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 e4 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likely that the peak at 85 cM is not entirely explained by the protein encoding variability at the ApoE locus (authors’ unpublished data).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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The primers we used were: 5′-TGACTGACTGACT-3′ (forward) and 5′-ACAGATGCTGGGAGCAGTG-3′ (reverse) for LXR1; 5′-ACAGATGCTGGGAGCAGTG-3′ (forward) and 5′-CCGCGATAACGTCTTTTCCT-3′ (reverse) for LXR2; 5′-ACCTATCGGCTCTCATCCCC-3′ (forward) and 5′-CGGAGGAGAGGACGCAC-3′ (reverse) for LXR3; 5′-GAAAAAGCACGATGATTGAGA-3′ (forward) and 5′-TAAGATGTCCGAGGCAC-3′ (reverse) for LXR4 (all the reverse primers are biotinylated).

Microfluidics of amplified DNA was then genotyped for the four NR1H2 SNPs via pyrosequencing procedure as we have previously described [18], using primers GTGACTGTGACTCTCTCTCC (for LXR1), AAAATGGACTACGAGC (for LXR2), CCCTATCGGCTCTCATCCCC (for LXR3), and GCGTGAAGTGGGATGAT (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 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.


Table 1

| 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* |

Table 2

| 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.
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 LXR2 LXR4 Exon6) (see Table 2).

Table 2
Haplotype permutation test in the USA sibpair series

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

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

* Significant p-value.

References


