Int J Mol Epidemiol Genet 2010;1(2):104-113
www.ijmeg.org /IJMEG1002002

Original Article

LRP-1 variation is not associated with risk of Alzheimer's disease

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Received February 8, 2010, accepted February 15, 2010, available online: February 20, 2010

Abstract: Alzheimer's disease (AD) is characterised by the extensive deposition of amyloid beta (Aβ) within the parenchyma and vasculature of the brain. It is hypothesised that a dysfunction in Aβ degradation and/or its removal from the brain may result in accumulation as plaques. Low density lipoprotein receptor-related protein-1 (LRP-1) is a multifunctional receptor shown to be involved in cholesterol metabolism but also the removal of Aβ from the brain. Its ability to transport Aβ from the brain to the periphery has made it an attractive candidate for involvement in Alzheimer's disease (AD). We have assessed the frequencies of 9 tag- SNPs and the commonly studied synonymous SNP within exon 3 (rs1799986) in a multi-centre AD/control cohort and performed haplotype analysis. We found no evidence from a combined total of 412 controls and 1057 AD patients to support the involvement of *LRP-1* variation, including the most commonly studied variant in rs1799986 in conferring genetic susceptibility to increased risk of AD.

Keywords: LRP-1, Alzheimer's disease, association analysis

Introduction

Alzheimer's disease (AD) (OMIM #104300) is the most common form of dementia, distinguishable from other dementias by the presence of extracellular deposits of Aβ peptide (neuritic plaques) and intraneuronal neurofibrillary tangles composed mainly of hyperphosphorylated tau protein. In the majority of subjects with AD there is also accumulation of Aβ in the cerebral vasculature [1].

The majority of AD cases are sporadic (i.e. have no apparent familial patterns of inheritance) compared with fewer than 5% of cases that are caused by autosomal dominant inheritance of mutations in *PSEN1*, *PSEN2* or *APP* [2]. These rarer familial forms of AD are characterised by elevated production of Aβ1-42 relative to Aβ1-40, or an overall increase in production of both forms of Aβ, which are derived from the amyloid precursor protein (APP) by sequential cleavages by β- and γ-secretases. Aβ1-42 also accumulates in sporadic AD but the mechanisms are unclear and may include various combinations of increased Aβ production, reduced degradation [3-7] and impaired clearance of Aβ into the cerebrospinal fluid [8-9] and across vessel walls into the bloodstream. Several genes have been identified as possible risk factors for the development of AD. In 1993, Corder *et al.* showed that there was a strongly correlation between the gene dose of apolipoprotein E (*APOE) ε4* alleles and an increased risk of developing AD [10]; whilst two recent genome-wide association (GWA) studies have identified variations within PICALM, CLU [11] and CR1 [12] as potential risk factors replicated in addition to the established *APOE* relationship.

In general, clearance of Aβ across vessel walls involves its binding to chaperones such as APOE

Table 1. Demographic information for each of the four cohorts

Cohort	Diagnosis	No. of cases	No. of females	Age range (yrs) *	Mean (±SD) age (yrs) *
Total	AD	1057	638	-	-
	Control	412	229	-	-
Bristol	AD	283	163	57-99	80.7±8.0
	Control	84	33	62-96	79.1±7.8
Belfast	AD	644	405[a]	54-95[b]	76.4±7.8
	Control	215	144[c]	40-100[d]	74.4±9.0
Italy I	AD	15	6	53-84	70.5±10.4
	Control	18	9	59-85	77.3±5.9
Italy II	AD	115	64	51-82	69.9±7.1
	Control	95	43	60-100	73.2±8.5

* For the Bristol cohort the ages indicated are the age at death. For the three clinical cohorts the age is age at disease onset for the AD cases and age that blood was taken for analysis for control cases. [a] Gender information missing from 11 cases; [b] Age information missing from 84 cases; [c] Gender information missing from 1 control; [d] Age information missing from 30 controls.

[13] and alpha-2 macroglobulin [14] and subsequent docking with receptors including low density lipoprotein receptor-related protein-1 (LRP-1) [13] and p-glycoprotein [15]. Aβ has also been shown to bind directly to LRP-1[16] and LRP-1 has been observed to co-localise with neuritic plaques [17].

LRP-1 has other functions which may influence the pathogenesis of AD. It has been shown to modulate APP trafficking [18-19] and also plays an important role in cholesterol metabolism through the internalisation of APOE-cholesterol complexes [20]. Cholesterol, and specifically its distribution in lipid rafts, has been shown to be influential in APP cleavage and Aβ production [21-24].Thus alterations in the function of LRP-1 have the potential to affect several aspects of Aβ metabolism within the brain.

Some genetic linkage studies [25] but not others [26-27] have found evidence that a region of chromosome 12 (12q1-q14) that includes the LRP-1 gene (*LRP-1*) (ncbi gene ID: 4035) was linked to late-onset AD [27-28]. *LRP-1* is a large gene with 89 exons and extensive intronic regions. Numerous synonymous, non-synonymous and frame-shift single nucleotide polymorphisms (SNPs) as well as some microsatellite variants are observed. Despite wide variability in *LRP-1* the extent to which variants have been studied with respect to AD has been relatively limited. According to Alzgene (www.alzgene.org), the online meta-analysis resource and database of published genetic association studies in AD, the synonymous C/T SNP within exon 3 of *LRP-1* (rs1799986) previously reported to be associated with AD [29] has been the most studied in the context of AD (Alzgene gene ID: 4035). However to date the findings remain inconclusive. Meta-analyses conducted by Pritchard *et al*. [30] and Alzgene do not show association between any of the most studied *LRP-1* variants and AD.

Most genetic studies of *LRP-1* variants to date have assessed only one or two SNPs. In the present study we have used haplotype approaches to investigate the candidacy of *LRP-1* variation in AD susceptibility. To achieve this we analysed 10 SNPs, based on tagging methods, within *LRP-1*, in four European case-control cohorts, making this one of the largest and most comprehensive studies of *LRP-1* variants and susceptibility in AD to date.

Methods

Study cohort

Brain tissue was obtained from the South West Dementia Brain Bank (SWDBB) in Bristol, UK from 84 normal controls and 283 cases (**Table 1**) with a post-mortem diagnosis of probable or definite AD according to CERAD criteria [31]. Clinical samples were obtained from three other centres (Belfast, Northern Ireland; Bari, Italy (Italy I) and San Giovanni Rotondo, Italy (Italy II)) to comprise a total of 328 clinical controls and 774 samples from patients with a NINCDS-ADRDA [32] diagnosis of probable AD (**Table 1**). Together 412 controls and 1057 cases with AD were investigated for *LRP-1* variation and AD risk.

Other relevant data retrieved for each case in-

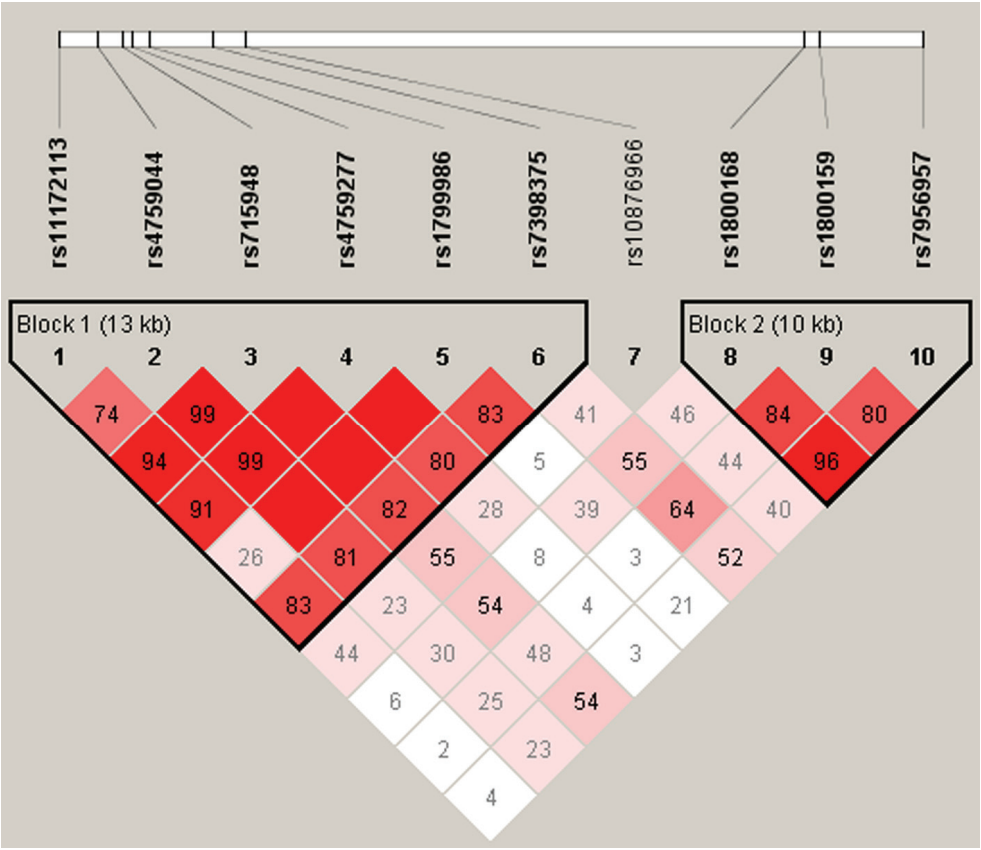

Figure 1. Linkage disequilibrium plot across *LRP-1*

cluded gender, age and *APOE* genotype. The recording of age differed for the post-mortem and clinical cohorts. For the post-mortem cohort the age at which the patient died was recorded whilst for the three clinical cohorts (Belfast and Italy I and II) both the age of disease onset for the AD cases and the age at which blood was taken for DNA analysis from control cases were recorded.

Genetic analysis

Genomic DNA was extracted from brain tissue and blood by use of a commercial DNA extraction kit (Nucleon ST Extraction kit, Nucleon Biosciences, Manchester, UK) and diluted to 10ng/µl in Sigma® water (Sigma Aldrich, St Louis, USA). 100µl of DNA was pipetted into each well of 96-well plates and dispatched to KBiosciences (www.kbioscience.co.uk) for SNP genotyping. We selected 10 SNPs (listed in **Table 2**) across *LRP-1*, 9 of which served as tagged SNPs according to HAPMAP (build 35) (www.hapmap.org), and with the exception of SNP 5 (exon 3 - rs1799986), were all intronic. All reported SNPs can be found in the dbSNP database under their respective rs numbers (**Table 2**).

KBiosciences designed the primers for each SNP according to the sequence information that we sent for each of the SNPs. They validated the assays and performed the genotyping on all the DNA samples using their in-house patented single plex KASPar technology.

Statistical analyses

The chi-squared statistic (χ^2) was used to assess deviation from Hardy–Weinberg equilibrium (HWE) for alleles at individual loci as well as differences in genotype and haplotype distributions between demented and non-demented groups. Logistic regression was also performed with gender, age and *APOE e4* and cohort (Italian, UK/Northern Ireland) as co-variants. A-

Table 2. Details of SNPs and genotype and allele frequencies

SNP no.	rs no.	Exon/ intron	Chromosome position	AD/ control	Genotype frequencies			Allele frequencies		Genotype	Allele	Logistic
					TT	CT	CC	T	C			
SNP 1	rs11172113	intron 1	55813550	Control	142 (.35)	188 (.47)	71 (.18)	472 (.59)	330 (.41)	p=0.70	p=0.87	p=0.24
				AD	356 (.35)	505 (.49)	167 (.16)	1217 (.59)	839 (.41)			
					TT	CT	CC	T	C			
SNP 2	rs4759044	intron 1	55816937	Control	120 (.32)	181 (.48)	77 (.20)	421 (.56)	335 (.44)	p=0.59	p=0.30	p=0.90
				AD	284 (.29)	466 (.48)	217 (.22)	1034 (.53)	900 (.47)			
					GG	AG	AA	G	A			
SNP3	rs715948	intron 2	55819249	Control	212 (.52)	162 (.40)	34 (.08)	586 (.72)	230 (.28)	p=0.92	p=0.73	p=0.40
				AD	528 (.51)	410 (.40)	92 (.09)	1466 (.71)	594 (.29)			
					AA	AC	CC	A	C			
SNP4	rs4759277	intron 2	55819957	Control	56 (.14)	180 (.45)	167 (.41)	292 (.36)	514 (.64)	p=0.96	p=0.81	p=0.30
				AD	147 (.14)	471 (.45)	424 (.41)	765 (.37)	1319 (.63)			
					TT	CT	CC	T	C			
SNP5	rs1799986	exon 3	55821533	Control	15 (.04)	105 (.26)	291 (.71)	135 (.16)	687 (.84)	p=0.10	p=0.12	p=0.24
				AD	19 (.02)	256 (.25)	765 (.74)	294 (.14)	786 (.86)			
					GG	GC	CC	G	C			
SNP6	rs7398375	intron 6	55827115	Control	26 (.07)	151 (.38)	223 (.56)	203 (.25)	597 (.75)	p=0.99	p=0.94	p=0.59

				AD	66 (.07)	386 (.38)	563 (55)	518 (.26)	1512 (.74)			
					TT	CT	CC	T	C			
SNP7	rs10876966	intron 6	55829839	Control	22 (.05)	143 (.35)	246 (.60)	187 (.23)	635 (.77)	p=0.31	p=0.18	p=0.04
				AD	53 (.05)	319 (.31)	664 (.64)	425 (.21)	1647 (.79)			
					TT	CT	CC	T	C			
SNP8	rs1800168	intron 60	55878824	Control	187 (.48)	161 (.42)	38 (.10)	535 (.69)	237 (.31)	p=0.52	p=0.48	p=0.59
				AD	479 (.48)	403 (.40)	120 (.12)	1361 (.68)	643 (.32)			
					GG	AG	AA	G	A			
SNP9	rs1800159	intron 62	55880161	Control	184 (.46)	180 (.45)	35 (.09)	548 (.69)	250 (.31)	p=0.16	p=0.53	p=0.90
				AD	484 (.47)	426 (.41)	123 (.12)	1394 (.67)	672 (.33)			
					GG	GC	CC	G	C			
SNP10	rs7956957	intron 78	55889082	Control	165 (.42)	181 (.46)	50 (.12)	511 (.65)	381 (.35)	p=0.50	p=0.91	p=0.85
				AD	449 (.44)	437 (.42)	145 (.14)	1335 (.65)	727 (.35)			

Table 3. Haplotype frequency analysis

Block	Haplotype frequency	Case, control frequencies	χ^2	p value
Block 1				
CCGACC	0.326	0.326, 0.325	0.001	0.9718
TTACCG	0.224	0.224, 0.225	0.009	0.9242
TTGCCC	0.099	0.099, 0.099	0.005	0.9461
TTGCTC	0.093	0.091, 0.097	0.242	0.6229
TCGCTC	0.084	0.087, 0.077	0.799	0.3715
TTACCC	0.059	0.062, 0.052	1.039	0.3081
CTGCTC	0.048	0.044, 0.060	3.348	0.0673
TCGACC	0.019	0.019, 0.021	0.1	0.7512
CCGACG	0.019	0.021, 0.012	2.608	0.1063
Block 2				
TGG	0.606	0.604, 0.609	0.071	0.79
CAC	0.281	0.282, 0.279	0.024	0.8773
TGC	0.038	0.037, 0.043	0.738	0.3902
TAG	0.035	0.035, 0.034	0.055	0.815
CGC	0.032	0.033, 0.030	0.221	0.6383

priori sample size calculations were performed to ensure that the cohort was of adequate size to detect a 20% shift in allele frequency at a $p<0.05$. Bonferroni post-testing was applied to adjust for multiple comparisons. Haplotype frequencies in the *LRP-1* region were estimated after linkage disequilibrium (LD) block definition in individual blocks using Haploview v4.1 [33] (**Figure 1**)). LD blocks were defined by solid splines. LD between marker pairs within *LRP-1* was estimated using the r^2 metric [34]. The calculation of empirical p-values for haplotypes in case-control tests was performed using 1000 permutations (**Table 3** and **Figure 2**).

Results

Combining one post-mortem and 3 clinical cohorts, 1057 AD and 412 control cases were genotyped for 9 tagging-SNPs throughout *LRP-1* and a SNP (rs1799986) within exon 3 which had been previously associated with elevated AD risk. The age range, mean age at death (Bristol) and mean age of disease onset (Belfast and Italy) of cases are listed in **Table 1**. The AD patients from the Belfast cohort were significantly older than those in the Italian I (p=0.003)

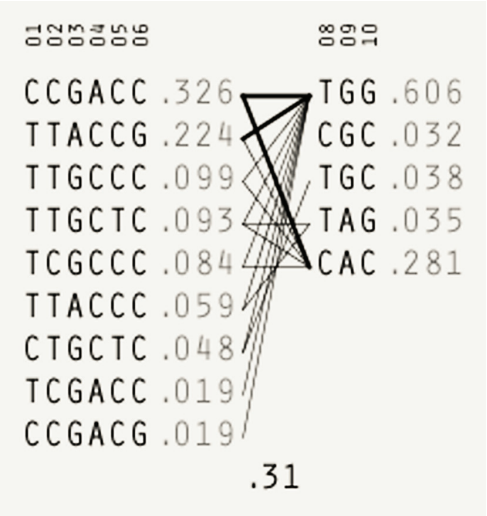

Figure 2. Haplotype frequencies

and Italian II ($p<0.0001$) cohorts however there was no difference between the two Italian groups. None of the control groups differed significantly in age.

Genotype frequencies for each SNP are displayed in **Table 2**. None of the SNPs deviated significantly from HWE and the sample size had 80% power to detect an odds ratio of approximately 1.7 at an alpha of 0.05 and assuming a risk allele frequency of 20%.

Neither the genotype nor allele frequencies differed between AD and control cases for any of the SNPs assessed (**Table 2**). Analyses using logistic regression did suggest that with gender, age and *APOE e*4 as co-variates, and a cohort identifier to control for population stratification that allele frequencies for SNP 7 (rs10876966) did differ marginally between AD and controls ($p=0.04$) (**Table 2**). However, this difference no longer remained significant after adjustment for multiple testing across the study.

Haploview v4.1 was used to construct an LD plot across *LRP-1* and is illustrated in **Figure 1**. The LD plot generated as a solid spline of LD indicates that there are two distinct LD blocks and these are outlined in red with the number in each square being an r^2 metric (darkest red representing perfect LD with $r^2 = 1$). The LD plot was created using both cases and controls combined. SNP 7 (rs10876966), located between the 2 blocks, was not observed to be in strong LD with any of the suggested tagging SNPs and was therefore excluded from haplotype analyses.

Haplotype frequencies are displayed in **Table 3** and illustrates, in agreement with single marker analyses, that there was no evidence of a significance distortion between cases and controls in either of the two LD blocks around *LRP-1*. **Figure 2** illustrates the association between the haplotypes in the LD blocks and the lack of average correlation between the various haplotypes (connecting lines represent a measure of multiallelic D' where thicker lines reflect a higher level of recombination).

Discussion

AD is widely regarded as a disease of multifactorial pathogenesis, with inflammation, reduced cerebral blood flow, Aβ toxicity and cytoskeletal alterations all contributing to the neurodegeneration. *APOE e*4 is the only robust genetic risk factor for the development of AD [10]. Many other genetic risk factors have been proposed; however, replication studies and meta-analyses have not consistently supported these (see Alzgene). Recent findings from two GWA studies however, have provided evidence to support the involvement of variations in *PICALM*, *CLU* [11] and *CR1* [12] with AD susceptibility.

LRP-1 is closely involved in the metabolism of APOE, cholesterol and APP production and the clearance of Aβ from the brain [13, 18, 20-24] and it is therefore conceivable that altered expression or function of LRP-1 could contribute to the pathogenesis of AD. Although numerous studies have assessed *LRP-1* variants as potential risk factors for AD, most have only considered one or two SNPs (for up-to date overview see Alzgene gene ID=46). To our knowledge this is one of only a few studies to look at a range of SNPs that span *LRP-1*. We selected 9 tagged SNPs and the exon 3 SNP (rs1799986) to look for association with AD but initial comparison of genotype and allele frequencies did not reveal differences between AD and control populations for any of the SNPs tested. Genotype and allele frequencies for the most commonly studied exon 3 SNP (rs1799986) were commensurate with those found by a number of other groups (see Alzgene). On the evidence of our data and results of previous meta-analyses by Pritchard *et al*. [30] and Alzgene it seems reasonable to conclude that the T/C SNP rs1799986 (SNP5 in our dataset) of *LRP-1* is unlikely to contribute to increased risk of AD. Furthermore we have also shown that other variations within the gene to do not appear to be associated with the development of AD.

This study did not test whether any of the variants were associated with the severity of AD neuropathology or the rate of disease progression and thus we cannot exclude a role for variations in *LRP-1* in these processes. We also have to acknowledge the limitation of our study, that contrary to the use of reported tag SNPs to give our intended coverage across *LRP-1*, the subsequent LD plots demonstrated that full coverage was not entirely achieved. Furthermore, within the region of low coverage we identified weak evidence of association for increased risk of AD with SNP 7 (rs10876966)

although this association did not survive correction for multiple testing.

This highlights two things. First that reliance on tag SNPs for SNP selection, although very useful, can still have its limitations, particularly for age-related diseases where the ageing might influence the frequencies of various alleles compared to the generally younger populations from which the HAPMAP data has been derived. Second, although the evidence from the majority of SNPs investigated here and previous studies suggests that genetic variation within *LRP-1* may not be an important contributor to the development of AD, for *LRP-1* to be fully excluded as a candidate gene, the region in and around SNP 7 (rs10876966) linking up to the two main LD blocks demonstrated here, may still be worthy of some study.

Indeed, the findings in the two recent GWA studies by Harold *et al*. and Lambert and colleagues that provided evidence that clusterin (*CLU*) may be associated with AD [11-12] still point to Aβ processing being important in the disease. Clusterin, also known as APOJ, has been shown to bind Aβ [35-36] and clear Aβ across the blood brain barrier [37-38] which may be mediated in part via binding of APOJ-Aβ complexes to LRP-1 [39]. It is therefore possible, and remains to be shown, whether variations within *CLU* may influence the accumulation of Aβ either independently or in interaction with variants of *LRP-1* in the AD brain.

Acknowledgements

This work was supported by the ART (Alzheimer's Research Trust), BRACE (Bristol Research into Alzheimer's and Care of the Elderly), the Alzheimer's Society and the Northern Ireland R&D office. Also "Ministero della Salute", IRCCS Research Program, Ricerca Corrente 2009-2011, Linea n. 2 "Malattie Complesse". We thank all patients and relatives who have been involved in this study.

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