Assessment of Alzheimer's Disease Risk Genes with CSF-Biomarker Levels

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Background

Alzheimer's disease (AD) is genetically complex, resulting in neurodegeneration and progressive cognitive decline. Recently, a number of genome-wide association studies (GWAS) have implicated several loci as novel Alzheimer's disease risk genes in addition to *APOE* (see www.alzgene.org). Eleven additional putative AD loci are reported at this meeting (Lambert et al. 2013 [F1-01-01]). In work related to the project presented here, we showed that the AD-associated risk alleles near *PICALM*, but not those near *CLU* or *CR1*, correlate with reduced levels of Aβ42 in the cerebrospinal fluid (CSF) of AD patients and healthy controls (1). We have now extended these CSF-biomarker analyses to include single-nucleotide polymorphisms (SNPs) in a number of additional GWAS loci – including those that emerged from a recent GWAS using CSF tau levels as quantitative trait (2) – in a substantially extended dataset, currently including more than 1,600 individuals.

Methods

Study Subjects. All subjects were recruited at the respective collaborative sites (see Table 1).

SNP Selection. The SNPs investigated in this study were chosen based on several GWAS published in AD since 2009. The *CD33* locus (here analyzing SNP rs3865444) was originally identified by Bertram et al (3); the *ABCA7* locus (rs3764650 and rs3752246) and the *MS4A* locus (rs610932) were identified in Hollingworth et al (4); *CD2AP* (rs1485780) and *EPHA1* (rs11767557) were identified in Naj et al (5); and *BIN1* (rs744373) was identified in Seshadri et al (6). We also included four SNPs on chromosomes 3, 6 and 9 rs9877502 (near SNAR-I), rs6922617 (near *NCR2* and a number of TREM and TREM-like genes incl. *TREM2*), rs514716 and rs624290 (in *GLIS3*) found to be associated with CSF-tau levels in Cruchaga et al (2).

Genotyping. Genotyping was performed at MPIMG on DNA samples contributed by the collaborating sites derived from blood or saliva using standard extraction procedures. All SNPs were genotyped in 384-well format using TaqMan singleplex assays according to manufacturer's instructions. Genotyping efficiency was 99.5% with an error rate below <0.1% based on genotyping samples from the International HapMap Consortium (~5% on each 384-well plate). None of the markers violated HWE at P≤0.05 in the control samples.

Measurements of CSF biomarkers. CSF collection and pre-analytic processing was performed at each respective clinic of origin (see Table 1 for more details).

Statistical analysis. Prior to the association analyses, CSF Aβ42 and t-tau values were transformed to normal distributions after adjusting for age at examination (AAE) and *APOE*-ε4 status (0,1,2) using an inverse normal transformation. The actual association analyses between the transformed CSF traits and genotypes at each SNP were performed per site per DX group using a linear regression model assuming additive transmission. Within DX groups, site-specific association results were combined via fixed-effect meta-analyses. The effective number of independent tests was 11 per trait (calculated by SimpleM*), which yields a nominal experiment-wide significance level of α= 0.0008.

Table 1: Demographic characteristics of the populations studied.

DX / Site	TOTAL	Munich - LMU Germany[5]	Munich - TU Germany[5]	Rostock Germany[5]	Ulm Germany[5]	Barcelona Spain[4]	Uppsala Sweden[6]	Zagreb Croatia[3]
Healthy	315	12	-	7	256	28	-	12
AAE (SD)	62.9 (7.8)	64.2 (6.3)	-	69.7 (1.2)	63.0 (10.8)	61.5 (9.2)	-	56.1 (11.4)
Women (%)	182 (51%)	4 (33%)	-	4 (57%)	131 (51%)	15 (54%)	-	8 (67%)
CSF Aβ42 (SD)[2]	864.0 (273.7)	789.5 (184.0)	-	998.6 (237.3)	936.3 (316.1)	769.7 (172.3)	-	845.8 (458.6)
CSF t-tau (SD)[2]	229.8 (132.5)	264.1 (141.8)	-	125.9 (25.0)	258.9 (134.0)	202.7 (67.4)	-	297.5 (294.5)
AD	1010	360	58	55	324	57	53	103
AAE (SD)	70.6 (8.0)	72.7 (8.6)	64.0 (9.4)	72.8 (7.8)	72.7 (7.4)	70.7 (7.5)	68.5 (7.5)	72.5 (7.5)
Women (%)	622 (62%)	230 (64%)	30 (52%)	37 (67%)	202 (62%)	35 (61%)	34 (64%)	94 (52%)
CSF Aβ42 (SD)[2]	472.6 (204.3)	465.9 (215.1)	570.4 (227.8)	413.2 (175.0)	493.0 (205.1)	379.8 (179.4)	367.2 (132.3)	618.9 (295.0)
CSF t-tau (SD)[2]	589.1 (399.4)	665.4 (378.6)	589.8 (596.0)	482.0 (310.3)	617.4 (373.1)	675.7 (354.0)	636.2 (394.2)	457.3 (389.7)
MCI	290	164	-	13	-	-	57	56
AAE (SD)	68.9 (8.2)	70.1 (8.5)	-	75.5 (4.8)	-	-	63.7 (8.4)	66.4 (10.9)
Women (%)	138 (48%)	82 (50%)	-	3 (23%)	-	-	24 (42%)	29 (52%)
CSF Aβ42 (SD)[2]	619.6 (305.3)	692.8 (291.5)	-	535.6 (313.6)	-	-	526.5 (238.4)	723.4 (377.7)
CSF t-tau (SD)[2]	386.7 (241.4)	451.1 (311.2)	-	379.8 (216.5)	-	-	478.9 (275.9)	237.1 (162.1)
Total	1615	536	58	75	580	85	110	171
Women (%)	922 (57%)	316 (59%)	30 (52%)	44 (59%)	333 (57%)	50 (59%)	58 (53%)	91 (53%)

Legend: SD= Standard Deviation, AAE = Age at Examination, AD= Alzheimer's Disease, MCI=Mild Cognitive Impairment, Healthy controls were free of any impairment in cognitive function, AD was diagnosed according to NINCDS-ADRDA criteria and DSM-IV, classifications of MCI were based on the Petersen criteria (Munich-LMU, Rostock, Uppsala) and the NINCDS-ADRDA criteria (Zagreb). [1]CSF Aβ42/tau levels quantified using the Innogenetics ELISA kit (Innotest® "β-amyloid(1-42)" and "hTau Ag" kits). [2]CSF Aβ42/tau levels quantified using xMAP Luminex bead-based assay methodology.

Results

Figure 1. Association analyses of SNPs in *APOE* and CSF-Aβ42 & CSF-t-tau levels.

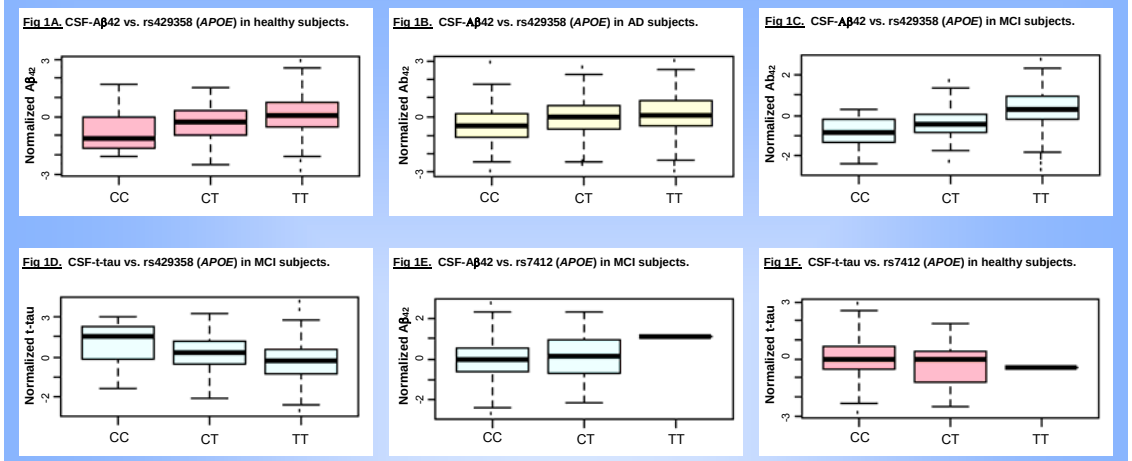
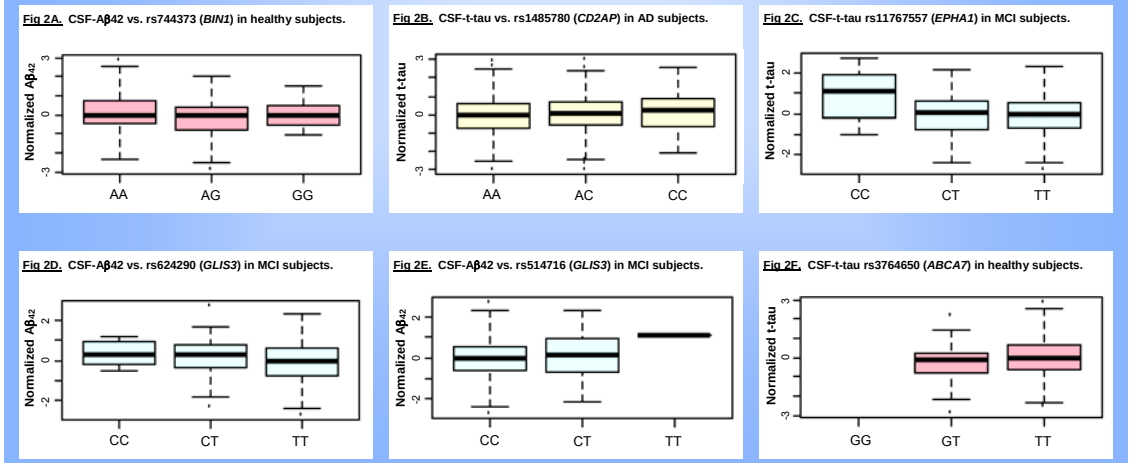


Figure 2. Association analyses of SNPs in non-*APOE* GWAS loci and CSF-Aβ42 & CSF-t-tau levels.



Results (continued)

Table 2: Results of the most significant association analyses between CSF-Aβ42 or CSF-t-tau and cognitive phenotype.

SNP (rs-ID)	Chr.	Gene	A1	A2	Effect (β)	SE	P	Trait	DX
rs744373	2	*BIN1*	G	A	-0.1928	0.0966	**0.0461**	Aβ42	Healthy
rs744373	2	*BIN1*	G	A	0.1688	0.0974	0.0831	t-tau	MCI
rs9877502	3	*GMNC*	A	G	0.158	0.0876	0.07132	t-tau	Healthy
rs6922617	6	*NCR2*	A	G	0.338	0.1804	0.0609	Aβ42	Healthy
rs1485780	6	*CD2AP*	C	A	0.1114	0.0509	**0.02867**	t-tau	AD
rs11767557	7	*EPHA1*	C	T	-0.2027	0.106	0.05575	Aβ42	MCI
rs11767557	7	*EPHA1*	C	T	0.2237	0.1035	**0.0306**	t-tau	MCI
rs11767557	7	*EPHA1*	C	T	0.1873	0.1083	0.0838	t-tau	Healthy
rs624290	9	*GLIS3*	C	T	0.2934	0.1349	**0.0297**	Aβ42	MCI
rs514716	9	*GLIS3*	C	T	0.2863	0.1213	**0.0183**	Aβ42	MCI
rs3764650	19	*ABCA7*	G	T	-0.372	0.1662	**0.0252**	t-tau	Healthy
rs3764650	19	*ABCA7*	G	T	-0.1394	0.0752	0.06394	t-tau	AD
rs3752246	19	*ABCA7*	G	C	0.2006	0.1026	0.0505	t-tau	MCI
rs429358	19	*APOE* (ε4)	C	T	-0.4628	0.1157	**6.34E-05**	Aβ42	Healthy
rs429358	19	*APOE* (ε4)	C	T	-0.317	0.0453	**2.56E-12**	Aβ42	AD
rs429358	19	*APOE* (ε4)	C	T	-0.6644	0.086	**1.10E-14**	Aβ42	MCI
rs429358	19	*APOE* (ε4)	C	T	0.4519	0.0911	**7.12E-07**	t-tau	MCI
rs7412	19	*APOE* (ε2)	T	C	0.4098	0.1771	**0.02066**	Aβ42	MCI
rs7412	19	*APOE* (ε2)	T	C	-0.3953	0.1607	**0.01387**	t-tau	Healthy
rs7412	19	*APOE* (ε2)	T	C	-0.2136	0.1308	0.1000	t-tau	AD

Legend: SNP = single nucleotide polymorphism, Chr = chromosome, A1/A2 = SNP alleles 1/2, SE = standard error, DX = Diagnosis. **Bold font** = nominally significant (P ≤0.05) results. Adjusted nominal significance threshold for this study P=0.0008 (see methods). Results with P-values ≤0.1 are displayed in Figures 1 &2.

Conclusions

- We have assembled a large, independent sample of **>1,600 subjects** (healthy, AD, MCI) with **DNA & CSF biomarker data**
- While multiple nominally significant associations were observed here, **only SNPs in *APOE*** surpassed the experiment-wide threshold (P≤0.0008, Table 2)
- The strongest **non-*APOE* signal** using **CSF-Aβ42** was elicited by ***GLIS3*** (P=0.02)
- The strongest **non-*APOE* signal** using **CSF-t-tau** was elicited by ***ABCA7*** (P=0.025)

References & Acknowledgements

References.
(1) Schjeide BM, et al. *Arch Gen Psychiatry.* 2011;68(2):207-13; (2) Cruchaga C, et al.. *Neuron.* 2013;78:256-68; (3) Bertram L. et al. *Am J Hum Genet.* 2008 Nov;83(5):623-32; (4) Hollingworth P, et al. *Nat. Genet.* 2011;43(5):429-435. (5) Naj A, et al. *Nat. Genet.* 2011;43(5):436-441. (6) Seshadri S, et al. *JAMA.* 2010;303(18):1832-40.

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