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Recent Positive Selection Drives the Expansion of a Schizophrenia Risk Nonsynonymous Variant at *SLC39A8* in Europeans

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Natural selection has played important roles in optimizing complex human adaptations. However, schizophrenia poses an evolutionary paradox during human evolution, as the illness has strongly negative effects on fitness, but persists with a prevalence of ~0.5% across global populations. Recent studies have identified numerous risk variations in diverse populations, which might be able to explain the stable and high rate of schizophrenia morbidity in different cultures and regions, but the questions about why the risk alleles derived and maintained in human gene pool still remain unsolved. Here, we studied the evolutionary pattern of a schizophrenia risk variant rs13107325 ($P < 5.0 \times 10^{-8}$ in Europeans) in the *SLC39A8* gene. We found the SNP is monomorphic in Asians and Africans with risk (derived) T-allele totally absent, and further evolutionary analyses showed the T-allele has experienced recent positive selection in Europeans. Subsequent exploratory analyses implicated that the colder environment in Europe was the likely selective pressures, ie, when modern humans migrated "out of Africa" and moved to Europe mainland (a colder and cooler continent than Africa), new alleles derived due to positive selection and protected humans from risk of hypertension and also helped them adapt to the cold environment. The hypothesis was supported by our pleiotropic analyses with hypertension and energy intake as well as obesity in Europeans. Our data thus provides an intriguing example to illustrate a possible mechanism for maintaining schizophrenia risk alleles in the human gene pool, and further supported that schizophrenia is likely a product caused by pleiotropic effect during human evolution.

Key words: *SLC39A8*/nonsynonymous SNP/schizophrenia/positive selection/pleiotropic effects/Europe

Introduction

Schizophrenia is a severe chronic neuropsychiatric disorder which affects about 0.5% of the world populations.[1] Family, twin, and adoption studies have revealed a strong genetic component with the estimated heritability about 80%.[2] During the past decades, genetic analyses including genome-wide scan have implicated a number of common and rare single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and other types of variations in schizophrenia.[3–5] However, many of the identified variants showed population-specific susceptibility and cannot be replicated across different ethnic groups.[6–9] Furthermore, partial of the risk variants in single population were even not polymorphic in other specific ethnic groups, suggesting potential population differentiation caused by natural selection or genetic drift.[10]

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As one of the most complex mental disorders, the evolutionary origin of schizophrenia remains largely unknown. Considering that schizophrenia is associated with reduced evolutionary fitness (eg, reduced fecundity, reproductive disadvantages, and increased mortality),[11–13] it has long been speculated that schizophrenia risk alleles will be eliminated from human gene pool gradually by natural selection. However, accumulating data clearly indicates that schizophrenia is maintained at relatively high prevalence in diverse human populations and many susceptibility variants are widely spread throughout worldwide human populations.[9,14,15] The high prevalence, high heritability and reduced evolutionary fitness of the illness raises an evolutionary paradox,[16] ie, why and how could schizophrenia risk alleles be stably preserved in the human gene pool?

A prevalent hypothesis about the origin of schizophrenia is the "trade-off" theory, which proposed that under Darwinian positive selection, the frequency of advantageous mutations (eg, protecting humans from infection diseases or adapting to the changed environment better) can increase rapidly in human populations.[16–18] However, as a consequence of pleiotropic effect,[19–24] some of the mutations may also increase susceptibility to other disorders such as schizophrenia.[25]

Here, we studied the evolutionary pattern of a schizophrenia associated SNP rs13107325 in the *SLC39A8* gene and found that its risk T-allele is mostly dominant in Europeans and has experienced positive selection when modern humans migrated to Europe. The cold temperature of climate in Europe seems to be the selective pressure and resulted in an increased T-allele frequency, which enabled humans to better adapt to the changed environment after the ancestors of modern human had migrated "out of Africa," while at the same time, this allele would also increase risk for schizophrenia.

Material and Methods

Samples for Population Genetic Analyses

We used a total of 1092 unrelated individuals from the 1000-Human-Genome Project,[26] including 379 Europeans (including 85 Utah Residents with Northern and Western European ancestry; 88 Toscana in Italia; 14 Iberian in Spain; 93 Finnish in Finland; and 89 in England and Scotland), 286 Asians (consisted of 97 Han Chinese in Beijing, China; 100 Southern Han Chinese; and 89 Japanese in Tokyo, Japan), 181 Americans (comprised of 66 Mexican Ancestry from Los Angeles, United States; 55 Puerto Ricans from Puerto Rico; and 60 Colombians from Medellin, Colombia), and 246 Africans (containing 88 Yoruba in Ibadan, Nigeria; 97 Luhya in Webuye, Kenya; 61 African Ancestry in Southwest United States), to perform the following population genetic analyses: calculations of integrated haplotype score (iHS), long-range haplotype (LRH) test and haplotype network construction.

To analyze the frequency distribution of rs13107325 in different geographic populations, we also obtained the genotype data from the HGDP-CEPH dataset,[27] which included 1043 unrelated individuals from 53 world populations genotyped by the Illumina HuHap 650k platform.[28] This sample was also used to calculate the iHS and to perform LRH test of the candidate region.

Tests of Natural Selection

Long-Range Haplotype. The LRH test was first developed by Sabeti *et al.*[29] In brief, the rationale for the LRH test is that, under the assumption of neutral evolution, new alleles usually need a long time to reach relative high frequency in the population and LD surrounding the alleles will decay substantially because of recombination and mutation. Accordingly, common alleles will be relatively old and will have only short-range LD. However, when a SNP is under positive selection, it increases in frequency more rapidly than would be expected by random genetic drift and other SNPs adjacent to the selected SNP also increase in frequency due to the hitchhiking effect. In this case, we would tend to observe a high-frequency haplotype that became common over a short period of time, such that recombination has not had sufficient time to break down the positive selected haplotype.

Based on the LD pattern surrounding the studied SNP (rs13107325), we applied the extended haplotype homozygosity (EHH) and relative EHH (REHH) to identify signatures of recent positive selection.[29] We measured the decay of LD for a given core SNP by calculating EHH for the core SNP and the surrounding SNPs in the order of increasing distances by starting with the core SNP (EHH = 1) and following with the next closest neighboring SNPs to the core SNP at both proximal and distal sides. The estimated EHH and REHH values of the core and neighboring SNPs were plotted against their genetic distances for the derived and ancestral alleles for a given core site. Ancestral alleles for each SNP were obtained from 1000-Human-Genome, which were deduced through comparing the alleles with the sequences from the chimpanzee and other nonhuman primate. EHH and REHH were plotted using the program Sweep 1.1.[29]

Integrated Haplotype Score. The iHS is an EHH-based test for detection of recent positive selection which is typically designed to detect incomplete selective sweep. The iHS test statistic, calculated for common SNPs (minor allele frequency > 0.05) in the candidate region, could reflect the differences in the long-range LD patterns containing the ancestral vs derived alleles. The genotype data were first phased, and then the iHS was calculated using software Selscan according to the methods implemented in previous studies.[29–32] The criteria for SNP under positive selection should have $|iHS| > 2$ ($iHS < -2$ means the derived allele undergoes positive selection and $iHS > 2$ indicates the ancestral allele undergoes positive selection), which corresponds to the most extreme 5% of iHS values across the genome with minor allele frequency >0.05.

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Haplotype Network Analysis. For haplotype network analysis, the 54 shared SNPs within 4kb covering rs13107325 among European, American, Asian and African populations were used, and haplotype inferring was conducted by PHASE implemented in DnaSP (version 5).[33] A median-joining network was constructed following the method described in Bandelt *et al.*[34] To simplify the network, a maximum parsimony calculation was performed to eliminate superfluous links between haplotypes with the default settings.

Genetic Association Between rs13107325 and Schizophrenia

Summary statistics of SNPs located in chr4:102900000–103300000 (hg19) were extracted from the Psychiatric Genomics Consortium (PGC2) genome-wide association study (GWAS).[3] In brief, the study represents a meta-analysis of multiple GWAS datasets with most of the samples were of European ancestry. The study ("PGC2") comprised up to 36989 schizophrenia cases and 113075 controls (the discovery sample includes 35476 cases and 46839 controls) and identified 108 independent genetic risk loci.[3] Detailed information about sample ascertainment, diagnosis, genotyping quality control, and statistical analyses can be found in the original report and PGC website.[3]

In addition, we also obtained the association results of rs13107325 from another study including 4545 cases and 15575 controls (samples were Santiago, Spain, and SGENE-Plus),[35] with one of the replication samples (SGENE-Plus) has partial overlap with the PGC2 study.[3] Detailed information about sample ascertainment, diagnosis, genotyping quality control, and statistical analyses can be found in the original study.[35]

Meta-analysis was performed with a fixed-effect model by PLINK.[36] As PGC2 GWAS contained SGENE-Plus samples, we carried out a meta-analysis using Santiago (476 cases and 447 controls), Spain (932 cases and 1033 controls) and PGC2 discovery (35476 cases and 46839 controls) samples.

Alignment, Secondary Structure Prediction and 3D Modeling of the SLC39A8 Protein

Function prediction of the residue affected by rs13107325 was carried out by using Polyphen2.[37] Protein sequences of SLC39A8 were obtained and aligned using UCSC genome browser.[38] Secondary prediction was extracted from Uniprot (Web Resources). We also conducted secondary structure prediction using PSIPRED.[39,40] Structural models of complete protein sequence of SLC39A8 were generated using the intensive model algorithm of phyre2 and drawn by POLYVIEW-3D.[41]

Analyses on Pleiotropic Effects of rs13107325

We used 3 strategies to explore the pleiotropic effects of rs13107325 on human traits and diseases. (1) We searched NCBI PubMed with the search terms "rs13107325" or "*SLC39A8*." (2) We queried GWAS Catalog (as of January 05, 2015) from the National Human Genome Research Institute's (NHGRI) using "rs13107325" or "*SLC39A8*" as a keyword. (3) With an *in prior* assumption, we analyzed the results of rs13107325 in several GWASs (dietary macronutrient intake, coronary artery disease, and type 2 diabetes, etc.). Brief descriptions about the included studies can be found in the original reports or shown below.

Blood Pressure and Hypertension. The International Consortium for Blood Pressure (ICBP) performed a multi-stage GWAS meta-analysis in >200000 individuals. In brief, the study utilized a primary GWAS screening sample including 69395 individuals of European ancestry and 133661 additional individuals as validations, and identified 29 loci associated with systolic blood pressure (SBP), diastolic blood pressure (DBP), and hypertension.[42]

In another study, the ICBP conducted a meta-analysis of GWAS datasets (N = 74064 for discovery stage and N = 48607 for follow-up replication) on 2 further blood pressure phenotypes,[43] pulse pressure (PP, the difference between SBP and DBP), a measure of stiffness of the main arteries and mean arterial pressure (MAP), a weighted average of SBP and DBP. Both PP and MAP are predictive of hypertension[44] and cardiovascular diseases.[45] Summary statistics of SNP associations (including rs13107325 and its surrounding SNPs located on chr4:102900000–103300000 [hg19]) were extracted from the ICBP GWAS.[42]

Body Mass Index and Obesity. Obesity is a prevalent and highly heritable disorder in global populations.[46] As a noninvasive and inexpensive measure of obesity, body mass index (BMI) is used extensively to predict the risk of obesity-related complications. To better understand the biological basis of obesity, Speliotes *et al.*[47] conducted genetic association analyses between BMI about 2.8 million SNPs in up to 123865 individuals of European ancestry. They followed up 42 SNPs in up to 125931 additional subjects and identified 18 new loci that showed genome-wide level of significance. Detailed information about sample ascertainment and statistical analyses can be found in the original study and GIANT website.[47]

Energy Intake. Dietary intake of macronutrients (carbohydrate, protein, and fat) is associated with risk of chronic conditions such as obesity and diabetes.[48] Two GWASs (discovery sample size N > 30000 in each study) have been conducted in independent samples of European descent to identify common genetic variants that are associated with macronutrient intake,[49,50] and they reported variants in *FGF21* and *FTO* showing genome-wide significant associations.

Blood Lipids. Blood lipids, including high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL)

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cholesterol, triglycerides, and total cholesterol are important risk factors for coronary artery disease.[51] To identify the genetic variants associated with blood lipids, Teslovich *et al*.[52] performed a GWAS in >100 000 individuals of European ancestry, and they identified 95 loci significantly associated with blood lipids. In another study, the Global Lipids Genetics Consortium performed a GWAS in 188 577 subjects,[53] and they identified 157 loci associated with blood lipids at genome-wide level of significance.

Results

A Nonsynonymous SNP (rs13107325) in SLC39A8 is Significantly Associated With Schizophrenia in Europeans

In 2011, Carrera *et al*.[35] has performed a genetic association study to test the associations between 5100 common nonsynonymous SNPs (cnsSNPs) and schizophrenia in 476 cases and 447 controls of European origin. They identified rs13107325 in *SLC39A8* as one of most significant SNPs ($P = 3.20 \times 10^{-4}$, OR = 1.76), and independent follow-up replication analyses further confirmed the associations ($P < .05$, Table 1). In the combined samples including a total of 4545 cases and 15 575 controls, rs13107325 showed the most significant association with schizophrenia among all 5100 cnsSNPs ($P = 2.70 \times 10^{-6}$, OR = 1.25).

In 2014, the latest and largest GWAS on schizophrenia by PGC2 has reported 108 independent risk loci in a total of 36 989 cases and 113 075 controls (the discovery sample includes 35 476 cases and 46 839 controls).[3] In their study, rs13107325 is again significantly associated with schizophrenia, reaching the genome-wide level of statistical significance ($P = 1.54 \times 10^{-12}$, OR = 1.16). Although there is a partial overlap between the replication samples (SGENE-Plus) in Carrera *et al*.[35] and PGC2 GWAS,[3] the dramatic decreasing *P*-value along with the increasing sample size clearly indicated that rs13107325 is likely an authentic risk SNP for schizophrenia in populations of Europeans ancestry.

We also conducted a joint analysis by combining the samples from Carrera *et al*.[35] and PGC2 discovery GWAS,[3] the SGENE-plus was not included due to the overlapped samples. The meta-analysis showed a stronger association between rs13107325 and schizophrenia ($P = 5.30 \times 10^{-15}$, OR = 1.17, Table 1). However, in genetic association studies, it is difficult to precisely identify the causal variant as an associated SNP most likely points to a larger region of correlated variants that showed high degree of linkage disequilibrium (LD). To investigate if there are SNPs linked with rs13107325, we explored the LD between rs13107325 and its surrounding SNPs. A proxy search for SNPs of LD with rs13107325 was performed on the SNAP website with the European panel from the 1000-Human-Genomes (pilot 1) dataset. This search found that there is no SNP in relative high LD ($r^2 > .85$) with rs13107325 (figure 1A).

Potential Functional Consequences of rs13107325

SNP rs13107325 is a nonsynonymous variant located in amino acid position 391 of SLC39A8. This polymorphism leads to an amino acid change from alanine (major C-allele, the ancestral allele) to threonine (minor T-allele, the derived and schizophrenia risk allele). Alanine is a hydrophobic amino acid while threonine is a hydrophilic polar amino acid, and a polarity-status change might result in functional consequence. We therefore performed functional predictions using Polyphen2 and the results showed that alanine and threonine residues at rs13107325 may have differences for the function of SLC39A8. Alignment of protein sequence across multiple species implied that rs13107325 is highly conserved with all of the included species showing alanine at this amino acid site (figure 2A). Interestingly, the protein sequence surrounding rs13107325 is also completely conserved, suggesting that rs13107325 is likely located in a functional important region (figure 2A).

We further explored the potential impact of rs13107325 by predicting the secondary structure of SLC39A8 using Uniprot, and we found this protein has 7 transmembrane domains (figure 2B) and the amino acid encoded by rs13107325 is located in the sixth transmembrane domain. We also conducted the secondary structure prediction using PSIPRED and obtained similar results.[39,40] A 3-dimensional

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Table 1. Association Results of rs13107325 with Schizophrenia in Europeans

Study	Sample	Sample Size		Allele	Frequency[a]	OR[b]	*P*-value
		Case	Control				
Carrera *et al.*[35]	Santiago	476	447	T	0.08	1.76	3.20E-04
	Spain	932	1033	T	0.08	1.40	5.50E-03
	SGENE-Plus	3137	14 095	T	0.08	1.11	1.30E-02
Ripke *et al.*[3]	PGC2	35 476	46 839	T	0.08	1.16	1.54E-12
Meta-analysis	Santiago, Spain, and PGC2	36 454	48 319	T	—	1.17	5.30E-15

[a]The frequency of rs13107325 was derived from European populations in 1000-Human-Genome.
[b]The OR in Carrera *et al.*[35] study was calculated based on heterozygous model (CT vs CC), and OR in PGC2 GWAS[3] was calculated based on additive model.

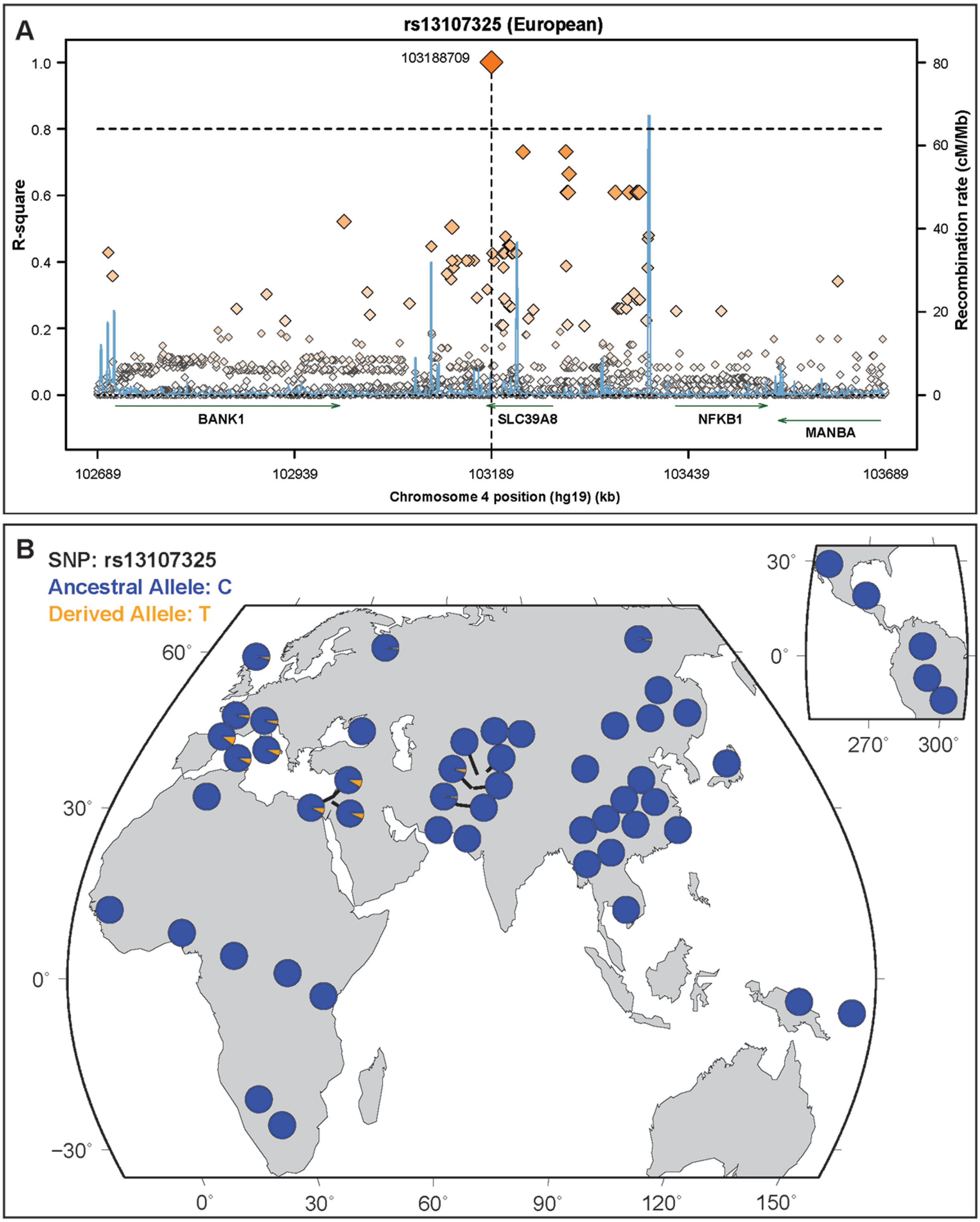

Fig. 1. (A) Plot of chromosome region showing a genomic area of LD with rs13107325 in European populations. (B) Global distributions of rs13107325 in 53 world populations.

protein model of human SLC39A8 predicted the spatial position of the amino acid encoded by rs13107325 (figure 2C). This data suggest that rs13107325 might affect the function of SLC39A8, probably through impacting the function or structure of the transmembrane domain.

Rs13107325 is Monomorphic in African and Asian Populations

Replicating the risk associations in different ethnics is a plausible way to confirm if the SNP of interest is a

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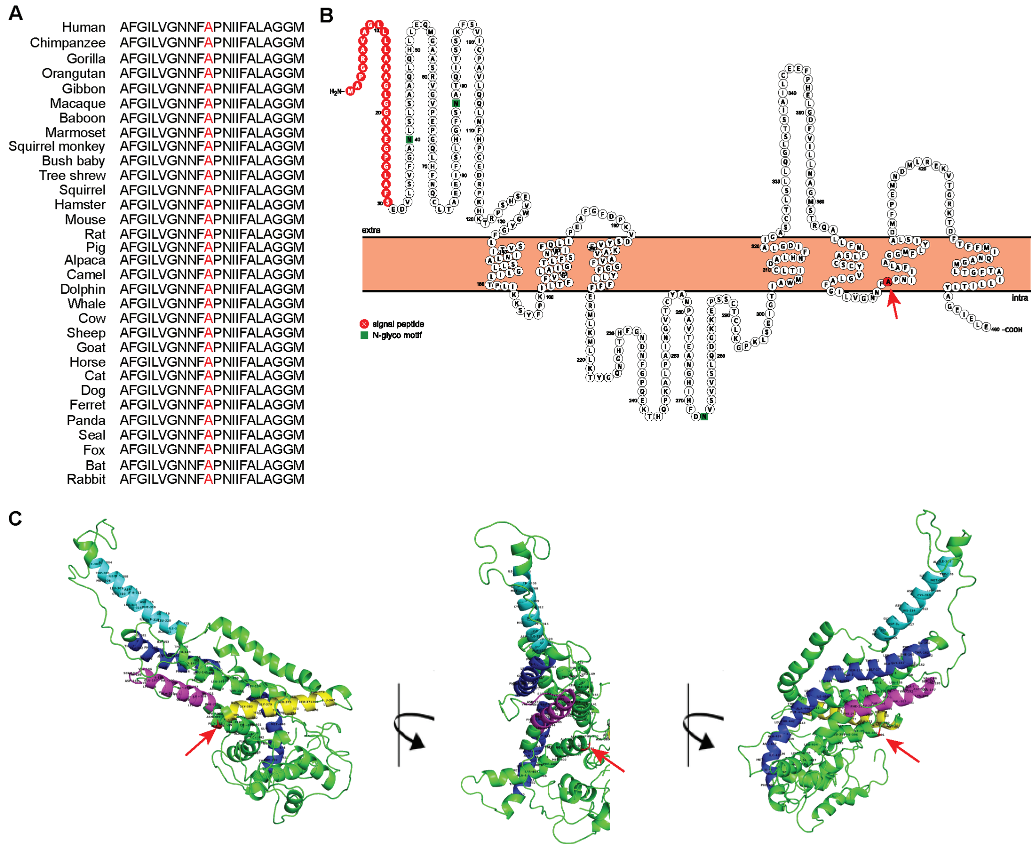

Fig. 2. Localization of the amino acid (p391) encoded by rs13107325 at the protein level. (A) The amino acid encoded by rs1310735 is completely conserved in vertebrates, with all of the species showing alanine (corresponding to ancestral C-allele). (B) The amino acid corresponds to rs13107325 is located in the sixth transmembrane domain of SLC39A8. (C) 3D protein structure modeling reveals the spatial position of the amino acid encoded by rs13107325.

common risk for schizophrenia in general populations. To test if rs13107325 is also associated with schizophrenia in non-European populations, we first examined the frequency distribution of rs13107325 in Asian and African populations. Strikingly, rs13107325 is monomorphic in Africans and Asians in data from 1000-Human-Genome project, with the schizophrenia risk T-allele completely absent. In populations of Americans, the SNP is polymorphic with a lower frequency of T-allele (0.041) compared with Europeans (0.078), possibly reflects its origin of European.

To further explore the detailed global distributions of rs13107325, we analyzed the allele frequencies of this SNP from 53 world populations in HGDP-CEPH dataset. Intriguingly, the SNP again showed a regional enrichment with the highest frequencies (~10%) in Europe and the Middle East, followed by Central Asia and Egypt, rare in Siberia and totally absent in East Asia, South Asia, Southeast Asia, Oceania, and most area in Africa (figure 1B). This regional distribution pattern suggested that the T-allele occurred after the out-of-Africa scenario and might be originated in the Middle East or Europe, with a recent origin (less than 40 000 years before present).[54]

The Schizophrenia Risk T-allele of rs13107325 is Under Positive Selection in Europeans

The regional enrichment of T-allele of rs13107325 in Europeans can be explained either by population substructure due to random genetic drift, or by recent positive selection in regional populations such as Europeans. LRH tests are useful for detecting partial selective sweeps, particularly with allele frequencies as low as ~10%,[55] which are suitable for the SNP rs13107325. Therefore, we calculated iHS of rs13107325, which is a statistic based on the extent of decay of LD surrounding a variant subjected to natural selection.[29–32] The iHS analysis in 379 European individuals from 1000-Human-Genome revealed that the derived T-allele has experienced recent positive selection (iHS = −2.24, $P < .05$), and located the second strongest signal among 322 common SNPs (minor allele frequency > 0.05) in this entire genomic region (figure 3).

We then compared the patterns of LD decay in Europeans from 1000-Human-Genome using plots depicting the EHH and REHH by defining rs13107325 as the core SNP. EHH analysis revealed that the haplotypes carrying the derived T-allele of rs13107325 decay slower than the ancestral allele, indicating unusually long haplotypes carrying this derived allele ($P < .05$, by using

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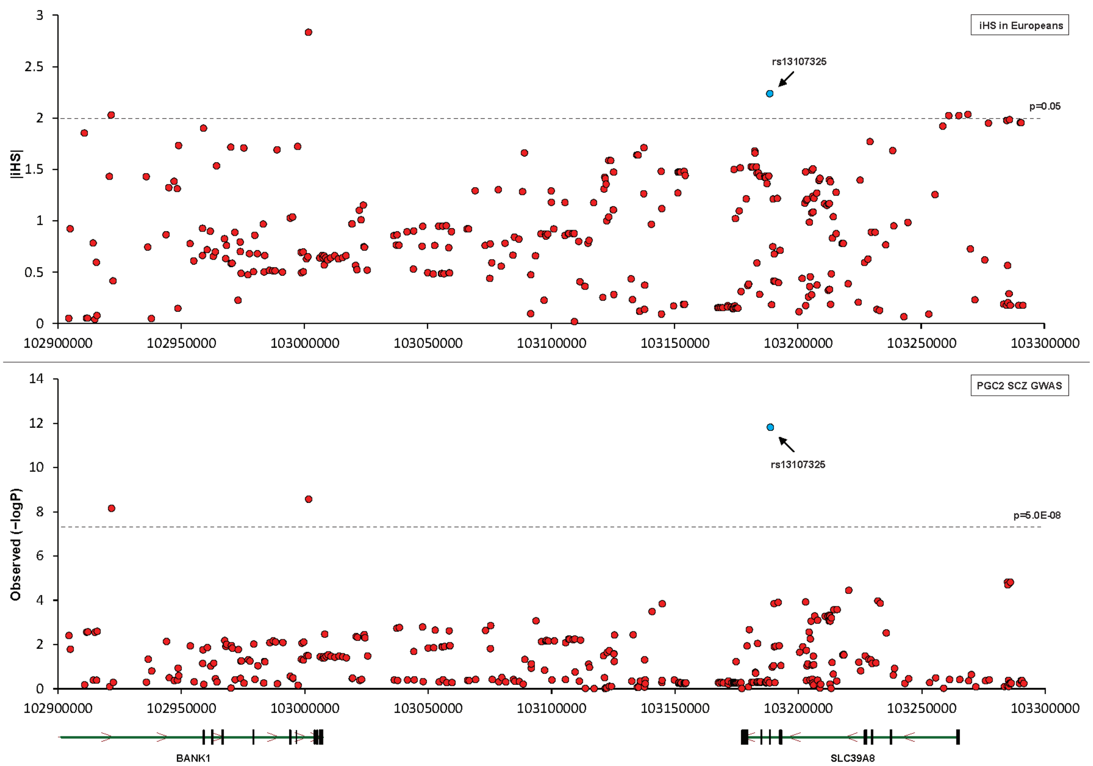

Fig. 3. The iHS and association results with schizophrenia in PGC2 study for the SNPs in the genomic region of chr4:102900000–103300000 in European populations.

1-NORMSDIST) (figure 4A). Consistently, the REHH values of haplotypes carrying the T-allele continue to rise to high levels with increasing distance from the core SNP rs13107325, and reaches 10 to the downstream and upstream in a short distance, which is an indication of positive selection ($P < .03$, by using 1-NORMSDIST) (figure 4B). In contrast, the REHH value of the other haplotype carrying the C-allele is sustained at a similar level (~0) across the genomic distance and shows no sign of selection.

The positive selection of rs13107325 was also supported by analysis in independent HGDP-CEPH populations. In this dataset, the iHS of rs13107325 are −2.43 ($P < .05$) and −3.23 ($P < .01$) in populations from Europe mainland (118 subjects) and Middle East (176 subjects), respectively, and the EHH and REHH analyses got similar results with 1000-Human-Genome and further confirmed the action of positive selection on the T-allele of rs13107325 ($P < .03$ in Middle East and $P < .1$ in Europe mainland, by using 1-NORMSDIST) (figure 4C–F).

Network analysis using *SLC39A8* haplotypes derived from the 54 SNPs spanning a 4-kb region encompassing rs13107325 also supported the action of positive selection. We observed a European-dominant haplotype (marked as "positive selected haplotype" in figure 5), which is common in Europeans (7.8%), but totally or almost absent in Asians and Africans. This European-dominant haplotype is defined by the T-allele of rs13107325. Collectively, the LRH test and haplotype network supported that the T-allele of rs13107325 is under recent positive selection, leading to its prevalence in Europeans.

Pleiotropic Effects of rs13107325 T-allele on Human Phenotypes

The risk allele in rs13107325 underwent recent positive selection is remarkable, given that schizophrenia is associated with reduced evolutionary fitness (eg, reproductive disadvantages). We speculated there might be pleiotropic effects of rs13107325 (or *SLC39A8*) on other human complex traits or diseases driving the positive selection and expansion of the risk T-allele. Intriguingly, our exploratory analyses revealed several striking findings (Table 2): (1) the T-allele of rs13107325 is significantly associated with reduced blood pressure phenotypes and decreased risk of hypertension (P-value ranges from 2.69×10^{-3} to 2.30×10^{-17});[42,43] (2) the T-allele is associated with higher BMI ($P = 1.50 \times 10^{-13}$) and increased risk of obesity ($P = 9.03 \times 10^{-5}$);[47] (3) the T-allele is associated with higher caloric intake from protein (Meta $P = .0027$ without BMI adjustment);[49,50] (4) the T-allele is associated with decreased HDL cholesterol ($P < 1.0 \times 10^{-10}$ in Willer *et al.* study[53] and $P = 7.0 \times 10^{-11}$ in Teslovich *et al.* study[52]). However, the SNP is not associated with coronary artery disease ($P = .91$) or type 2 diabetes ($P = .38$).[56–58]

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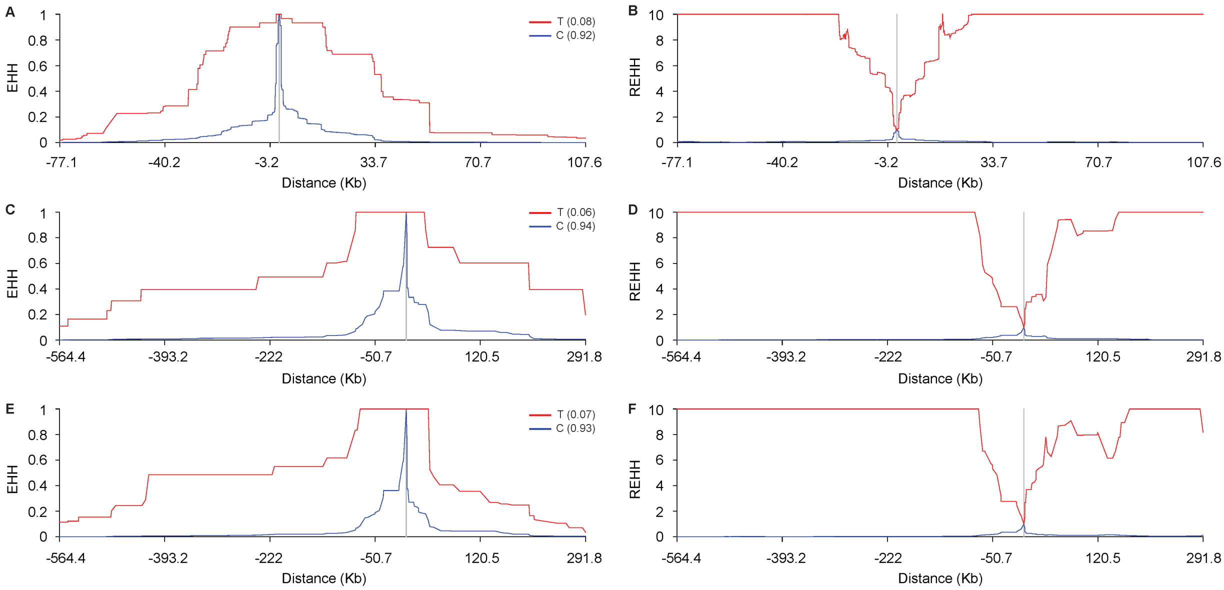

Fig. 4. Extended haplotype homozygosity (EHH, A) and relative EHH (REHH, B) plots of core SNP rs13107325 in European populations from 1000-Human-Genome; EHH and REHH plots of core SNP rs13107325 in populations from European mainland (C and D) and Middle East (E and F) from HGDP-CEPH databases. The EHH and REHH values are plotted against the physical distance extending both upstream and downstream of the selected core region.

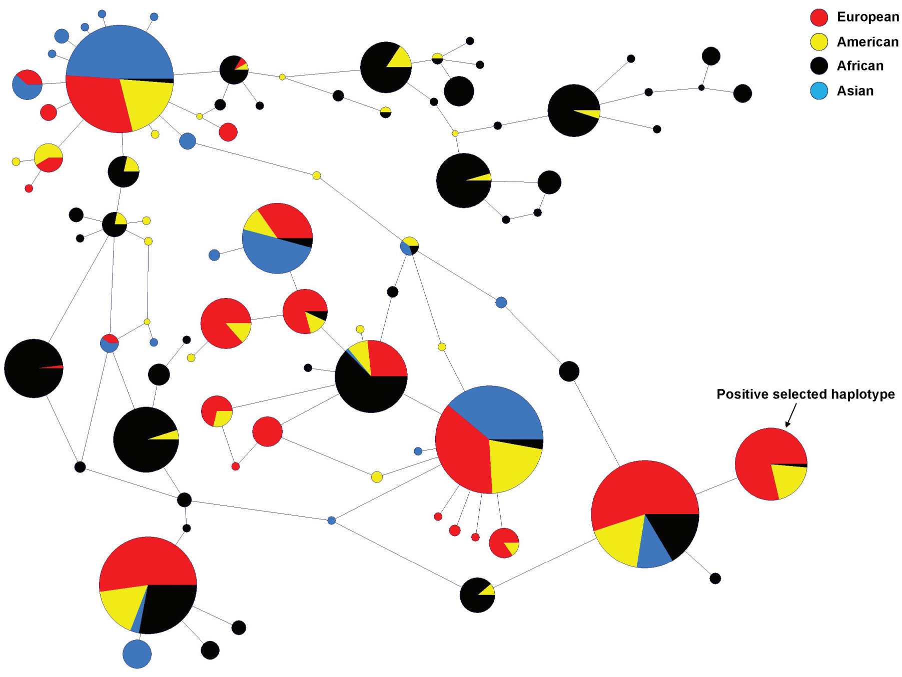

Fig. 5. Haplotype networks consisting of 54 SNPs spanning a 4-kb region encompassing rs13107325. Each node represents a haplotype, and the size is proportional to its frequency.

Further detailed analyses using extensive common SNPs (N = 322) in this genomic region found that rs13107325 is most significantly associated with SBP and DBP as well as BMI in European populations (figure 6), which further support the importance of this SNP in human development and diseases, and lead us to speculate that the T-allele of rs13107325 might be beneficial in some aspects during periods of human evolution.

Discussion

Primary Findings About rs13107325 and SLC39A8

In the present study, we observed recent positive selection on a nonsynonymous SNP rs13107325 in *SLC39A8* in populations of European ancestry, resulting in an increased frequency of the derived T-allele. As shown in figure 1B, the frequencies of rs13107325 T-allele was

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Table 2. Pleiotropic Effects of rs13107325 on Human Complex Traits in Europeans

Study	Traits	Sample Size		Allele	Frequency	Beta Coefficient (or OR)	*P*-value
		Initial	Replication				
Ehret *et al.*[42]	DBP	69 395	133 361	T	0.05	−0.684 mm Hg	2.30E-17
	SBP	69 395	133 361	T	0.05	−0.981 mm Hg	3.30E-14
	Hypertension	69 395	133 361	T	0.05	−0.105 unit	4.90E-07
Wain *et al.*[43]	MAP	63 443	46 594	T	0.117	−0.633 mm Hg	1.30E-10
	PP	63 443	46 534	T	0.117	−0.288 mm Hg	2.69E-03
Speliotes *et al.*[47]	BMI	123 348	122 030	T	0.07	0.19 kg/m^2	1.50E-13
	Obesity	78 427	—	T	0.07	1.098	9.03E-05
Willer *et al.*[53]	HDL cholesterol	94 595	93 982	T	0.08	−0.071 unit	1.10E-15
	Triglycerides	94 595	93 982	T	0.08	0.0309 unit	3.98E-05
Teslovich *et al.*[52]	HDL cholesterol	99 900		T	0.07	−0.84 mg dl^{-1}	7.00E-11
Tanaka *et al.*[50]	Energy intake[a]	38 360		T	0.0832	0.1081	3.17E-02
Chu *et al.*[49]	Energy intake[a]	33 532		T	0.0732	0.1011	3.66E-02
Meta-analysis	Energy intake[a]	71 892		T	—	0.1045	2.70E-03

Note: DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure (a weighted average of SBP and DBP); PP, pulse pressure (the difference between SBP and DBP, a measure of stiffness of the main arteries); BMI, body mass index; HDL, high-density lipoprotein.

[a]Percentage of total caloric intake from protein; statistical analysis was performed by covaring sex, age, and sub-population stratification.

maintained at ~10% in Europeans, but rare or absent in most other world populations, indicating a clear regional distribution pattern and a relatively young origin. Considering that modern humans migrated to Europe mainland about 40 000 years ago,[54] we speculate this positive selection occurred after the ancestors of modern humans have arrived to Europe mainland, since the LRH test finds evidence for a more recent positive selection event (<30 000 years old), and thus the driving force of this selection might be related to European-specific historical events.

SLC39A8 encodes transmembrane transporter protein ZIP8, a member of the family 39 of solute carrier transporters (SLC39).[59,60] ZIP8 transports the divalent cations such as Zn^{2+}, Fe^{2+}, and Mn^{2+} into cells. Zn^{2+}, Fe^{2+}, and Mn^{2+} are essential metals which play important roles in the development and functioning of many organs, including the brain. Loss of SLC39A8 in mice lead to complete preweaning lethality.[61] Besecker *et al.*[62] showed that ZIP8 plays a critical role in zinc-mediated cytoprotection in lung epithelia. In addition to transporting Zn^{2+}, Fe^{2+}, and Mn^{2+} into cells, recent studies also found that SLC39A8 is responsible for cadmium-induced toxicity in the testis.[63,64] Cadmium is a carcinogenic and toxic nonessential metal, ie, abundantly present in cigarette smoke. Long-term exposure to cadmium results in many diseases, including kidney disease, lung disease, and osteomalacia. Expression of ZIP8 in cultured mouse fibroblasts leads to dramatic increase in the rate of intracellular cadmium influx and accumulation, which greatly increased cell death.[59,60]

Speculations About the Potential Selective Pressures

It is well known that one of the most changes of environment when modern humans migrated to Europe mainland is the decreased temperature of climates compared with Africa continent, and some ancestral alleles which had been beneficial in Africa became deleterious to humans in Europe.[65] In such changed conditions, some new alleles arose because of positive selection and protected humans from developing disease or helped humans adapt to the new environment.[66] In this study, it is remarkable to observe that the derived T-allele could reduce blood pressures and risk of hypertension in Europeans, as one of the most prevalent evolutionary hypotheses about hypertension (and blood pressure) is the "sodium hypothesis,"[65,67] which argued that natural selection for sodium conservation in the hot, dry savannah climate (eg, Africa) into which humans first emerged could have resulted in sodium avidity that today is maladaptive. As humans migrated out of Africa, selection pressure for sodium conservation would be expected to decline in the cooler and wetter climates of the northern latitudes (eg, Europe). However, ancestral sodium-conserving genotypes (the called "thrifty genotype") would be expected to persist as the result of genetic drift, and might lead to an increased risk of hypertension in a changed environment of sodium abundance (eg, Europe) if they changed the set point of the renal pressure-natriuresis to a higher blood pressure range. In such case, the new alleles arose and were exposed to positive selection in the cooler and wetter climates of environment (eg, Europe) and could reduce risk of hypertension; this hypothesis has been

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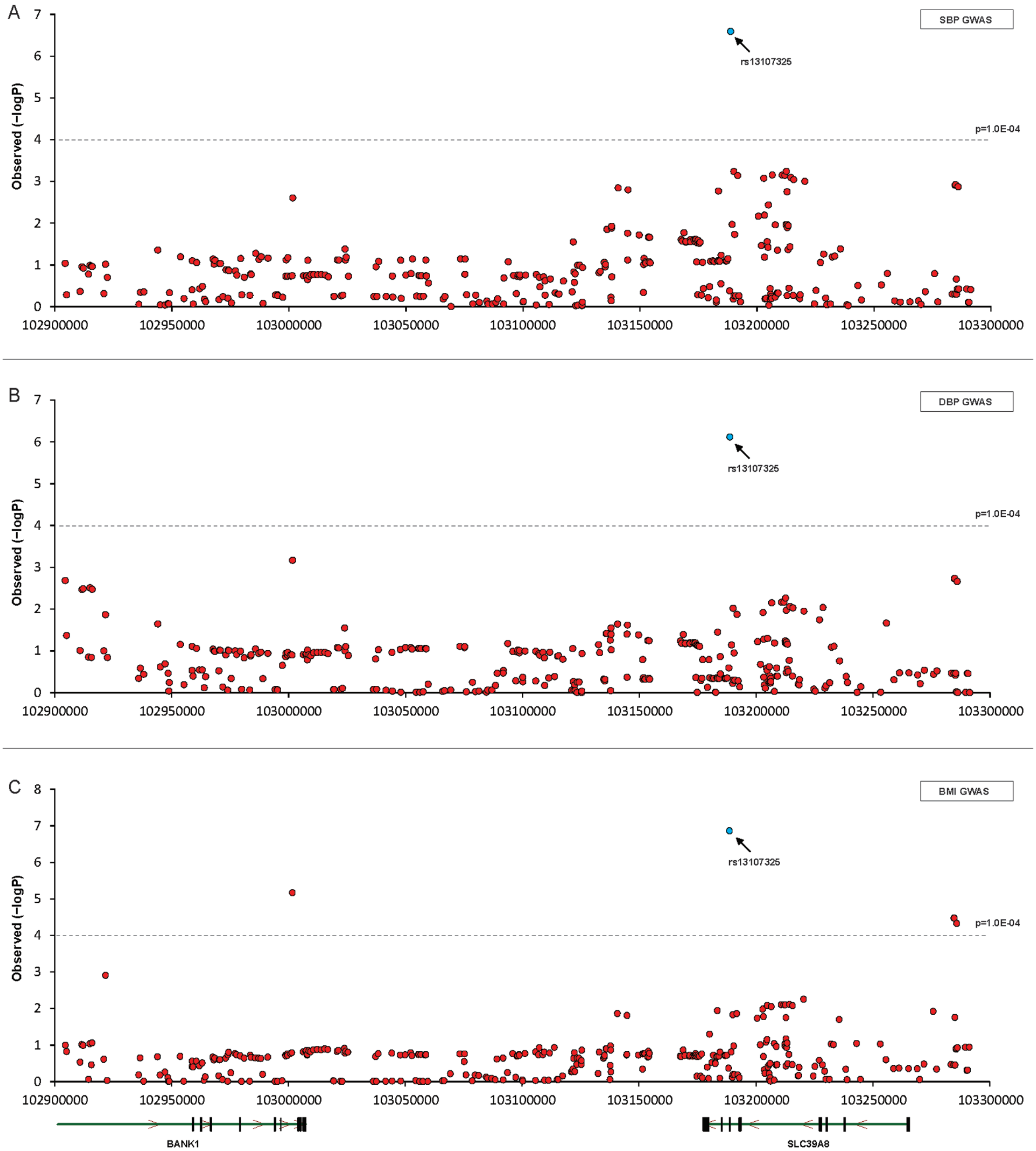

Fig. 6. The association results of SNPs in the genomic region of chr4:102900000–103300000 with human traits. (A) association of SBP in the discovery sample of Ehret *et al.*,[42] (B) association of SBP in the discovery sample of Ehret *et al.*,[42] (C) association of BMI in the discovery sample of Speliotes *et al.*[47]

validated by previous observations on several hypertension candidate genes (eg, *CYP3A* and *AGT*),[68,69] and rs13107325 in *SLC39A8* seems to be another example to fit such positive selection. Therefore, the protective effects of T-allele in rs13107325 from hypertension and blood pressures might reflect one of the driving forces for this positive selection.

A second speculated selective pressure derives from its effects on obesity and energy intake. Although the association of T-allele and increased risk of obesity and related metabolic traits (eg, lower HDL) seems to be harmful for humans, however, considering that Europe mainland is much colder than Africa continent and when modern humans have migrated to this changed environment, the positive selected alleles (eg, rs13107325 T-allele) arose and helped humans to increase their energy intake and expenditure to maintain thermal homeostasis (ie, an optimal body temperature that is most often above their

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ambient temperature) and undergo the long cold temperature period, while the same allele would also contribute to the contemporary increase in obesity rates and related metabolic traits. This speculation is supported by the significant associations with rs13107325 T-allele with higher caloric intake from protein in Europeans (Meta $P = .0027$). We also cannot exclude the possibility that the effect of rs13107325 on higher dietary macronutrient intake reflects another selective pressure, while its association with obesity is likely a by-product of higher energy intake. However, we are a bit cautious in the interpretation of this hypothesis because it might not be able to fully explain why the derived T-allele is rare in Siberia (extreme cold), but is common in the Middle East area, and further studies are needed to improve this hypothesis.

Conclusions and Implications

Our data provide evidence of positive selection on a schizophrenia risk SNP rs13107325 in the *SLC39A8* gene, and we propose a hypothesis about the relationship among positive selection of host alleles, schizophrenia, hypertension, energy intake, and the unique history of Europeans (figure 7). The positive selected risk T-allele might be beneficial for humans to better adapt to the Europe environment, however, as a by-product of pleiotropic effect, ie, increased susceptibility to schizophrenia among populations carrying the same allele, which further support the hypothesis that schizophrenia is likely the by-product of human evolution. To beyond, we believe that the evolutionary advantages of schizophrenia risk alleles caused by positive selection would not be restricted within single population, and there may also be additional selective pressures to drive the expansion of the risk alleles.

It is noteworthy to observe that the schizophrenia risk T-allele at rs13107325 could reduce risk of several metabolic syndromes (eg, hypertension and blood pressure), because metabolic syndromes are highly prevalent in individuals with schizophrenia.[70] This pleiotropic effects implied that *SLC39A8* (T-allele) might be a good drug target for metabolic syndromes, especially in schizophrenia patients. On the other hand, side effects related to metabolic syndromes should also be considered when developing antipsychotics drugs and therapies targeting *SLC39A8* in schizophrenia patients.

In summary, our evolutionary and genetic analyses may offer a unique and powerful opportunity to bring proximate and ultimate approaches together to discover how and why human diseases (eg, schizophrenia) risks have evolved.[19] It is likely that schizophrenia risk variants acted as a double-edged sword in the evolutionary history of humans, ie, genetic variants that contribute to schizophrenia risk may also bring compensatory advantages to humans. The primary goals of medicine are the prevention, alleviation, or repair of the phenotypes or diseases that humans consider maladaptive, via well-substantiated therapies. As such, the uncertainties of the most purported evolutionary insights into human health concerns usually preclude consideration serious enough to warrant clinical evaluation, and provide guidance for future researches and therapies.

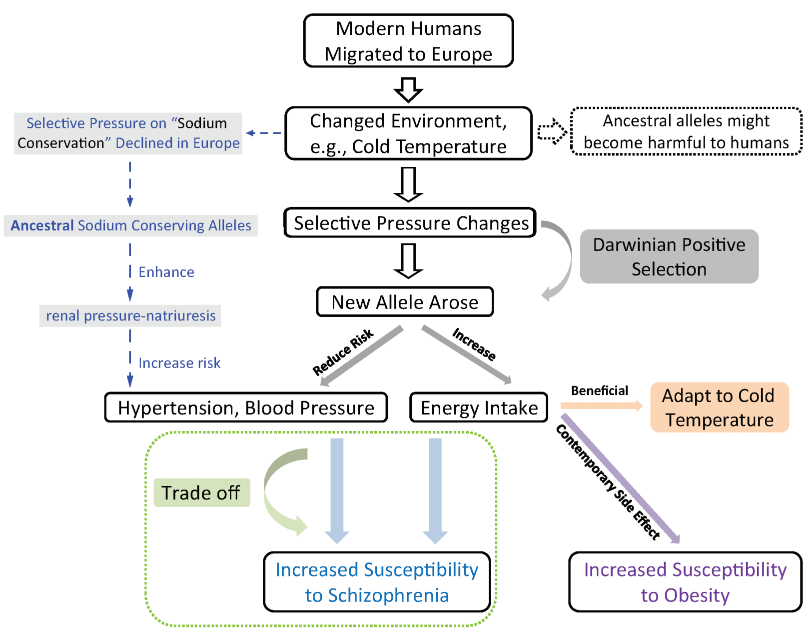

Fig. 7. Hypothesis about positive selection and schizophrenia in European populations.

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