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Replication of the association between variants in the *IDE-KIF11-HHEX* harboring region on chromosome 10q and plasma amyloid β levels in Alzheimer's disease

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Abstract

Background and Objective—Genetic linkage and association studies in late-onset Alzheimer's disease (LOAD) or LOAD endophenotypes have pointed to several candidate regions on chromosome 10q, among these the ~250kb LD block harboring the three genes *IDE*, *KIF11* and *HHEX*. We explored the association between variants in the genomic region harboring the *IDE-KIF11-HHEX* complex with plasma Aβ40 and Aβ42 levels in a case-control cohort of Caribbean Hispanics.

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HUMAN SUBJECTS: Appropriate approval and procedures were used concerning human subjects and animals.

Methods—First, we performed single marker multivariate linear regression analysis relating the individual SNPs with plasma Aβ40 and Aβ42 levels. Then we performed 3-SNP sliding window haplotype analyses, correcting all analyses for multiple testing

Results—Out of 32 SNPs in this region, three SNPs in *IDE* (rs2421943, rs12264682, rs11187060) were significantly associated with plasma Aß40 or Aß42 levels in single marker and haplotype analyses after correction for multiple testing. As described above, all these SNPs lie within the same linkage disequilibrium block, and are in linkage disequilibrium with the previously reported haplotypes.

Conclusion—Our findings provide modest support for an association in the *IDE* harboring region on chromosome 10q with Aβ 40 and 42 levels.

Keywords

amyloid beta; Alzheimer's disease; genetics; insulin-degrading enzyme

INTRODUCTION

Risk loci for late-onset Alzheimer's disease (LOAD) may be present on chromosome 10q having been identified using case-control status, age-of onset, or plasma Aβ levels as the phenotype. One of the functionally plausible candidate genes lying within the genetic region showing evidence for association or linkage reported by these studies is *IDE* occurring in a ~250kb haplotype block with *KIFF11* and *HHEX*. Following the initial report on linkage and association of markers around *IDE* with LOAD (Bertram et al., 2000), some studies that used LOAD as the phenotype did not find an association (Cousin et al., 2009; Reiman et al., 2007) but other independent studies identified haplotypes spanning the *IDE-KIFF-HHEX* complex that show association with LOAD risk or intermediate LOAD phenotypes (Ertekin-Taner et al., 2004; Prince et al., 2003), including CSF tau levels, MMSE scores, senile plaque and neurofibrillary tangle density, and age-at-onset (Prince et al., 2003). The same haplotypes were associated with plasma Aβ levels in 24 extended Caucasian LOAD families, and with LOAD status in two independent case control series (Ertekin-Taner et al., 2004). Three SNPs in *IDE*, that are in linkage disequilibrium (LD) with these haplotypes, have been shown to influence *IDE* expression in LOAD brains (Zou et al., 2010). The objective in the present paper was to confirm or refute a role of genetic variation in the *IDE-KIF11-HHEX* complex on chromosome 10q in variation of plasma Aβ40 and Aβ42 levels, the main putative culprits in LOAD.

METHODS

Participants

We selected unrelated affected and unaffected individuals from Caribbean Hispanics participating in a population-based study in northern Manhattan (Tang et al., 1998) and single individuals from Caribbean Hispanic families multiply affected by LOAD (Romas et al., 2002). The final analytic sample consisted of 454 Caribbean Hispanic subjects (160 cases, 294 controls) with information on plasma Aβ40 and 42 levels. Dementia diagnosis was based on DSM-IV criteria and NINCDS/ADRDA criteria.

Plasma Aß40 and Aß42 levels

Plasma was obtained at baseline and follow-up, and was stored within 2 hours after collection at −70 °C. Aß40 and Aß42 levels were measured using a combination of monoclonal antibody 6E10 (specific to an epitope present on 1 to 16 amino acid residues of Aß) and rabbit antibodies specific for Aß40 (R162) and Aß42 (R165) in a double-antibody

sandwich ELISA. The detection limit for this assay was 5pg/mL for Aß40 and 10pg/mL for Aß42.

Genotyping

Genotyping was performed using the HumanHap650Y BeadChip from Illumina, Inc. Included in the present analyses were 32 SNPs spanning the *IDE-KIF11-HHEX* region (Supplemental table 1).

Statistical Methods

Regression analyses were performed individually relating the 32 SNPs with Aβ40 and Aβ42 levels, adjusting for sex, age-at-onset or age-at-examination, APOE genotype, education, and population stratification. Finally, we performed three-SNP sliding window haplotype analyses for Aβ40 and 42 levels. We did not correct for multiple testing as all explored SNPs are in strong LD (Figure) and marker-phenotype associations are therefore not independent (Nyholt, 2001).

RESULTS

All SNPs were in HWE. The man age of the sample was 82.2±6.3 years. The A-allele of *IDE* SNP rs2421943 was associated with significantly higher Aß40 levels and the A allele of *IDE* SNP rs12264682 was associated with significantly lower Aß40 levels (Table 1, Supplemental table 2). While the association of rs2421943 with Aß40 was driven by LOAD cases, the association of rs12264682 was driven by the unaffected persons.

Haplotype analyses confirmed these findings. In 3-SNP sliding window haplotype analyses, haplotypes that included the A alleles of SNP rs2421943 (rs11187062|rs11187064|rs2421943: TTA, ß=7.8, p=0.04; rs11187064|rs2421943|rs7908111: TAG, ß=7.9, p=0.03; and rs2421943|rs7908111|rs1999763: AGG, ß=7.8, p=0.03) were associated with higher Aß40 levels, and halotypes that included the A allele of SNP rs12264682 (rs7908111|rs1999763|rs12264682: GGA, ß=−20.5, p=0.02; rs1999763|rs12264682|rs7100623: GAC, ß=−20.5, p=0.02; and rs12264682|rs7100623|rs6583826: ACG, ß=−22.6, p=0.01) were associated with lower Aβ40 levels.

The association with Aß42 levels differed (Table 1). In these analyses, the T allele of *IDE* SNP rs11187060 was associated with lower Aß42 levels in LOAD cases. Also this finding was confirmed by haplotype analyses (rs11187025|rs7078413|rs11187060: CCT, ß=-−9.5, p=0.02; rs7078413|rs11187060|rs11187062: CTT, p=0.07; ß=-−5.6, rs11187060|rs11187062|rs11187064: TTT, ß=-−8.1, p=0.01). Of note, the directions of effects for all three SNPs were similar for Aβ40 and 42 levels, and all three SNPs lie in the same haplotype block as the previously reported SNPs (Figure) (Ertekin-Taner et al., 2004;Prince et al., 2003;Zou et al., 2010). Adjustment for disease duration did not change the associations. When we used AD as the phenotype to explore whether any of the identified SNPs is also associated with the disease phenotype, the C allele of SNP rs11187062 was associated with significantly lower AD risk (OR=0.54±0.3, p=0.03). Of note, this SNP is adjacent and only 2kb apart from rs11187060 that is associated with Aβ 42 levels.

DISCUSSION

Three *IDE* SNPs (rs2421943, rs12264682, rs11187060) were significantly associated with changes in plasma Aß40 or Aß42 levels in single marker and haplotype analyses. We used nominal p-values as all assessed SNPs are in strong LD and marker-phenotype associations are therefore not independent (Nyholt, 2001). Of note, while these SNPs have not been assessed in previous studies, they also lie within the same LD block as the haplotypes

reported in previous Caucasian studies (Ertekin-Taner et al., 2004; Prince et al., 2003). In addition, they are in strong LD (D' >90) with the three SNPs that have previously been shown to influence IDE expression in brain samples of 200 LOAD cases (Figure) (Zou et al., 2010).

While SNPs rs2421943 and rs12264682 were associated with changes in Aβ 40 levels, rs11187060 was associated with changes in Aβ42 levels. The directions of associations of all three SNPs were consistent for Aβ 40 and Aβ 42 levels. A likely explanation for the differences in the strengths of the associations of the individual SNPs with Aβ 40 and 42 levels is, that Aβ 42 is a stronger surrogate of pathological changes underlying AD than Aβ 40 which is rather a marker of aging. This note is supported by the fact that the association of SNP rs11187060 with Aβ 42 levels is 10-fold stronger in cases than controls (β: −5.6 vs. −0.5), and that this SNP is in close proximity (~2kb) and strong LD with SNP rs11187062 that is associated with AD. Alternative explanations for differences in the strengths of the associations with Aβ 40 and 42 levels are differences in allele frequencies or power.

IDE binds and degrades Aβ40 and Aβ42 (Perez et al., 2000), and this Aβ degrading activity has been shown to be lower in AD brains than in controls (Perez et al., 2000). In *IDE*-knock–out mice, brain Aβ levels are elevated (Farris et al., 2003), suggesting that IDE activity is one of several factors determining the amount of brain Aβ in vivo. Enhanced IDE activity in *IDE* and *APP* double transgenic mice decreases their brain Aβ levels, and reduces the formation of AD pathology (Leissring et al., 2003). Finally, polymorphisms in *IDE* may also contribute to the risk of type 2 diabetes (Rudovich et al., 2009), which itself is associated with LOAD. Taken together the findings reported here support the possibility that the *IDE-KIF11-HHEX* region on chromosome 10q may contain genetic variants that modify Aβ40 and 42 levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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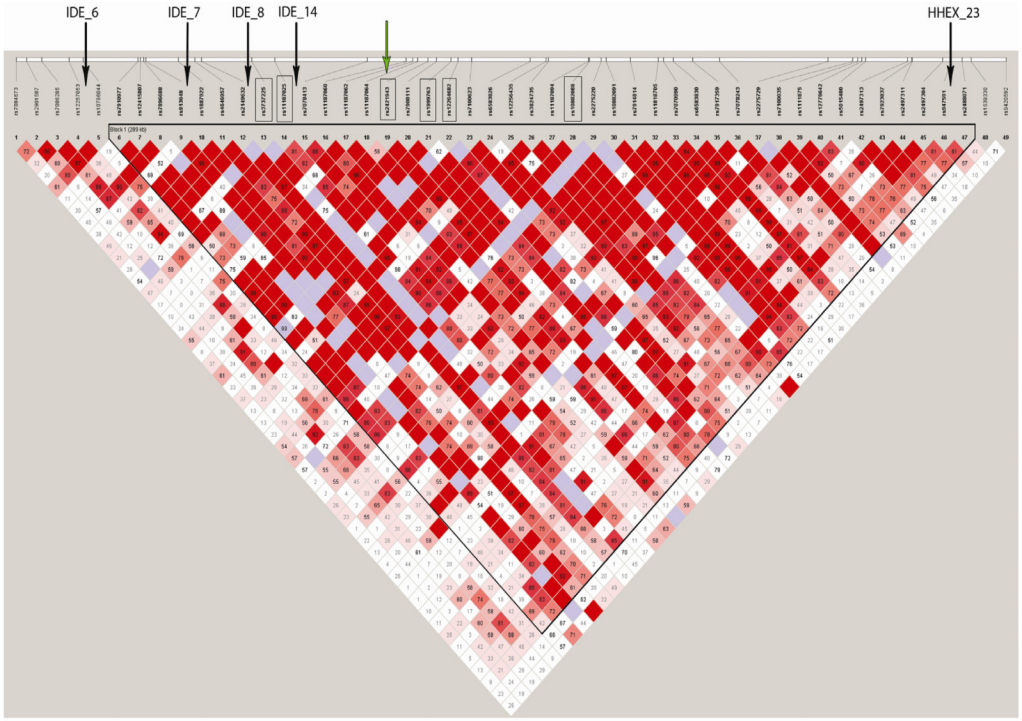

Figure.

LD pattern of the 10q region spanning *IDE*, *KIF11* and *HHEX* in the present sample. The boxes indicate SNPs significant in the present analyses, the black arrows indicate SNPs significant in the study by Ertekin-Taner (2004).(Ertekin-Taner et al., 2004) The green arrow indicates the SNPs that have been shown to influence *IDE*expression in LOAD brain samples inthe study by Zou et al.(Zou et al., 2010)

Table 1

Association between SNPs in the *IDE-KIF11-HHEX* complex and plasma Aβ40 and Aβ42 levels

				ALL (n=454)				Cases (n=160)				Controls (n=294)			
Gene	**SNP**	**BP**	**A1**	**Aβ (mean) by genotype**	**BETA**	**SE**	**P**	**Aβ (mean) by genotype**	**BETA**	**SE**	**P**	**Aβ (mean) by genotype**	**BETA**	**SE**	**P**
Aβ40															
IDE	rs11187060	94294112	T	T/T, T/C, C/C (88.7, 86.8, 94.5)	**−4.5**	**3.9**	**0.3**	T/T, T/C, C/C (100.7, 90.1, 108)	**−8.1**	**6.3**	**0.2**	T/T, T/C, C/C (79.9, 85.1, 86.7)	−2.4	5.0	0.6
IDE	rs2421943	94301795	A	A/A, A/G, G/G (92.62 , 98.1, 83)	**9.4**	**3.8**	**0.01**	A/A, A/G, G/G (112.2, 105.8, 91.43)	**12.3**	**6.5**	**0.05**	A/A, A/G, G/G (83.58 , 93.53, 78.66)	6.8	4.6	0.14
IDE	rs12264682	94312407	A	A/A, A/C, C/C (60.55, 71.1, 92)	**−21.8**	**9.1**	**0.02**	A/A, A/C, C/C (--, 82.75, 101.5)	−21.9	14.9	0.15	A/A, A/C, C/C (60.55, 61.91, 87.02)	**−22.7**	**11.7**	**0.05**
Aβ42															
IDE	rs11187060	94294112	T	T/T, T/C, C/C (36.23, 36.39, 39.83)	−2.3	1.8	0.2	T/T, T/C, C/C (37.02, 38.65, 46.19)	**−5.6**	**2.9**	**0.05**	T/T, T/C, C/C (79.93, 85.1, 86.66)	−0.5	2.3	0.82
IDE	rs2421943	94301795	A	A/A, A/G, G/G (40.4, 37.7, 37.4)	1.3	1.7	0.4	A/A, A/G, G/G (46.7, 43.6, 39.7)	**3.9**	**2.9**	**0.1**	A/A, A/G, G/G (37.4, 34.3, 36.2)	0.2	2.1	0.9
IDE	rs12264682	94312407	A	A/A, A/C, C/C (39.1, 30.9, 41.4)	−7.1	4.2	0.09	A/A, A/C, C/C (-, 32.2, 42.9)	**−11.5**	**6.8**	**0.09**	A/A, A/C, C/C (39.1, 30.0, 40.1)	−4.6	5.3	0.3

For all 3 models, gender, age-at-onset/examination, education, population stratification and APOE genotype (presence vs. absence) were included as covariates. For the model combining cases and controls, affection status was included as an additional covariate.