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Genetic Variation in Imprinted Genes is Associated with Risk of Late-Onset Alzheimer's Disease

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Abstract.
Epigenetic changes including genomic imprinting may affect risk of late-onset Alzheimer's disease (LOAD). There are >100 known imprinted genes and most of them are expressed in human brain. In this study, we examined the association of single nucleotide polymorphisms (SNPs) in 93 imprinted genes with LOAD risk in 1291 LOAD cases and 958 cognitively normal controls. We performed single-site, gene-based, and haplotype analyses. Single-site analysis showed 14 significant associations at $p < 0.01$. The most significant SNP (rs11770199; $p = 0.0003$) in single-site analysis was located on chromosome 7 in the GRB10 gene. Gene-based analyses revealed four significant associations in the *WT1, ZC3H12C, DLGAP2,* and *GPR1* genes at $p < 0.05$. The haplotype analysis also revealed significant associations with three genes (*ZC3H12C, DLGAP2,* and *GPR1*). These findings suggest a possible role of imprinted genes in AD pathogenesis that show specific expression in the brain.

Keywords: Brain, gene expression, imprinting, late onset Alzheimer's disease

INTRODUCTION

Late-onset Alzheimer's disease (LOAD) is a major form of dementia. Both genetic and environmental factors determine its etiology [1]. Until recently, *APOE* was the only established susceptibility gene for LOAD [2]. Since 2009, large scale genome wide association studies (GWAS) have identified more than twenty non-*APOE* susceptibility loci for LOAD (*CR1, BIN1, INPP5D, MEF2C, TREM2, CD2AP, HLA-DRB1/HLA- DRB5, EPHA1, NME8, ZCWPW1, CLU, PTK2B, PICALM, SORL1, CELF1, MS4A4/MS4A6E, SLC24A4/RIN3, FER- MT2, CD33, ABCA7, CASS4*) [3–6]. However, common variation in known LOAD genes explain only ∼24–33% of total phenotypic variance of AD [7, 8]. This indicates the involvement of additional genes that affect the risk of LOAD. An attractive group of candidate genes that might affect the risk of LOAD include those that participate in genomic imprinting. To date, approximately 150 imprinted genes have been reported in mice and humans, representing less than 1% of the genome [9–11]. Imprinted genes are highly expressed in placenta and the brain [12]. In brain, imprinted genes are associated with neurodevelopment and behavior [13].

Genomic imprinting leads to parental origin specific gene expression, also called parent of origin effect [14]. A number of studies have reported parent of origin

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effect in LOAD [15–20]. Epigenetic studies in AD have also shown that methylation process involved in imprinting is altered in AD cases compared to controls [21–24]. Given the role of imprinting in neurodevelopment and also in the pathogenesis of LOAD, the present study examined the association of genetic variation in 93 imprinted genes with the risk of LOAD in a large case-control sample. As all of these genes are known for their temporal expression in brain, we hypothesized that genetic variation in these genes may be associated with LOAD risk due to dysregulation of epigenetic mechanisms.

MATERIALS AND METHODS

Subjects

The case-control sample used in this study is described elsewhere [25]. All subjects were European Americans. The mean age-at-onset (AAO) for LOAD cases ($n = 1{,}440$) was 72.6 ± 6.4 years (65.6% women and 23.5% autopsy-confirmed). Control subjects ($n = 1{,}000$) were 60 years or older (mean age 74.07 ± 6.20 years; 59.8% women and 0.2% autopsy-confirmed). LOAD cases were recruited from the University of Pittsburgh Alzheimer's Disease Research Center (ADRC), all of whom met the National Institute of Neurological and Communication Disorders and Stroke (NINCDS)/Alzheimer's Disease and Related Disorders Association (ADRDA) criteria for probable or definite AD. The University of Pittsburgh ADRC follows a standard evaluation protocol, including medical history, general medical and neurological examinations, psychiatric interview, neuro-psychological testing, and magnetic resonance imaging scan. Controls were cognitively normal subjects that were derived from the same geographical area as the cases. Following standard quality control and exclusion criteria, 2,249 subjects (1,291 LOAD cases and 958 controls) were included in the final analysis. All subjects were recruited with informed consent, and the study was approved by the University of Pittsburgh Institutional Review Board.

Genotyping

A list of 107 human imprinted genes was developed from imprinted gene database (http://www.geneimprint.com) and catalogue of imprinted genes (http://igc.otago.ac.nz). Only 93 imprinted genes were selected for final association analysis based on their representation in refgene database and our genotyped database. The Illumina Omni1-Quad chip was used for genotyping as described by Kamboh et al. [25]. There were a total of 3,401 single-nucleotide polymorphisms (SNPs) present on the Illumina chip in the examined 93 candidate genes.

Statistical analyses

Association of 3,401 SNPs located in 93 imprinted genes was tested using the logistic regression under an additive model adjusting for age, gender, and the first four principal components as covariates using PLINK [25]. Further adjustment was made for *APOE* genotype following initial association tests. Nominal p-values less than 0.05 were considered suggestive evidence of association in these candidate genes

Versatile Gene-Based Association (VEGA) analysis was performed to determine the association of imprinted gene with LOAD. Tagger analysis was performed in Haploview with the following parameters: $r^2 = 0.80$, minor-allele frequency (MAF) <0.01 to identify tagSNPs in each gene [26] (http://www.broadinstitute.org/haploview). This information was used to calculate p-value threshold for significance (Supplementary Table 1).

Haplotype analysis within each gene was done using a sliding-windows approach, including four SNPs per window. The global p-value less than 0.05 was considered evidence for association between the trait and the haplotype window. Only SNPs with allele frequencies of 0.01 and higher in the pooled case-control sample were included in the haplotype analysis. Sliding-window haplotype analysis was performed using *haplo.glm* function in Haplo.Stats R package (version 1.5.5).

RESULTS

Single-locus analysis

We examined the association of 3,401 SNPs in 93 candidate genes with the risk of LOAD. A total of 40 SNPs yielded nominal association ($p < 0.05$) and 14 of them were significant at $p < 0.01$ (Table 1). The most significant SNP, rs11770199 ($p = 0.0003$) was located in the *GRB10* gene on chromosome 7 followed by rs11161237 ($p = 0.0008$) in *ATP10A* and rs6992443 ($p = 0.0009$) in *DLGAP2* on chromosome 8. Among the 14 significant SNPs at $p < 0.01$, 8 revealed protection against AD risk (odds ratio (OR) range = 0.63–0.83) while the remaining 6 were associated with AD risk (OR range = 1.21–1.68).

Table 1
Most significant SNPs in 14 genes in single-locus analysis

Gene	CHR[1]	Base pair position	Most significant SNP	*p*-value	OR[2] (95% CI)[3]
GRB10	7	50653096	rs11770199	0.0003	0.748 (0.64–0.87)
ATP10A	15	23633521	rs11161237	0.0008	1.689 (1.24–2.29)
DLGAP2	8	1637345	rs6992443	0.0009	1.537 (1.19–1.98)
GLIS3	9	4121657	rs1340252	0.0015	1.241 (1.09–1.41)
KCNQ1	11	2504751	rs2283155	0.0017	1.218 (1.07–1.37)
PHACTR2	6	144083752	rs6915880	0.0029	0.829 (0.73–0.93)
ANO1	11	69711744	rs3740722	0.003	0.764 (0.64–0.91)
MAGI2	7	78013532	rs1978326	0.0043	0.658 (0.49–0.87)
WT1	11	32366048	rs5030328	0.0054	0.632 (0.45–0.87)
GPR1	2	206775748	rs16838070	0.0058	1.245 (1.06–1.45)
ZNF331	19	58720819	rs2708771	0.006	0.803 (0.69–0.94)
NTM	11	131287579	rs656644	0.0067	0.812 (0.69–0.94)
ZC3H12C	11	109547367	rs4754425	0.0084	0.836 (0.73–0.95)
SLC22A2	6	160558735	rs2450974	0.0088	1.331 (1.07–1.65)

[1]Chromosomal location. [2]Odds ratio. [3]Confidence Interval.

Gene-based analysis

Gene-based analyses including all SNPs in a given gene, revealed four significant associations (*WT1, ZC3H12C, DLGAP2,* and *GPR1*) at $p < 0.05$ (Table 2). Interestingly, all of these four genes (*WT1, ZC3H12C, DLGAP2,* and *GPR1*) also revealed associations at $p < 0.01$ in single-locus analysis. The most significant association was seen with *WT1* ($p = 0.010$). However, this association did not remain significant after correcting for gene-based multiple comparisons.

Haplotype analysis

Haplotype analysis was performed within four genes (*WT1, ZC3H12C, DLGAP2,* and *GPR1*) that showed significant gene-based associations. Three of the four genes examined (*ZC3H12C, DLGAP2,* and *GPR1*) showed significant haplotype window associations with LOAD (Fig. 1). *ZC3H12C* had 8 significant windows and the best association was observed for window 21 (SNPs rs10891068-rs10891069-rs622822-rs17110877; $p = 0.009801$). *DLGAP2* showed 23 significant windows and the best association was observed for window 58 (SNPs rs4256621-rs4876115-rs6558504-rs17064153; $p = 0.003875$). Only one window was significant in *GPR1* (SNPs rs2010572-rs16838070-rs6715398-rs16838083; $p = 0.024761$). No significant haplotype window was observed in the *WT1* gene (Fig. 1).

DISCUSSION

In this study, we have tested the hypothesis that genetic variation in imprinted genes is associated with LOAD risk. We performed single-locus, gene-based and haplotype analyses on 93 imprinted genes. Single-locus analysis demonstrated 14 significant loci at $p < 0.01$ and four of them also revealed significant associations in gene-based analysis. Four genes that showed significant association in gene-based and single locus analysis include *WT1, ZC3H12C*, *DLGAP2,* and *GPR1*. Although the association signals in these genes do not survive after multiple testings, they deserve attention as they are expressed in the brain and thus are potential candidates for AD. Our single-locus analysis showed that SNPs in two of these genes (*DLGAP2*/rs6992443; *GPR1*/rs16838070) are associated with increased risk for LOAD (OR = 1.24–1.53). Also the significant haplotype windows within these genes contained best SNPs from single-locus analysis. These two genes have been reported as maternally imprinted. *DLGAP2* (disks large-associated protein 2) is highly expressed in the

Table 2
Imprinted gene loci associated with Alzheimer Disease in VEGA analysis

Gene	CHR[1]	SNP	Base-pair location	Total SNPs	No. of tagging SNPs	*p*-value Threshold for significance	*p*-value
WT1	11	rs5030328	32365897–32413657	29	17	0.002941	0.01
ZC3H12C	11	rs4754425	109469296–109547776	30	11	0.004545	0.0227
DLGAP2	8	rs6992443	1436975–1644049	95	60	0.000833	0.0364
GPR1	2	rs16838070	206748284–206791016	17	14	0.003571	0.0371

[1]Chromosomal location.

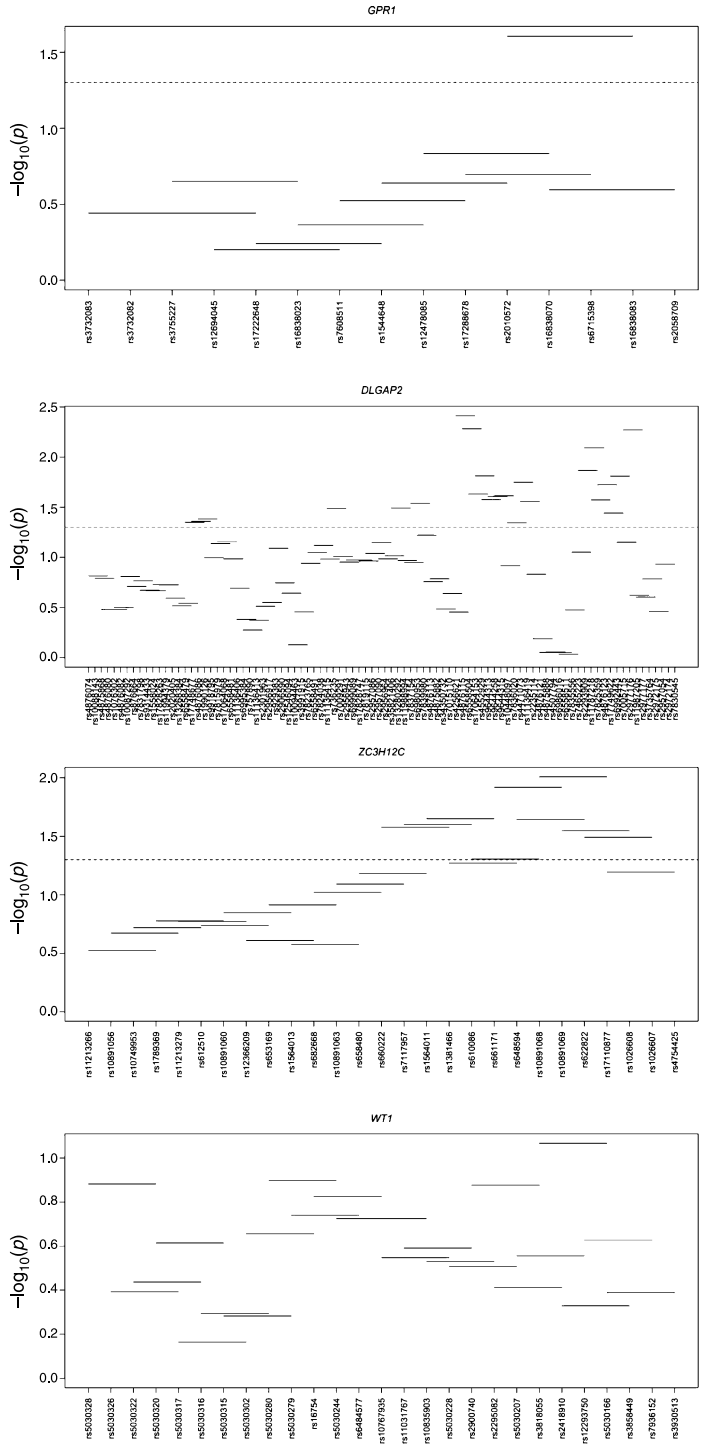

Fig. 1. Haplotype windows showing association with AD. Horizontal lines show the windows tested, with the corresponding SNP rs number along the horizontal axis and global p-value on the vertical axis. Significant association fall above the dotted line at $-\log_{10}(0.05) = 1.3$.

brain [27] and it encodes membrane-associated guanylate kinases that plays a role in molecular organization of synapses and signaling of neuronal cells. *GPR1* (G Protein-Coupled Receptor 1) encodes for a receptor that has a role in regulation of inflammation and is expressed in hippocampus [28].

The other two genes that showed decreased risk for AD were *ZC3H12C*/rs4754425 (OR = 0.84) and *WT1*/rs5030328 (OR = 0.63). *ZC3H12C* showed 8 significant haplotype windows; however, we did not find any significant haplotype window for *WT1*. The *WT1* (Wilms Tumor 1) gene encodes a transcription factor. It plays an important role in cellular development and cell survival. *WT1* has been reported as paternally imprinted in placenta and fetal brain but maternally imprinted in fibroblasts and lymphocytes. It is suggested that neuronal apoptosis plays an important role in the pathogenesis of AD and relevant to this is the observation that levels of Wilms tumor 1 protein are increased during neuronal apoptosis [29]. Zinc Finger CCCH-Type containing 12C (*ZC3H12C*) functions as RNase and it regulates the levels of target RNA species [30].

All the four significant genes in our gene-based analysis are maternally imprinted and thus may provide a mechanism responsible for increased maternal inheritance in AD patients [19]. In this regard, maternally expressed imprinted genes in mice generally, but not always, show their expression in cerebral cortex/hippocampal regions that are associated with cognition and executive functioning [31]. So, it is quite possible that silencing or imprinting of maternal alleles disrupt the cognitive functioning of the brain, and it could lead to neurodegenerative disorders like AD. This is in line with many imprinting disorders where imprinting status of a gene is associated with abnormal brain development and function [32].

Although the imprinted genes in the brain are candidate for AD, the current study does not tell us about the parental origin of single-base changes. However, finding significant associations with selected number of imprinted genes implies that imprinting may be an important mechanism for AD. Additional genetic studies in independent samples are needed to confirm our findings and to comprehensively examine the role of genetic variation in imprinted genes in relation to LOAD risk.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-142106.

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