89% (PGC2 plus Chinese); for $P_T = 0.05$, it is increased from 2.34% (EUR49), 3.09% (PGC2) and 6.16% (Chinese) to 7.86% (PGC2 plus Chinese). To evaluate the increased variance explained by the newly identified GWS loci, we performed additional polygenic risk-score profiling trained on the full data set (GWAS excluding the target sample and with replication) and restricted to the newly identified loci with the Chinese sample included. These novel loci explained 1.34% of the variance, 30% of which was contributed by the loci from the Chinese-only analysis.

Correlations between two psychiatric disorders in the Chinese sample

Strong evidence of a shared genetic etiology between schizophrenia and other psychiatric disorders (such as bipolar disorder and major depressive disorder) has been observed in European samples[49,50]. The degree of shared variation across psychiatric disorders in the Chinese population has been unclear. We estimated the genetic correlation between schizophrenia and major depressive disorder, two diseases for which Chinese GWAS data with large sample sizes are available, by using LD-score regression[14]. We observed a statistically significant genetic correlation between schizophrenia and major depressive disorder in the Chinese sample ($r_g = 0.43$, s.e. = 0.08, LD-score regression $P = 5.87 \times 10^{-8}$), in agreement with findings ($r_g = \sim 0.40$) in the European samples[49].

DISCUSSION

In the large GWAS analysis of schizophrenia in subjects of Chinese ancestry, we identified seven GWS loci, four of which were novel. In general, alleles identified as being associated at subthreshold levels of significance in the Chinese data set were also enriched in schizophrenia cases in the GWAS from PGC2, thus supporting the validity of combining the two data sets. The transancestry meta-analyses of the Chinese and PGC2 data identified 109 GWS risk loci, three of which were GWS in the Chinese-only analysis. Our analyses confirmed most of the previously identified schizophrenia loci and identified 30 novel loci.

We observed a significant excess in the directional consistency of schizophrenia risk alleles across ancestry groups, even at SNPs demonstrating only weak evidence of an association. These findings indicated that most schizophrenia risk loci were shared across these two ancestral populations, and transancestry meta-analysis provided a powerful means for identifying new loci and narrowing the association intervals. Polygenic scoring analysis also demonstrated notable increases in the explained variance in case–control status (PGC2-plus-Chinese training to Chinese target compared with PGC2 to Chinese target or Chinese training to Chinese target). However, this analysis also suggested that variants identified in European samples partially explained the genetic variance of schizophrenia in Chinese populations. Notably, estimates of the proportion of explained variance in liability were lower than those in European populations[7], similarly to previous reports on transethnic analyses[4,51]. Such lower estimates might be a result of differences in the allele frequencies and LD patterns between different populations[4].

It has been suggested that there are also population-specific risk alleles for schizophrenia[6] and that, if so, cross-ancestry analyses might have less power than that of studies of individuals with a recent shared ancestry. We found that some GWS loci in the PGC2 report were not GWS in the PGC2-plus-Chinese combined analysis. Moreover, most of the GWS SNPs identified in the analysis of Chinese subjects showed strong heterogeneity only across ancestries, though three of them achieved nominal significance with the same sign in the PGC2 data set. Another SNP fell within the previous PGC2-identified locus, but it was rare (MAF <1%) in European populations. Thus, further transancestry fine-mapping, by leveraging the differences in the LD structure among diverse populations, may be an efficient approach to identify the causal variants underlying such associations and may also distinguish population-specific loci. Indeed, we also observed considerable improvements in the fine-mapping resolution at several susceptibility loci.

Our use of fine-mapping tools and functional annotations to analyze schizophrenia-associated loci identified numerous candidate genes with several lines of supporting evidence, including genes that have previously been implicated in schizophrenia (for example, *FYN* and *MAGI2*) and novel genes (for example, *EMX1* and *BNIP3L*) within the novel loci. Moreover, pathway analyses highlighted several pathways that contribute to schizophrenia pathogenesis, including previously described pathways (the voltage-gated calcium-channel pathway and postsynaptic density) and a new pathway (regulation of insulin secretion by glucagon-like peptide 1). The latter has not been highlighted in previous genetic studies of schizophrenia, but evidence from other investigation types has linked insulin signaling to the pathophysiology of schizophrenia. Previous epidemiological data have suggested that individuals with schizophrenia, compared with the general population or healthy controls, have a higher prevalence of metabolic syndrome[52,53]. Moreover, high prevalence rates of impaired glucose metabolism have been observed in drug-naive patients with schizophrenia[54]. A proteomic analysis has shown that levels of several proteins involved in energy metabolism are altered in the brains of schizophrenic people[55]. Our results provided further support for a role for insulin-related energy metabolism in the etiology of schizophrenia.

In summary, the Chinese ($n = 36,180$) and multiancestry ($n = 118,495$) GWAS meta-analysis and follow-up replication studies identified

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

113 GWS risk loci for schizophrenia, 30 of which are novel. Our results demonstrated added value from transancestry meta-analysis for fine-mapping of loci associated with schizophrenia and highlighted the existence of shared genetic risk across populations. In addition to confirming known genetic architectures, our comprehensive analyses provide further biological insights into the etiology of schizophrenia, thus potentially facilitating further mechanistic studies to assess the pathogenesis of this complex disorder.

URLs. PGC, http://pgc.unc.edu/; EIGENSTRAT, https://github.com/DReichLab/EIG/tree/master/EIGENSTRAT/; SHAPEIT, https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html; IMPUTE2, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html; 1000 Genomes Project, http://www.1000genomes.org/; The NIH Roadmap Epigenomics Mapping Consortium, http://www.roadmapepigenomics.org/; HaploReg v4.1, http://archive.broadinstitute.org/mammals/haploreg/haploreg_v4.1.php/; PLINK, https://www.cog-genomics.org/plink2/; PubMed, http://www.ncbi.nlm.nih.gov/pubmed/; NHGRI-EBI GWAS Catalog, https://www.ebi.ac.uk/gwas/; UCSC, http://genome.ucsc.edu/; GeneCards, http://www.genecards.org/; LDSC, https://github.com/bulik/ldsc/; A. Price laboratory, https://www.hsph.harvard.edu/alkes-price/software.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank all of the participants in the study and the international Psychiatric GWAS Consortium (PGC) for the large-scale data resources that made this research possible. We also appreciate H. Huang, B. Neale and M. Daly for their valuable suggestions for data analysis and manuscript organization. This work was supported by the 973 Program (2015CB559100 to Y.S.), the National Key R&D Program of China (2016YFC0903402 to Y.S. and Z.L., and 2016YFC1201701 to X.L.), the Natural Science Foundation of China (31325014 to Y.S., 81130022 to Y.S., 81421061 to L.H. and 81701321 to Z.L.), the Program of Shanghai Subject Chief Scientist (15XD1502200 to Y.S.), the National Program for Support of Top-Notch Young Professionals to Y.S., the Shanghai Key Laboratory of Psychotic Disorders (13dz2260500 to Y.X.), the 'Shu Guang' project supported by the Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17 to Y.S.), the China Postdoctoral Science Foundation (2016M590615 to Z.L.), the Shandong Postdoctoral Innovation Foundation (201601015 to Z.L.), the Qingdao Postdoctoral Application Research Project (2016048 to Z.L.), the Shanghai Hospital Development Center (SHDC12016115 to Y.X.), the US NIMH and NIDA (U01 MH109528 to P.F.S. and U01 MH1095320 to P.F.S.), and the Swedish Research Council (Vetenskapsrådet, award D0886501 to P.F.S.).

AUTHOR CONTRIBUTIONS

Y.S. conceived and designed the experiments, and supervised all aspects of the work; J.C., Y.X., L.H., D.Z., W.Y., P.W., P.Y., B. Liu, W.S., Q.X., W.J., G.F., Q.Y., C.L. and X.L. performed sample collection and phenotyping; J.C., H.Y., J.Z., B.C., Y.L., J.W., J.J., M.W., Q.W., Z.W., Wenjin Li, K.L., F.H., J.Z., G.H., Weidong Li, C.W. and B. Li performed the experiments and data management; Z.L., H.Y., Z.S., J.S., S.R., P.F.S. and M.C.O'D. performed bioinformatics and statistical analyses; Y.S. and Z.L. interpreted the main findings; Y.S. and Z.L. drafted the manuscript; Y.S., L.H., Z.L., Y.X., X.L. and P.F.S. obtained the funding support; all authors revised and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

1. Knapp, M., Mangalore, R. & Simon, J. The global costs of schizophrenia. *Schizophr. Bull.* **30**, 279–293 (2004).
2. Montgomery, W. *et al.* The personal, societal, and economic burden of schizophrenia in the People's Republic of China: implications for antipsychotic therapy. *Clinicoecon. Outcomes Res.* **5**, 407–418 (2013).
3. Burmeister, M., McInnis, M.G. & Zöllner, S. Psychiatric genetics: progress amid controversy. *Nat. Rev. Genet.* **9**, 527–540 (2008).
4. Purcell, S.M. *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748–752 (2009).
5. Ripke, S. *et al.* Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat. Genet.* **45**, 1150–1159 (2013).
6. de Candia, T.R. *et al.* Additive genetic variation in schizophrenia risk is shared by populations of African and European descent. *Am. J. Hum. Genet.* **93**, 463–470 (2013).
7. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427 (2014).
8. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* **43**, 969–976 (2011).
9. Shi, J. *et al.* Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* **460**, 753–757 (2009).
10. Shi, Y. *et al.* Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. *Nat. Genet.* **43**, 1224–1227 (2011).
11. Stefansson, H. *et al.* Common variants conferring risk of schizophrenia. *Nature* **460**, 744–747 (2009).
12. Steinberg, S. *et al.* Common variants at VRK2 and TCF4 conferring risk of schizophrenia. *Hum. Mol. Genet.* **20**, 4076–4081 (2011).
13. Yue, W.-H. *et al.* Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. *Nat. Genet.* **43**, 1228–1231 (2011).
14. Bulik-Sullivan, B.K. *et al.* LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat. Genet.* **47**, 291–295 (2015).
15. Pocklington, A.J., O'Donovan, M. & Owen, M.J. The synapse in schizophrenia. *Eur. J. Neurosci.* **39**, 1059–1067 (2014).
16. Lango Allen, H. *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* **467**, 832–838 (2010).
17. Winkler, T.W. *et al.* Quality control and conduct of genome-wide association meta-analyses. *Nat. Protoc.* **9**, 1192–1212 (2014).
18. Yang, J. *et al.* Genomic inflation factors under polygenic inheritance. *Eur. J. Hum. Genet.* **19**, 807–812 (2011).
19. Yu, H. *et al.* Common variants on 2p16.1, 6p22.1 and 10q24.32 are associated with schizophrenia in Han Chinese population. *Mol. Psychiatry* **22**, 954–960 (2017).
20. Han, B. & Eskin, E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am. J. Hum. Genet.* **88**, 586–598 (2011).
21. Li, Z. *et al.* Loci with genome-wide associations with schizophrenia in the Han Chinese population. *Br. J. Psychiatry* **207**, 490–494 (2015).
22. Nyholt, D.R. SECA: SNP effect concordance analysis using genome-wide association summary results. *Bioinformatics* **30**, 2086–2088 (2014).
23. Ward, L.D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* **40**, D930–D934 (2012).
24. Pers, T.H. *et al.* Biological interpretation of genome-wide association studies using predicted gene functions. *Nat. Commun.* **6**, 5890 (2015).
25. GTEx Consortium. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* **348**, 648–660 (2015).
26. Gibbs, J.R. *et al.* Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet.* **6**, e1000952 (2010).
27. Westra, H.-J. *et al.* Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* **45**, 1238–1243 (2013).
28. Fromer, M. *et al.* Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nat. Neurosci.* **19**, 1442–1453 (2016).
29. Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat. Genet.* **48**, 481–487 (2016).
30. Catapano, L.A. & Manji, H.K. G protein-coupled receptors in major psychiatric disorders. *Biochim. Biophys. Acta* **1768**, 976–993 (2007).
31. Adkins, D.E. *et al.* Genomewide pharmacogenomic study of metabolic side effects to antipsychotic drugs. *Mol. Psychiatry* **16**, 321–332 (2011).
32. Ali, D.W. & Salter, M.W. NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. *Curr. Opin. Neurobiol.* **11**, 336–342 (2001).
33. Du, C.P., Tan, R. & Hou, X.Y. Fyn kinases play a critical role in neuronal apoptosis induced by oxygen and glucose deprivation or amyloid-β peptide treatment. *CNS Neurosci. Ther.* **18**, 754–761 (2012).
34. Hattori, K. *et al.* Decreased expression of Fyn protein and disbalanced alternative splicing patterns in platelets from patients with schizophrenia. *Psychiatry Res.* **168**, 119–128 (2009).
35. Bauß, K. *et al.* Phosphorylation of the Usher syndrome 1G protein SANS controls Magi2-mediated endocytosis. *Hum. Mol. Genet.* **23**, 3923–3942 (2014).

36. Calabrese, F., Riva, M.A. & Molteni, R. Synaptic alterations associated with depression and schizophrenia: potential as a therapeutic target. *Expert Opin. Ther. Targets* **20**, 1195–1207 (2016).
37. Koide, T. *et al.* Common variants in MAGI2 gene are associated with increased risk for cognitive impairment in schizophrenic patients. *PLoS One* **7**, e36836 (2012).
38. Gaulton, K.J. *et al.* Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat. Genet.* **47**, 1415–1425 (2015).
39. Morris, D.L. *et al.* Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat. Genet.* **48**, 940–946 (2016).
40. Farh, K.K.-H. *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* **518**, 337–343 (2015).
41. Gusev, A. *et al.* Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. *Am. J. Hum. Genet.* **95**, 535–552 (2014).
42. Kichaev, G. & Pasaniuc, B. Leveraging functional-annotation data in trans-ethnic fine-mapping studies. *Am. J. Hum. Genet.* **97**, 260–271 (2015).
43. de Anda, F.C. *et al.* Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat. Neurosci.* **15**, 1022–1031 (2012).
44. Boonen, M. *et al.* Cathepsin D and its newly identified transport receptor SEZ6L2 can modulate neurite outgrowth. *J. Cell Sci.* **129**, 557–568 (2016).
45. Kundaje, A. *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–330 (2015).
46. de Leeuw, C.A., Mooij, J.M., Heskes, T. & Posthuma, D. MAGMA: generalized gene-set analysis of GWAS data. *PLOS Comput. Biol.* **11**, e1004219 (2015).
47. Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. *Nat. Neurosci.* **18**, 199–209 (2015).
48. Lee, S.H., Goddard, M.E., Wray, N.R. & Visscher, P.M. A better coefficient of determination for genetic profile analysis. *Genet. Epidemiol.* **36**, 214–224 (2012).
49. Bulik-Sullivan, B. *et al.* An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* **47**, 1236–1241 (2015).
50. Lee, S.H. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat. Genet.* **45**, 984–994 (2013).
51. Ikeda, M. *et al.* Genome-wide association study of schizophrenia in a Japanese population. *Biol. Psychiatry* **69**, 472–478 (2011).
52. Citrome, L. & Volavka, J. Consensus development conference on antipsychotic drugs and obesity and diabetes: response to consensus statement. *Diabetes Care* **27**, 2087–2088, author reply 2089–2090 (2004).
53. Mitchell, A.J. *et al.* Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders: a systematic review and meta-analysis. *Schizophr. Bull.* **39**, 306–318 (2013).
54. Steiner, J. *et al.* Immune system and glucose metabolism interaction in schizophrenia: a chicken-egg dilemma. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **48**, 287–294 (2014).
55. Gottschalk, M.G., Wesseling, H., Guest, P.C. & Bahn, S. Proteomic enrichment analysis of psychotic and affective disorders reveals common signatures in presynaptic glutamatergic signaling and energy metabolism. *Int. J. Neuropsychopharmacol.* **18**, pyu019 (2014).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

ONLINE METHODS

Recruitment of research subjects. As in our previous study[10], all cases of Chinese ancestry were inpatients or outpatients with a history of more than 2 years of schizophrenia, who were recruited from mental-health centers in China, interviewed by two independent psychiatrists and diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. All cases met the following two criteria: preoccupation with one or more delusions and frequent auditory hallucinations. However, none of the following symptoms were prominent: disorganized speech, disorganized or catatonic behavior, or flat or inappropriate effects. The controls were randomly selected from Chinese volunteers (from hospitals and a community survey) who were asked to reply to a written invitation to evaluate their medical histories. Lists of potential control subjects were screened for suitability as volunteers by excluding subjects with major mental illnesses. All participants provided written informed consent. The study was approved by the Ethics Committee of Human Genetic Resources at the Bio-X Institutes of Shanghai Jiao Tong University, in accordance with the tenets of the Declaration of Helsinki. We confirm that our study is compliant with the Guidance of the Ministry of Science and Technology (MOST) for the Review and Approval of Human Genetic Resources.

Genotyping, quality control and genotype imputation of the Chinese GWAS data. Several different genome-wide genotyping platforms were used in this study: Affymetrix Genome-Wide Human SNP Array 6.0 (SNP6.0), Affymetrix Axiom Genome-Wide CHB1 Array Plate and Illumina 1M Array.

For the SNP6.0 chips, the genotype calls were generated together by using Affymetrix Axiom Analysis according to the Best Practices Workflow for SNP6.0. Sample QC filtering of the GWAS data was first performed by excluding arrays with Contrast QC measurements (a metric developed by Affymetrix for SNP6.0 QC, n = 197) that were <0.4. Step 1 genotyping was run on all CEL files passing QC over a subset of 20,000 SNPs, and samples with a call rate ≤97% were excluded (n = 285). The remaining samples were used for step 2 genotyping analysis. SNP polisher was then used for SNP QC, and the SNPs in the recommended categories (PolyHighRes, MonoHighRes, NoMinorHom and Hemizygous) were retained. Sex was established via genotyping and evaluated for each of the subjects, and samples with inconsistent sex (compared with the sample record) were removed (n = 79). Heterozygosity rates were calculated with the intent of removing deviations that exceeded 6 s.d. from the mean (n = 0). PLINK's identity-by-descent analysis was used to detect cryptic relatedness[56] (URLs). When a pair of individuals had PI_HAT >0.2, the member of the pair with the lower call rate was excluded from the analysis (n = 259). SNPs with call rates <97% (n = 28,040), MAF <1% (n = 185,439) or significant deviation from Hardy–Weinberg equilibrium (HWE) in controls (HWE $P \leq 1 \times 10^{-6}$, n = 20,344) were excluded. We also excluded population outliers on the basis of PCA. After application of quality-control criteria, a set of 590,413 SNPs for 14,645 individuals was generated for genotype imputation.

For the CHB1 chips, the genotype calls were generated together according to the Axiom Genotyping Solution Data Analysis Guide. Briefly, arrays with dish QC (DQC), a single-sample metric developed by Affymetrix for Axiom QC) values <0.82 were first excluded (n = 181). Samples that surpassed the DQC values were used for genotype calling with a subset of probe sets. Samples with a call rate <97% or in a nonpassing plate (an average call rate of passing samples <98.5%) were also excluded (n = 276). The post-QC samples were then coclustered, and genotype calls were produced with the Axiom Genotyping Algorithm v1 (Axiom GT1). SNP QC was also executed with the SNP polisher procedure, and the SNPs in the recommended categories were retained. Verification procedures for sex, relatedness and PCA outliers were also conducted in sample QC as described above (n = 289). SNPs with call rates <97% (n = 56,735), MAF <1% (n = 206) or significant deviations from HWE in controls (HWE $P \leq 1 \times 10^{-6}$, n = 18,849) were excluded. After application of QC criteria, a set of 555,058 SNPs for 9,580 individuals was generated for genotype imputation.

For Illumina 1M chips, SNP genotypes were generated from normalized bead intensity data with Genome Studio. Samples with a call rate <97% were excluded (n = 35). Regular sample QC procedures for parameters including sex, relatedness, heterozygosity rate and PCA outlier checking, were performed as described above (n = 231). SNPs with call rates <97% (n = 35,743), MAF <1% (n = 89,032) or HWE $P \leq 1 \times 10^{-6}$ (n = 954) were excluded. After application of QC criteria, a set of 716,466 SNPs for 1,823 individuals was generated for genotype imputation.

For each GWAS data set, the entire set was imputed together as follows: the genotypes were phased with SHAPEIT (URLs)[57,58] for each chromosome, and imputation was performed for each 5-Mb chromosome interval with IMPUTE2 (URLs)[59]. The haplotypes derived from the 1000 Genomes Project Phase 1 (release v3, URLs) were used as reference data[60]. Because two genotyping platforms were used for GWAS set 3, we used two phased reference panels in this special case, as proposed by Howie *et al.*[59]. For each platform, the prephased data from the other platform were used as the second reference panel. The variants with INFO >0.8, MAF >0.01, a call rate ≥97% and HWE $P \geq 1 \times 10^{-6}$ in the controls were saved for further analysis. Those present in at least two data sets were saved for the meta-analysis. A set of 5,107,227 genetic variants for 7,699 cases and 18,327 controls remained in the final analysis.

PGC2 GWAS data set. The PGC2 GWAS data set[7] comprised 49 case–control samples (34,241 cases and 45,604 controls) and three family-based samples (1,235 parent–affected offspring trios). All of the samples were from subjects of European ancestry, excluding three case–control samples from subjects of East Asian ancestry (1,836 cases and 3,383 controls). The summary results for the PGC2 data set and European only data set (EUR49) were downloaded from the PGC website (URLs).

Replication data set. The replication sample consisted of 4,384 cases and 5,770 controls of Han Chinese ancestry. More details of the general characteristics and genotyping have been presented in our previous research[19]. For the Chinese-only analyses, the independent SNPs with $P < 1 \times 10^{-5}$ in the Chinese GWAS analysis of pre- or postcorrection with the inflation factor were selected. For the transancestry analysis, the independent SNPs with $P < 5 \times 10^{-7}$ in the Chinese (pre- or postcorrection) and PGC2 GWAS meta-analyses were selected. The precorrection data sets were used only for including more candidate SNPs for replication. All the association results in this article were based on the postcorrection data sets, wherein the global inflations were controlled. A total of 295 SNPs were analyzed in the Chinese replication analysis.

Power calculations. Power calculations were performed with the GAS Power Calculator[61] with a range of genotype relative risks and disease-allele frequencies, assuming a population prevalence of 0.01 and a significance level of 5×10^{-8}. For the Chinese-only (n = 36,180) and transancestry (n = 118,495) analyses, we had adequate power (>80%) to detect variants with low risk-allele frequencies (RAFs) of 0.03 with genotypic relative risks of 1.318 and 1.161, respectively. This sample size in Chinese-only analyses was large enough to achieve adequate power for risk variants with genotypic relative risks of 1.150 and RAFs of 0.14 to 0.85, and the transancestry analyses achieved adequate power for risk variants with 1.075 and RAFs of 0.15 to 0.84.

Statistical methods and bioinformatics analysis. Population substructure was evaluated through a PCA with EIGENSTRAT software (URLs), on the basis of LD-pruned autosomal SNP genotypes[62,63]. Two rounds PCA were performed. One round with samples from the HapMap Project phase 3 (HapMap3) was performed to identify admixed samples, and the other round was performed for each subset of cases and controls, wherein individual outliers (>6 s.d. from the mean on any one of the top ten PCs) were identified and removed for five iterations, and final PCs reflecting subtle ancestry information for each sample were generated for further correction. In the Chinese GWAS stage, the association was analyzed for subsets by using a logistic regression model involving covariates for PCs to adjust for possible population stratification. We evaluated the effects of the 20 PCs on genome-wide test statistics to determine the PC inclusion in the final association analysis for each data set. In the Chinese replication stage, the associations between SNPs and schizophrenia risk were evaluated on the basis of logistic regression with SNPTEST[64]. The Higgins and Thompson I^2 index was used for assessing heterogeneity across data sets[65]. Both fixed-effects-model and random-effects-model meta-analyses were used in this study. The variants with pronounced heterogeneity (I^2 >75%) were combined in a random-effects model in the transancestry meta-analysis[17].

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

We assessed the genome-wide congruence of risk alleles across samples by using binomial sign tests that compared the direction of the effect sizes of independent SNPs between PGC2 and Chinese GWAS results. *P* values were generated under the null hypothesis (H_0: $P = 0.50$). The proportion of variance in liability to schizophrenia explained by the common SNPs was estimated by using genome-wide complex-trait analysis[66], and the PCs were included in the analysis as covariates. For each of the associated loci (except the eMHC region, owing to the complexity of this region[7]), we calculated an approximate Bayes factor per Wakefield, as well as the posterior probability of driving the association for each SNP within a 2-Mb window, and then created 99% credibility sets[38,39,67]. We created credibility sets by using the Chinese, PGC2 (European) and combined data sets separately. We conducted the transancestry fine-mapping in the presence of functional information by using PAINTOR according to the suggested pipeline, as well as PGC2-only and Chinese-only analyses for comparison. The primary functional annotations for SNPs proposed by Gusev *et al.*[41] were obtained from the A. Price laboratory website (URLs). The reference epigenomes of 127 human tissues and cell types[45] were obtained from the NIH Roadmap Epigenomics Mapping Consortium (URLs). We included seven highlighted epigenomic marks (H3K4me3, H3K4me1, H3K36me3, H3K27me3, H3K9me3, H3K27ac and H3K9ac)[45] in our analyses. Enrichment analyses of the schizophrenia associations in the current and PGC2 analyses with the epigenomic features were performed with the genomic regulatory elements and GWAS overlap algorithm (GREGOR)[68], and the top 100 enriched annotations were selected for further PAINTOR analyses. The online tool HaploReg[23] (v4.1; URLs) was used to explore the genes nearest to the index SNPs, and genes containing a missense mutation in high LD ($r^2 > 0.8$, on the basis of the 1000 Genomes Phase 1 CEU or ASI population for the LD calculation) with the GWS SNPs. The effects of GWS SNPs on expression in eQTL studies of different tissues (including blood and brain tissues[25–27]) were extracted from the query results of HaploReg[23] and the CommonMind Consortium Knowledge Portal[28]. A significant eQTL was reported as having a false discovery rate of 0.05 in the original studies[25–28] and being located in the credible interval covered by the 99% credible set for the regulated gene for the data sets in which detailed results were available for establishing the credible sets[25,27]. We used DEPICT[24] to identify the most likely causal genes for the schizophrenia-associated loci, on the basis of the functional similarity among genes from associated regions. We carried out SMR analysis[29] for the blood and brain tissue eQTL data sets[25,27], using the 1000 Genomes Project data as reference files. For the gene prioritization analysis at the GWS loci (excluding the eMHC region, owing to the complexity of this region[29]), only probes with at least one *cis*-eQTL at $P < 5.0 \times 10^{-8}$ were considered for SMR analysis, and a significance threshold was set as $P_{SMR} < 5.20 \times 10^{-5}$ corresponding to a Bonferroni correction for 960 tests (960 probes with *cis*-eQTL at $P < 5.0 \times 10^{-8}$ across the GWS loci)[29]. The heterogeneity in dependent instruments (HEIDI) test was also performed, and $P < 0.05$ was considered to indicate significant heterogeneity. The genes prioritized by the GWS index SNP or its high LD ($r^2 > 0.8$) proxies were listed. In addition, the SMR analysis was also performed for some specific SNPs and genes. Here, the *P*-value threshold for selecting eQTL was not applicable, and the details are shown in the results. We searched the published literature for these genes with respect to schizophrenia in PubMed (URLs) and the NHGRI-EBI GWAS Catalog (URLs), and we obtained additional functional evidence for these SNPs and genes from the published literature, the UCSC genome database (URLs) and GeneCards (URLs).

LD-score regression for Chinese GWAS data. We estimated Chinese LD scores from the Chinese subjects in the 1000 Genomes Project Phase 3, using the LD Score (LDSC; URLs) software package[14]. We used a window size of 1 cM to estimate LD scores, excluded singletons and did not set an r^2 cutoff. The LD-score regression intercept from the Chinese GWAS data was estimated according to application notes for real data from the LDSC developers[14]. As Bulik-Sullivan *et al.* have proposed[14], correcting test statistics with the LD-score regression intercept is a robust way for controlling the confounding bias from inflation. Correction was applied to the Chinese GWAS meta-analysis results by multiplying the standard errors by the square root of the correction factor[16].

Polygenic scoring analysis. Approximately 1,000 cases and 1,000 controls from the Chinese sample were randomly selected as the target sample. Risk-profile SNPs ($P_T = 5 \times 10^{-8}$, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1) from the training GWAS data sets (the PGC2 European-only (EUR49) and full data sets, the Chinese GWAS data set excluding the target sample and the Chinese plus PGC2 combined data set) were selected with the PLINK '--clumped' function, and SNPs within 500 kb or with $r^2 \geq 0.1$ were discarded. The risk-profile SNPs were then used to generate scores for the target samples by using the PLINK '--score' function. The case–control status was then predicted by logistic regression analysis of polygenic scores plus PC covariates. Nagelkerke's R^2 was used for the full model, using the polygenic score plus the covariates minus R^2 for the covariates alone, thus yielding an estimate of the explained variance. The R^2 was then transformed into a liability scale[48], assuming a population prevalence of 1% for schizophrenia[7].

Pathway analysis. MAGMA[46] was used to explore pathway-based associations in the genome-wide meta-analysis data set. An *F* test was used to compute the gene *P* value, and the gene *P* values and gene correlation matrix were then used for the gene-set analysis with a regression model[46]. We defined gene boundaries 35 kb upstream and 10 kb downstream for assigning SNPs to a gene, as adopted in a recent psychiatric-disorder pathway analysis[47]. Each gene was then assigned pathways in the Gene Ontology (GO), PANTHER, Ingenuity, Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome and BioCarta gene set databases[69]. A total of 2,981 pathways or gene sets were used in this analysis.

Data availability. Summary statistics for the meta-analyses will be made available at http://gwas.bio-x.cn/. A **Life Sciences Reporting Summary** is available.

56. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
57. Delaneau, O., Marchini, J. & Zagury, J.-F. A linear complexity phasing method for thousands of genomes. *Nat. Methods* **9**, 179–181 (2011).
58. Delaneau, O., Zagury, J.-F. & Marchini, J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat. Methods* **10**, 5–6 (2013).
59. Howie, B.N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529 (2009).
60. 1000 Genomes Project Consortium. *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
61. Skol, A.D., Scott, L.J., Abecasis, G.R. & Boehnke, M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat. Genet.* **38**, 209–213 (2006).
62. Price, A.L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
63. Patterson, N., Price, A.L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
64. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913 (2007).
65. Higgins, J.P.T., Thompson, S.G., Deeks, J.J. & Altman, D.G. Measuring inconsistency in meta-analyses. *Br. Med. J.* **327**, 557–560 (2003).
66. Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M. GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76–82 (2011).
67. Wakefield, J. Bayes factors for genome-wide association studies: comparison with P-values. *Genet. Epidemiol.* **33**, 79–86 (2009).
68. Schmidt, E.M. *et al.* GREGOR: evaluating global enrichment of trait-associated variants in epigenomic features using a systematic, data-driven approach. *Bioinformatics* **31**, 2601–2606 (2015).
69. Segre, A.V. *et al.* Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLoS Genet.* **6**, e1001058 (2010).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

doi:10.1038/ng.3973

nature research

Corresponding author(s): Zhiqiang Li

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No initial power analysis was done to determine the sample size. Our post hoc power analysis indicated that our sample size was large enough to achieve adequate power for detecting variants of low risk allele frequencies of 0.03 with genotypic relative risks of 1.161.

2. Data exclusions

Describe any data exclusions.

Typical quality control was performed for our GWAS data sets. Arrays with low quality data were excluded. Samples failed in the sex, relatedness, heterozygosity rate and PCA outlier checking procedures were also excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We performed Chinese and multi-ancestry GWAS meta-analyses and follow-up replication analyses, and the identified loci were reliably reproduced with genome-wide significant evidence.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The samples were grouped by disease status.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The researchers were not blinded to group allocation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☒	☐	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☒	☐	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Nature Genetics: doi:10.1038/ng.3973

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	The URLs for the software used were provided.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The materials were commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Not applicable.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Not applicable.
b. Describe the method of cell line authentication used.	Not applicable.
c. Report whether the cell lines were tested for mycoplasma contamination.	Not applicable.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	Not applicable.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Not applicable.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	For privacy concerns, we can't provide detailed information for the participants. These information were not used as covariates in our analysis.

Nature Genetics: doi:10.1038/ng.3973

Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia

Zhiqiang Li[1–4,22], Jianhua Chen[2,5,22], Hao Yu[6–8], Lin He[2,4], Yifeng Xu[5], Dai Zhang[6,7,9], Qizhong Yi[10], Changgui Li[11], Xingwang Li[2], Jiawei Shen[2], Zhijian Song[2], Weidong Ji[4,12], Meng Wang[2], Juan Zhou[2], Boyu Chen[2], Yahui Liu[2], Jiqiang Wang[2], Peng Wang[13], Ping Yang[13], Qingzhong Wang[2], Guoyin Feng[5], Benxiu Liu[14], Wensheng Sun[14], Baojie Li[2], Guang He[2], Weidong Li[2], Chunling Wan[2], Qi Xu[15], Wenjin Li[2], Zujia Wen[2], Ke Liu[2], Fang Huang[2], Jue Ji[2], Stephan Ripke[16–18], Weihua Yue[6,7], Patrick F Sullivan[19,20], Michael C O'Donovan[21] & Yongyong Shi[1–4,10,12]

We conducted a genome-wide association study (GWAS) with replication in 36,180 Chinese individuals and performed further transancestry meta-analyses with data from the Psychiatry Genomics Consortium (PGC2). Approximately 95% of the genome-wide significant (GWS) index alleles (or their proxies) from the PGC2 study were overrepresented in Chinese schizophrenia cases, including ~50% that achieved nominal significance and ~75% that continued to be GWS in the transancestry analysis. The Chinese-only analysis identified seven GWS loci; three of these also were GWS in the transancestry analyses, which identified 109 GWS loci, thus yielding a total of 113 GWS loci (30 novel) in at least one of these analyses. We observed improvements in the fine-mapping resolution at many susceptibility loci. Our results provide several lines of evidence supporting candidate genes at many loci and highlight some pathways for further research. Together, our findings provide novel insight into the genetic architecture and biological etiology of schizophrenia.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Schizophrenia (MIM181500) is a chronic, severe and disabling brain disorder that affects approximately 1% of the worldwide population and imposes an enormous burden on society[1,2]. It is a highly heritable psychiatric disorder (with an estimated heritability of 70–85%[3]) with a substantial polygenic component including thousands of common alleles with small effects that contribute to disease risk[4]. Approximately 33–50% of the genetic risk of schizophrenia has been captured by common alleles in GWAS[5]. The evidence to date suggests that many risk alleles for common schizophrenia-associated genetic loci may be shared across ancestry groups, but others may be population specific because of differing causal variants or linkage disequilibrium (LD) patterns in populations of different ancestries[6]. Previous GWAS have identified more than 110 schizophrenia-associated loci and have substantially advanced understanding of this condition[5,7–13]. In particular, the most recent and largest schizophrenia GWAS (from the Schizophrenia Working group of the Psychiatric Genomics Consortium, PGC2) which, with discovery and extension, included a total of 36,989 schizophrenia cases and 113,075 controls, has identified 128 independent genome-wide significant associations spanning 108 loci[7].

However, a large proportion of the genetic factors underlying schizophrenia remain unknown. Most of the heritability of schizophrenia is not yet attributable to specific loci; only 3.5% of the liability can be explained by GWS loci[7]. Moreover, to date, most GWAS participants with schizophrenia are of European descent. Thus, although the PGC2 report includes samples from East Asia, the proportions are small (approximately 5% and 3% for cases and controls, respectively). Large-scale GWAS including individuals of non-European descent

[1]Affiliated Hospital of Qingdao University and Biomedical Sciences Institute of Qingdao University (Qingdao Branch of SJTU Bio-X Institutes), Qingdao University, Qingdao, China. [2]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Collaborative Innovation Center for Brain Science, Shanghai Jiao Tong University, Shanghai, China. [3]Institute of Social Cognitive and Behavioral Sciences, Shanghai Jiao Tong University, Shanghai, China. [4]Institute of Neuropsychiatric Science and Systems Biological Medicine, Shanghai Jiao Tong University, Shanghai, China. [5]Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [6]Tsinghua-Peking Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing, China. [7]Institute of Mental Health, Sixth Hospital, Peking University, Beijing, China. [8]Department of Psychiatry, Jining Medical University, Jining, China. [9]Peking-Tsinghua Joint Center for Life Sciences/PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing, China. [10]Department of Psychiatry, First Teaching Hospital of Xinjiang Medical University, Urumqi, China. [11]Shandong Provincial Key Laboratory of Metabolic Disease and Metabolic Disease Institute of Qingdao University, Qingdao, China. [12]Changning Mental Health Center, Shanghai, China. [13]Wuhu Fourth People's Hospital, Wuhu, China. [14]Longquan Mountain Hospital of Guangxi Province, Liuzhou, China. [15]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [16]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, Massachusetts, USA. [17]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. [18]Department of Psychiatry and Psychotherapy, Charité-Universitätsmedizin, Berlin, Germany. [19]Departments of Genetics and Psychiatry, University of North Carolina, Chapel Hill, North Carolina, USA. [20]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden. [21]MRC Centre for Neuropsychiatric Genetics and Genomics, Division of Psychological Medicine and Clinical Neurosciences, School of Medicine, Cardiff University, Cardiff, UK. [22]These authors contributed equally to this work. Correspondence should be addressed to Y.S. (shiyongyong@gmail.com).

Received 17 April; accepted 19 September; published online 9 October 2017; doi:10.1038/ng.3973

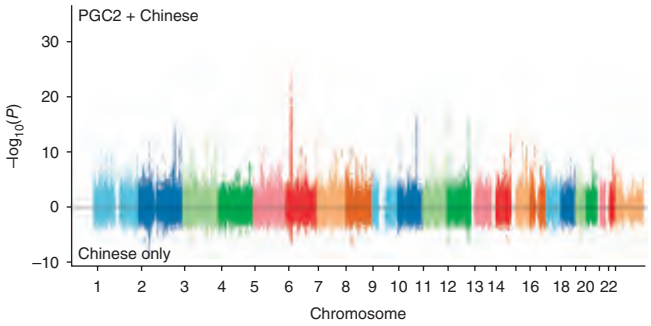


Figure 1 Comparison of Manhattan plots for the Chinese and transancestry analyses. Manhattan plot of results from the Chinese-only (7,699 schizophrenia cases and 18,327 controls) and PGC2-plus-Chinese transancestry (43,175 cases and 65,166 controls) analyses. $-\log_{10}P$ values for PGC2 plus Chinese transancestry analyses and $\log_{10}P$ values for Chinese-only analyses are shown.

are essential for extending understanding of the genetic architecture of schizophrenia in the human population as a whole, for testing the generalizability of the results from European populations regarding this global disorder[6] and for identifying population-specific risk factors, should they exist.

To identify additional schizophrenia susceptibility loci and to gain a better understanding of the genes and biological pathways implicated in schizophrenia, we performed a GWAS including 7,699 cases and 18,327 controls of Chinese ancestry, as well as a transancestry GWAS meta-analysis with PGC2 (43,175 cases and 65,166 controls in total). The candidate loci found in each analysis were then studied in an independent replication sample of 4,384 schizophrenia cases and 5,770 controls of Chinese ancestry.

RESULTS

Results of GWAS screening in the Chinese population

We first conducted a GWAS for schizophrenia in the Chinese population (in comparison to the discovery phase of our prior GWAS report[10], the number of cases was doubled, and the number of controls was tripled). After systematic quality control (QC) analysis and imputation to the 1000 Genomes Project data (Online Methods), we assessed the associations of 5,107,227 genetic variants in 7,699 schizophrenia cases and 18,327 controls (**Supplementary Table 1**). The primary GWAS comprised three samples that were genotyped on different platforms: 4,175 cases and 10,470 controls genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0 (SNP6.0); 2,472 cases and 5,928 controls genotyped with the Affymetrix Axiom Genome-Wide CHB1 Array Plate (CHB1); and 1,052 cases and 1,929 controls genotyped with CHB1 or the Illumina 1M Array (1M). Principal component analysis (PCA) was used to assess population substructure (**Supplementary Fig. 1** and Online Methods). For each subset, association testing was conducted with logistic regression including ancestry principal components (PCs) as covariates to adjust for population stratification. The results were combined with inverse-variance-weighted meta-analysis (based on a fixed-effects model). The genomic inflation factor (λ_{GC}) was 1.22, and the $\lambda_{1,000}$ (a scaled value to 1,000 cases and 1,000 controls) was 1.02. We conducted LD-score regression analysis[14] to distinguish the relative contributions of confounding bias and polygenicity. The LD-score regression intercept was 1.07 (s.e. = 0.01), and the slope was greater than zero, thus suggesting that most of the increase in the mean χ^2 statistic was from polygenic architecture rather than population stratification, in agreement with the previously documented polygenic nature of schizophrenia inheritance[7,15]. However, given this modest elevation in the intercept, we further corrected the meta-analysis statistics for residual test-statistic inflation[14,16] (Online Methods). Quantile–quantile and Manhattan plots are shown in **Supplementary Figures 2** and **3**. In this analysis (**Fig. 1**), we observed 66 GWS variants in a region previously reported to be associated with schizophrenia (2p16.1)[11,12].

The proportion of variance in susceptibility to schizophrenia explained by genome-wide SNP genotypes for individuals of Han Chinese ancestry (Online Methods) was estimated to be 31.5% (s.e. = 1.9%), assuming a population risk of 0.01. This result was similar to the corresponding estimate for European samples (33%)[5], thus providing further evidence of the highly polygenic nature of schizophrenia beyond that in previous studies[7,15].

Results of the Chinese and PGC2 genome-wide meta-analysis

We performed a meta-analysis of the Chinese GWAS samples (7,699 schizophrenia cases and 18,327 controls) (denoted Chinese GWAS) and PGC2 GWAS samples (35,476 schizophrenia cases and 46,839 controls) to explore the effects of power and heterogeneity. A total of 4,303,606 genetic variants were common to the two data sets and were retained in the combined analysis. For combining the data, we used a fixed-effects model, but for variants with pronounced heterogeneity (I^2 >75%)[17], we used a random-effects model to allow for the possibility that the presence of heterogeneity might result in test-statistic inflation. In our final result, the λ_{GC} was 1.50, and the $\lambda_{1,000}$ was less than 1.01. The deviation of the observed statistics from the null hypothesis was less than that expected under a polygenic model for schizophrenia[7,18]. Quantile–quantile and Manhattan plots are shown in **Supplementary Figures 4** and **5**. In the combined analysis, we detected 5,618 SNPs surpassing the threshold for GWS for association with schizophrenia. These SNPs mapped to 104 physically distinct associated regions, as defined by clumping the variants by using r^2 >0.1 and merging the LD-independent variants within 250 kb (**Fig. 1** and **Supplementary Table 2**).

Results of the combined analysis with replication samples

We then obtained association results from an independent Chinese cohort of 4,384 schizophrenia cases and 5,770 controls[19] (**Supplementary Table 1**) for LD-independent SNPs with $P < 1 \times 10^{-5}$ in the Chinese-only GWAS meta-analysis or with $P < 5 \times 10^{-7}$ in the Chinese and PGC2 GWAS meta-analysis (Online Methods).

The combined analysis of the Chinese GWAS and replication samples resulted in a data set of 12,083 cases and 24,097 controls. Seven loci were GWS for association with schizophrenia in the meta-analysis of individuals of Chinese ancestry. Of those loci, three have been previously reported to be associated with schizophrenia (**Supplementary Fig. 6**), and the other four are novel: rs2073499 at 3p21.31 (odds ratio (OR) = 0.899, fixed-effects meta-analysis $P = 2.61 \times 10^{-8}$), rs7757969 at 6q21 (OR = 1.110, $P = 4.82 \times 10^{-8}$), rs4479915 at 6q27 (OR = 0.876, $P = 4.82 \times 10^{-9}$) and rs11534004 at 7q31.1 (OR = 0.890, $P = 1.71 \times 10^{-8}$) (**Fig. 2**). Four additional loci were significant at $P < 1 \times 10^{-5}$ in the Chinese GWAS meta-analysis and showed nominal evidence of replication (P <0.05) but were not GWS in the combined analysis. Results for all tested SNPs are presented in **Supplementary Table 3**.

The combined results of the transancestry meta-analysis (43,175 schizophrenia cases and 65,166 controls) and replication samples (4,384 schizophrenia cases and 5,770 controls) identified a total of 109 GWS loci (**Supplementary Table 4** and **Supplementary Data 1**). Of the 109 loci, 83 had previously been reported, and 26 loci were novel.

Together, the above results identified 124 SNPs that were GWS and were associated with schizophrenia. The SNPs mapped to 113

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

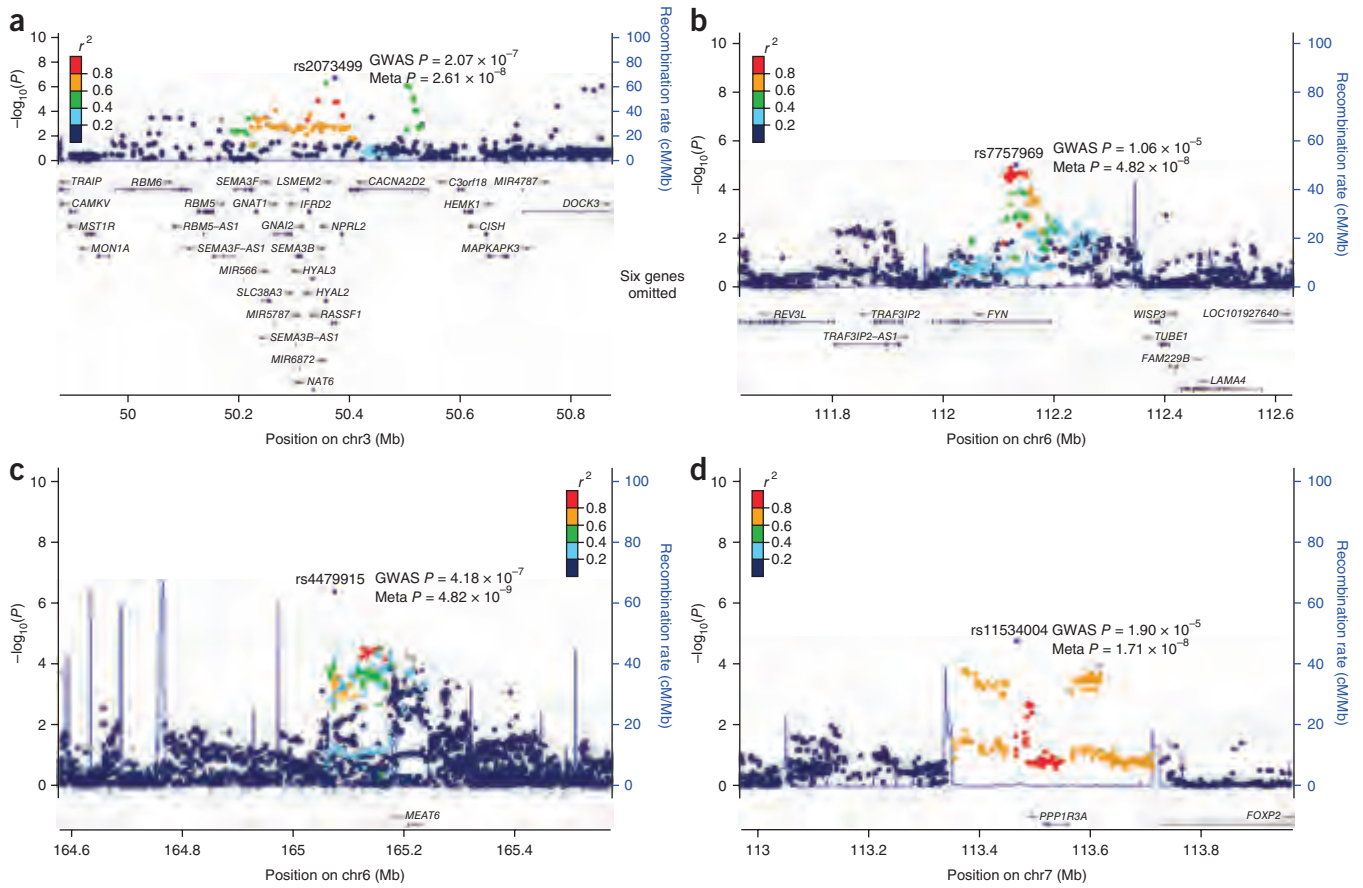


Figure 2 Regional plots for novel GWS loci in Chinese people. (**a**) rs2073499 at 3p21.31. (**b**) rs7757969 at 6q21. (**c**) rs4479915 at 6q27. (**d**) rs11534004 at 7q31.1. Meta, meta-analysis; chr, chromosome. $-\log_{10}P$ values are shown for SNPs for the region 500 kb on either side of the marker SNPs. The index SNP is shown in purple, and the r^2 values of the other SNPs are indicated by color. The r^2 values were established on the basis of 1000 Genomes data (November 2014). *P* values for the GWAS stage are shown with circles, and *P* values for the meta-analysis combining all data sets are shown with text. The genes within the relevant regions are annotated and shown as arrows.

physically distinct loci: four loci were GWS only in the Chinese-only analysis, 106 were GWS only in the transancestry analysis, and three were present in both analyses (**Supplementary Table 5**). Of the 113 associated loci, 30 have not been previously reported (**Table 1**), four of which were GWS in the Chinese sample but not the transancestry analysis. In addition, at three of the previously reported loci, the GWS SNPs in the present study were in low LD with the previously identified GWS SNPs (r^2 <0.1 in both the European and Chinese populations), thus possibly suggesting independent signals in these regions (**Supplementary Table 5**).

Similarities and differences across ancestries

Of the 108 loci (128 index SNPs) identified in the PGC2 report[7], we were able to investigate 103 loci (117 index SNPs or their proxies) that were in common between PGC2 and Chinese data sets (**Supplementary Table 6**). Of these, the PGC2-associated risk alleles were overrepresented in Chinese cases at 109 SNPs (from 98 loci), and at 58 SNPs (from 56 loci) this overrepresentation achieved nominal significance (*P* <0.05). In transancestry meta-analyses, 85 SNPs at 78 loci continued to be GWS. It is known that the random-effects model might be overly conservative[20], and therefore on an exploratory basis, we performed a fixed-effects model meta-analysis for all these SNPs regardless of the existence of heterogeneity. Under the fixed-effects model, an additional eight SNPs (93 in total) at eight loci (86 loci in total) were GWS in the combined analysis. However, the results for the GWS SNPs indicated by fixed-effects meta-analysis and with evidence of heterogeneity should be interpreted with caution. Nevertheless, this finding suggested that the schizophrenia susceptibility loci identified in European samples were applicable to the Chinese sample. Moreover, the transancestry meta-analyses also confirmed two GWS loci (8p12 and 7q11.22) identified in our previous reports[10,21].

Regarding the seven GWS index SNPs analyzed in the Chinese-only analysis in this study, three replicated at *P* <0.05 in the PGC2 data set but showed significant heterogeneity (Higgins and Thompson I^2 index >75%) across populations and were not GWS in transancestry meta-analyses. In addition, one of the index SNPs (rs78681500) was absent in the PGC2 data set, owing to its rarity (minor allele frequency (MAF)<1%). Of the 117 GWS index SNPs identified in the transancestry analysis, all showed the same direction of effect across ancestries, and the I^2 was less than 75%.

We next assessed the genome-wide congruence of risk alleles across the PGC2 and Chinese GWAS data sets for LD-clumped independent SNPs[22]. For the schizophrenia-associated SNPs ($P \leq 0.0001$) identified in the Chinese data set, we observed a highly significant excess of directional concordance in the PGC2 data set (67.7%, binomial test $P = 3.06 \times 10^{-7}$). For the SNPs demonstrating weaker evidence of an association with schizophrenia ($0.0001 < P \leq 0.05$), we also observed an excess of consistency in the direction of effect. In contrast, for the

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Table 1 Novel schizophrenia GWS loci and notable genes

Chromosome	SNP	Position	*P* value	Notable gene(s)[a]
2	rs999494	73157395	2.40×10^{-10}	*EMX1* (N, D)
2	rs62152284	104984387	5.86×10^{-9}	*LOC100287010* (N)
2	rs6430491	134840967	9.55×10^{-10}	*MIR3679* (N)
3	rs10510653	32058559	2.54×10^{-8}	*GPD1L* (Q), *ZNF860* (N)
3	rs2073499	50374293	2.61×10^{-8}	*HYAL3* (Q), *RASSF1* (N)
4	rs11722779	103827488	3.40×10^{-8}	*BDH2* (Q), *CENPE* (Q), *CISD2* (Q), *KRT8P46* (Q), *LRRC37A15P* (Q), *NHEDC1* (N), *SLC9B1* (Q)
5	rs10940346	49806042	1.11×10^{-8}	*EMB* (N, Q)
5	rs2247870	90151589	2.54×10^{-8}	*ADGRV1* (N, M, D)
5	rs2764766	127213625	1.94×10^{-8}	*LINC01184* (N)
6	rs6903570	64866857	2.70×10^{-8}	*EYS* (N), *PHF3* (D), *PTP4A1* (D)
6	rs160593	105466332	7.69×10^{-9}	*HACE1* (Q), *LIN28B* (N, Q)
6	rs7757969	112132032	4.82×10^{-8}	*FYN* (N, Q)
6	rs4479915	165075601	4.82×10^{-9}	*C6ORF118* (N)
7	rs323167	78336677	4.47×10^{-8}	*MAGI2* (N, D)
7	rs11534004	113467444	1.71×10^{-8}	*PPP1R3A* (N, M)
8	rs17687067	17036201	3.39×10^{-12}	*MTMR7* (Q), *VPS37A* (Q), *ZDHHC2* (N, D, Q)
8	rs73219805	26272768	1.94×10^{-11}	*BNIP3L* (N, D), *PPP2R2A* (D), *SDAD1P1* (Q)
10	rs111364339	64857872	5.37×10^{-9}	*JMJD1C* (D), *NRBF2* (N)
12	rs28607014	117708611	1.75×10^{-8}	*NOS1* (N)
14	rs10148671	29469373	4.46×10^{-8}	*LINC01551* (N)
14	rs2383377	33257914	2.36×10^{-8}	*AKAP6* (N, D), *NPAS3* (D)
14	rs8012642	84669481	4.66×10^{-8}	*FLRT2* (N)
15	rs783540	83254708	3.05×10^{-8}	*AP3B2* (D, Q), *CPEB1* (N, Q)
15	rs758129	89900887	2.87×10^{-8}	*MIR9-3* (N), *POLG* (D), *RLBP1* (Q)
16	rs6500596	4470027	5.24×10^{-9}	*CDIP1* (Q), *CORO7* (N, D, Q), *DNAJA3* (M, Q), *NMRAL1* (Q, S)
16	rs8058130	64371163	4.77×10^{-8}	*CDH11* (N)
17	rs56007784	1290950	1.16×10^{-9}	*YWHAE* (N)
17	rs72843506	19946287	3.73×10^{-8}	*AKAP10* (D), *CCDC144CP* (Q), *SPECC1* (N, D, Q), *USP32P3* (Q)
17	rs35065479	55736735	2.31×10^{-8}	*TSPOAP1-AS1* (Q), *MSI2* (N)
18	rs56775891	77575613	1.85×10^{-8}	*KCNG2* (N, Q, S)
18	rs28735056	77622879	4.60×10^{-10}	*KCNG2* (N)

Genomic position is based on the UCSC hg19/NCBI build 37. [a]Notable genes are indicated as follows: gene nearest to the index SNP (N); schizophrenia-associated variant in strong LD ($r^2 \geq 0.8$) with a missense variant in the indicated gene (M); gene prioritized by DEPICT (D); gene with mRNA levels in *cis* genetic linkage with the index SNPs (Q); and gene prioritized by SMR analysis (S).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

SNPs with no evidence of association ($P > 0.5$), there was no enrichment in coincident risk alleles across ancestry groups (**Supplementary Table 7**). We repeated this analysis by identifying the schizophrenia risk alleles at SNPs in the PGC2 data set and assessing concordance in the direction of the effect in the Chinese data set, and we found a very similar pattern (**Supplementary Table 7**). We concluded that there was a significant excess in directional concordance across ancestry groups for the SNPs with evidence of a schizophrenia association.

Potential biological mechanisms of the associated loci

To determine the likely causal genes of the schizophrenia-associated genetic loci, we considered each of the following to represent evidence supporting a gene's causality within a locus (Online Methods): (i) being the gene nearest the index SNP[23]; (ii) containing a missense mutation and being in high LD ($r^2 > 0.8$) with the GWS SNPs[23]; (iii) showing prioritization with DEPICT[24]; (iv) being *cis*-acting expression quantitative trait loci (*cis*-eQTL) genes for the index SNPs[23,25–28]; or (v) showing prioritization in summary-data-based Mendelian randomization (SMR) analysis[29]. Using these criteria, we prioritized 247 genes from the schizophrenia risk loci and found that 85 had more than one line of supporting evidence (defined as 'prioritized candidate genes') (**Supplementary Table 8**). We first focused on those genes in the newly identified loci (**Table 1**). As expected, some of those genes were plausibly biologically relevant. The index SNP rs2247870 (NP_115495.3, p.Val5876Ile) at 5q14.3 (GWS locus no. 37) is a missense variant in *ADGRV1* (also known as *GPR98*), which encodes a member of the G-protein-coupled-receptor superfamily and is expressed in the central nervous system. Multiple lines of evidence suggest that G-protein-coupled receptors play critical roles in major psychiatric disorders (including schizophrenia) and their treatment[30]. A variant in *GPR98* has been found to be associated with the response to antipsychotic treatment[31]. *FYN* (GWS locus no. 49) encodes a membrane-associated tyrosine kinase. FYN plays a critical role in neuronal apoptosis and is involved in brain development and synaptic transmission[32,33]. Lower expression of FYN protein has been observed in the platelets of schizophrenic patients compared with controls[34]. The results from whole-blood eQTL analysis[27] indicated that the schizophrenia risk allele identified in this study (rs7757969[C]) was correlated with a lower expression of *FYN* ($P = 1.71 \times 10^{-7}$, with a false discovery rate <0.05 and in the credible interval covered by the 99% credible set), in agreement with previous findings. The estimate (b_{XY}) for the effect of gene expression on schizophrenia risk under the SMR analysis was −0.70 ($P_{SMR} = 7.55 \times 10^{-4}$). *MAGI2* (GWS locus no. 54) encodes a synaptic scaffolding molecule that is essential for the development and maintenance of synapses[35]. Synaptic dysfunction has been suggested to play an important role in schizophrenia[36]. Common variants in *MAGI2* have been found to be associated with cognitive impairment in people with schizophrenia[37]. Although it is currently difficult to pinpoint a causal gene that is responsible for a given locus, the prioritized genes may be considered as favorable candidates for further research to unravel the plausible biological mechanisms underlying the associations.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Improved fine-mapping resolution at the associated loci

We sought to refine the localization of likely functional variants in the schizophrenia-associated loci by using a previously published approach[38,39]. We derived Bayesian credibility sets in different data sets and evaluated the evidence for improved fine-mapping resolution through transancestry meta-analysis. For the 99% credible SNP sets, the transancestry data set produced the smallest spanned regions for ~80% (n = 88) of the tested loci (**Supplementary Table 9**), including 11 loci with a spanned region less than 30 kb. At 53 of the 88 loci, the number of genes that overlapped with the transancestry interval defined by a credible set was two or fewer. Of those overlapped genes mapping to the credible intervals in the 53 loci, 49.1% was were in the list of prioritized candidate genes, whereas the proportion was 12.4% and 5.1% for the analysis of PGC2 and Chinese data, respectively.

We also conducted fine-mapping analysis with PAINTOR by leveraging the functional annotation data and LD information in multi-ancestry cohorts[40–42]. We integrated the primary functional categories (coding, UTR, promoter, DNase-hypersensitivity site, intronic and intergenic) proposed by Gusev *et al.*[41]. A total of 62 variants achieved a posterior probability of >0.80 in at least one of the single-population and transancestry analyses (**Supplementary Table 10**). Of them, 38 variants had a higher posterior probability in the transethnic analysis than in the single-population analyses, including an additional 16 variants that achieved a transancestry posterior probability of >0.80 but had a posterior probability <0.80 in the single-population analyses. Eleven (68.8%) of these 16 variants had at least one hit in the selected eQTL studies in HaploReg v4.1 (ref. 23 and **Supplementary Table 11**). For example, at GWS locus no. 80, rs12541 with a posterior probability of 0.926 (**Supplementary Fig. 7a**) is in the UTR region of *ESAM* and correlated with its expression in whole blood ($P = 3.62 \times 10^{-8}$ and in the 99% credible-set interval)[27]. A further example is GWS locus no. 103, rs3814883, which had a posterior probability of 0.911 (**Supplementary Fig. 7b**) and is a synonymous variant of *TAOK2* and also an eQTL SNP for several genes in different tissues[23] (**Supplementary Table 12**). It might also be correlated with the expression of *SEZ6L2* in the brain cerebellum and frontal cortex ($P = 2.37 \times 10^{-8}$ and 5.03×10^{-8}, respectively)[26]. TAOK2 has been found to affect basal-dendrite development in cortical neurons[43]. SEZ6L2 has been found to be a Cathepsin D transport receptor involved in neurite outgrowth[44]. To further explore the regulatory nature in the context of the cell-type-specific epigenome, we also integrated the reference epigenomes of seven highlighted marks for 127 human tissues and cell types produced by the Roadmap Epigenomics Project[45] (Online Methods). Of the top 100 enriched cell-type-specific epigenomic annotations for schizophrenia associations in the current and PGC2 analyses[7], over 40 were related to the brain and nervous system (**Supplementary Table 13**). In the further PAINTOR fine-mapping analyses with the top 100 epigenomic annotations, many SNPs had higher posterior probabilities, some of which increased to a value >0.80, thus indicating potential biologically relevant cell types for these associations (**Supplementary Table 14**). For example, rs6670165, a candidate causal SNP at GWS locus no. 7, mapped to enhancers and promoters active in several brain regions. The identification of these SNPs suggested an important benefit of the transancestry fine-mapping signal in functional annotation data. However, 14 variants had a posterior probability >0.80 in the single-population analyses, which decreased to <0.8 in the transancestry analysis (**Supplementary Table 10**).

Biological pathways and gene sets

To identify pathways and gene sets in the transancestry meta-analysis, we performed an enrichment analysis with MAGMA[46]. We identified

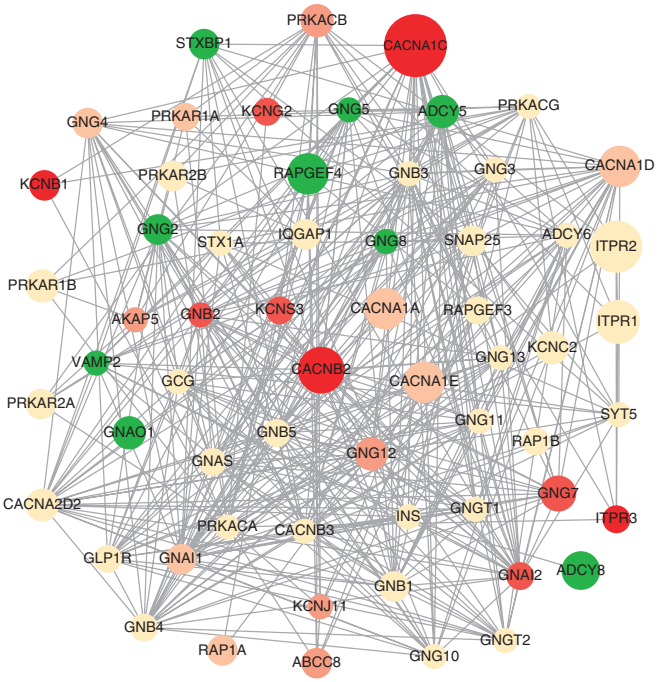


Figure 3 Interaction network of the schizophrenia-associated pathway 'glucagon-like peptide-1 regulates insulin secretion'. The network shows functional interactions for the genes in the pathway 'glucagon-like peptide-1 regulates insulin secretion' from the Reactome database. Each node represents a gene, and each edge represents a functional interaction. The node size corresponds to the gene size. The node color corresponds to the significance of the gene on the basis of the MAGMA analysis, and the green-to-red gradient corresponds to nonsignificance to high significance.

one gene set, 'regulation of insulin secretion by glucagon-like peptide 1' (from the Reactome database) that was significantly enriched (MAGMA competitive $P = 5.14 \times 10^{-7}$; **Fig. 3**). The MAGMA pathway analysis also highlighted several other pathways. Two of the previously highlighted schizophrenia-associated pathways, 'postsynaptic density'[47] and 'voltage-gated calcium channel complex'[7], also ranked highly in our analysis, with P values of 9.01×10^{-4} and 1.32×10^{-3}, respectively (**Supplementary Table 15**).

Polygenic risk-score profiling

Polygenic scoring analyses have been proposed to predict the case–control status in a target data set, on the basis of the results from a training GWAS[4]. To assess the overlap between the common-variant signal in the European and Chinese populations and to provide estimates of the proportions of variance additionally explained by the Chinese sample, we conducted a polygenic scoring analysis. We randomly selected approximately 1,000 schizophrenia cases and 1,000 controls from the Chinese sample as the target sample and used four training data sets: (i) the PGC2 European-only data set (EUR49); (ii) the full PGC2 data set; (iii) the Chinese sample, excluding the target sample; and (iv) the Chinese plus PGC2 combined data set (**Fig. 4**). The risk-profile SNPs (P thresholds (P_T) = 5×10^{-8}, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1) from the European-only data set alone explained approximately 1.11% to 2.34% of the variance in the case–control status of the Chinese sample on the liability scale[48] (assuming a population risk of 0.01). When the Asian samples were included, the PGC2 data set explained approximately 1.52% to 3.51% of the variance. The Chinese data set alone explained approxi-

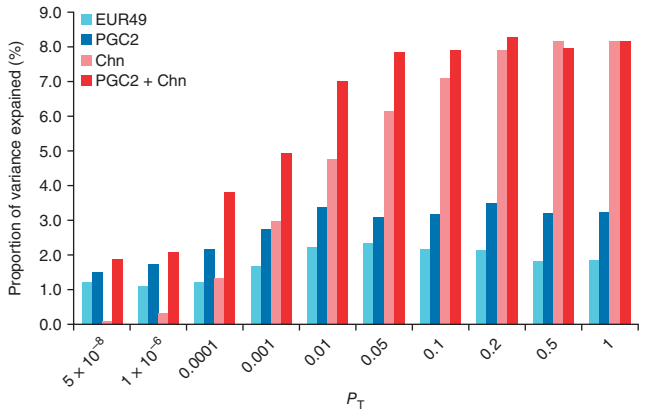


Figure 4 Polygenic risk-score profiling analysis. Polygenic risk-score profiling analysis using approximately 1,000 randomly selected schizophrenia cases and 1,000 controls from the Chinese sample as a target and deriving risk alleles from three training data sets: the PGC2 European-only (EUR49) data set (light blue); the full PGC2 data set (blue); the Chinese (Chn) sample excluding the target sample (light red); and the Chinese and PGC2 data sets combined (red). The *x* axis shows ten *P*-value thresholds ($P_T = 5 \times 10^{-8}$, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1). The *y* axis is the estimate of the proportion of variance explained on the liability scale, which is converted from Nagelkerke's pseudo R^2 (computed by comparison of a full model including covariates and polygenic risk scores to a reduced model including covariates only).

mately 0.10% to 8.15% of the variance. In almost all situations, the combined data set (PGC2 plus Chinese) explained larger proportions of the variance (approximately 1.89% to 8.28%). For $P_T = 5 \times 10^{-8}$, the proportion of the explained variance increased from 1.20% (EUR49), 1.52% (PGC2) and 0.10% (Chinese) to 1.89% (PGC2 plus Chinese); for $P_T = 0.05$, it is increased from 2.34% (EUR49), 3.09% (PGC2) and 6.16% (Chinese) to 7.86% (PGC2 plus Chinese). To evaluate the increased variance explained by the newly identified GWS loci, we performed additional polygenic risk-score profiling trained on the full data set (GWAS excluding the target sample and with replication) and restricted to the newly identified loci with the Chinese sample included. These novel loci explained 1.34% of the variance, 30% of which was contributed by the loci from the Chinese-only analysis.

Correlations between two psychiatric disorders in the Chinese sample

Strong evidence of a shared genetic etiology between schizophrenia and other psychiatric disorders (such as bipolar disorder and major depressive disorder) has been observed in European samples[49,50]. The degree of shared variation across psychiatric disorders in the Chinese population has been unclear. We estimated the genetic correlation between schizophrenia and major depressive disorder, two diseases for which Chinese GWAS data with large sample sizes are available, by using LD-score regression[14]. We observed a statistically significant genetic correlation between schizophrenia and major depressive disorder in the Chinese sample ($r_g = 0.43$, s.e. = 0.08, LD-score regression $P = 5.87 \times 10^{-8}$), in agreement with findings ($r_g = \sim 0.40$) in the European samples[49].

DISCUSSION

In the large GWAS analysis of schizophrenia in subjects of Chinese ancestry, we identified seven GWS loci, four of which were novel. In general, alleles identified as being associated at subthreshold levels of significance in the Chinese data set were also enriched in schizophrenia cases in the GWAS from PGC2, thus supporting the validity of combining the two data sets. The transancestry meta-analyses of the Chinese and PGC2 data identified 109 GWS risk loci, three of which were GWS in the Chinese-only analysis. Our analyses confirmed most of the previously identified schizophrenia loci and identified 30 novel loci.

We observed a significant excess in the directional consistency of schizophrenia risk alleles across ancestry groups, even at SNPs demonstrating only weak evidence of an association. These findings indicated that most schizophrenia risk loci were shared across these two ancestral populations, and transancestry meta-analysis provided a powerful means for identifying new loci and narrowing the association intervals. Polygenic scoring analysis also demonstrated notable increases in the explained variance in case–control status (PGC2-plus-Chinese training to Chinese target compared with PGC2 to Chinese target or Chinese training to Chinese target). However, this analysis also suggested that variants identified in European samples partially explained the genetic variance of schizophrenia in Chinese populations. Notably, estimates of the proportion of explained variance in liability were lower than those in European populations[7], similarly to previous reports on transethnic analyses[4,51]. Such lower estimates might be a result of differences in the allele frequencies and LD patterns between different populations[4].

It has been suggested that there are also population-specific risk alleles for schizophrenia[6] and that, if so, cross-ancestry analyses might have less power than that of studies of individuals with a recent shared ancestry. We found that some GWS loci in the PGC2 report were not GWS in the PGC2-plus-Chinese combined analysis. Moreover, most of the GWS SNPs identified in the analysis of Chinese subjects showed strong heterogeneity only across ancestries, though three of them achieved nominal significance with the same sign in the PGC2 data set. Another SNP fell within the previous PGC2-identified locus, but it was rare (MAF <1%) in European populations. Thus, further transancestry fine-mapping, by leveraging the differences in the LD structure among diverse populations, may be an efficient approach to identify the causal variants underlying such associations and may also distinguish population-specific loci. Indeed, we also observed considerable improvements in the fine-mapping resolution at several susceptibility loci.

Our use of fine-mapping tools and functional annotations to analyze schizophrenia-associated loci identified numerous candidate genes with several lines of supporting evidence, including genes that have previously been implicated in schizophrenia (for example, *FYN* and *MAGI2*) and novel genes (for example, *EMX1* and *BNIP3L*) within the novel loci. Moreover, pathway analyses highlighted several pathways that contribute to schizophrenia pathogenesis, including previously described pathways (the voltage-gated calcium-channel pathway and postsynaptic density) and a new pathway (regulation of insulin secretion by glucagon-like peptide 1). The latter has not been highlighted in previous genetic studies of schizophrenia, but evidence from other investigation types has linked insulin signaling to the pathophysiology of schizophrenia. Previous epidemiological data have suggested that individuals with schizophrenia, compared with the general population or healthy controls, have a higher prevalence of metabolic syndrome[52,53]. Moreover, high prevalence rates of impaired glucose metabolism have been observed in drug-naive patients with schizophrenia[54]. A proteomic analysis has shown that levels of several proteins involved in energy metabolism are altered in the brains of schizophrenic people[55]. Our results provided further support for a role for insulin-related energy metabolism in the etiology of schizophrenia.

In summary, the Chinese ($n = 36,180$) and multiancestry ($n = 118,495$) GWAS meta-analysis and follow-up replication studies identified

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

113 GWS risk loci for schizophrenia, 30 of which are novel. Our results demonstrated added value from transancestry meta-analysis for fine-mapping of loci associated with schizophrenia and highlighted the existence of shared genetic risk across populations. In addition to confirming known genetic architectures, our comprehensive analyses provide further biological insights into the etiology of schizophrenia, thus potentially facilitating further mechanistic studies to assess the pathogenesis of this complex disorder.

URLs. PGC, http://pgc.unc.edu/; EIGENSTRAT, https://github.com/DReichLab/EIG/tree/master/EIGENSTRAT/; SHAPEIT, https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html; IMPUTE2, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html; 1000 Genomes Project, http://www.1000genomes.org/; The NIH Roadmap Epigenomics Mapping Consortium, http://www.roadmapepigenomics.org/; HaploReg v4.1, http://archive.broadinstitute.org/mammals/haploreg/haploreg_v4.1.php/; PLINK, https://www.cog-genomics.org/plink2/; PubMed, http://www.ncbi.nlm.nih.gov/pubmed/; NHGRI-EBI GWAS Catalog, https://www.ebi.ac.uk/gwas/; UCSC, http://genome.ucsc.edu/; GeneCards, http://www.genecards.org/; LDSC, https://github.com/bulik/ldsc/; A. Price laboratory, https://www.hsph.harvard.edu/alkes-price/software.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank all of the participants in the study and the international Psychiatric GWAS Consortium (PGC) for the large-scale data resources that made this research possible. We also appreciate H. Huang, B. Neale and M. Daly for their valuable suggestions for data analysis and manuscript organization. This work was supported by the 973 Program (2015CB559100 to Y.S.), the National Key R&D Program of China (2016YFC0903402 to Y.S. and Z.L., and 2016YFC1201701 to X.L.), the Natural Science Foundation of China (31325014 to Y.S., 81130022 to Y.S., 81421061 to L.H. and 81701321 to Z.L.), the Program of Shanghai Subject Chief Scientist (15XD1502200 to Y.S.), the National Program for Support of Top-Notch Young Professionals to Y.S., the Shanghai Key Laboratory of Psychotic Disorders (13dz2260500 to Y.X.), the 'Shu Guang' project supported by the Shanghai Municipal Education Commission and Shanghai Education Development Foundation (12SG17 to Y.S.), the China Postdoctoral Science Foundation (2016M590615 to Z.L.), the Shandong Postdoctoral Innovation Foundation (201601015 to Z.L.), the Qingdao Postdoctoral Application Research Project (2016048 to Z.L.), the Shanghai Hospital Development Center (SHDC12016115 to Y.X.), the US NIMH and NIDA (U01 MH109528 to P.F.S. and U01 MH1095320 to P.F.S.), and the Swedish Research Council (Vetenskapsrådet, award D0886501 to P.F.S.).

AUTHOR CONTRIBUTIONS

Y.S. conceived and designed the experiments, and supervised all aspects of the work; J.C., Y.X., L.H., D.Z., W.Y., P.W., P.Y., B. Liu, W.S., Q.X., W.J., G.F., Q.Y., C.L. and X.L. performed sample collection and phenotyping; J.C., H.Y., J.Z., B.C., Y.L., J.W., J.J., M.W., Q.W., Z.W., Wenjin Li, K.L., F.H., J.Z., G.H., Weidong Li, C.W. and B. Li performed the experiments and data management; Z.L., H.Y., Z.S., J.S., S.R., P.F.S. and M.C.O'D. performed bioinformatics and statistical analyses; Y.S. and Z.L. interpreted the main findings; Y.S. and Z.L. drafted the manuscript; Y.S., L.H., Z.L., Y.X., X.L. and P.F.S. obtained the funding support; all authors revised and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html. Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

1. Knapp, M., Mangalore, R. & Simon, J. The global costs of schizophrenia. *Schizophr. Bull.* **30**, 279–293 (2004).
2. Montgomery, W. *et al.* The personal, societal, and economic burden of schizophrenia in the People's Republic of China: implications for antipsychotic therapy. *Clinicoecon. Outcomes Res.* **5**, 407–418 (2013).
3. Burmeister, M., McInnis, M.G. & Zöllner, S. Psychiatric genetics: progress amid controversy. *Nat. Rev. Genet.* **9**, 527–540 (2008).
4. Purcell, S.M. *et al.* Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748–752 (2009).
5. Ripke, S. *et al.* Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat. Genet.* **45**, 1150–1159 (2013).
6. de Candia, T.R. *et al.* Additive genetic variation in schizophrenia risk is shared by populations of African and European descent. *Am. J. Hum. Genet.* **93**, 463–470 (2013).
7. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421–427 (2014).
8. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* **43**, 969–976 (2011).
9. Shi, J. *et al.* Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* **460**, 753–757 (2009).
10. Shi, Y. *et al.* Common variants on 8p12 and 1q24.2 confer risk of schizophrenia. *Nat. Genet.* **43**, 1224–1227 (2011).
11. Stefansson, H. *et al.* Common variants conferring risk of schizophrenia. *Nature* **460**, 744–747 (2009).
12. Steinberg, S. *et al.* Common variants at VRK2 and TCF4 conferring risk of schizophrenia. *Hum. Mol. Genet.* **20**, 4076–4081 (2011).
13. Yue, W.-H. *et al.* Genome-wide association study identifies a susceptibility locus for schizophrenia in Han Chinese at 11p11.2. *Nat. Genet.* **43**, 1228–1231 (2011).
14. Bulik-Sullivan, B.K. *et al.* LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat. Genet.* **47**, 291–295 (2015).
15. Pocklington, A.J., O'Donovan, M. & Owen, M.J. The synapse in schizophrenia. *Eur. J. Neurosci.* **39**, 1059–1067 (2014).
16. Lango Allen, H. *et al.* Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature* **467**, 832–838 (2010).
17. Winkler, T.W. *et al.* Quality control and conduct of genome-wide association meta-analyses. *Nat. Protoc.* **9**, 1192–1212 (2014).
18. Yang, J. *et al.* Genomic inflation factors under polygenic inheritance. *Eur. J. Hum. Genet.* **19**, 807–812 (2011).
19. Yu, H. *et al.* Common variants on 2p16.1, 6p22.1 and 10q24.32 are associated with schizophrenia in Han Chinese population. *Mol. Psychiatry* **22**, 954–960 (2017).
20. Han, B. & Eskin, E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am. J. Hum. Genet.* **88**, 586–598 (2011).
21. Li, Z. *et al.* Loci with genome-wide associations with schizophrenia in the Han Chinese population. *Br. J. Psychiatry* **207**, 490–494 (2015).
22. Nyholt, D.R. SECA: SNP effect concordance analysis using genome-wide association summary results. *Bioinformatics* **30**, 2086–2088 (2014).
23. Ward, L.D. & Kellis, M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res.* **40**, D930–D934 (2012).
24. Pers, T.H. *et al.* Biological interpretation of genome-wide association studies using predicted gene functions. *Nat. Commun.* **6**, 5890 (2015).
25. GTEx Consortium. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* **348**, 648–660 (2015).
26. Gibbs, J.R. *et al.* Abundant quantitative trait loci exist for DNA methylation and gene expression in human brain. *PLoS Genet.* **6**, e1000952 (2010).
27. Westra, H.-J. *et al.* Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat. Genet.* **45**, 1238–1243 (2013).
28. Fromer, M. *et al.* Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nat. Neurosci.* **19**, 1442–1453 (2016).
29. Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat. Genet.* **48**, 481–487 (2016).
30. Catapano, L.A. & Manji, H.K. G protein-coupled receptors in major psychiatric disorders. *Biochim. Biophys. Acta* **1768**, 976–993 (2007).
31. Adkins, D.E. *et al.* Genomewide pharmacogenomic study of metabolic side effects to antipsychotic drugs. *Mol. Psychiatry* **16**, 321–332 (2011).
32. Ali, D.W. & Salter, M.W. NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. *Curr. Opin. Neurobiol.* **11**, 336–342 (2001).
33. Du, C.P., Tan, R. & Hou, X.Y. Fyn kinases play a critical role in neuronal apoptosis induced by oxygen and glucose deprivation or amyloid-β peptide treatment. *CNS Neurosci. Ther.* **18**, 754–761 (2012).
34. Hattori, K. *et al.* Decreased expression of Fyn protein and disbalanced alternative splicing patterns in platelets from patients with schizophrenia. *Psychiatry Res.* **168**, 119–128 (2009).
35. Bauß, K. *et al.* Phosphorylation of the Usher syndrome 1G protein SANS controls Magi2-mediated endocytosis. *Hum. Mol. Genet.* **23**, 3923–3942 (2014).

36. Calabrese, F., Riva, M.A. & Molteni, R. Synaptic alterations associated with depression and schizophrenia: potential as a therapeutic target. *Expert Opin. Ther. Targets* **20**, 1195–1207 (2016).
37. Koide, T. *et al.* Common variants in MAGI2 gene are associated with increased risk for cognitive impairment in schizophrenic patients. *PLoS One* **7**, e36836 (2012).
38. Gaulton, K.J. *et al.* Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. *Nat. Genet.* **47**, 1415–1425 (2015).
39. Morris, D.L. *et al.* Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat. Genet.* **48**, 940–946 (2016).
40. Farh, K.K.-H. *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* **518**, 337–343 (2015).
41. Gusev, A. *et al.* Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. *Am. J. Hum. Genet.* **95**, 535–552 (2014).
42. Kichaev, G. & Pasaniuc, B. Leveraging functional-annotation data in trans-ethnic fine-mapping studies. *Am. J. Hum. Genet.* **97**, 260–271 (2015).
43. de Anda, F.C. *et al.* Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex. *Nat. Neurosci.* **15**, 1022–1031 (2012).
44. Boonen, M. *et al.* Cathepsin D and its newly identified transport receptor SEZ6L2 can modulate neurite outgrowth. *J. Cell Sci.* **129**, 557–568 (2016).
45. Kundaje, A. *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–330 (2015).
46. de Leeuw, C.A., Mooij, J.M., Heskes, T. & Posthuma, D. MAGMA: generalized gene-set analysis of GWAS data. *PLOS Comput. Biol.* **11**, e1004219 (2015).
47. Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium. Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. *Nat. Neurosci.* **18**, 199–209 (2015).
48. Lee, S.H., Goddard, M.E., Wray, N.R. & Visscher, P.M. A better coefficient of determination for genetic profile analysis. *Genet. Epidemiol.* **36**, 214–224 (2012).
49. Bulik-Sullivan, B. *et al.* An atlas of genetic correlations across human diseases and traits. *Nat. Genet.* **47**, 1236–1241 (2015).
50. Lee, S.H. *et al.* Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat. Genet.* **45**, 984–994 (2013).
51. Ikeda, M. *et al.* Genome-wide association study of schizophrenia in a Japanese population. *Biol. Psychiatry* **69**, 472–478 (2011).
52. Citrome, L. & Volavka, J. Consensus development conference on antipsychotic drugs and obesity and diabetes: response to consensus statement. *Diabetes Care* **27**, 2087–2088, author reply 2089–2090 (2004).
53. Mitchell, A.J. *et al.* Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders: a systematic review and meta-analysis. *Schizophr. Bull.* **39**, 306–318 (2013).
54. Steiner, J. *et al.* Immune system and glucose metabolism interaction in schizophrenia: a chicken-egg dilemma. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **48**, 287–294 (2014).
55. Gottschalk, M.G., Wesseling, H., Guest, P.C. & Bahn, S. Proteomic enrichment analysis of psychotic and affective disorders reveals common signatures in presynaptic glutamatergic signaling and energy metabolism. *Int. J. Neuropsychopharmacol.* **18**, pyu019 (2014).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

ONLINE METHODS

Recruitment of research subjects. As in our previous study[10], all cases of Chinese ancestry were inpatients or outpatients with a history of more than 2 years of schizophrenia, who were recruited from mental-health centers in China, interviewed by two independent psychiatrists and diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. All cases met the following two criteria: preoccupation with one or more delusions and frequent auditory hallucinations. However, none of the following symptoms were prominent: disorganized speech, disorganized or catatonic behavior, or flat or inappropriate effects. The controls were randomly selected from Chinese volunteers (from hospitals and a community survey) who were asked to reply to a written invitation to evaluate their medical histories. Lists of potential control subjects were screened for suitability as volunteers by excluding subjects with major mental illnesses. All participants provided written informed consent. The study was approved by the Ethics Committee of Human Genetic Resources at the Bio-X Institutes of Shanghai Jiao Tong University, in accordance with the tenets of the Declaration of Helsinki. We confirm that our study is compliant with the Guidance of the Ministry of Science and Technology (MOST) for the Review and Approval of Human Genetic Resources.

Genotyping, quality control and genotype imputation of the Chinese GWAS data. Several different genome-wide genotyping platforms were used in this study: Affymetrix Genome-Wide Human SNP Array 6.0 (SNP6.0), Affymetrix Axiom Genome-Wide CHB1 Array Plate and Illumina 1M Array.

For the SNP6.0 chips, the genotype calls were generated together by using Affymetrix Axiom Analysis according to the Best Practices Workflow for SNP6.0. Sample QC filtering of the GWAS data was first performed by excluding arrays with Contrast QC measurements (a metric developed by Affymetrix for SNP6.0 QC, n = 197) that were <0.4. Step 1 genotyping was run on all CEL files passing QC over a subset of 20,000 SNPs, and samples with a call rate ≤97% were excluded (n = 285). The remaining samples were used for step 2 genotyping analysis. SNP polisher was then used for SNP QC, and the SNPs in the recommended categories (PolyHighRes, MonoHighRes, NoMinorHom and Hemizygous) were retained. Sex was established via genotyping and evaluated for each of the subjects, and samples with inconsistent sex (compared with the sample record) were removed (n = 79). Heterozygosity rates were calculated with the intent of removing deviations that exceeded 6 s.d. from the mean (n = 0). PLINK's identity-by-descent analysis was used to detect cryptic relatedness[56] (URLs). When a pair of individuals had PI_HAT >0.2, the member of the pair with the lower call rate was excluded from the analysis (n = 259). SNPs with call rates <97% (n = 28,040), MAF <1% (n = 185,439) or significant deviation from Hardy–Weinberg equilibrium (HWE) in controls (HWE $P \leq 1 \times 10^{-6}$, n = 20,344) were excluded. We also excluded population outliers on the basis of PCA. After application of quality-control criteria, a set of 590,413 SNPs for 14,645 individuals was generated for genotype imputation.

For the CHB1 chips, the genotype calls were generated together according to the Axiom Genotyping Solution Data Analysis Guide. Briefly, arrays with dish QC (DQC), a single-sample metric developed by Affymetrix for Axiom QC) values <0.82 were first excluded (n = 181). Samples that surpassed the DQC values were used for genotype calling with a subset of probe sets. Samples with a call rate <97% or in a nonpassing plate (an average call rate of passing samples <98.5%) were also excluded (n = 276). The post-QC samples were then coclustered, and genotype calls were produced with the Axiom Genotyping Algorithm v1 (Axiom GT1). SNP QC was also executed with the SNP polisher procedure, and the SNPs in the recommended categories were retained. Verification procedures for sex, relatedness and PCA outliers were also conducted in sample QC as described above (n = 289). SNPs with call rates <97% (n = 56,735), MAF <1% (n = 206) or significant deviations from HWE in controls (HWE $P \leq 1 \times 10^{-6}$, n = 18,849) were excluded. After application of QC criteria, a set of 555,058 SNPs for 9,580 individuals was generated for genotype imputation.

For Illumina 1M chips, SNP genotypes were generated from normalized bead intensity data with Genome Studio. Samples with a call rate <97% were excluded (n = 35). Regular sample QC procedures for parameters including sex, relatedness, heterozygosity rate and PCA outlier checking, were performed as described above (n = 231). SNPs with call rates <97% (n = 35,743), MAF <1% (n = 89,032) or HWE $P \leq 1 \times 10^{-6}$ (n = 954) were excluded. After application of QC criteria, a set of 716,466 SNPs for 1,823 individuals was generated for genotype imputation.

For each GWAS data set, the entire set was imputed together as follows: the genotypes were phased with SHAPEIT (URLs)[57,58] for each chromosome, and imputation was performed for each 5-Mb chromosome interval with IMPUTE2 (URLs)[59]. The haplotypes derived from the 1000 Genomes Project Phase 1 (release v3, URLs) were used as reference data[60]. Because two genotyping platforms were used for GWAS set 3, we used two phased reference panels in this special case, as proposed by Howie *et al.*[59]. For each platform, the prephased data from the other platform were used as the second reference panel. The variants with INFO >0.8, MAF >0.01, a call rate ≥97% and HWE $P \geq 1 \times 10^{-6}$ in the controls were saved for further analysis. Those present in at least two data sets were saved for the meta-analysis. A set of 5,107,227 genetic variants for 7,699 cases and 18,327 controls remained in the final analysis.

PGC2 GWAS data set. The PGC2 GWAS data set[7] comprised 49 case–control samples (34,241 cases and 45,604 controls) and three family-based samples (1,235 parent–affected offspring trios). All of the samples were from subjects of European ancestry, excluding three case–control samples from subjects of East Asian ancestry (1,836 cases and 3,383 controls). The summary results for the PGC2 data set and European only data set (EUR49) were downloaded from the PGC website (URLs).

Replication data set. The replication sample consisted of 4,384 cases and 5,770 controls of Han Chinese ancestry. More details of the general characteristics and genotyping have been presented in our previous research[19]. For the Chinese-only analyses, the independent SNPs with $P < 1 \times 10^{-5}$ in the Chinese GWAS analysis of pre- or postcorrection with the inflation factor were selected. For the transancestry analysis, the independent SNPs with $P < 5 \times 10^{-7}$ in the Chinese (pre- or postcorrection) and PGC2 GWAS meta-analyses were selected. The precorrection data sets were used only for including more candidate SNPs for replication. All the association results in this article were based on the postcorrection data sets, wherein the global inflations were controlled. A total of 295 SNPs were analyzed in the Chinese replication analysis.

Power calculations. Power calculations were performed with the GAS Power Calculator[61] with a range of genotype relative risks and disease-allele frequencies, assuming a population prevalence of 0.01 and a significance level of 5×10^{-8}. For the Chinese-only (n = 36,180) and transancestry (n = 118,495) analyses, we had adequate power (>80%) to detect variants with low risk-allele frequencies (RAFs) of 0.03 with genotypic relative risks of 1.318 and 1.161, respectively. This sample size in Chinese-only analyses was large enough to achieve adequate power for risk variants with genotypic relative risks of 1.150 and RAFs of 0.14 to 0.85, and the transancestry analyses achieved adequate power for risk variants with 1.075 and RAFs of 0.15 to 0.84.

Statistical methods and bioinformatics analysis. Population substructure was evaluated through a PCA with EIGENSTRAT software (URLs), on the basis of LD-pruned autosomal SNP genotypes[62,63]. Two rounds PCA were performed. One round with samples from the HapMap Project phase 3 (HapMap3) was performed to identify admixed samples, and the other round was performed for each subset of cases and controls, wherein individual outliers (>6 s.d. from the mean on any one of the top ten PCs) were identified and removed for five iterations, and final PCs reflecting subtle ancestry information for each sample were generated for further correction. In the Chinese GWAS stage, the association was analyzed for subsets by using a logistic regression model involving covariates for PCs to adjust for possible population stratification. We evaluated the effects of the 20 PCs on genome-wide test statistics to determine the PC inclusion in the final association analysis for each data set. In the Chinese replication stage, the associations between SNPs and schizophrenia risk were evaluated on the basis of logistic regression with SNPTEST[64]. The Higgins and Thompson I^2 index was used for assessing heterogeneity across data sets[65]. Both fixed-effects-model and random-effects-model meta-analyses were used in this study. The variants with pronounced heterogeneity (I^2 >75%) were combined in a random-effects model in the transancestry meta-analysis[17].

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

We assessed the genome-wide congruence of risk alleles across samples by using binomial sign tests that compared the direction of the effect sizes of independent SNPs between PGC2 and Chinese GWAS results. *P* values were generated under the null hypothesis (H_0: $P = 0.50$). The proportion of variance in liability to schizophrenia explained by the common SNPs was estimated by using genome-wide complex-trait analysis[66], and the PCs were included in the analysis as covariates. For each of the associated loci (except the eMHC region, owing to the complexity of this region[7]), we calculated an approximate Bayes factor per Wakefield, as well as the posterior probability of driving the association for each SNP within a 2-Mb window, and then created 99% credibility sets[38,39,67]. We created credibility sets by using the Chinese, PGC2 (European) and combined data sets separately. We conducted the transancestry fine-mapping in the presence of functional information by using PAINTOR according to the suggested pipeline, as well as PGC2-only and Chinese-only analyses for comparison. The primary functional annotations for SNPs proposed by Gusev *et al.*[41] were obtained from the A. Price laboratory website (URLs). The reference epigenomes of 127 human tissues and cell types[45] were obtained from the NIH Roadmap Epigenomics Mapping Consortium (URLs). We included seven highlighted epigenomic marks (H3K4me3, H3K4me1, H3K36me3, H3K27me3, H3K9me3, H3K27ac and H3K9ac)[45] in our analyses. Enrichment analyses of the schizophrenia associations in the current and PGC2 analyses with the epigenomic features were performed with the genomic regulatory elements and GWAS overlap algorithm (GREGOR)[68], and the top 100 enriched annotations were selected for further PAINTOR analyses. The online tool HaploReg[23] (v4.1; URLs) was used to explore the genes nearest to the index SNPs, and genes containing a missense mutation in high LD ($r^2 > 0.8$, on the basis of the 1000 Genomes Phase 1 CEU or ASI population for the LD calculation) with the GWS SNPs. The effects of GWS SNPs on expression in eQTL studies of different tissues (including blood and brain tissues[25–27]) were extracted from the query results of HaploReg[23] and the CommonMind Consortium Knowledge Portal[28]. A significant eQTL was reported as having a false discovery rate of 0.05 in the original studies[25–28] and being located in the credible interval covered by the 99% credible set for the regulated gene for the data sets in which detailed results were available for establishing the credible sets[25,27]. We used DEPICT[24] to identify the most likely causal genes for the schizophrenia-associated loci, on the basis of the functional similarity among genes from associated regions. We carried out SMR analysis[29] for the blood and brain tissue eQTL data sets[25,27], using the 1000 Genomes Project data as reference files. For the gene prioritization analysis at the GWS loci (excluding the eMHC region, owing to the complexity of this region[29]), only probes with at least one *cis*-eQTL at $P < 5.0 \times 10^{-8}$ were considered for SMR analysis, and a significance threshold was set as $P_{SMR} < 5.20 \times 10^{-5}$ corresponding to a Bonferroni correction for 960 tests (960 probes with *cis*-eQTL at $P < 5.0 \times 10^{-8}$ across the GWS loci)[29]. The heterogeneity in dependent instruments (HEIDI) test was also performed, and $P < 0.05$ was considered to indicate significant heterogeneity. The genes prioritized by the GWS index SNP or its high LD ($r^2 > 0.8$) proxies were listed. In addition, the SMR analysis was also performed for some specific SNPs and genes. Here, the *P*-value threshold for selecting eQTL was not applicable, and the details are shown in the results. We searched the published literature for these genes with respect to schizophrenia in PubMed (URLs) and the NHGRI-EBI GWAS Catalog (URLs), and we obtained additional functional evidence for these SNPs and genes from the published literature, the UCSC genome database (URLs) and GeneCards (URLs).

LD-score regression for Chinese GWAS data. We estimated Chinese LD scores from the Chinese subjects in the 1000 Genomes Project Phase 3, using the LD Score (LDSC; URLs) software package[14]. We used a window size of 1 cM to estimate LD scores, excluded singletons and did not set an r^2 cutoff. The LD-score regression intercept from the Chinese GWAS data was estimated according to application notes for real data from the LDSC developers[14]. As Bulik-Sullivan *et al.* have proposed[14], correcting test statistics with the LD-score regression intercept is a robust way for controlling the confounding bias from inflation. Correction was applied to the Chinese GWAS meta-analysis results by multiplying the standard errors by the square root of the correction factor[16].

Polygenic scoring analysis. Approximately 1,000 cases and 1,000 controls from the Chinese sample were randomly selected as the target sample. Risk-profile SNPs ($P_T = 5 \times 10^{-8}$, 1×10^{-6}, 0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 0.5 and 1) from the training GWAS data sets (the PGC2 European-only (EUR49) and full data sets, the Chinese GWAS data set excluding the target sample and the Chinese plus PGC2 combined data set) were selected with the PLINK '--clumped' function, and SNPs within 500 kb or with $r^2 \geq 0.1$ were discarded. The risk-profile SNPs were then used to generate scores for the target samples by using the PLINK '--score' function. The case–control status was then predicted by logistic regression analysis of polygenic scores plus PC covariates. Nagelkerke's R^2 was used for the full model, using the polygenic score plus the covariates minus R^2 for the covariates alone, thus yielding an estimate of the explained variance. The R^2 was then transformed into a liability scale[48], assuming a population prevalence of 1% for schizophrenia[7].

Pathway analysis. MAGMA[46] was used to explore pathway-based associations in the genome-wide meta-analysis data set. An *F* test was used to compute the gene *P* value, and the gene *P* values and gene correlation matrix were then used for the gene-set analysis with a regression model[46]. We defined gene boundaries 35 kb upstream and 10 kb downstream for assigning SNPs to a gene, as adopted in a recent psychiatric-disorder pathway analysis[47]. Each gene was then assigned pathways in the Gene Ontology (GO), PANTHER, Ingenuity, Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome and BioCarta gene set databases[69]. A total of 2,981 pathways or gene sets were used in this analysis.

Data availability. Summary statistics for the meta-analyses will be made available at http://gwas.bio-x.cn/. A **Life Sciences Reporting Summary** is available.

56. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
57. Delaneau, O., Marchini, J. & Zagury, J.-F. A linear complexity phasing method for thousands of genomes. *Nat. Methods* **9**, 179–181 (2011).
58. Delaneau, O., Zagury, J.-F. & Marchini, J. Improved whole-chromosome phasing for disease and population genetic studies. *Nat. Methods* **10**, 5–6 (2013).
59. Howie, B.N., Donnelly, P. & Marchini, J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* **5**, e1000529 (2009).
60. 1000 Genomes Project Consortium. *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012).
61. Skol, A.D., Scott, L.J., Abecasis, G.R. & Boehnke, M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat. Genet.* **38**, 209–213 (2006).
62. Price, A.L. *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* **38**, 904–909 (2006).
63. Patterson, N., Price, A.L. & Reich, D. Population structure and eigenanalysis. *PLoS Genet.* **2**, e190 (2006).
64. Marchini, J., Howie, B., Myers, S., McVean, G. & Donnelly, P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat. Genet.* **39**, 906–913 (2007).
65. Higgins, J.P.T., Thompson, S.G., Deeks, J.J. & Altman, D.G. Measuring inconsistency in meta-analyses. *Br. Med. J.* **327**, 557–560 (2003).
66. Yang, J., Lee, S.H., Goddard, M.E. & Visscher, P.M. GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76–82 (2011).
67. Wakefield, J. Bayes factors for genome-wide association studies: comparison with P-values. *Genet. Epidemiol.* **33**, 79–86 (2009).
68. Schmidt, E.M. *et al.* GREGOR: evaluating global enrichment of trait-associated variants in epigenomic features using a systematic, data-driven approach. *Bioinformatics* **31**, 2601–2606 (2015).
69. Segre, A.V. *et al.* Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLoS Genet.* **6**, e1001058 (2010).

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

doi:10.1038/ng.3973

nature research

Corresponding author(s): Zhiqiang Li

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No initial power analysis was done to determine the sample size. Our post hoc power analysis indicated that our sample size was large enough to achieve adequate power for detecting variants of low risk allele frequencies of 0.03 with genotypic relative risks of 1.161.

2. Data exclusions

Describe any data exclusions.

Typical quality control was performed for our GWAS data sets. Arrays with low quality data were excluded. Samples failed in the sex, relatedness, heterozygosity rate and PCA outlier checking procedures were also excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

We performed Chinese and multi-ancestry GWAS meta-analyses and follow-up replication analyses, and the identified loci were reliably reproduced with genome-wide significant evidence.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The samples were grouped by disease status.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The researchers were not blinded to group allocation.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☒	☐	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☒	☐	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Nature Genetics: doi:10.1038/ng.3973

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	The URLs for the software used were provided.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The materials were commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Not applicable.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Not applicable.
b. Describe the method of cell line authentication used.	Not applicable.
c. Report whether the cell lines were tested for mycoplasma contamination.	Not applicable.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	Not applicable.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Not applicable.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	For privacy concerns, we can't provide detailed information for the participants. These information were not used as covariates in our analysis.

Nature Genetics: doi:10.1038/ng.3973