No association between a previously reported OLR1 3’ UTR polymorphism and Alzheimer’s disease in a large family sample

L Bertram, M Parkinson, K Mullin, R Menon, D Blacker, R E Tanzi

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cently, two studies reported independent evidence of genetic association between a 3’ UTR single nucleotide polymorphism (SNP; rs1050283, also known as “+1073”) in the oxidised LDL receptor 1 gene (OLR1) on chromosome 12p13.2 and risk for Alzheimer’s disease (AD). The implied chromosomal area is a highly promising AD candidate region because both genetic linkage and association studies have reported significant signals to two different locations separated by about 40 Mb (about 44 cM): the more proximal region is located near 10 Mb, on 12p13, and contains OLR1 as well as the gene encoding α2-macroglobulin (A2M), while the more distal region near 50 Mb, on 12q13, maps close to the genes encoding LRPI (low density lipoprotein-related protein 1) and TCP2 (transcription factor CP2). All four of these genes have shown independent associations with AD risk, although the results for A2M and LRPI have been controversial (for recent reviews see Bertram and Tanzi and Saunders et al).

For OLR1, Luedecking-Zimmer and colleagues first reported evidence of association with the 3’ UTR SNP (rs1050283) in a white North American case-control sample of more than 1500 subjects. This association showed a strong interaction with APOE (apolipoprotein E) ε4-genotype, and led to opposite effects in subgroups stratified by APOE ε4. Specifically, APOE ε4-positive carriers of the TT-genotype had a 1.7-fold increased AD risk, whereas APOE ε4-negative carriers of the same genotype showed a significantly reduced (OR = 0.7) risk for AD. Performing electrophoretic mobility shift assays (EMSAs) indicated reduced binding of nuclear proteins related to the T-allele of this variant.

The more recently published results by Lambert et al were obtained on a large French case-control sample and a considerably smaller North American family-based sample, and also showed significant effects with this polymorphism. However, no APOE ε4-dependence was observed in either of the samples investigated in the latter study. Furthermore, these authors also saw a significant under-representation of the TT-genotype v the CC/CT-genotypes, and this finding was interpreted as a risk effect in carriers of the ε-allele v non-carriers. Similar to the previous analyses, these genetic results were also supported by some functional data suggesting reduced binding of the T-allele to nuclear and cytoplasmic proteins as compared to the ε-allele as determined by EMSA. While no correlation between this polymorphism and cerebral Aβ or tau load was observed in this study, there was evidence of a significantly reduced expression of OLR1 in carriers of the ε-allele carriers v non-carriers.

Our laboratory has previously tested several AD candidate genes on chromosome 12 for association with AD in one of the largest AD family samples collected to date, the NIMH Genetics Initiative Study sample. Of these, the strongest and most consistent signals observed were obtained with polymorphisms in A2M, which show a strong effect on AD risk, predominantly in families with late-onset AD. Although A2M maps about 1 Mb pter of OLR1, it is conceivable that at least part of the independent association signals observed across our and the OLR1-positive studies is due to polymorphisms in linkage disequilibrium (LD) with genetic variants in these two genes. Thus, we have tested the OLR1 SNP in the full NIMH sample, which is comprised of 1439 of individuals from 437 families in which all affected individuals had disease onset at >65 years, and “early/mixed” otherwise. These include 994 affected individuals (mean age of onset 72.4 ± 7.7 years, range 50–97), 411 unaffected, and 34 with phenotype unknown. Pedigrees were classified as “late-onset” (320 pedigrees) when all sampled affecteds in each pedigree had onset ages of >65 years, and “early/mixed” otherwise. APOE genotyping was performed as described previously, and pedigrees were classified as “APOE-ε4/4 positive” when at least one affected individual per pedigree carried the ε4/4 genotype (120 pedigrees), and as “APOE-ε4/4 negative” otherwise, and as “APOE-ε4 positive” when at least one affected individual per pedigree carried the ε4-allele (358

Key points

- Chromosome 12 has been implicated to harbour one or more Alzheimer’s disease (AD) loci by several laboratories using both linkage and association methods.
- Two studies recently observed genetic association with a 3’ UTR single nucleotide polymorphism (SNP) in the gene encoding OLR1, which is involved in the binding, internalisation, and degradation of oxidised lipoproteins.
- In a large sample of 437 multiplex AD families (1439 subjects) no evidence was observed favouring a genetic involvement of the same OLR1 SNP in AD, nor was any linkage disequilibrium observed between this SNP and polymorphisms in the nearby A2M gene, which show a strong disease association in the same sample.
- Our results suggest that the contribution of OLR1 to AD risk in the general population may be considerably smaller than previously suggested.

Abbreviations: A2M, α2-macroglobulin; AD, Alzheimer’s disease; APOE, apolipoprotein E; CLR, conditional logistic regression; EMSAs, electrophoretic mobility shift assays; LD, linkage disequilibrium; LRPI, low density lipoprotein-related protein 1; OLR1, oxidised LDL receptor 1
pedigrees), and as “APOE-e4 negative” otherwise (for more details on the NIMH sample see Blacker et al1,4).

Genotyping of rs1050283 was performed on all individuals using a high-efficiency fluorescence polarisation single-base extension (FP-SBE) detection assay, similar to that used for genotyping of most A2M SNPs.7 Genotyping efficiency was at 96% and showed no discrepancies based on ~10% internally duplicated samples. To test for association of this SNP with AD, we used “FBAT” (v.1.4.2), a program for family-based association testing that allows for missing parental genotypes. Like the transmission-disequilibrium test (TDT) and other family-based association tests, this method is not susceptible to bias due to population admixture. For all analyses we used the empirical variance function (EV-FBAT) to account for the presence of linkage in the area, and an equal-weight offset correction to incorporate genotypes from both affected and unaffected individuals (see the FBAT website http://www.biostat.harvard.edu/~fbat/default.html for more details). The results of these analyses are shown in table 1 and did not reveal any evidence of association with either alleles or genotypes, nor did it detect any significant interactions of the SNP with either alleles or genotypes, nor did it detect any significant alleles or genotypes. Finally, none of four haplotype tagging variants in A2M displayed any significant degree of pairwise LD with the OLR1 SNPs (parameterised as D’), suggesting that LD is most likely not responsible for the previously observed independent AD associations reported for these genes.1,3,4,5 This is in agreement with recent estimates indicating that detectable LD in outbred and heterogeneous populations such as these extends from one to several hundred kb, but rarely beyond.10

Unfortunately, the reason for the conflicting findings regarding our negative and the previous positive results remains unclear. While our sample clearly has the power to detect effects of the magnitude discussed for OLR1, we were still unable to observe any evidence supporting a role of the analysed OLR1 SNP (rs1050283) in our AD families. The same families, however, demonstrate strong and consistent association with several variants in the nearby A2M gene.4 Finally, we did not detect evidence of LD between the OLR1 SNP and four polymorphisms in A2M. There are at least three possible explanations for the discrepancies across studies. First, the previous positive associations between OLR1 and/or A2M and AD are chance findings (type 1 errors). This seems unlikely because replications in multiple independent samples have been published for both genes. Second, both genes are in fact genuine AD susceptibility factors, but only exert small effects in the general population which are detectable in some study samples but not in others.1,4,5 This is supported by the fairly small effect sizes estimated for both genes (for example OLR1 ORs about 2 for risk-allele carriers,1 4 and A2M ORs between 1.8 and 3.5 in our sample) as compared to the average ORs of about 3 for heterozygous and about 15 for homozygous carriers of the APOE e4-allele.11) Third, as we do not find evidence of significant LD between the two genes, the independent findings with OLR1 in some AD populations, and A2M in others could also reflect LD with variant(s) in a third gene located somewhere in the vicinity of these two candidates. In this context it is interesting to notice that several other members of the C-type lectin superfamily (to which OLR1 belongs as well)—for example CLEC1 and C-type lectin-like receptors-1 and -2, CLL-1 (C-type lectin protein CLL-1), or LTL1 (lectin-like NK cell receptor-1)—map between OLR1 and A2M (see: “USC Genome Browser” [July 2003 freeze]; http://genome.usc.edu/). More systematic LD-based mapping in additional AD samples is required to further elucidate the role of these and other proposed AD candidate genes on chromosome 12.

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### Table 2

Pairwise linkage disequilibrium of polymorphisms in A2M and OLR1

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>A2M-UTR</th>
<th>A2M-12e</th>
<th>A2M-15i</th>
<th>A2M-18i</th>
<th>OLR1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2M-UTR</td>
<td>−</td>
<td>0.72</td>
<td>1</td>
<td>0.94</td>
<td>0.02</td>
</tr>
<tr>
<td>A2M-12e</td>
<td>***</td>
<td>−</td>
<td>0.86</td>
<td>0.78</td>
<td>0.11</td>
</tr>
<tr>
<td>A2M-15i</td>
<td>***</td>
<td>***</td>
<td>−</td>
<td>1</td>
<td>0.05</td>
</tr>
<tr>
<td>A2M-18i</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>−</td>
<td>0.06</td>
</tr>
<tr>
<td>OLR1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

For a key to A2M polymorphisms see Saunders et al. Cells above the diagonal represent D’ values, and cells below the diagonal represent P values (Fisher’s exact test, two-sided: “NS” indicates P > 0.05; *** indicates P < 0.001). LD statistics calculated with “2by2” (http://linkage.rockefeller.edu/ott/linkutil.html), based on haplotype frequencies in one affected individual per pedigree estimated by the FBAT program.

### Table 1

Association statistics for OLR1 rs1050283 and AD in families of the NIMH sample

<table>
<thead>
<tr>
<th>Sample size, n (families)</th>
<th>Association (FBAT), z score (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>437 (1439)</td>
</tr>
<tr>
<td>APOE e4/4-pos</td>
<td>120 (453)</td>
</tr>
<tr>
<td>APOE e4-pos†</td>
<td>358 (1213)</td>
</tr>
<tr>
<td>APOE e4/4-neg‡</td>
<td>317 (986)</td>
</tr>
<tr>
<td>APOE e4-neg‡</td>
<td>79 (226)</td>
</tr>
<tr>
<td>Late-onset</td>
<td>120 (996)</td>
</tr>
<tr>
<td>Early/mixed-onset</td>
<td>317 (443)</td>
</tr>
</tbody>
</table>

* z score for risk allele (C) of previous studies: frequency in total sample = 0.52. No significant interaction was observed between APOE e4 or e4/4 status and the OLR1 SNP (rs1050283) on chromosome 12 (data not shown); the undertransmission of the C-allele in this stratum did not confer any significant decrease in risk for AD as estimated by CLR [ORs (95% CI): ORC/C-C vs. T/T = 0.2 (0.02 to 2.1); ORC/C-C vs. T/T = 0.7 (0.2 to 2.5); ORC/C-C vs. C/C = 0.7 (0.2 to 2.5)].

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### REFERENCES


The number of adenomatous polyps in somebody with familial adenomatous polyposis (FAP) is influenced by the position of the germline mutation in the APC gene. This, however, does not explain the variability within families which is consistent with the effect of modifier genes. Such genes are known to affect disease severity in a mouse model. Several common polymorphisms may be associated with increased risk of colorectal cancer and could act as APC modifier genes. Researchers in London, UK have studied polymorphisms in eight genes (MTHFR, NAT1, NAT2, GSTM, GSTT, cyclin D1, E-cadherin, and APC) in relation to severity of FAP (number of polyps per colectomy specimen standardised for colon size).

DNA samples (from blood, established cell line, or fixed normal tissue) were obtained from 151 patients from 51 families with established classical FAP. The relationship between polyp count and genotype at each polymorphic site was assessed, making allowance for the position of the germline APC mutation. Among all 151 patients severe disease (high polyp count) was associated with variations in the N-acetyltransferase genes; more specifically with the absence of the NAT1*10 genotype and the presence of both NAT1*non-10 and NAT2*fast genotypes. There was a weak association between severe disease and the APCT1493C allele. None of the other polymorphisms analysed was associated with disease severity. Among patients with germline mutations in the “mutation cluster region” (MCR) severe disease was associated with the presence of NAT2*fast alleles.

Alleles at NAT1 and/or NAT2 loci on chromosome 8p22 may act as disease severity modifiers in patients with FAP and may explain a twofold variation in polyp number.


Modifier genes and familial adenomatous polyposis coli

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