LETTER TO JMG

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

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J Med Genet 2004;**41**:286–288. doi: 10.1136/jmg.2003.016980

J Med Genet: first published as on 1 April 2004. Downloaded from http://jmg.bmj.com/ on September 21, 2019 at MIT Libraries. Protected by copyright.

Recently, two studies[1 2] 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 *LRP1* (low density lipoprotein-related protein 1) and *TFCP2* (transcription factor CP2). All four of these genes have shown independent associations with AD risk, although the results for *A2M* and *LRP1* have been controversial (for recent reviews see Bertram and Tanzi[3] and Saunders *et al*[4]).

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.[1] 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.[2] 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 C-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 C-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 C-allele carriers *v* non-carriers.[2]

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.[3–6] 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.[4 5] 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 >50 years. These include 994 affected individuals (mean age of onset 72.4±7.7 years, range 50–97), 411 unaffecteds, 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,[7] 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; LRP1, low density lipoprotein-related protein 1; OLR1, oxidised LDL receptor 1

pedigrees), and as "*APOE*-ε4 negative" otherwise (for more details on the NIMH sample see Blacker *et al*[7 8]).

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.[4] 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.[9] 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 between this *OLR1* SNP and AD in the full NIMH sample, or in strata based on onset age or *APOE* genotype. In accordance with these findings, conditional logistic regression (CLR) analysis stratified on family showed no significant effects with either alleles or genotypes, nor did it detect any significant interactions of the *OLR1* SNP with age or *APOE* genotype (data not shown). Finally, none of four haplotype tagging variants in *A2M* displayed any significant degree of pairwise LD with the *OLR1* SNPs (parameterised as D′, table 2), suggesting that LD is most likely not responsible for the previously observed independent AD associations reported for these genes.[1 2 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 I 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 2 4] This is supported by the fairly small effect sizes estimated for both genes (for example *OLR1* ORs about 2 for risk-allele carriers,[1 2] and *A2M* ORs between 1.8 and 3.5 in our sample)[4] as compared to the average ORs of about 3 for heterozygous and about 15 for homozygous carriers of the *APOE* ε4-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 *2* (C-type lectin-like receptors-1 and -2), *CLL-1* (C-type lectin protein CLL-1),or *LLT1* (lectin-like NK cell receptor-1)—map between *OLR1* and *A2M* (see: "UCSC Genome Browser" [July 2003 freeze]: http://genome.ucsc.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.

Table 1 Association statistics for *OLR1rs1050283* and AD in families of the NIMH sample

	Sample size, $n_{families}$ ($n_{individuals}$)	Association (FBAT), z score (P)*
Total	437 (1439)	0.4 (0.655)
APOE ε4/4-pos†	120 (453)	0.6 (0.568)
APOE ε4-pos†	358 (1213)	1.0 (0.313)
APOE ε4/4-neg†	317 (986)	0.1 (0.905)
APOE ε4-neg†‡	79 (226)	−1.8 (0.074)
Late-onset	120 (996)	0.2 (0.856)
Early/mixed-onset	317 (443)	0.5 (0.615)

*z score for risk allele (C) of previous studies: frequency in total sample = 0.52; †no significant interaction was observed between *APOE*-ε4 or ε4/4 status and the *OLR1* SNP using CLR stratified on family (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]): $OR_{C/C\ v\ T/T}$ = 0.2 [0.02 to 2.1]; $OR_{C/T\ v\ T/T}$ = 0.7 [0.2 to 2.5]; $OR_{any\text{-}C\ v\ T/T}$ 0.7 [0.2 to 2.5].

Table 2 Pairwise linkage disequilibrium of polymorphisms in *A2M* and *OLR1*

	A2M-UTR	*A2M*-12e	*A2M*-15i	*A2M*-18i	*OLR1*
***A2M*-UTR**	–	0.72	1	0.94	0.02
***A2M*-12e**	***	–	0.86	0.78	0.11
***A2M*-15i**	***	**	–	1	0.05
***A2M*-18i**	***	***	***	–	0.06
OLR1	NS	NS	NS	NS	–

For a key to *A2M* polymorphisms see Saunders *et al*[4].
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⩽0.01; ***indicates P⩽0.001). LD statistics calculated with "2by2" (http://linkage.rockefeller.edu/ott/linkutil.htm), based on haplotype frequencies in one affected individual per pedigree estimated by the FBAT program.

ACKNOWLEDGEMENTS

This work was sponsored by grants from the NIMH, NIA (ADRC) and the Alzheimer Association. LB was a fellow of the Deutsche Forschungsgemeinschaft (DFG) and now receives a translational fellowship of the Harvard Center for Neurodegeneration and Repair (HCNR), Core A.

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Received 28 November 2003
Accepted for publication 15 December 2003

REFERENCES

1 **Luedecking-Zimmer E**, DeKosky ST, Chen Q, Barmada MM, Kamboh MI. Investigation of oxidized LDL-receptor 1 (OLR1) as the candidate gene for Alzheimer's disease on chromosome 12. *Hum Genet* 2002;**111**(4–5):443–51.
2 **Lambert JC**, Luedecking-Zimmer E, Merrot S, Hayes A, Thaker U, Desai P, Houzet A, Hermant X, Cottel D, Pritchard A, Iwatsubo T, Pasquier F, Frigard B,

J Med Genet: first published as on 1 April 2004. Downloaded from http://jmg.bmj.com/ on September 21, 2019 at MIT Libraries. Protected by copyright.

Conneally PM, Chartier-Harlin MC, DeKosky ST, Lendon C, Mann D, Kamboh MI, Amouyel P. Association of 3′-UTR polymorphisms of the oxidised LDL receptor 1 (OLR1) gene with Alzheimer's disease. *J Med Genet* 2003;**40**(6):424–30.
3 **Bertram L**, Tanzi RE. Dancing in the dark? The status of late-onset Alzheimer's disease genetics. *J Mol Neurosci* 2001;**17**(2):127–36.
4 **Saunders AJ**, Bertram L, Mullin K, Sampson AJ, Latifzai K, Basu S, Jones J, Kinney D, MacKenzie-Ingano L, Yu S, Albert MS, Moscarillo TJ, Go RC, Bassett SS, Daly MJ, Laird NM, Wang X, Velicelebi G, Wagner SL, Becker DK, Tanzi RE, Blacker D. Genetic association of Alzheimer's disease with multiple polymorphisms in alpha-2-macroglobulin. *Hum Mol Genet* 2003;**12**(21):2765–76.
5 **Blacker D**, Wilcox MA, Laird NM, Rodes L, Horvath SM, Go RC, Perry R, Watson B, Bassett SS, McInnis MG, Albert MS, Hyman BT, Tanzi RE. Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat Genet* 1998;**19**(4):357–60.
6 **Bertram L**, Blacker D, Crystal A, Mullin K, Keeney D, Jones J, Basu S, Yhu S, Guenette S, McInnis M, Go R, Tanzi R. Candidate genes showing no evidence for association or linkage with Alzheimer's disease using family-based methodologies. *Exp Gerontol* 2000;**35**(9–10):1353–1361.
7 **Blacker D**, Haines JL, Rodes L, Terwedow H, Go RC, Harrell LE, Perry RT, Bassett SS, Chase G, Meyers D, Albert MS, Tanzi R. ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. *Neurology* 1997;**48**(1):139–47.
8 **Blacker D**, Bertram L, Saunders AJ, Moscarillo TJ, Albert MS, Wiener H, Perry RT, Collins JS, Harrell LE, Go RC, Mahoney A, Beaty T, Fallin MD, Avramopoulos D, Chase GA, Folstein MF, McInnis MG, Bassett SS, Doheny KJ, Pugh EW, Tanzi RE. Results of a high-resolution genome screen of 437 Alzheimer's disease families. *Hum Mol Genet* 2003;**12**(1):23–32.
9 **Rabinowitz D**, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered* 2000;**50**(4):211–23.
10 **Wall JD**, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. *Nat Rev Genet* 2003;**4**(8):587–97.
11 **Farrer LA**, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* 1997;**278**(16):1349–56.

ECHO

Modifier genes and familial adenomatous polyposis coli

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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.

▲ *Gut* 2004;**53**:271–276.

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