NIH-PA Author Manuscript

ABCA1 POLYMORPHISMS AND ALZHEIMER'S DISEASE

Fabienne Wavrant-De Vrièze[1,#], **Danielle Compton**[1], **Meridith Womick**[1], **Sampath Arepalli**[1], **Omanma Adighibe**[1], **Ling Li**[2], **Jordi Pèrez-Tur**[3], and **John Hardy**[1]

1 Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, 35 Convent Drive, Room 1A1015 MSC 3707, Bethesda, MD 20892-3707 – USA

2 Department of Medicine, Arteriosclerosis Research Unit, UAB Medical Center, 1808 7th Avenue South, Birmingham, AL35294, USA

3Unitat de Genètica Molecular, Departament de Genòmica i Proteòmica, Institut de Biomedicina de València-CSIC, C/Jaume Roig, 11, E46010 València, Spain

Abstract

In our search for genetic factors related to the development of Alzheimer's disease, we have genotyped 332 pedigrees for 3 coding polymorphisms in the *ABCA1* gene, two of which are known to alter plasma cholesterol levels, as well as a non-coding polymorphism within the promoter. We show an apparent weak association of rs2230806 (p-value= 0.01) with the disease in a sibpair series of Alzheimer's disease that had shown previously evidence for linkage to the chromosome 9 locus where *ABCA1* maps.

Keywords

ABCA1; Alzheimer's disease; polymorphisms

Introduction

Despite intensive effort, the only universally acknowledged risk factor locus for late onset Alzheimer's disease is the apolipoprotein E gene on chromosome 19 [6,11]. The known biological function of apolipoprotein E is in cholesterol metabolism and there has been much interest about the role of this metabolism in Alzheimer pathogenesis [4]. The ABCA1 transporter is a membrane-associated protein that functions as a cholesterol efflux pump in the cellular lipid removal pathway. The gene encoding ABCA1 is located near the previously detected AD linkage peak on chromosome 9 at position q22 [1,13,17,18]. With this background, *ABCA1* makes a good positional and functional candidate gene for Alzheimer's disease though previous studies at this locus in Alzheimer's disease show some confliction. Some articles found positive association [12,22,26], while others reported a negative association [15], or even had suggested that it influenced the age at onset, but not the risk of disease [12,26]. Nevertheless, most of those studies being done on case-controls series, we decided to investigate the impact of this gene in our sibpairs series of Alzheimer's disease.

[#] Correspondence to Fabienne Wavrant-De Vrièze, NIH/NIA/LNG, 35 Convent Drive, Room 1A1015 MSC 3707, Bethesda, MD 20892-3707 – USA. Tel: 301-451-3830, Fax: 301-280-4830, Email: wavrant@mail.nih.gov

Homozygous mutations in the *ABCA1* gene are associated with Tangier's disease [2,3,21] and heterozygosity for mutant alleles has been reported to influence peripheral cholesterol metabolism [5]. With this in mind, we analyzed the effect that genetic variability, both frequent coding and promoter variants, at the *ABCA1* gene had on the risk for Alzheimer's disease. We selected variants which were shown as potential risk factors for coronary atherosclerosis in the general population [16] further reinforcing our hypothesis as atherosclerosis and cerebrovascular disease have been related to the pathogenesis of Alzheimer disease because of a strong association with frequent neuritic plaques [10]

Materials and Methods

We selected 4 SNPS among the most informative ones for which restriction digest assays could be easily designed and ran those through the sample series in which we had observed linkage to markers on chromosome 9 [1,13,17]; in addition to supplementary families that became available subsequent to this analysis. Genotypes were generated for 332 pedigrees, represented by 899 persons, which are largely described in our genetic linkage study [17]. In summary, sibpairs affected with the late onset form of AD (680 confirmed affected samples with 71.8% female, mean age at onset=73.7y ±8.26y) were selected from the National Institute of Mental Health (NIMH) and the National Cell Repository for Alzheimer's Disease (NCRAD; grant number (U24 AG21886); as well as their unaffected siblings when available (125 individuals with 55.2% female, mean age= 75.8y ± 6.77y).

The SNPs used were rs2230806 (also designated as rs2234884) which encodes variant R219K (c.969A>G, numbering according to GenBank reference NM_005502) with a reported ~25% minor allele frequency in Caucasian populations [5,7]; rs2066718 which is a V771M (c.2624A>G) variant with a reported ~3% minor allele frequency [5,7]; rs2230808 which encodes a R1587K (c.5073A>G) variant with a reported 26% minor allele frequency [5,7], and finally rs2422493 which is a 5−UTR polymorphism located at −477 in the promoter with a reported 46% minor allele frequency [23,25]. Subsequent to us choosing these SNPs, rs2230808 and rs2066718 variants were reported to be associated with variability in plasma HDL levels [7–9] and with plasma cholesterol levels; which further validated their use in this association study. Further information on those polymorphisms can be found at Alzforum website (http://www.alzforum.org/res/com/gen/alzgene/geneoverview.asp?geneid=88). Genotyping was performed by RFLP method using the primers described [5] Tests of association were performed using FBAT program (version 1.5.1) with an additive model (http://www.biostat.harvard.edu/~fbat/default.html) for all four polymorphisms. The genotype frequencies were consistent with Hardy-Weinberg equilibrium. Given that there have been several reports of linkage in this region on chromosome 9 [1,17], we used the empirical variance option in FBAT (test distribution under the null hypothesis of linkage but no association) that adjusts for the correlation among sibling marker genotypes and for different nuclear families within a single pedigree.

Results and Discussion

Only T/T genotype of RS2422493 showed an association with the disease in the whole series (p=0.02), while both A/G and G/G genotypes of RS2230806 were associated with the disease only in the ApoE-ε4 non carriers (p=0.01 and p=0.02 respectively (Table 1). None of the analyzed polymorphisms showed significant linkage disequilibrium with the exception of RS2230806 and RS2230808 which showed evidence of Linkage Disequilibrium in the cases group (D′=0.237; p=1.99×10^{-5}).

Subsidiary analyses of the different haplotypes in either the presence or absence of the apolipoprotein E4 allele gave different results (Table 2). The haplotype were created with the

following SNPs order: RS2422493- RS2230806- RS2066718- RS2230808. A weak association of the haplotype C-A-G-G (p=0.02) with the disease is present in the ApoE-ε4 carrier; while haplotypes T-G-G-A (p=0.01) and T-A-G-G (p=0.01) showed an association with the disease in the ApoE-ε4 non-carrier; though, the unimpressive overall p-value for this analysis (p=0.03) suggests that the result can not be relied on because the number of informative families for the second haplotype is too low (2.6). However, the fact, that the genotypes G/G and A/G of RS2230806 also shows an association with the disease in the same sub-group (E4 negative), and that the first haplotype occurs in a reasonable number of informative families (7.1), might support the existence of an association for the haplotype T-G-G-A with the disease. It is of interest to note that this result is concordant with the findings of Shibata, *et al.* who also observed a significant association with the G allele of RS2230806 in the absence of the ApoE-ε4 allele [22]. The overall result suggests there might be a genetic influence of the ABCA1 polymorphisms on Alzheimer's disease, as shown by other groups [12]. The results shown here have not been corrected for multiple testing. Indeed, since the tests performed were done under the null hypothesis, reducing the type I error for null association increases the type II errors for those association that are not null [19,20]. A correction such as the Bonferroni test would make those results non-significant at the p=0.05 level, while this is a very conservative correction it highlights that the data here should be considered as preliminary and further studies are necessary in order to make any conclusion.

Nevertheless, these results are of interest since it was recently showed that targeted disruption of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP transgenic mice [14]; while lack of ABCA1 causes poor lipidation of ApoE, which become strongly amyloidogenic *in vivo* [24]. These previous findings emphasize the role of cholesterol in AD pathology which has been described as a risk factor for several years.

Our data is most consistent with the view that ABCA1 variability has a weak contribution to Alzheimer risk, but even though our study is reasonably large, a study of the order of one magnitude larger than we describe would be required to demonstrate or refute this possibility completely.

References

1. 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;12:23–32. [PubMed: 12490529]

2. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 1999;22:347–51. [PubMed: 10431237]

3. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest J Jr, Hayden MR. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999;22:336–45. [PubMed: 10431236]

4. Burns M, Duff K. Use of in vivo models to study the role of cholesterol in the etiology of Alzheimer's disease. Neurochem Res 2003;28:979–86. [PubMed: 12737522]

5. Clee SM, Zwinderman AH, Engert JC, Zwarts KY, Molhuizen HO, Roomp K, Jukema JW, van Wijland M, van Dam M, Hudson TJ, Brooks-Wilson A, Genest J Jr, Kastelein JJ, Hayden MR. Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 2001;103:1198–205. [PubMed: 11238261]

6. Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261:921–3. [PubMed: 8346443]

7. Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. J Clin Invest 2004;114:1343–53. [PubMed: 15520867]

8. Hodoglugil U, Williamson DW, Huang Y, Mahley RW. Common polymorphisms of ATP binding cassette transporter A1, including a functional promoter polymorphism, associated with plasma high density lipoprotein cholesterol levels in Turks. Atherosclerosis 2005;183:199–212. [PubMed: 15935359]

9. Hong SH, Rhyne J, Zeller K, Miller M. ABCA1(Alabama): a novel variant associated with HDL deficiency and premature coronary artery disease. Atherosclerosis 2002;164:245–50. [PubMed: 12204794]

10. Honig LS, Kukull W, Mayeux R. Atherosclerosis and AD: analysis of data from the US National Alzheimer's Coordinating Center. Neurology 2005;64:494–500. [PubMed: 15699381]

11. Kamboh MI. Molecular genetics of late-onset Alzheimer's disease. Ann Hum Genet 2004;68:381–404. [PubMed: 15225164]

12. Katzov H, Chalmers K, Palmgren J, Andreasen N, Johansson B, Cairns NJ, Gatz M, Wilcock GK, Love S, Pedersen NL, Brookes AJ, Blennow K, Kehoe PG, Prince JA. Genetic variants of ABCA1 modify Alzheimer disease risk and quantitative traits related to beta-amyloid metabolism. Hum Mutat 2004;23:358–67. [PubMed: 15024730]

13. Kehoe P, Wavrant-De Vrieze F, Crook R, Wu WS, Holmans P, Fenton I, Spurlock G, Norton N, Williams H, Williams N, Lovestone S, Perez-Tur J, Hutton M, Chartier-Harlin MC, Shears S, Roehl K, Booth J, Van Voorst W, Ramic D, Williams J, Goate A, Hardy J, Owen MJ. A full genome scan for late onset Alzheimer's disease. Hum Mol Genet 1999;8:237–45. [PubMed: 9931331]

14. Koldamova R, Staufenbiel M, Lefterov I. Lack of ABCA1 considerably decreases brain ApoE level and increases amyloid deposition in APP23 mice. J Biol Chem 2005;280:43224–35. [PubMed: 16207713]

15. Li Y, Tacey K, Doil L, van Luchene R, Garcia V, Rowland C, Schrodi S, Leong D, Lau K, Catanese J, Sninsky J, Nowotny P, Holmans P, Hardy J, Powell J, Lovestone S, Thal L, Owen M, Williams J, Goate A, Grupe A. Association of ABCA1 with late-onset Alzheimer's disease is not observed in a case-control study. Neurosci Lett 2004;366:268–71. [PubMed: 15288432]

16. Lutucuta S, Ballantyne CM, Elghannam H, Gotto AM Jr, Marian AJ. Novel polymorphisms in promoter region of atp binding cassette transporter gene and plasma lipids, severity, progression, and regression of coronary atherosclerosis and response to therapy. Circ Res 2001;88:969–73. [PubMed: 11349008]

17. Myers A, Wavrant De-Vrieze F, Holmans P, Hamshere M, Crook R, Compton D, Marshall H, Meyer D, Shears S, Booth J, Ramic D, Knowles H, Morris JC, Williams N, Norton N, Abraham R, Kehoe P, Williams H, Rudrasingham V, Rice F, Giles P, Tunstall N, Jones L, Lovestone S, Williams J, Owen MJ, Hardy J, Goate A. Full genome screen for Alzheimer disease: stage II analysis. Am J Med Genet 2002;114:235–44. [PubMed: 11857588]

18. Pericak-Vance MA, Grubber J, Bailey LR, Hedges D, West S, Santoro L, Kemmerer B, Hall JL, Saunders AM, Roses AD, Small GW, Scott WK, Conneally PM, Vance JM, Haines JL. Identification of novel genes in late-onset Alzheimer's disease. Exp Gerontol 2000;35:1343–52. [PubMed: 11113612]

19. Perneger TV. What's wrong with Bonferroni adjustments. Bmj 1998;316:1236–8. [PubMed: 9553006]

20. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1:43–6. [PubMed: 2081237]

21. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 1999;22:352–5. [PubMed: 10431238]

22. Shibata N, Kawarai T, Lee JH, Lee HS, Shibata E, Sato C, Liang Y, Duara R, Mayeux RP, St George-Hyslop PH, Rogaeva E. Association studies of cholesterol metabolism genes (CH25H, ABCA1 and CH24H) in Alzheimer's disease. Neurosci Lett 2006;391:142–6. [PubMed: 16157450]

23. Souverein OW, Jukema JW, Boekholdt SM, Zwinderman AH, Tanck MW. Polymorphisms in APOA1 and LPL genes are statistically independently associated with fasting TG in men with CAD. Eur J Hum Genet 2005;13:445–51. [PubMed: 15657615]

24. Wahrle SE, Jiang H, Parsadanian M, Hartman RE, Bales KR, Paul SM, Holtzman DM. Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. J Biol Chem 2005;280:43236–42. [PubMed: 16207708]

25. Woll PS, Hanson NQ, Arends VL, Tsai MY. Effect of two common polymorphisms in the ATP binding cassette transporter A1 gene on HDL-cholesterol concentration. Clin Chem 2005;51:907–9. [PubMed: 15855668]

26. Wollmer MA, Streffer JR, Lutjohann D, Tsolaki M, Iakovidou V, Hegi T, Pasch T, Jung HH, Bergmann K, Nitsch RM, Hock C, Papassotiropoulos A. ABCA1 modulates CSF cholesterol levels and influences the age at onset of Alzheimer's disease. Neurobiol Aging 2003;24:421–6. [PubMed: 12600718]

NIH-PA Author Manuscript   NIH-PA Author Manuscript   NIH-PA Author Manuscript

Table 1

Genotype Frequency of *ABCA1* polymorphisms in USA Sib-pair Series

SNP ID (Function)		Total (Nf=331)			ApoE-ε4- (Nf=76)			ApoE-ε4+ (Nf=227)		
		Fam#	Freq	P-Value	Fam#	Freq	P-Value	Fam#	Freq	P-Value
rs2422493 *(promoter)*	C/C	45	0.266	0.62	5	0.245	N/A	25	0.284	0.62
	T/C	79	0.494	0.17	14	0.494	0.29	38	0.489	0.35
	T/T	44	0.241	0.02*	10	0.261	0.30	21	0.227	0.08
rs2230806 (R219K)	A/A	17	0.095	0.93	2	0.058	N/A	10	0.111	0.86
	A/G	66	0.399	0.23	12	0.483	0.01*	31	0.388	0.70
	G/G	56	0.507	0.21	11	0.459	0.02*	25	0.500	0.59
rs2066718 (V771M)	A/A	0	0	N/A	0	0	N/A	0	0	N/A
	A/G	14	0.100	0.69	2	0.162	N/A	6	0.102	N/A
	G/G	14	0.900	0.69	2	0.838	N/A	6	0.898	N/A
rs2230808 (R1587K)	A/A	15	0.066	0.06	5	0.044	N/A	8	0.064	N/A
	A/G	62	0.426	0.69	14	0.505	0.64	32	0.420	0.90
	G/G	56	0.509	0.55	13	0.451	0.57	29	0.515	0.67

*: significant p-value

Fam#: Number of informative families

Freq: Frequency

Nf: Number of Nuclear Families

N/A: non applicable

Table 2

Haplotype permutation test of *ABCA1* polymorphisms (rs2422493- rs2230806- rs2066718- rs2230808) in the USA Sib-pair Series

Haplotype	Total (Nf=331)			ApoE-ε4- (Nf=76)			ApoE-ε4+ (Nf=227)		
	Fam#	Freq	P-value	Fam#	Freq	P-value	Fam#	Freq	P-value
T-G-G-G	63.8	0.318	0.65	13.1	0.332	0.89	32.5	0.307	0.54
C-G-G-G	64.1	0.294	0.77	9.1	0.211	0.40	35.6	0.338	0.47
C-G-G-A	28.3	0.082	0.81	6.1	0.134	0.93	15.1	0.069	0.90
C-A-G-G	22.4	0.071	0.19	4.6	0.076	0.27	10.1	0.059	0.02[*]
T-A-G-G	23.4	0.069	0.59	2.6	0.062	0.01[*]	12.7	0.069	0.52
T-G-G-A	27.7	0.061	0.06	7.1	0.08	0.01[*]	14.9	0.047	0.08
C-A-G-A	13.1	0.044	0.31	3.3	0.054	0.12	8.4	0.04	0.80
T-A-G-A	11.7	0.038	0.63	2.3	0.029	0.76	6.2	0.045	0.83
C-A-A-G	6.1	0.011	0.88	1	0.004	0.50	3	0.015	0.69
T-A-A-G	1.9	0.004	0.72		0.004	N/A	1	0.004	0.49
C-G-A-A	2	0.003	0.43	1	0.004	0.34	1	0.003	0.17
T-A-A-A	few	0.002	N/A	few	0.004	N/A		0.002	N/A
C-G-A-G	few	0.002	N/A		N/A		few	0.002	N/A
C-A-A-A	1	0.002	0.16	few	0.004	N/A		N/A	
T-G-A-A	few	0.001	N/A		N/A		few	0.002	N/A

[*]
: significant p-value

Fam#: Number of informative families

Freq: Frequency

Nf: Number of Nuclear Families