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Brief communication

Genetic variability at the LXR gene (*NR1H2*) may contribute to the risk of Alzheimer's disease

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Abstract

We have initiated a systematic analysis of the role of cholesterol metabolizing genes as risk factors for Alzheimer's disease pathogenesis. As part of this analysis, we have assessed the *NR1H2* gene on chromosome 19 and report here a modest association with the locus in sibpairs with late onset disease.

Keywords: Alzheimer's disease; Genetics; Cholesterol; LXR gene

Apolipoprotein ε4 allele (*ApoE*) is the only established risk variant for occurrence of late onset Alzheimer's disease (AD) [2,6]. While the mechanism by which it increases risk is not clear, one likely possibility is that it does so through its effect on cholesterol metabolism [19]: circumstantial evidence for this suggestion has come from the observations that cholesterol lowering drugs reduce Aβ42 production [20] and that statin use has been associated with reduced incidence of AD in retrospective studies [23]. With this background, we have been assessing other genes whose products may affect brain cholesterol metabolism to see whether genetic variability within them may influence risk for developing AD [5,13].

A number of nuclear receptors have been identified as key regulators of cholesterol homeostasis and one that has been shown to also play a role in regulation of Aβ production is the liver X receptor (LXR) encoded by the nuclear receptor 1 type H2 (*NR1H2*) [8,21]. The *NR1H2* gene is located on chromosome 19 at ∼80 cM. Genome screen on AD series have, of course, shown a high-LOD score for chromosome 19 [3,10] close to the *ApoE* [6] gene at 85 cM as well as one on the 'p' arm at about 30 cM [22]. It is likely that the peak at 85 cM is not entirely explained by the protein encoding variability at the *ApoE* locus (authors' unpublished data). Thus, *NR1H2* gene is a potential candidate gene for AD. LXR is expressed in two isoforms, LXRα and LXRβ, of which only LXRβ is expressed in the brain [7]. Oxidized cholesterol activates LXR to form a heterodimer with the retinoid X receptor and in turn regulates ATP-binding cassette transporters (ABCA1), which mediate cholesterol efflux and secretion of excess cholesterol from cells to lipid-poor apolipoproteins such as ApoE [14]. Some studies have shown that in *NR1H2* knockout mice, there was a failure to adapt metabolically when challenged with high cholesterol diets [1]. Other studies have also shown that treatment of mice with LXR activators resulted in a decrease of Aβ formation [21]. Since LXRβ plays such a key role in Aβ and cholesterol modulation [15], variation in its cognate gene could have an associated functional implication for the risk of AD. Thus, we investigated the allelic polymorphisms of *NR1H2* and their association to AD risk in sibpairs with late onset form of AD (age at onset after 60 years old), samples provided by the National Institute of Mental Health (NIMH) and the National Cell Repository for Alzheimer's Disease (NCRAD; grant no. U24 AG21886). This series contains a large subset which we genotyped in our stage II genome screen [16], in addition we

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included supplementary families that became available since this analysis.

The NCBI database search was used to identify four single nucleotide polymorphisms (SNP) at the *NR1H2* locus (transcript# ENST00000253727); LXR1 = rs1052533 in intron 5, LXR2 = rs1405655 in intron 7, LXR3 = rs1802589 in the 3′ UTR and LXR4 = rs2695121 in intron 2. PCR assay was then performed on our DNA series consisting of 458 nuclear families, giving a total of 1327 persons. Among those were 931 patients with an average age at onset of 73 years old (73% female) and their unaffected sibling when available The primers we used were: 5′-CCTACAGGCGTCCTTTCTGA-3′ (forward) and 5′-TCCCACAGGTCGATTGTCG-3′(reverse) for LXR 1; 5′-ACAGATGCTGGGAGCAGAGT-3′(forward) and 5′-CCGCGATAACGTCTCTTTTC-3′ (reverse) for LXR2; 5′ACCTATCGGCTCTCATCCCT-3′ (forward) and 5′-CGGAAGGAGAAGGAGACGAA-3′(reverse) for LXR3; 5′-GAAAAAGCAGGTGGATTGGA-3′ (forward) and 5′-TAAGATGTCCGAGGCAC-3′ (reverse) for LXR4 (all the reverse primers are biotinylated). Five microliters of amplified DNA was then genotyped for the four *NR1H2* SNPs via pyrosequencing procedure as we have previously described [18], using primers GTGACTGTGACTCCTG for LXR1, AAAATTGAGGATCAGGC for LXR2, CCACTGACCCTTCCC for LXR3, and GGCTGAGAGTAGGGTCT for LXR4. Based on the genotyping data, statistical analysis using the Family Based Association Test program (FBAT; version 1.5.1) was then performed on only the most polymorphic of the four *NR1H2* SNPs (LXR2 and LXR4). Indeed, all samples were homozygotes for LXR1 and LXR3. The FBAT statistical analysis (http://www.biostat.harvard.edu/~fbat/default.html) was done as three, two point analyses: analysis of sibpair genotype in total dataset, segregation analysis based on the presence or absence of *ApoE*-ε4 allele and haplotype association analysis also in relation to the presence or absence of *ApoE*-ε4 allele. We analyzed the sample by using an additive model as a whole and two strata based on *ApoE*-ε4 carrier status. Given that there have been several reports of linkage in this region on chromosome 19 [10,16,22], we used the empirical variance calculation for the estimation of *p*-values which adjusts for the correlation among sibling marker genotypes and for different nuclear families within a single pedigree. The association test is done under the null hypothesis of "no association in the presence of linkage" (Table 1).

To analyze the haplotype transmission, we used the same software which is able to deal with multilocus haplotypes, even in the presence of phase uncertainty and missing parental genotypes. We used the haplotype permutation test in order to account for haplotypes with a frequency of less than 5%. Multiple nuclear families from extended pedigrees were allowed (Table 2).

Results of the FBAT statistical analysis on the genotype results are illustrated in Table 1. Within the total sibpair series population there were significant *p*-values for the C/C genotype of LXR2 and LXR4 ($p = 0.05$ for both). Segregation analysis of the sibpair series population according to the presence or absence of *ApoE*-ε4 allele showed that in the *ApoE*-ε4 allele positive population, there was a significant LXR4 C/C genotypic association ($p = 0.02$). Because we obtained significant associations, DNA sequencing using Big Dye Terminator Cycle sequence (Applied Biosystems) was then performed in *NR1H2* exonic regions in twelve random AD cases to further search for any undescribed polymorphisms. During this analysis we identified an insertion/deletion polymorphism of a CAG (glutamine) residue at codon 175 in exon 6 obtained from sequencing these 12 AD cases. The reference sequence (NCBI 56699414; transcript# ENST00000253727) was, in fact the minor allele and ~95% Caucasians chromosomes have the extra CAG (unpublished data). A duplication of 16 nucleotides, 23 bp before exon 9 was also observed but did not seem to affect splicing, therefore it most likely does

Table 1
Genotype frequency in USA sibpair series

	Total (Nf = 458)			*ApoE*-ε4− (Nf = 127)			*ApoE*-ε4+ (Nf = 311)		
	Fam#	Freq	*p*-Value	Fam#	Freq	*p*-Value	Fam#	Freq	*p*-Value
LXR2									
T/T	132	0.396	0.94	30	0.399	0.51	82	0.405	0.12
T/C	152	0.556	0.22	36	0.529	0.23	89	0.553	0.40
C/C	50	0.048	0.05*	11	0.072	0.30	27	0.043	0.28
LXR4									
T/T	89	0.142	0.50	18	0.138	0.62	52	0.157	0.13
T/C	167	0.598	0.24	38	0.599	0.87	99	0.576	0.42
C/C	121	0.260	0.05*	31	0.263	0.85	71	0.266	0.02*
Exon6									
Ins/Ins	40	0.880	0.50	9	0.858	0.87	25	0.868	0.41
Ins/WT	40	0.120	0.50	9	0.142	0.87	25	0.132	0.41
WT/WT	0	0	NA	0	0	NA	0	0	NA

Fam#: number of informative families, Freq: frequency, Ins: CAG insertion compared to the database, Nf: nuclear families, WT: wild type.
* Significant *p*-value.

Table 2
Haplotype permutation test in the USA sibpair series

LXR2	LXR4	Exon6	Total (Nf = 458)			*ApoE*-ε4− (Nf = 127)			*ApoE*-ε4+ (Nf = 311)		
			Fam#	Freq	*p*-Value	Fam#	Freq	*p*-Value	Fam#	Freq	*p*-Value
T	T	Ins	171.8	0.397	0.19	42.0	0.399	0.84	109.7	0.394	0.01*
C	C	Ins	154.3	0.282	0.11	37.3	0.291	0.97	96.3	0.271	0.0006*
T	C	Ins	137.9	0.274	0.89	34.5	0.262	0.97	88.9	0.285	0.77
T	T	WT	24.6	0.031	0.70	2.3	0.017	0.44	19.5	0.035	0.84
C	C	WT	5.4	0.010	0.34	1.7	0.015	0.57	3.4	0.009	0.98
T	C	WT	4.2	0.005	0.28	4.1	0.016	0.93	4.3	0.005	0.007*
C	T	Ins	1.1	0.001	0.74	NA	NA	NA	1.1	0.001	0.79

Fam#: number of informative families, Freq: frequency, Ins: CAG insertion compared to the database, Nf: nuclear families, WT: wild type.
* Significant *p*-value.

not affect LXRβ protein function. We added the data from our CAG insertion/deletion analysis and redid our FBAT analysis for the haplotypes using all the polymorphisms (LXR2, LXR4 and Exon6) (see Table 2).

Interestingly, there were significant associations for the haplotype C-C-Insertion (for SNP LXR2–LXR4–Exon6) ($p = 0.0006$), as well as a more moderate association for the haplotype T–T–insertion ($p = 0.01$: in *ApoE*-ε4+ population). To note, the T–C–wild type haplotype in the *ApoE*-ε4+ population also shows a significant result ($p = 0.007$) but the low number of informative families (4.3) indicates that this result is probably a false positive.

These data suggest that genetic variability at the *NR1H2* locus may be a risk factor for AD. This risk is not encoded at the ins/del polymorphism and we did not find any other coding changes in the gene in our sequencing of 12 individuals affected with late onset AD. This suggests that any effect must either be modulated by genetic variability in the expression levels of LXR or by alterations in the alternative splicing which seems to be the case for LXR4. EST alignment of BI771247, BQ932071 and BX338682 against human reference assembly shows that LXR4 SNP resides in either the coding region or the splicing junction. Therefore, it is likely that LXR4 is a functional SNP. LXR2 does not seem to have any known biological function. However, it may be in linkage disequilibrium with some functional element nearby. Of course, all these predications remain to be validated with RT-PCR and/or other relevant functional assays. Nevertheless, we believe such mechanisms are plausible given that LXR activators have been shown to reduce Aβ production [4,11,12,21]. One hypothesis is that low expressing *NR1H2* haplotypes would be more susceptible to AD. Another suggestion is that induction of the LXR system may increase the risk of amyloid deposition associated with high cholesterol [9,17] since LXR can effectively mediate cholesterol homeostasis in astrocytes [14]. Indeed, activation of LXR leads to a dramatic increase in *ApoE* mRNA and protein expression; with *ApoE*-ε4 allele being a risk factor for AD. In such a scheme, LXR maybe upstream of ApoE and potentiates the risk associated effects of the ε4 allele.

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