Neuroscience Letters 468 (2010) 202–206

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Association of the aromatase gene with Alzheimer's disease in women

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ARTICLE INFO

Article history:
Received 19 September 2009
Received in revised form 15 October 2009
Accepted 27 October 2009

Keywords:
CYP19A1
Indel
Microsatellite
SNP
Linkage disequilibrium
Menopause
Neurosteroid

ABSTRACT

Associations have been reported of aromatase polymorphisms with Alzheimer's disease (AD). We studied nine polymorphisms in 207 cases of AD, 23 cases of mild cognitive impairment (MCI) and 233 controls, all from the OPTIMA cohort. We replicated two reported associations and found others. Our findings were consistent between AD and MCI. Further, our results were sex-specific, i.e. there were significant interactions between certain polymorphisms and gender, and the associations with AD were almost entirely in women. Aromatase catalyses the conversion of androgens to estrogens. It is expressed in the human brain. In the hippocampus, it is upregulated in postmenopausal women and is lowered in AD. These sex-specific results are therefore plausible. However, our results now need to be replicated in a larger dataset.

Aromatase catalyses the production of aromatic C18 estrogens from C19 androgens. It is expressed in the human brain [31,32,51,54]; its hippocampal expression is raised in postmenopausal women and is lowered in Alzheimer's disease (AD) [31]. Brain production of certain neurosteroids, e.g. of estrogen, has been shown to be neuroprotective (reviewed in [6]) and may therefore influence the risk of AD. Variants of the aromatase gene may thus affect that risk.

Iivonen et al. [30] reported associations of certain single nucleotide polymorphisms (SNPs) in and around the aromatase gene (*CYP19A1*), i.e. SNPs 4–9 in their terminology (which we have adopted), with the risk of sporadic AD. But these were not replicated by Huang and Poduslo [29], who however proposed an association with AD only in carriers of the ε4 allele of apolipoprotein E (*APOE*4). Corbo et al. [15] studied three other SNPs than the above and reported an association of rs4646 with onset age of AD in women. Further reports, by Combarros et al. [12,13], proposed interactions between an aromatase SNP, rs1062033, and the genes for butyrylcholinesterase [12] and interleukin-10 [13]. The ongoing AlzGene meta-analysis of rs1062033 [5] (http://www.alzforum.org/res/com/gen/alzgene/), based on five studies, including two large genome-wide association studies, found no association with AD (17 September 2009): odds ratio = 0.95 (95% confidence interval: 0.86–1.04). We set out to replicate some of the above associations and to investigate possible sex differences in those associations.

We examined seven SNPs studied by Iivonen et al. [30] and/or by Huang and Poduslo (2006) [29] and also an indel and a microsatellite, i.e. a TTTA repeat (Table 1). We used 463 samples of DNA from 207 cases of sporadic AD (116 women), 23 cases of mild cognitive impairment (MCI) (10 women) and 233 elderly controls (122 women). They were all Caucasians from the longitudinal cohort of the Oxford Project to Investigate Memory and Ageing (OPTIMA) [10], drawn from the Oxford region. Protocols were approved by the Central Oxford Ethics Committee number 1656. Mean age of onset of AD was 70.3 (±9.1) years, of MCI was 81.3 (±4.7) years and of death or last examination of controls was 78.9 (±8.8) years. Of the AD cases, 148 were neuropathologically confirmed by CERAD criteria (128 "definite' and 20 'probable") and 59 were diagnosed "probable AD" by NINCDS-ADRDA criteria. Possible autosomal dominant cases were excluded, based on family history. MCI cases were diagnosed by the criteria of Petersen et al. [42]. All 233 controls were without cognitive impairment and with CAMCOG scores >80.

DNA was extracted from whole blood using the Promega, Wizard DNA Purification kit and from frozen brain tissue using the QIAgen, QIAamp DNA mini kit and then normalised to a concentration of 50 ng μl^{-1}. The microsatellite (TTTA repeat) and indel (TCT insertion/deletion) were assayed simultaneously using the forward primer 5′-GCAGGTACTTAGTTAGCTAC fluorescently labelled with FAM and the reverse primer 5′-TTACAGTGAGCCAAGGTCGT. The

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doi:10.1016/j.neulet.2009.10.089

Table 1
Studied polymorphisms.

Polymorphism	SNP 1	SNP 3	SNP 4	SNP 5	SNP 7	Indel	Microsatellite	SNP 8.3	SNP 9
Rs number	1004984	1902586	1008805	767199	1065778	11575899		700519	10046
Location	Intron 1	Intron 1	Intron 1	Intron 1	Intron 3	Intron 4	Intron 4	Exon 7	3′UTR
Variation	C/T	C/T	T/C	A/G	G/A	TCT/-	$(TTTA)_n$	C/T = Arg264Cys	C/T
MAF[a]	36% (T)	4.5% (T)	46% (C)	48% (A)	47% (G)	41% (del)	47% (long[b])	3% (T)	49% (C)
Studied by:									
Iivonen et al. [30]	√	√	√	√	√				√
Huang and Poduslo [29]			√	√	√			√	√

[a] Minor allele frequency, in OPTIMA controls.
[b] 8–13 TTTA repeats.

protocol was modified from Baghaei et al. [3]. The PCR reaction contained 100 ng of DNA with 2 U of AmpliTaq Gold DNA Polymerase (PerkinElmer, Stockholm, Sweden) and 400 nM of each primer, in a 25 μl reaction volume. There was an initial 12 min cycle at 95 °C followed by 25 cycles of 92 °C for 32 s, 60 °C for 30 s and 72 °C for 30 s followed by 72 °C for 7 min. The fragments were run on either an Applied Biosystems 3700 or 3730xl DNA Analyser Instrument alongside ROX 400HD size standard (PerkinElmer, Stockholm, Sweden) and the fragments analysed using Genotyper software (PerkinElmer, Stockholm, Sweden). The SNPs were analysed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry on the Sequenom (GmbH) MassARRAY platform using either hME or iPLEX assays. Standard PCR methods were used for the apolipoprotein E gene. All genotyping was undertaken blind to diagnosis.

Statistical analysis used PASW Statistics 17.0.2 and R Version 2.6.1. Association analysis was by Fisher's exact test. Synergy factor analysis used the method of Cortina-Borja et al. [11,17]. The microsatellite consisted of a common or short allele (7 TTTA repeats, 53.2% frequency in controls) and six longer alleles (8–13 repeats). We therefore adopted a biallelic system for all analyses, comprising short (7 repeats) and long alleles (8–13). We applied a correction factor of 2 to the analyses stratified by sex.

All polymorphisms were in Hardy–Weinberg equilibrium ($p > 0.05$), both in controls and in AD cases. Most polymorphisms were in strong linkage disequilibrium (LD) (Table 2). The main exceptions were: SNP 4, which was generally in weaker LD ($D' < 0.8$), and SNP 1, which was only in LD, weakly, with the indel. Previous studies from Finland [30] and the USA [29] found a broadly similar pattern of LD between the SNPs.

Table 3 shows the results of the association analysis of the nine polymorphisms with AD and with MCI. SNPs 7 and 9, the indel and the microsatellite were all associated with AD; SNPs 5 and 7, the indel and the microsatellite were associated with MCI ($p < 0.05$). Table 4 shows the sex-specific nature of the associations with AD. SNPs 5, 7 and 9, the indel and the microsatellite were only associated with AD in women. The synergy factors for the interaction between each polymorphism and sex were significant in the cases of SNPs 3 and 9. The interactions tended to be in the same direction for all polymorphisms ($p < 0.2$) except for SNPs 1 and 4. For SNP 9, in the 3′ untranslated region of the gene, the odds ratios of AD for genotypes TT + CT versus CC were 4.1 (95% confidence interval: 1.8–9.3) in women and 0.8 (0.4–1.6) in men; the synergy factor for SNP 9 × sex was 5.2 (1.7–15.8) (Table 4).

Stratification by age ±75 years and by *APOE*4 status suggested that the associations might be stronger in the younger subset and in *APOE*4-negatives (data not shown), but the differences were not significant. We found no associations with onset age of AD either overall or in women. We did not attempt to replicate the proposed interactions of Combarros et al. [12,13] with butyrylcholinesterase and interleukin-10, since a larger dataset is needed to replicate such interactions. We intend to examine these interactions in the much larger Epistasis Project [14].

However, we replicated the reported associations of SNPs 7 and 9 with AD [30] and also found associations of the indel and the microsatellite with AD (Table 3). Our results for MCI were consistent with those for AD (Table 3); several of the same SNPs were significantly associated with both conditions, in spite of the small number of cases of MCI. There was strong LD between all the above five polymorphisms (Table 2). This suggests that most of these associations were due to this LD. In addition, these effects were sex-specific, i.e. found only or mainly in women, e.g. SNP 9 (Table 4). The association with onset age reported by Corbo et al. [15] was also only found in women. Interestingly, two studies [44,47] have found sex-specific associations with hypertension of aromatase variants, including SNP 9 in both studies, i.e. the polymorphism with the strongest sex-specific association with AD in our study. The larger of these studies [44], with 3448 subjects, found associations of both the indel and SNP 9 with diastolic hypertension in non-obese women. Hypertension in mid-to-late life increases the risk of later cognitive decline [20,52] and AD [33]. Sex differences are commonly found in AD: in incidence [22,36], in pathology [9,16], in glucose metabolism and brain reserve [41], in risk factors [1] and in genetics [21,23,35].

The synthesis of neurosteroids such as estrogen in the human brain is well established [50]. Aromatase is expressed in various regions of the brain [31,32,51,54], with a changed pattern in AD

Table 2
Linkage disequilibrium of aromatase polymorphisms in OPTIMA controls.

	SNP 1	SNP 3	SNP 4	SNP 5	SNP 7	Indel	Microsatellite	SNP 8.3	SNP 9
SNP 1		–	–	–	–	0.409	–	–	–
SNP 3	–		–	**0.998**	**0.998**	**0.998**	**0.998**	**0.883**	–
SNP 4	–	0.007		0.752	0.768	0.578	0.765	–	0.664
SNP 5	–	0.044	0.447		**0.975**	**0.971**	**0.977**	**0.996**	**0.966**
SNP 7	–	0.042	0.451	**0.918**		**1.000**	**0.991**	**0.996**	**0.991**
Indel	0.066	0.033	0.268	**0.598**	**0.612**		**1.000**	**0.996**	**0.981**
Microsatellite	–	0.042	0.441	**0.910**	**0.970**	**0.605**		**0.996**	**0.991**
SNP 8.3	–	**0.521**	–	0.029	0.028	0.022	0.028		**0.997**
SNP 9	–	0.001	0.397	**0.820**	**0.833**	**0.694**	**0.824**	0.033	

The upper right section gives D' values and the lower left gives r^2.
Data are only given where $p < 0.01$; results in bold indicate either $D' > 0.85$ (upper right) or $r^2 > 0.5$ (lower left).