Genetic association of the \textit{EGR2} gene with bipolar disorder in Korea

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Abbreviations: ANK3, ankyrin G; BD, bipolar disorder; CNS, central nervous system; DIGS, Diagnostic Interview for Genetic Studies; EGR, early growth response gene; HWE, Hardy-Weinberg equilibrium; LD, linkage disequilibrium; LTD, long-term depression; LTP, long-term potentiation; MAF, minor allele frequency; MeCP2, methyl-CpG-binding protein 2; NMDA, N-methyl D-aspartate; SPR, schizophrenia

Abstract

The \textit{early growth response gene 2 (EGR2)} is located at chromosome 10q21, one of the susceptibility loci in bipolar disorder (BD). \textit{EGR2} is involved in cognitive function, myelination, and signal transduction related to neuregulin-ErbB receptor, Bcl-2 family proteins, and brain-derived neurotrophic factor. This study investigated the genetic association of the \textit{EGR2} gene with BD and schizophrenia (SPR) in Korea. In 946 subjects (350 healthy controls, 352 patients with BD, and 244 with SPR), nine single nucleotide polymorphisms (SNPs) in the \textit{EGR2} gene region were genotyped. Five SNPs showed nominally significant allelic associations with BD (rs2295814, rs61865882, rs10995315, rs2297488, and rs2297489), and the positive associations of all except rs2297488 remained significant after multiple testing correction. Linkage disequilibrium structure analysis revealed two haplotype blocks. Among the common identified haplotypes (frequency > 5%), 'T-G-A-C-T (block 1)' and 'A-A-G-C (block 2)' haplotypes were over-represented, while 'C-G-G-T-T (block 1)' haplotype was under-represented in BD. In contrast, no significant associations were found with SPR. Although an extended analysis with a larger sample size or independent replication is required, these findings suggest a genetic association of \textit{EGR2} with BD. Combined with a plausible biological function of \textit{EGR2}, the \textit{EGR2} gene is a possible susceptibility gene in BD.

Keywords: bipolar disorder; EGR2 protein, human; genes, immediate early; genetic association studies; schizophrenia

Introduction

\textit{Early growth response (EGR)} genes, which include \textit{EGR1}, \textit{EGR2}, \textit{EGR3}, and \textit{EGR4}, encode DNA-binding transcription factors that contain cys\textsubscript{2}-his\textsubscript{2} zinc fingers (Beckmann and Wilce, 1997; O’Donovan et al., 1999). The \textit{EGR} genes encode a family of immediate early gene transcription factors that mediate the transcription of various genes related to neuronal development and plasticity, cognition, circadian rhythm, and social behaviors (Beckmann and Wilce, 1997; Morris et al., 1998; O’Donovan et al., 1999; Davis et al., 2003; Knapska and Kaczmarek, 2004; James et al., 2006; Baumgarten et al., 2008; Poirier et al., 2008), which have been implicated in the pathophysiology of schizophrenia (SPR) and bipolar disorder (BD). \textit{EGRs} are involved in rodent models of psychotic disorders...
(Gallitano-Mendel et al., 2007, 2008), as well as in the action mechanisms of antipsychotics and electroconvulsive seizures (Yamagata et al., 1994; Jung et al., 1996; Verma et al., 2007).

Moreover, downregulation of EGR1, EGR2, and EGR3 transcripts in the postmortem brains of patients with SPR has been reported (Yamada et al., 2007). In particular, EGR3 has a strong, consistent association with SPR in both family-based and case-control association studies in Japanese cohorts (Yamada et al., 2007). We replicated this in an independent association study in Korea (Kim et al., 2010b), while a negative association has been reported in a Chinese population (Liu et al., 2010). Additionally, a genetic association between EGR3 and bipolar disorder was recently reported (Mansour et al., 2009).

Although EGRs encode closely related transcription factors that contain cysteine-zinc fingers that can bind to the same cognate GC-rich consensus DNA binding motif, individual EGR genes have little sequence homology outside the common DNA-binding domain. Furthermore, they are regulated by different signal pathways and serve different functions (Beckmann and Wilce, 1997; Herdegen and Leah, 1998; Poirier et al., 2008). In certain circumstances, they may even have antagonistic functions; for example, Egr1 and Egr2 exert opposing influences on adipocyte differentiation (Boyle et al., 2009) and T cell receptor-induced changes in T cell function (Collins et al., 2008). Additionally, Egr2 has been reported to have different effects on cognitive function than other Egr genes (Poirier et al., 2007).

The human EGR2 gene resides at chromosomal location 10q21.3, which the linkage analysis has identified as a susceptibility locus for BD (Venken et al., 2010). The linked regions could contain multiple susceptibility genes showing association (Straub and Weinberger, 2006). For example, ankyrin G (ANK3), one of the most promising susceptibility genes for BD, is also located at chromosome 10q21 (Ferreira et al., 2008; Schulze et al., 2009; Smith et al., 2009). On the other hand, Egr2 is involved in regulating Bcl-2 family protein and mitochondrial function (Unoki and Nakamura, 2003; Lauritsen et al., 2008), which are thought to be involved in the pathophysiology of BD (Kato, 2006; Marmol, 2008; Andreazza et al., 2010; Kim et al., 2010a). These findings suggest the possible involvement of EGR2 in BD.

EGR2 plays important roles in peripheral nerve myelination, T cell maturation, hindbrain segmentation, and lipid biosynthesis (Schneider-Maunoury et al., 1993; Swiatek and Gridley, 1993; Topilko et al., 1994; Leblanc et al., 2005). As noted above, the involvement of Egr2 in cognitive behavioral phenotypes have been reported. An attention set shifting task induced the expression of Egr2, but not Egr1 or Egr3, in the frontal cortex of mice (DeSteno and Schmauss, 2008). Forebrain-specific Egr2-deficient mice demonstrated enhanced long-term object recognition memory and superior implicit motor skill learning abilities (Poirier et al., 2007), in sharp contrast to the behavioral phenotypes of Egr3-deficient mice, which had poor long- and short-term memory (Gallitano-Mendel et al., 2007). These findings suggest that EGR2 has different effects on brain functions than other EGR genes.

Given the possible relationship between EGR2 and BD, we investigated the association of EGR2 with BD in a Korean population. We also extended the investigation to the association of EGR2 with SPR, which showed negative associations in previous Korean and Chinese case control association studies (Kim et al., 2010b; Liu et al., 2010) and positive findings in a family-based association study done in Japanese population (Yamada et al., 2007).

Results

Nine SNPs within the 15-kb region surrounding the EGR2 gene were selected as described in Methods. SNP information, including the relative location of the SNPs on the EGR2 gene, is presented in Figure 1. Of the nine investigated SNPs of EGR2, rs2295814 (SNP3), rs61865882 (SNP4), rs10995315 (SNP6), rs2297488 (SNP7), and rs2297489 (SNP8) showed significant allelic associations with BD ($P = 0.0027, 0.0008, 0.0020, 0.0103, and 0.0011$ for each SNP, respectively). These SNPs also showed genotypic associations with BD ($P = 0.0026, 0.0011, 0.0068, 0.0240, and 0.0048$ for each SNP, respectively). The frequencies of the A, C, A, A, and G alleles in SNP 3, 4, 6, 7, and 8, respectively, were significantly increased in the BD group compared to the controls, and all withstood multiple testing correction with 10,000 permutations ($P_{\text{permutation}} = 0.0164, 0.0056, 0.0127, 0.0460, and 0.0074$ for each SNP, respectively). Additionally, the positive associations with BD in SNP 3, 4, 6, and 8 remained significant after the Bonferroni correction ($P < 0.0028$). The other four SNPs of EGR2 [rs9990 (SNP1), rs1509963 (SNP2), rs45602133 (SNP5), and rs224278 (SNP9)] did not show significant associations with BD in allele-wise or genotype-wise analyses. Additionally, no significant association was found between the examined EGR2 SNPs and the SPR group. Association results for rs2295814
| SNP    | Group | N   | Allele 1/2 | Allele frequency (%) | OR   | $\chi^2$ | $P$ | $P_{\text{permutation}}$ | Genotype 1/2/3 | Genotype frequency (%) | $\chi^2$ | $P$ | HWE |
|--------|-------|-----|------------|----------------------|------|---------|----|--------------------------|-----------------|------------------------|---------|-----|-----|
| SNP1   | CTR   | 350 | C/T        | 58.3                 | 41.7 |         |    |                          | CC/CT/TT        | 34.4                   | 47.9     | 17.8 | 0.5513 |
| rs9990 | BD    | 352 | C/T        | 54.0                 | 46.0 | 1.193   | 2.6714 | 0.1022 | 0.4195 | GG/GA/AA | 45.7 | 44.3 | 12.1 | 0.3995 |
| SNP2   | CTR   | 350 | G/A        | 65.8                 | 34.2 |         |    |                          | GG/GA/AA        | 64.9                   | 35.1     | 1.040 | 0.1224 | 0.7265 | 0.9994 |
| rs150963 | BD   | 352 | G/A        | 64.9                 | 35.1 | 1.040   | 0.1224 | 0.7265 | 0.9994 | GG/GA/AA | 42.6 | 44.6 | 12.8 | 0.1228 | 0.9405 | 0.8819 |
| SNP3   | CTR   | 350 | G/A        | 92.9                 | 7.1  |         |    |                          | GG/GA/AA        | 92.9                   | 7.1      | 1.748 | 8.9817 | 0.0027 | 0.0164 |
| rs2295814 | BD | 350 | G/A        | 88.2                 | 11.8 | 1.748   | 8.9817 | 0.0027 | 0.0164 | GG/GA/AA | 76.6 | 23.1 | 0.3 | 11.8989 | 0.0026 | 0.9037 |
| SNP4   | CTR   | 350 | T/C        | 92.3                 | 7.7  |         |    |                          | TT/TC/CC        | 92.3                   | 7.7      | 1.451 | 3.1172 | 0.0775 | 0.3337 |
| rs6186582 | BD | 352 | T/C        | 86.8                 | 13.2 | 1.451   | 3.1172 | 0.0775 | 0.3337 | GA/GA/AA | 86.3 | 13.1 | 0.6 | 0.4710 |
| SNP5   | CTR   | 350 | T/G        | 88.1                 | 11.9 |         |    |                          | GG/GA/AA        | 88.1                   | 11.9     | 1.270 | 1.2985 | 0.2545 | 0.7578 |
| rs45602133 | BD | 352 | T/G        | 87.5                 | 12.5 | 1.270   | 1.2985 | 0.2545 | 0.7578 | GA/GA/AA | 78.2 | 19.8 | 2.0 | 0.3969 |
| SNP6   | CTR   | 349 | G/A        | 90.4                 | 9.6  |         |    |                          | GG/GA/AA        | 90.4                   | 9.6      | 1.046 | 3.0117 | 0.0807 | 0.9006 |
| rs10995315 | BD | 352 | G/A        | 84.9                 | 15.1 | 1.046   | 3.0117 | 0.0807 | 0.9006 | GA/GA/AA | 74.4 | 24.7 | 0.9 | 13.5684 | 0.0011 | 1.0000 |
| SNP7   | CTR   | 350 | G/A        | 90.1                 | 9.9  |         |    |                          | GA/GA/AA        | 90.1                   | 9.9      | 1.166 | 9.5297 | 0.0020 | 0.0127 |
| rs2297488 | BD | 350 | G/A        | 85.7                 | 14.3 | 1.166   | 9.5297 | 0.0020 | 0.0127 | GA/GA/AA | 79.2 | 20.4 | 0.4 | 2.8926 | 0.2354 | 1.0000 |
| SNP8   | CTR   | 350 | C/G        | 90.4                 | 9.6  |         |    |                          | CC/CG/CC        | 90.4                   | 9.6      | 1.527 | 6.4911 | 0.0103 | 0.0460 |
| rs2297489 | BD | 352 | C/G        | 84.7                 | 15.3 | 1.527   | 6.4911 | 0.0103 | 0.0460 | CC/CG/CC | 71.9 | 25.6 | 2.6 | 10.6791 | 0.0048 | 0.7613 |
| SNP9   | CTR   | 350 | T/C        | 70.1                 | 29.9 |         |    |                          | TT/TC/CC        | 70.1                   | 29.9     | 1.121 | 0.9756 | 0.3233 | 0.8547 |
| rs224278 | BD   | 352 | T/C        | 67.6                 | 32.4 | 1.121   | 0.9756 | 0.3233 | 0.8547 | GA/GA/AA | 45.5 | 44.3 | 10.2 | 1.3887 | 0.4994 | 0.3985 |
| SP2    | 242   |     |            | 70.5                 | 29.5 | 0.979   | 0.0268 | 0.8698 | 1.0000 | GA/GA/AA | 50.6 | 39.7 | 9.6 | 0.0339 | 0.9832 | 0.1477 |

Significant $P$-values are shown in bold. CTR: control, BD: bipolar disorder, SPR: schizophrenia.
Figure 1. Location of the SNPs on the EGR2 gene and the linkage disequilibrium structure of the locus. Graphic representation of the genomic structure and location of the EGR2 SNP sites examined and the linkage disequilibrium (LD) structure of the EGR2 haplotype block. Exons are denoted by boxes, with untranslated regions in white and translated regions in gray. The LD between SNPs was analyzed using the pair-wise LD measures $D'$ and $r^2$ and the haplotype block was constructed using the solid spine haplotype algorithm ($D' > 0.8$). Each diamond either represents the $D'$ or $r^2$ measure of LD ($D'$: darker shades of red represent greater $D'$ values, $r^2$: darker shades of black represent greater $r^2$ values).

Table 2. Analysis of common haplotypes of the EGR2 gene in the patients with BD

| Marker | Frequency Case | Frequency Control | OR | $\chi^2$ | P |
|--------|----------------|-------------------|----|---------|---|
| Block 1 |                |                   |    |         |   |
| rs9990 | C G G T T      | 0.509 0.563 0.805 | 4.126 | 0.0422 |
| rs1509963 | T A A G        | 0.221 0.227 0.966 | 0.075 | 0.7843 |
| rs2295814 | T G A A        | 0.107 0.105 1.021 | 0.017 | 0.8973 |
| rs61865882 | T G A C       | 0.114 0.069 1.736 | 8.548 | 0.0035 |
| rs45602133 | T G A T       | - 0.658 0.696 0.840 | 2.290 | 0.1302 |
| Block 2 |                |                   |    |         |   |
| rs10995315 | G G G C       | 0.182 0.204 0.868 | 1.171 | 0.2792 |
| rs2297488 | G C C T       | 0.138 0.094 1.543 | 6.807 | 0.0091 |

The common haplotypes (frequency > 5%) are shown with significant P-values in bold.
shown). The frequencies of the EGR2 haplotypes in the control and BD groups are summarized in Table 2.

Discussion

In this study, we found a genetic association between EGR2 and BD in Korea. Of the nine EGR2 SNPs examined, five [SNP3 (rs2295814), SNP4 (rs61865882), SNP6 (rs10995315), SNP7 (rs2297-488), and SNP8 (rs2297489)] showed significant allelic and genotypic associations with BD. Additionally, the ‘T-G-A-C-T (SNP1-5)’ and ‘A-A-G-C (SNP6-9)’ haplotypes were over-represented, while the ‘C-G-G-T-T (SNP1-5)’ haplotype was under-represented in the BD subjects. In contrast, there was no association between the examined SNPs of EGR2 and SPR. These findings provide provisional evidence that EGR2 may be a susceptibility gene for BD.

To our knowledge, this is the first report of a positive association of EGR2 with BD. Five SNPs in the EGR2 gene, which showed a LD trend, were found to be associated with BD. Significant positive associations of four EGR2 SNPs (SNPs 3, 4, 6, and 8) remained after the multiple testing correction with permutation analysis and the Bonferroni correction. Minor alleles of the associated SNPs, A, C, A, A, G in SNP 3, 4, 6, 7, and 8, respectively, were over-represented in the BD group. Consistently, the haplotypes containing these alleles, ‘T-G-A-C-T (SNP1-5)’ in haplotype block 1 and ‘A-A-G-C (SNP6-9)’ in haplotype block 2, were significantly over-represented in the BD group. Although an extended analysis with a larger sample size or independent replication is required, these findings suggest that EGR2 may be related to the development of BD.

Of the associated EGR2 SNPs, SNPs 7 and 8 (rs2297488 and rs2297489) shown on intron1 of EGR2. EGR2 expression is upregulated through intronic CpG islands methylation (Unoki and Nakamura, 2003), and the binding of methyl-CpG-binding protein 2 (MeCP2), which plays important roles in neurodevelopment (Gonzales and LaSalle, 2010), to this region upregulates EGR2 expression (Swanberg et al., 2009). The C/G polymorphism in SNP8 is associated with a sequence change from CC to CG [5’-CCACCC(G/C)CCATC-3’], which can add a CpG site for methylation. Changes in nucleotide sequences related to polymorphisms creating additional CpG sites can affect the DNA methylation and transcriptional activity (Moser et al., 2009). These findings suggest a possible function of BD-associated intronic SNPs of EGR2 regulating gene expression via alterations in DNA methylation, which requires further examination.

In the central nervous system (CNS), Egr2 expression is abundant in neurons (Herdegen et al., 1993), and it is expressed strongly in layers II and III of the cortical regions of the brain (Beckmann and Wilce, 1997), where the reduction in neuronal density is most prominent in the postmortem brains of patients with mood disorders (Rajkowska, 2000). Forebrain-specific knockout of Egr2 in mice induced enhanced motor skill learning and long-term memory, indicating an inhibitory role of Egr2 in certain cognitive functions (Poirier et al., 2007, 2008). Egr2 is induced in the prefrontal cortex of mice performing attention set-shifting (DeSteno and Schmauss, 2008), dysfunction of which has been implicated as an endophenotype of BD (Clark et al., 2005). Egr2 is also involved in Nmethyl D-aspartate (NMDA) receptor-mediated long-term potentiation (LTP) and long-term depression (LTD) in the mouse hippocampus (Coba et al., 2008). These findings suggest the involvement of EGR2 dysregulation in the cognitive dysfunction of BD through altered levels in the brain.

The function of Egr2 as an intracellular signaling molecule supports its potential role in BD. Egr2 is involved in the signal pathways possibly responsible for the pathogenesis of psychotic disorders, such as SPR and BD, including intracellular signal transduction related to the neuregulin-ErbB receptor (Jacobson et al., 2004; He et al., 2010), calcineurin (Hildeman et al., 2003; Kao et al., 2009), and brain-derived neurotrophic factor (Glorioso et al., 2006). Neuregulin 1 (NRG1) gene has been reported to be associated with SPR and BD (Stefansson et al., 2002, 2003; Cassidy et al., 2006; Thomson et al., 2007; Georgieva et al., 2008). Egr2 acts as a downstream effector of the neuregulin-ErbB receptor signal, which plays a critical role in peripheral nerve myelination in Schwann cells (Kao et al., 2009; He et al., 2010). In the CNS, a different role of the neuregulin signal in brain development has been suggested, such as guiding axon pathfinding (Birchmeier, 2009). These findings suggest a different role of Egr2 in the CNS as a downstream effector of neuregulin signals, which requires further investigation. Egr2 is also involved in apoptosis and mitochondrial functions via the regulation of Bcl-2 family proteins (Unoki and Nakamura, 2003; Lauritsen et al., 2008), and the involvement of dysregulation of the Bcl-2 family proteins or mitochondrial function in the pathogenesis of BD has been suggested (Kato, 2006; Marmol, 2008; Andreadza et al., 2010; Kim et al., 2010a).
In this study, no association was found with SPR. Recently, we reported the association of EGR3 with SPR in a case-control association study in Korea (Kim et al., 2010b), replicating a previous Japanese report (Yamada et al., 2007). However, two EGR2 SNPs (rs2295814 and rs2297488) did not show positive associations with SPR (Kim et al., 2010b), although they were associated with SPR in a Japanese family-based association analysis (Yamada et al., 2007). Additionally, in the current study, seven more examined EGR2 SNPs did not show associations with SPR. A recent case-control association study in a Han Chinese population also demonstrated that EGR2 did not show a significant association with SPR (Liu et al., 2010). The possible association of EGR2 with SPR and the common and differential involvement of EGR genes, especially EGR2 and EGR3, require further clarification.

As our study had a limited sample size, further extended analysis with a larger sample size or an independent replication study is required to confirm the positive association of EGR2 with BD. The strong association of EGR2 SNPs with BD, withstanding multiple testing corrections and a P value < 0.001 (in the case of rs61865882), are noteworthy, and reduce the chance of this being a false-positive finding and predict future replication (Lohmueller et al., 2003). Although the possible involvement of population stratification was not tested in this study, it seems unlikely in our sample because the Korean population is relatively homogeneous.

In conclusion, we found a genetic association between EGR2 and BD in Korea. Of the nine SNPs of EGR2 examined, five showed nominal significant allelic and genotypic associations with BD, and the positive association of four SNPs remained significant after multiple testing correction. Additionally, the corresponding haplotypes were found to be associated with BD. The human EGR2 gene is located at chromosome 10q21, one of the loci related to the pathophysiology of BD. EGR2 plays important roles in cognitive function, the myelination process, and intracellular signaling, making involvement in the pathogenesis of BD plausible. These findings suggest that the EGR2 gene is related to the pathogenesis of BD.

Methods

Subjects

This study enrolled 946 individuals: 350 controls (174 men, 176 women; mean age 25.9 ± 6.5 years), 352 patients with BD (149 men, 203 women; average age 33.5 ± 12.0 years), and 244 patients with SPR (147 men, 97 women; average age 32.7 ± 7.8 years). The participants were all ethnic Koreans. All patients met the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), criteria for the diagnosis of BD or SPR. Patients were interviewed individually by trained researchers using the Korean translation of the Diagnostic Interview for Genetic Studies (DIGS) (Joo et al., 2004). Meetings with at least two psychiatrists were held regularly to arrive at consensus decisions about the participants’ final diagnoses and to eliminate subjects with organic brain disease, alcohol or drug problems, or other general medical conditions possibly manifesting as psychiatric symptoms. Healthy subjects included in the control group were recruited from hospital staff members and college students who volunteered to participate. Interviews conducted by a psychiatrist enabled the exclusion of subjects with histories or current evidence of psychiatric illnesses, organic mental disorders, substance abuse, or any medical conditions that might lead to mental symptoms. Control subjects who had any first-degree relatives with suspected psychiatric illnesses were also excluded. All subjects in this study were unrelated.

The study protocol was approved by the ethics committee of Seoul National University Hospital, and this study was conducted in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from each patient before enrollment, and only subjects able to provide informed consent were included in the study.

SNPs selection and genotyping

Tag SNPs within the 15-kb region surrounding the EGR2 gene (from 64237 to 64252 kb) were selected in the international HapMap database (Data release #27, http://hapmap.ncbi.nlm.nih.gov). Based on the close genetic backgrounds of the Korean and Japanese populations (Kim et al., 2005; Tian et al., 2008), the genotypes downloaded were restricted to those of the Japanese population of Tokyo, Japan (JPT). Five tag SNPs (rs9990, rs1509963, rs2295814, rs10995315, and rs2242728) with minor allele frequencies (MAF) above 10% and a minimum value of 0.8 for the r² parameter were selected using the tagger algorithm (de Bakker et al., 2005) implemented in Haploview (ver. 4.2) (Barrett et al., 2005). SNP rs2297488, which was included in previous studies of SPR in Japan (Yamada et al., 2007), Korea (Kim et al., 2010b), and China (Liu et al., 2010) was included. Three additional EGR2 SNPs (rs61865882, rs45602133, and rs2297489) with MAF above 10% in East Asian populations in the NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP/) were included in the analysis. SNP information, including the relative location of the SNPs on the EGR2 gene, is presented in Figure 1. DNA was extracted from whole blood samples using a DNA isolation kit (Roche Applied Science, Indianapolis, IN). Genotyping assays were performed using the TaqMan method (Applied Biosystems, Foster City, CA), and high genotyping rates for all markers were achieved. The genotyping failure rates for all markers were less than 1% for the control, BD, and SPR groups. SNPs rs2295814 and rs2297488 in the control and SPR groups were genotyped in our previous study (Kim et al., 2010b),
and are presented together with the current findings.

Statistical analyses

Contingency chi-square tests or Fisher's exact test were performed to compare allele and genotype frequencies between patients and controls. Correction for multiple testing was performed using a correction with 10,000 permutations with the haplotype data. We used Bonferroni correction to avoid false-positive results due to multiple testing. Pair-wise LD of Haplotypes were excluded from the analysis, while frequencies exceeding 5% were included for the analysis. Pair-wise LD statistics and HWE were also assessed with Haploview (ver. 4.2).

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