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Genetic variability at the amyloid-β precursor protein locus may contribute to the risk of late-onset Alzheimer's disease

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Abstract

In a series of sibpairs with late onset Alzheimer's disease, we have examined the segregation of the loci involved in the early onset, autosomal dominant form of the disorder by using flanking microsatellite repeat markers: thus we have used APP-PCR3 and D21S210 to examine the segregation of the amyloid-β precursor protein (APP) gene, the markers Dl 4S77 and D14S284 to examine the segregation of the presenilin 1 (PSI) gene and the markers D1S227, D1S249 and D1S419 to examine the segregation of presenilin 2 (PS2). We carried out our analyses on the whole dataset of 291 affected sibpairs, and on subsets comprising those sibpairs in which neither had an apolipoprotein E4 allele (65 affected sibpairs) and those in which both had an apolipoprotein E4 allele (165 affected sibpairs). We used the programs SPLINK to generate allele frequencies and MAPMAKER/SIBS to analyze our results. We examined the segregation of the markers D19S908 and D19S918 that are close to the apolipoprotein E (ApoE) gene as a positive control to assess whether the methods we are employing have the capability to identify known loci. The sibpair approach to the identification of genetic risk loci is relatively insensitive as indicated by the failure of the ApoE locus to reach statistical significance ($P = 0.06$). Nevertheless, these data suggest that neither the PS1 nor the PS2 gene is a major locus for late-onset AD, but that the APP gene cannot be ruled out as a risk locus in those sibships without an E4 allele ($P = 0.014$). The possibility that APP is indeed a locus for late onset disease will need confirmation in other series of familial cases.

Keywords: Alzheimer's disease; Genetics; Amyloid precursor protein; Apolipoprotein E; Presenilin 1; Presenilin 2; Sibpairs

Mutations in the amyloid-β precursor protein (APP), the presenilin 1 (PS1) and the presenilin 2 (PS2) genes lead to early onset autosomal dominant Alzheimer's disease (AD) [7,14,23] almost certainly through pathways which depend upon production of increased amounts of the amyloidogenic peptide Aβ42 [22]. In addition, it is likely that individuals with Down syndrome develop AD because they too, produce more Aβ42 as the APP gene is encoded on chromosome 21 [17,21]. Mutations in these genes have never been found in significant numbers of elderly individuals and the consensus has been that mutations in these genes do not contribute (with very rare exceptions) to late onset disease.

The only locus that is known to contribute to the risk of late onset AD is the apolipoprotein E (ApoE) gene. Relative to the common E3 allele of ApoE, the E4 allele is associated with increased risk of disease and the E2 allele with decreased risk of disease [3,4]. However, the possibility that the loci associated with early onset disease could contribute to late onset disease has, surprisingly, never been addressed in a systematic genetic fashion. Previous experiments have merely sought mutations in the open reading frame of these genes or in promoter or intronic elements

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Table 1
Primer sequence for loci at ApoE, APP, PS1 and PS21[a]

Marker	Dye	Size	Forward	Reverse
D19S908	TET	221–253	TGTAGTAAGCCAAGATCACTCC	AATTGTACNCACTGTGTGCCAG
D19S918	FAM	268–306	AAAGGCTTGATTACCCCCOA	ATTACAGACGTGGGAGACCA
APP.PCR3	FAM	124–162	GCCCATCAAGATGCTACTGA	ACATTCCTCTCTGCCTCTAC
D21S210	HEX	148–190	AGATATTGGATGCTCAACTC	CTGGAGAGAACTCTGATATG
D14S77	FAM	214–260	TTATAAGCGTGAGTCACTGTGCCCA	GGCTNCAGACAGAAATTAACCAGAO
D14S284	FAM	184–208	TACAGGTATOAGCCACCACT	GCCTACTTATCNTCCAAAGACAG
D1S227	TET	111–125	AGCTGTGTCGGGTCTTGAAGAA	GAGCGATTTCAGAATGTTGC
D1S249	FAM	316–346	TGAGGCTGAGGCAGGAGAAT	TGGTTGTAGATG;AGACTGGC
D1S419	TET	325–357	CAGGACTGTACATTGGGTACCA	AGCTCAGAAAGAATGAGCACGC

[a] Primer sequences and dye labeling used for assessing the genetic contributions of the known AD loci. These sequences can be paneled together for efficiency and run together on an ABI machine.

close to the open reading frame [20,27] and then tested these specific changes for association with disease with mixed and inconclusive results [15,27].

A priori however, one might expect that genetic variability controlling the expression of the genes encoded at these loci might contribute significantly to disease risk. Overexpression of the APP gene clearly leads to AD in Down syndrome [17], and recent experiments in which the mouse PS1gene was knocked out suggested that expression levels of this gene might also play a part in regulating Aβ production [5]. Moreover, recent characterization of the APP promoter reveals several functional domains that can regulate the expression of the APP gene [6,18]. For these reasons, we decided to test the hypotheses that sibpairs who were both affected by late onset AD would share alleles at the APP, PS1 and PS2 loci more frequently than the 50% one would expect by chance.

For each of these genetic loci and for ApoE we chose two informative genetic markers (Table 1) (three markers for PS2) which framed the gene and examined the segregation of the markers within 291 sibpairs from the NIMH series of late-onset AD families [2]. We developed primer sets (Table 1) which allowed these markers to be paneled together and run simultaneously on ABI DNA sequencers. The program SPLINK was used to generate marker allele frequencies from our dataset [9] and both SPLINK and MAPMAKER SIBS [11] were used to analyze our results. We carried out three, two point analyses: in sibpairs in which both had an

Table 2
Single locus lodscores for markers at the ApoE, APP, PSI and PS2 loci analyzed using MAPMAKER/SIBS2[a]

Gene	ApoE		APP		PS1		PS2		
Marker	D19S908	D19S918	APPPCR3	D21S210	D14S77	D14S284	D1S227	D1S249	D1S419
All pairs	0.49	0.71	0	0.1	0.06	0	0.04	0.06	0.01
Exclusion									
λ1.2	0.49	0.71	−1.25	0	−1.10	4.44	−0.41	0.16	0.46
λ1.4	0.24	0.36	Excluded	0.54	Excluded	Excluded	−1.35	0.93	−1.58
λ1.6	0.31	0.4l	Excluded	−1.28	Excluded	Excluded	Excluded	−1.91	Excluded
λ1.8	−0.98	−1.38	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
λ2.0	−1.69	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded	Excluded
E4-pairs	NA	NA	0.3	1.12	0	0	0	0	0
Exclusion									
λ1.2	NA	NA	0.23	0.58	−0.60	−0.66	−0.20	0.20	0.24
λ1.4	NA	NA	0.29	0.8	−1.31	−1.37	0.52	−0.54	−0.45
λ1.6	NA	NA	0.22	0.98	−1.82	−1.97	−0.78	−0.78	−0.93
λ1.8	NA	NA	0.14	1.01	Excluded	Excluded	−1.07	−1.07	−1.28
λ2.0	NA	NA	0.04	1.01	Excluded	Excluded	4.35	−1.35	−1.62
E4 + pairs	NA	NA	0	0	0.18	0	0.15	0.03	0.15
Exclusion									
λ1.2	NA	NA	−1.18	−0.37	−0.66	−0.89	0.13	−0.12	0.2
λ1.4	NA	NA	Excluded	−0.91	−1.7	−1.93	−0.10	−0.61	−0.18
λ1.6	NA	NA	Excluded	−1.49	Excluded	Excluded	−0.46	−1.24	−0.64
λ1.8	NA	NA	Excluded	Excluded	Excluded	Excluded	−0.87	4.89	−1.15
λ2.0	NA	NA	Excluded	Excluded	Excluded	Excluded	−1.28	Excluded	−1.68

[a] Two-point analysis of segregation analyzed by MAPMAKER/SIBS. NA, not analyzed. Exclusion analysis was taken down to λ at which it was <−2.0, the conventional criteria for exclusion of linkage. Thus, these data show (for example), that the PS 1 locus does not have λ in all affected sibpairs of >1.4, in E4− pairs of >I.8 and in E4+ >pairs of >1.6.

E4 allele (165 sibpairs), in sibpairs in which neither had an E4 allele (65 sibpairs) and in the total dataset (291 sibpairs). We carried out exclusion analysis at different values of λs where λs is defined as the ratio of the risk to siblings of a case relative to the risk of the trait in the general population attributable to the locus under study [19].

Our results are illustrated in Table 2. First of all these experiments illustrate both the strengths and weaknesses of the sibpair approach to the finding of pathogenic loci. Thus, this analysis (which used ~850 samples) barely has the power to identify the ApoE locus ($P = 0.06$ at D195918: SPLINK analysis). In contrast, simple association studies would need only 50 samples to show the association with this locus: however, in the approach we use here, loci can be identified solely by their chromosomal position. Secondly, and given the caveat that this is an insensitive method for identifying loci involved in disease, they suggest that in sibships without an E4 allele, the APP locus is more likely to be shared by affected sibs than by chance ($P = 0.014$ at D21S210: SPLINK analysis: analysis uncorrected for multiple comparisons). These analyses provided neither significant evidence for or against the notion that the presenilin genes may contribute to the risk of getting late onset AD. However, they do suggest that these loci are not the site of major risk for developing disease and therefore they are not concordant with the suggestion, based on association studies, that the PS1 gene is a major risk locus for late-onset disease [27]. Thus, our analyses show no positive lod scores with either PS1 or PS2 genetic markers in any group and exclude these loci from having a risk comparable in effect size with the ApoE locus.

The observation that APP may be a risk factor locus is not surprising. Studies of plasma Aβ have suggested that a subgroup of late onset disease cases have an increase in the concentration of this biomarker [22]. This 'high expression' subgroup may overlap with the group of individuals whose genetic susceptibility is, in part, encoded at the APP gene. The fact that the APP gene appears to be a risk locus only in individuals without an E4 allele is interesting and consistent with the view that ApoE is involved in the process of deposition of Aβ rather than in its production [1].

A parsimonious explanation of these results is that genetic variability in regulatory regions of the APP gene contributes to disease risk through the production of Aβ. We have previously failed to find any genetic variability in the APP promoter in AD [20]. However, our study dealt only with the promoter region of 334 bp, from −558 and −225 bp relative to the transcription start site of the gene. The APP locus is complex and there are many other regulatory regions that need to be examined for genetic variability. Within the mRNA there are elements in the three untranslated region that may contribute to message processing [28]. Within and upstream of the promoter, there are many regulatory elements at which genetic variability may occur. For example, one block extending from about −600 to −460 bp acts as a positive regulator and a second block of sequences extending from −450 to −150 bp acts as a negative regulator [13]. Two additional upstream regulatory elements that control the activity of the human APP promoter, are from −2257 to −2234 bp and from −489 to −452 bp [12]. Other novel APP transcriptional regulatory elements within the first 100 bp from the start site are the GC-elements [16], the zinc finger protein motif CTCF [26] and an upstream regulatory factor (USF) [10]. Detailed characterization of the relevant nuclear factors in different cell types has not been reported. Recently, a l7 kb DNA fragment containing the 5′-upstream regulatory region of the rhesus APP gene has been characterized [24]. The rhesus APP promoter gene is similar in sequence to the human promoter and it contains many inducible characteristics responsible for its regulated transcription. A functional study with a series of 5′-deletion APP gene regions extending as far upstream as −7900 bp relative to the start site indicates that a −75 to +104 bp region is an essential promoter element, that multiple positive and negative elements regulate the expression of the promoter and that the interaction of the USF and pyrimidine-rich initiator element at the proximal region is crucial for transcription [25]. The study with the rhesus promoter reveals an important upstream regulatory element at −76 to −48 bp that controls the activity of the rhesus promoter in a cell type-specific manner. These functional studies clearly show that there are many elements around the APP gene where genetic variability has not been sought and which could influence APP expression [8].

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[1] Bales, K.R., Verina, T., Dodel, R.C., Du, Y., Mtstiel, L., Bender, M., Hyslop, P., Jolinstone, E.M., Little, S.P., Cummins, D.J., Piccardo, P., Ghetti, B. and Paul, S.M., Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. Nat Genet., 17 (1997) 263–264.

[2] Blacker, D., Haines, J.L., Rodes, L., Terwedow, H., Go, R.C., Harrell, L.E., Perry, R.T., Bassett, S.S., Chase, G., Meyers, D., Albert, M.S. and Tanzi, R., ApoE-4 and age at onset of Alzheimer's disease: the NIMH genetics initiative. Neurology, 48 (1997) 139–147.

[3] Chartier-Harlin, M.C., Parfiff, M., Legrain, S., Pérez-Tur, J., Brousseau, T., Evans, A., Berr, C., Vidal, O., Roques, P. and Gourlet, V., et al., Apolipoprotein E, epsilon 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. Hum. Mol. Genet., 3 (1994) 569–574.

[4] Corder, E.H., Saunders, A.M., Stritimatter, W.J., Schinechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A., Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science, 261 (1993) 921–923.

[5] De Strooper, B., Saflig, P., Craessaerts, K., Vanderstichele,

H., Guhde, G., Annaert, W., Von Figura, K. and Van Leuven, F., Deficiency of presenilin-l inhibits the normal cleavage of amyloid precursor protein. Nature, 391 (1998) 387–390.
[6] Fox, N.W., Johnstone, E.M., Ward, K.E., Schrementi, J. and Little, S.P., APP gene promoter constructs are preferentially expressed in the CNS and testis of transgenic mice. Biochem. Biophys. Res. Commun., 240 (1997) 759–762.
[7] Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N. and James, L., et al., Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature, 349 (1991) 704–706.
[8] Hattori, M., Tsukahara, F., Furuhata, Y., Tanahashi, H., Hirose, M., Saito, M., Tsukuni, S. and Sakaki, Y., A novel method for making nested deletions and its application for sequencing of a 300 kb region of human APP locus. Nucl. Acids Res., 25 (1997) 1802–1808.
[9] Holmans, P. and Clayton, D., Efficiency of typing unaffected relatives in an affected-sib-pair linkage study with single-locus and multiple tightly linked markers. Am. J. Hum. Genet., 57 (1995) 1221–1232.
[10] Kovacs, D.M., Wasco, W., Witherby, J., Felsenstein, K.M., Brunel, F., Roeder, R.G. and Tanzi, R.E., The upstream stimulatory factor functionally interacts with the Alzheimer amyloid β-protein precursor gene. Hum. Mol. Genet., 4 (1995) 1527–1533.
[11] Krugylak, L. and Lander, E.S., Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am. J. Hum. Genet., 57 (1995) 1237–1241.
[12] Lahiri, D.K., An upstream region of the gene promoter for the beta-amyloid precursor protein interacts with proteins from nuclear extracts of the human brain and PC12 cells. Mol. Brain Res., 58 (1998) 112–122.
[13] Lahiri, D.K. and Robakis, N.K., The promoter activity of the gene encoding Alzheimer beta-amyloid precursor protein (APP) is regulated by two blocks of upstream sequences. Mol. Brain Res., 9 (1991) 253–257.
[14] Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C.E., Jondro, P.D., Schmidt, S.D. and Wang, K., et al., Candidate gene for the chromosome 1 familial Alzheimer' disease locus. Science, 269 (1995) 973–977.
[15] Pérez-Tur, J., Wavrant-De Vrièze, F., Lambert, J.C. and Chartier-Harlin, M.C., Presenilin-1 polymorphism and Alzheimer's disease. Lancet, 347 (1996) 1560–1561.
[16] Pollwein, P., Masters, C.L. and Beyreuther, K., The expression of the amyloid precursor protein (APP) is regulated by two GC-elements in the promoter. Nucl. Acids Res., 20 (1992) 63–68.
[17] Prasher, V.P., Farrer, M.J., Kessling, A.M., Fisher, E.M., West, R.J., Barber, P.C. and Butler, A.C., Molecular mapping of Alzheimer-type dementia in Down's syndrome. Ann. Neurol., 43 (1998) 380–383.
[18] Quitschke, W.W. and Goldgaber, D., The amyloid beta-protein precursor promoter. A region essential for transcriptional activity contains a nuclear factor binding domain. J. Biol. Chem., 267 (1992) 17362–17368.
[19] Risch, N., Linkage strategies for genetically complex traits. I. Multilocus models. Am. J. Hum. Genet., 46 (1990) 222–228.
[20] Rooke, K., Goate, A., Fidani, L., Mullan, M., Roques, P., Rossor, M., Hardy, J. and ChartierHarlin, M.C., Screening of the promoter and the β-amyloid sequence of the APP gene for polymorphism in families with late onset Alzheimer's disease. Neurodegeneration, 1 (1993) 237–240.
[21] Rumble, B., Retallack, R., Hilbich, C., Simms, G., Muithaup, G., Martins, R., Hockey, A., Montgomery, P., Beyreuther, K. and Masters, C.L., Amyloid A4 protein and its precursor in Down's syndrome and Alzheimer's disease. N. Engl. J. Med., 320 (1989) 1446–1452.
[22] Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Vutanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S., Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med., 2 (1996) 864–870.
[23] Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G. and Holman, K., et al., Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature, 375 (1995) 754–760.
[24] Song, W. and Lahiri, D.K., Molecular cloning of the gene encoding the Rbesus monkey β-amyloid precursor protein: structural characterization of the promoter and comparative study with other species. Gene, 217 (1998) 151–164.
[25] Song, W. and Lahri, D.K., Functional identification of the promoter of the gene encoding the Rhesus monkey β-amyloid precursor protein. Gene, 217 (1998) 165–176.
[26] Vostrov, A.A. and Quitschke, W.W., The zinc finger protein CTCF binds to the APB beta domain of the amyloid beta-protein precursor promoter. Evidence for a role in transcriptional activation. J. Biol. Chem., 272 (1997) 33353–33359.
[27] Wragg, M., Hutton, M. and Talbot, C., Alzheimer's Disease Collaborative Group. Genetic association between intronic polymorphism in presenilin-1gene and late-onset Alzheimer's disease. Lancet, 347 (1996) 509–512.
[28] Zaidi, S.H. and Malter, J.S., Nucleolin and heterogeneous nuclear ribonucleoprotein C proteins specifically interact with the 5′-untranslated region of amyloid protein precursor mRNA. J. Biol. Chem., 270 (1995) 17292–17298.