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The *SNAP-25* gene is associated with cognitive ability: evidence from a family-based study in two independent Dutch cohorts

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The synaptosomal-associated protein of 25 kDa (*SNAP-25*) gene plays an integral role in synaptic transmission, and is differentially expressed in the mammalian brain in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra and cerebellar granular cells. Recent studies have suggested a possible involvement of *SNAP-25* in learning and memory, both of which are key components of human intelligence. In addition, the *SNAP-25* gene lies in a linkage area implicated previously in human intelligence. In two independent family-based Dutch samples of 391 (mean age 12.4 years) and 276 (mean age 37.3 years) subjects, respectively, we genotyped 12 single-nucleotide polymorphisms (SNPs) in the *SNAP-25* gene on 20p12–20p11.2. From all individuals, standardized intelligence measures were available. Using a family-based association test, a strong association was found between three SNPs in the *SNAP-25* gene and intelligence, two of which showed association in both independent samples. The strongest, replicated association was found between SNP rs363050 and performance IQ (PIQ), where the A allele was associated with an increase of 2.84 PIQ points ($P = 0.0002$). Variance in this SNP accounts for 3.4 % of the phenotypic variance in PIQ.
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Introduction

Intelligence is one of the most heritable traits in humans, with heritability estimates ranging from 25 to 40% in early childhood[1] to 80% in adulthood.[2] Recently, the first genome-wide scan for intelligence was published, identifying two regions on chromosome 2q and 6p that showed significant linkage to intelligence, and several other regions showing suggestive linkage (4p, 7q, 20p, 21p).[3] Other scans followed shortly, replicating the 6p region, and also pointing to other regions (e.g. 14q).[4–7] An alternative approach to gene finding is to perform genetic association tests with candidate genes that are selected based on prior knowledge of biochemical functioning. We followed the latter approach and selected a putative candidate gene that was recently shown to be involved in learning and memory, which are two major components of intelligence. Several studies have demonstrated that the hippocampus plays a central role in learning and memory.[4–8] Damage to the hippocampus selectively impairs the ability to learn and remember.[8–15] The synaptosomal-associated protein of 25 kDa (*SNAP-25*) gene lies in an area of previous suggestive linkage to intelligence (20p12–p11.2),[3] and is highly expressed by neurons in the hippocampus.[16–18] The *SNAP-25* gene product is a presynaptic plasma membrane protein that is an integral component of the vesicle docking and fusion machinery that regulates neurotransmitter release.[17,19,20] It is also implicated in axonal growth and synaptic plasticity.[21] Three lines of evidence suggest a major role of *SNAP-25* in learning and memory in humans. Firstly, selective inhibition of *SNAP-25* expression prevents axonal elongation and the transformation of growth cones to synaptic terminals,[21] especially in hippocampal neurons.[22] Such remodeling of nerve terminals in the adult brain may serve as a morphological substrate of learning and memory.[21,23] Secondly, mRNA levels of *SNAP-25* are increased after the induction of long-term potentiation (LTP) in granule cells of the dentate gyrus.[24]

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Hippocampal LTP is thought to be a form of synaptic plasticity that underlies memory and learning.[25–28] Thirdly, inhibition of hippocampal *SNAP-25* leads to impaired long-term contextual fear memory, spatial memory, as well as decreased LTP.[23] The suggestive (according to the Lander and Kruglyak guidelines)[29] linkage finding of general intelligence (20p12–p11.2)[3] to the area containing *SNAP-25* renders this gene a putative candidate gene for human intelligence.

The present study aims to investigate whether *SNAP-25* gene plays a role in human intelligence. To this end, a family-based association approach is used in two independent cohorts of children (mean age 12.4 years) and adults (mean age 37.3 years).

Materials and methods

Subjects

All twins and their siblings were part of two larger cognitive studies and were recruited from the Netherlands Twin Registry.[30] Informed consent was obtained from the participants (adult cohort) or from their parents if they were under 18 (young cohort). The current study was approved by the institutional review board of the VU University Medical Center. None of the individuals tested suffered from severe physical or mental handicaps, as assessed through standard questionnaire.

Young cohort

The young cohort consisted of 177 twin pairs born between 1990 and 1992, and 55 siblings.[31] The twins were 12 (mean = 12.4, s.d. = 0.95) years of age and the siblings were between 8 and 15 years old at the time of testing. There were 41 monozygotic male twin pairs (MZM), 28 dizygotic male twin pairs (DZM), 56 monozygotic female twin pairs (MZF), 25 dizygotic female twin pairs (DZF), 27 dizygotic opposite-sex twin pairs (DOS), 28 male siblings and 27 female siblings. Participation in this study included a voluntary agreement to provide buccal swabs for DNA extraction.

Adult cohort

A total of 793 family members from 317 extended twin families participated in the adult cognition study.[2] Participation in this study did not automatically include DNA collection; however, part of the sample (276 subjects) returned to the lab to provide blood for DNA extraction. Mean age was 37.3 years (s.d. = 12.50). There were 20 MZM, 11 DZM, one DZM triplet, 14 MZF, 22 DZF and 17 DOS, 23 female siblings and 23 male siblings, and 59 subjects from incomplete twin pairs (18 males, 41 females).

Cognitive testing

In the young cohort, cognitive ability was assessed with the Dutch adaptation of the Wechsler Intelligence Scale for Children-Revised,[32] and consisted of four verbal subtests (similarities, vocabulary, arithmetic and digit span) and two performance subtests (block design and object assembly).

In the adult cohort, the Dutch adaptation of the Wechsler Adult Intelligence Scale III-Revised[33] assessed IQ and consisted of four verbal subtests (information, similarities, vocabulary and arithmetic) and four performance subtests (picture completion, block design, matrix reasoning and digit-symbol substitution). In both cohorts, verbal IQ (VIQ), performance IQ (PIQ) and full-scale IQ (FSIQ) were normally distributed. Correlations between FSIQ/VIQ, FSIQ/PIQ and PIQ/VIQ were 0.89, 0.81 and 0.45, respectively, in the young cohort, and 0.90, 0.84 and 0.55, respectively, in the adult cohort. Means and standard deviations of the full and genotyped cohorts are provided in Table 1.

DNA collection and genotyping

Buccal swabs were obtained from 391 children; blood was obtained from 276 adults. The DNA isolation from buccal swabs was performed using a chloroform/isopropanol extraction.[34] DNA was extracted from blood samples using the salting out protocol.[35]

Zygosity was assessed using 11 polymorphic microsatellite markers (Het > 0.80). Tagging single-nucleotide polymorphisms (*tag*-SNPs) selection criteria were defined as SNPs with a minor allele frequency (MAF) above 0.10 and genotypic correlation (ρ) across the genotypes of maximal 0.85 as obtained from a randomly selected Caucasian sample (http://www.celeradiagnostics.com/cdx/applera_genomics). MAF had to be > 0.10 in order to avoid the rare heterozygous genotypes and SNPs with a ρ above

Table 1 Means and standard deviations of PIQ, VIQ and FSIQ in the young and adult cohorts

	Young cohort		*Adult cohort*	
N	*Total sample* 409	*Genotyped* 391	*Total sample* 793	*Genotyped* 276
Age (s.d.)	12.37 (0.95)	12.36 (0.90)	37.60 (13.00)	37.40 (12.42)
Mean PIQ (s.d.)	101.40 (12.85)	101.66 (12.96)	100.96 (12.50)	100.04 (12.40)
Mean VIQ (s.d.)	98.42 (19.04)	98.90 (19.02)	92.78 (13.83)	93.03 (14.36)
Mean FSIQ (s.d.)	99.81 (15.20)	100.21 (15.21)	95.74 (11.62)	95.59 (12.04)

Abbreviations: FSIQ, full-scale IQ; PIQ, performance IQ; VIQ, verbal IQ.

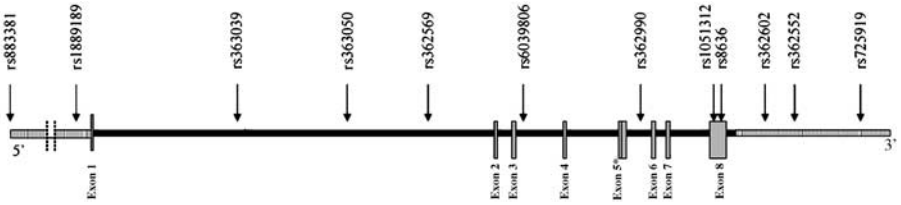

Figure 1 Location of *tag*-SNPs selected within the *SNAP-25* gene on chromosome 20 p12–p11.2.

0.85 with any of the other SNPs were not selected, to avoid redundancy. Twelve *tag*-SNPs in the *SNAP-25* gene were selected according to these criteria (http://www.appliedbiosystems.com/support/software/snplex/) using SNP Browser version 2.0.4 (NCBI build 34). Ranging from the 5′ untranslated region (5′ UTR) to 3′UTR region within the *SNAP-25* gene, the following SNPs were selected: rs883381, rs1889189, rs363039, rs363050, rs362569, rs6039806, rs362990, rs1051312, rs8636, rs362602, rs362552 and rs725919 (see Figure 1). Genotyping was performed blind to familial status and phenotypic data. Both MZ twins of a pair were included in genotyping, serving as additional controls.

The SNPlex assay was conducted following the manufacturer's recommendations (Applied Biosystems, Foster city, CA, USA). All pre-PCR steps were performed on a cooled block. Reactions were carried out in Gene Amp 9700 Thermocycler (Applied Biosystems, Foster city, CA, USA). PCR products were analyzed with ABI3730 Sequencer (Applied Biosystems, Foster city, CA, USA). Data were analyzed using Genemapper v3.7 (Applied Biosystems, Foster city, CA, USA).

Statistical analyses

Allele frequencies of the 12 selected *tag*-SNPs were estimated in both young and adult cohorts using Pedstats (http://www.sph.umich.edu/csg/abecasis/PedStats) in which a Hardy–Weinberg test is implemented, based on an exact calculation of the probability of observing a certain number of heterozygotes conditional on the number of copies of the minor SNP allele. MZ twins were considered as one genotype, when estimating allele frequencies.

Linkage disequilibrium (LD) parameters (D' and r^2) were calculated from the haplotype frequency estimates using Haploview 3.2 (http://www.broad.mit.edu/mpg/haploview). $D' = 1$ if, and only if, two SNPs have not been separated by recombination (or recurrent mutation). This LD parameter is sensitive to sample size, especially when SNPs with rare allele frequencies are considered. The value of $r^2 = 1$ if, and only if, the SNPs have not been separated by recombination and have the same allele frequency. For quantifying and comparing LD in the context of mapping, r^2 is slightly preferred.[36] Values of r^2 ranged from 0.001 to 0.680 in our sample, conforming relatively low LD between the separate *tag*-SNPs (see Table 2).

Haplotypes were estimated using SNPs that showed a significant association with IQ in both samples, using the expectation-maximization (EM) algorithm to obtain the maximum likelihood estimates of haplotype frequencies in each sample,[37] as implemented in the Allegro software package.[38] The EM algorithm allows for missing data and can be applied when no parental genotypes are available.

Genetic association tests were conducted using the program QTDT, which implements the orthogonal association model proposed by Abecasis *et al.*[39] (see also Fulker *et al.*;[40] extended by Posthuma *et al.*[41]). This model allows the decomposition of the genotypic association effect into orthogonal between- (β_b) and within- (β_w) family components, can incorporate fixed effects of covariates and can also model the residual sib-correlation as a function of polygenic or environmental factors. MZ twins can be included and are modeled as such, by adding zygosity status to the datafile. They are not informative to the within-family association component (unless they are paired with non-twin siblings), but are informative for the between-family component. The between-family association component is sensitive to population admixture, whereas the within-family component is significant only in the presence of LD owing to close linkage. If population stratification acts to create a false association, the test for association using the within-family component is still valid, and provides a conservative test of association. Testing for the equality of the β_b and β_w effects serves as a test of population stratification. If this test is not significant, the between- and within-family effects are equal and total association test that uses the whole population at once can be applied. It should be noted, however, that given the relatively modest sample size, both the within-family test and the population stratification test are not as powerful as the 'total' association test. As we tested multiple SNPs, a significance level of 0.01 was kept.

Results

Single SNP analysis

In total, 391 subjects for the young cohort and 276 subjects for the adult cohort were available for SNP genotyping. Based on blind controls and MZ checks, no genotyping errors were found. Eight SNPs out of the 12 selected were in Hardy–Weinberg equilibrium (HWE) in both cohorts. SNPs not in HWE (rs362990,

Table 2 Estimates of LD parameters r^2 (lower) and D'(upper) for *tag*-SNPs within the *SNAP-25* gene

	rs883381	*rs1889189*	*rs363039*	*rs363050*	*rs362569*	*rs6039806*	*rs362990*	*rs1051312*	*rs8636*	*rs362602*	*rs362552*	*rs725919*
rs883381	—	0.885	0.495	0.491	0.012	0.014	0.144	0.154	0.021	0.428	0.149	0.133
rs1889189	0.223	—	1.000	0.467	0.641	0.491	0.428	0.173	0.116	0.342	0.268	0.326
rs363039	0.211	0.242	—	0.828	0.072	0.071	0.195	0.184	0.066	0.316	0.177	0.234
rs363050	0.192	0.078	0.459	—	0.276	0.146	0.059	0.171	0.318	0.265	0.017	0.135
rs362569	0.000	0.124	0.004	0.039	—	0.909	0.938	0.078	0.453	0.036	0.234	0.177
rs6039806	0.000	0.098	0.002	0.015	0.630	—	0.971	0.099	0.394	0.037	0.248	0.165
rs362990	0.012	0.031	0.027	0.001	0.486	0.397	—	0.226	0.536	0.122	0.230	0.187
rs1051312	0.009	0.019	0.012	0.014	0.003	0.006	0.013	—	0.095	0.710	0.900	0.917
rs8636	0.000	0.010	0.004	0.074	0.078	0.079	0.062	0.002	—	0.098	0.811	0.888
rs362602	0.070	0.085	0.033	0.034	0.001	0.001	0.003	0.496	0.003	—	1.000	0.936
rs362552	0.015	0.013	0.024	0.000	0.032	0.028	0.048	0.240	0.155	0.258	—	0.977
rs725919	0.008	0.014	0.030	0.0070	0.013	0.009	0.027	0.176	0.134	0.158	0.682	—

Abbreviations: LD, linkage disequilibrium; *SNAP-25*, synaptosomal-associated protein of 25 kDa; *tag*-SNP, tagging single-nucleotide polymorphism.

rs6039806, rs362569 and rs1051312) were not included in further analyses. SNP rs883381 had a success rate of 80% in the young cohort; for all other SNPs in HWE, success rates were between 96.0 and 98.0% (see Table 3).

The models used in QTDT included effects of age and sex on the means and modeled additive allelic between- and within-family effects. Residual sib-correlations were modeled as a function of polygenic additive effects and non-shared environmental effects. Tests for the presence of population stratification were all nonsignificant, indicating that genotypic effects within families were not significantly different from those observed between families, suggesting that the more powerful total association test can be interpreted. Three SNPs (rs363039, rs363050, rs362602) showed significant associations with IQ. Two of these SNPs (rs363039, rs363050) were associated with IQ in both the young cohort and the independent adult cohort, showing association in the same direction and the same order of magnitude. The third SNP (rs362602) was seen as a trend to significant association only in the adult cohort. When we combined the two cohorts, the strongest association was seen between PIQ and rs363050, which is located on the 5′UTR of the *SNAP-25* gene ($\chi^2 = 13.56$, $P = 0.0002$). The increaser allele of this SNP was associated with an increase of 2.84 IQ points (see Tables 4 and 5 and Figure 2).

Within-family association tests are based on all siblings that are part of pairs with contrasting genotypes within a family and are thus less powerful than total association tests. The latter is preferred if there is no evidence of population stratification. It is, however, interesting to check whether the significant associations observed in the total association test are also present when looking only at the within-family association. In the within-association test, for SNP rs363039, a trend was seen in both cohorts separately, whereas the G allele was suggestive of association ($P < 0.05$) in the combined cohort. For SNP rs363050, the within-family association with the A allele was suggestive ($P = 0.06$) in the combined cohort. SNPs rs8636 and rs362602 were significant in the adult cohort ($P < 0.01$) when only considering the within-family test. These results support the results as found using the more powerful total association test.

Haplotype analysis

The two SNPs that showed a significant association with IQ in both cohorts were 13 kb apart. Because these SNPs are in LD with each other ($r^2 = 0.46$), these SNPs were used to estimate haplotypes within each sample. Haplotype analysis of SNPs that are in LD with each other is more powerful than single SNP analysis because the combination of SNPs into a haplotype can be considered as a multiallelic marker that is more informative than a biallelic marker. Nonsignificant SNPs were not used for further haplotype analysis, as all SNPs were selected on the basis of being tagging SNPs. From Table 2, it can be

Table 3 List of selected *tag*-SNP within the *SNAP*-25 gene with their estimated heterozygosity rates for the young/adult cohort

Name	*Position*	*Gene location*	*Obs HET*	*Pred HET*	*Success rate*	*MAF*	*HWE*
rs883381	10160727	5′UTR	0.41/0.54	0.47/0.46	80.10/94.80	0.38/0.37	OK/OK
rs1889189	10192086	5′UTR	0.40/0.37	0.43/0.44	100.00/99.30	0.32/0.33	OK/OK
rs363039	10215496	Intron 1	0.42/0.50	0.42/0.47	99.00/100.00	0.30/0.37	OK/OK
rs363050	10229257	Intron 1	0.52/0.47	0.49/0.50	100.00/100.00	0.42/0.47	OK/OK
rs362569	*10241733*	*Intron 1*	*0.37*/0.50	*0.46*/0.48	*90.70*/91.00	*0.36*/0.41	*Not in HWE*/OK
rs6039806	*10253654*	*Intron 3*	*0.38*/0.49	*0.50*/0.50	*92.80*/88.00	*0.46*/0.47	*Not in HWE*/OK
rs362990	*10271221*	*Intron 5*	*0.30*/0.41	*0.39*/0.40	*100.00*/96.30	*0.27*/*0.27*	*Not in HWE*/OK
rs1051312	*10282088*	*Exon 8*	*0.22*/*0.21*	*0.46*/*0.47*	*77.80*/*71.50*	*0.37*/*0.38*	*Not in HWE/Not in HWE*
rs8636	10282742	Exon 8	0.44/0.51	0.45/0.49	98.20/98.90	0.34/0.42	OK/OK
rs362602	10288528	3′UTR	0.42/0.47	0.47/0.47	100.00/98.50	0.39/0.38	OK/OK
rs362552	10291217	3′UTR	0.42/0.39	0.43/0.39	99.20/97.40	0.29/0.26	OK/OK
rs725919	10298094	3′UTR	0.34/0.34	0.36/0.33	99.00/98.90	0.23/0.21	OK/OK

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; Obs HET, observed heterozygosity; Pred HET, predicted heterozygosity; *SNAP*-25, synaptosomal-associated protein of 25 kDa; *tag*-SNP, tagging single-nucleotide polymorphism; UTR, untranslated region.
Note: *Tag*-SNPs were selected if allele frequency was >10% (18.0% heterozygosity) and a genotypic correlation (ρ) <0.85. SNPs not in HWE are in italics.

Table 4 Family-based association analysis for *SNAP-25 tag-SNPs* for young, adult and combined cohort

tag-*SNP position (bp)*		*Young cohort*				*Adult cohort*				*Combined*			
	Phenotype	N	χ^2	P	*Genotypic effect*	N	χ^2	P	*Genotypic effect*	N	χ^2	P	*Genotypic effect*
rs883381	PIQ	308	2.05	0.152	1.59 (G)	253	0.95	0.329	1.24 (G)	561	3.07	0.080	1.46 (G)
(10160727)	VIQ	306	0.00	0.981	0.04 (T)	254	2.24	0.134	2.00 (G)	560	0.55	0.457	0.80 (G)
	FSIQ	308	0.30	0.584	0.70 (G)	252	1.88	0.170	1.62 (G)	560	1.50	0.221	1.09 (G)
rs1889189	PIQ	385	0.07	0.788	0.30 (T)	268	1.13	0.287	1.22 (T)	653	0.63	0.427	0.63 (T)
(10192086)	VIQ	383	2.54	0.111	2.56 (C)	269	1.88	0.170	1.68 (T)	652	0.59	0.444	0.81 (C)
	FSIQ	385	0.94	0.332	1.25 (T)	267	1.91	0.166	1.49 (T)	652	0.03	0.861	0.15 (C)
rs363039	PIQ	381	**6.52**	**0.010**	**2.99 (G)**	271	3.28	0.070	2.08 (G)	652	**9.21**	**0.002**	**2.51 (G)**
(10215496)	VIQ	379	3.21	0.073	3.09 (G)	272	6.37	0.012	3.14 (G)	651	**7.88**	**0.005**	**3.12 (G)**
	FSIQ	381	5.83	0.016	3.34 (G)	270	6.18	0.013	2.68 (G)	651	**10.88**	**0.001**	**2.98 (G)**
rs363050	PIQ	385	**7.88**	**0.005**	**3.01 (A)**	267	5.44	0.020	2.55 (A)	652	**13.56**	**0.0002**	**2.84 (A)**
(10229257)	VIQ	383	1.94	0.164	2.21 (A)	268	6.03	0.014	2.89 (A)	651	**5.91**	**0.015**	**2.52 (A)**
	FSIQ	385	5.47	0.019	2.96 (A)	266	**6.90**	**0.009**	**2.69(A)**	651	**11.48**	**0.0007**	**2.86 (A)**
rs8636	PIQ	378	0.21	0.643	0.50 (T)	264	2.95	0.086	1.99 (C)	642	2.22	0.137	1.18 (T)
(10282742)	VIQ	376	0.30	0.587	0.86 (T)	265	1.92	0.166	1.71 (C)	641	0.07	0.785	0.29 (C)
	FSIQ	378	0.00	0.978	0.03 (T)	261	3.39	0.065	1.99 (C)	641	1.08	0.299	0.90 (C)
rs362602	PIQ	385	0.33	0.567	0.59 (A)	265	3.01	0.082	1.97(G)	650	0.30	0.583	0.42 (G)
(10288528)	VIQ	383	1.40	0.236	1.80 (A)	266	**7.91**	**0.005**	**3.49 (G)**	649	0.06	0.811	0.24 (G)
	FSIQ	385	1.16	0.281	1.31 (A)	264	**7.57**	**0.006**	**2.96 (G)**	649	0.22	0.639	0.40 (G)
rs362552	PIQ	382	1.01	0.314	1.09 (G)	260	0.30	0.587	0.73 (A)	642	0.32	0.572	0.47 (G)
(10291217)	VIQ	380	0.31	0.575	0.90 (G)	261	1.20	0.273	1.59 (A)	641	0.01	0.924	0.10 (G)
	FSIQ	382	0.67	0.415	1.04 (G)	259	1.01	0.315	1.27 (A)	641	0.04	0.832	0.19 (G)
rs725919	PIQ	381	1.87	0.172	1.63 (A)	267	0.45	0.505	0.94 (G)	648	0.60	0.439	0.70 (A)
(10298094)	VIQ	379	0.14	0.712	0.65 (A)	268	1.21	0.271	1.70 (G)	647	0.55	0.457	0.26 (A)
	FSIQ	381	0.65	0.420	1.13 (A)	266	1.11	0.292	1.40 (G)	647	0.05	0.831	0.21 (A)

Abbreviations: FSIQ, full-scale IQ; PIQ, performance IQ; *SNAP*-25, synaptosomal-associated protein of 25 kDa; *tag*-SNP, tagging single-nucleotide polymorphism; VIQ, verbal IQ.
Note: The genotypic effect is the increase in IQ points associated with the increaser allele. *P* values below <0.01 are in bold. Sex and age were included as covariates; residual variance was modeled as a function of polygenic effects and non-shared environmental effects.

Table 5 Means (s.d.) per genotype for PIQ, VIQ and FSIQ for young and adult cohorts in the four *tag*-SNPs within the *SNAP-25* gene that show association with a significant association

tag-*SNP position (bp)*	*Young cohort*					*Adult cohort*			
	Phenotype	*Genotype*			*Total* N	*Genotype frequency*			*Total* N
		GG	**AG**	**AA**		**GG**	**AG**	**AA**	
	Frequency	**0.49**	**0.42**	**0.09**		**0.38**	**0.50**	**0.12**	
rs363039	Mean PIQ (s.d.)	103.68 (13.72)	100.58 (12.30)	97.06 (10.79)	381	101.57 (12.46)	96.89 (11.69)	98.96 (13.71)	271
(10168496)	Mean VIQ (s.d.)	101.13 (19.28)	97.22 (19.14)	97.79 (16.19)	379	95.53 (14.86)	90.62 (13.66)	89.71 (14.15)	272
	Mean FSIQ (s.d.)	102.45 (16.09)	98.47 (14.63)	96.94 (13.54)	381	97.73 (12.66)	92.98 (11.28)	93.21 (13.27)	270
		AA	**AG**	**GG**		**AA**	**AG**	**GG**	
	Frequency	**0.32**	**0.52**	**0.16**		**0.29**	**0.47**	**0.24**	
rs363050	Mean PIQ (s.d.)	104.26 (13.26)	101.11 (13.08)	98.24 (11.29)	385	102.50 (13.58)	97.60 (11.95)	97.34 (11.01)	267
(10182257)	Mean VIQ (s.d.)	103.12 (18.82)	96.52 (19.08)	98.68 (17.92)	383	95.26 (15.65)	92.26 (13.05)	89.20 (15.12)	268
	Mean FSIQ (s.d.)	103.98 (15.33)	98.36 (15.44)	98.03 (13.92)	385	98.08 (13.72)	93.98 (11.42)	92.71 (11.45)	266
		AA	**AG**	**GG**		**AA**	**AG**	**GG**	
	Frequency	**0.40**	**0.42**	**0.18**		**0.39**	**0.47**	**0.14**	
rs362602	Mean PIQ (s.d.)	102.45 (11.74)	101.45 (13.11)	100.44 (15.34)	385	100.23 (12.45)	99.54 (12.82)	100.11 (11.06)	265
(10241528)	Mean VIQ (s.d.)	99.97 (16.86)	99.50 (19.62)	95.82 (21.78)	383	92.45 (14.98)	91.11 (14.40)	97.63 (12.40)	266
	Mean FSIQ (s.d.)	101.28 (12.81)	100.37 (15.77)	97.10 (19.01)	385	94.91 (12.59)	94.08 (12.43)	98.16 (10.99)	264

Abbreviations: FSIQ, full-scale IQ; MZ, monozygotic; PIQ, performance IQ; QTDT, quantitative transmission disequilibrium test; *SNAP*-25, synaptosomal-associated protein of 25 kDa; *tag*-SNP, tagging single-nucleotide polymorphism; VIQ, verbal IQ.
Note: *N* denotes the number of individuals.

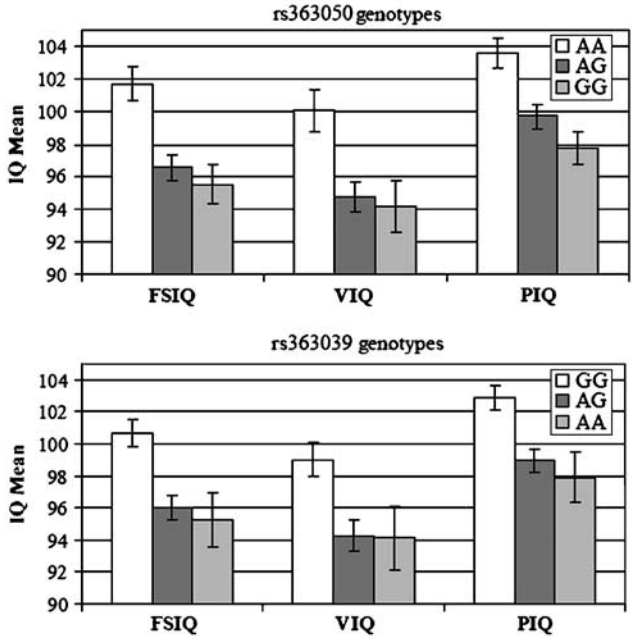

Figure 2 IQ means and standard error for the means for FSIQ, VIQ and PIQ for the combined cohort are plotted against the two most significant replicated *tag*-SNPs rs363050 and rs363039 genotypes.

seen that indeed LD among the *tag*-SNPs flanking the two most significant SNPs is very low, which would also be expected given the lack of association with these flanking SNPs and IQ.

Haplotypes were estimated using the two SNPs rs363039 and rs363050 that were associated with psychometric IQ scores. Four possible haplotypes were *G-A, A-G, G-G* and *A-A*, with haplotype frequencies 0.55, 0.29, 0.13 and 0.03, respectively, in the young cohort and 0.54, 0.31, 0.13 and 0.03, respectively, in the adult sample. Significant associations were found in both samples. When the data were combined, highly significant associations were observed with the *G-A* haplotype with FSIQ ($\chi^2(1) = 11.14$, $P = 0.0008$), VIQ ($\chi^2(1) = 7.15$, $P = 0.0074$) and PIQ ($\chi^2(1) = 10.61$, $P = 0.0011$) (see Table 6). These results confirm the single SNP association results.

Discussion

To investigate the possible role of the *SNAP-25* gene in intelligence, we employed a family-based genetic association test in two independent cohorts of 391 children (mean age 12.4 years) and 276 adults (mean age 37.3 years). Replicated association was found in the two cohorts for two SNPs in the *SNAP-25* gene. Strongest evidence was found for SNP rs363050 in intron 1 at the 5′UTR, showing an effect size of 2.84 IQ points ($P = 0.0002$) for the increaser allele. Haplotype analyses confirmed the region containing these two SNPs to be strongly associated with IQ.

Table 6 Family-based association analysis for *SNAP-25 tagging* haplotype for young, adult and combined cohort

Haplotype	*Haplotype frequency*		*Phenotype*	*Young*				*Adult*				*Combined*			
	Young	*Adult*		*N*	χ^2	P	*Genotypic effect*	*N*	χ^2	P	*Genotypic effect*	*N*	χ^2	P	*Genotypic effect*
G-A	0.55	0.54	PIQ	380	6.53	0.011	2.34	263	3.82	0.051	1.86	643	10.61	0.001	2.18
			VIQ	378	2.98	0.084	2.33	264	5.47	0.019	2.44	642	7.15	0.007	2.41
			FSIQ	380	5.91	0.015	2.64	262	5.75	0.016	2.15	642	11.14	0.001	2.44
A-G	0.29	0.31	PIQ	380	2.54	0.111	−1.98	263	4.19	0.041	−2.50	643	6.45	0.011	−2.24
			VIQ	378	0.82	0.365	−1.65	264	5.63	0.018	−3.17	642	4.24	0.040	−2.44
			FSIQ	380	1.98	0.159	−2.07	262	6.56	0.010	−2.96	642	6.81	0.009	−2.53
G-G	0.13	0.13	PIQ	380	1.23	0.267	−1.48	263	0.17	0.680	−0.65	643	1.49	0.222	−1.24
			VIQ	378	0.16	0.689	−0.79	264	0.09	0.764	−0.51	642	0.23	0.632	−0.66
			FSIQ	380	0.78	0.377	−1.32	262	0.06	0.806	−0.36	642	0.78	0.377	−0.98
A-A	0.03	0.03	PIQ	380	1.27	0.260	−1.97	263	0.07	0.791	−0.65	643	1.14	0.286	−1.50
			VIQ	378	2.13	0.144	−3.74	264	0.58	0.446	−1.96	642	2.65	0.104	−3.07
			FSIQ	380	2.31	0.129	−3.17	262	0.42	0.517	−1.46	642	2.58	0.108	−2.48

Abbreviations: FSIQ, full-scale IQ; PIQ, performance IQ; *SNAP-25*, synaptosomal-associated protein of 25 kDa; VIQ, verbal IQ.

The *SNAP-25* gene, located on chromosome 20 p12–12p11.2, encodes a presynaptic terminal protein. In the mature brain, the *SNAP-25* gene product forms a complex with syntaxin and the synaptic vesicle proteins (synaptobrevin and synaptotagmin) that mediates exocytosis of neurotransmitter from the synaptic vesicle into the synaptic cleft (see Horikawa *et al.*,[19] Seagar *et al.*,[20] Bark *et al.*,[42] Low *et al.*[43]). During development, *SNAP-25* is also involved in synaptogenesis, forming presynaptic sites and neuritic outgrowth.[17,21] *SNAP-25* is thought to be differentially expressed in the brain, and is primarily present in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra and cerebellar granular cells. In the mature brain, expression is mainly seen at presynaptic terminals.[17]

SNAP-25 exists in two splicing variants in relation to exon 5, *SNAP-25a* and *SNAP-25b*. Both isoforms differ in only nine out of 39 amino acids encoded by the alternative spliced exons,[44] resulting in a differentiated membrane anchoring relative to cysteine residues involved in post-transcriptional fatty acylation.[45] Both isoforms are thought to be equally important but at different time points for both neuronal maturation and neurotransmitter release.[21,22,42,46] Roberts *et al.*[24] demonstrated that mRNA levels of both isoforms are elevated after induction of LTP, suggesting a role of *SNAP-25* in synaptic plasticity. A recent study involving antisense oligonucleotides against *SNAP-25* at the hippocampal CA1 region reported the possible involvement of *SNAP-25* in learning and memory, particularly memory consolidation.[23] Steffensen *et al.*[47] found that hippocampal LTP is attenuated in hemizygous mice from the *Coloboma* mice strain. The *Coloboma* mice strain is characterized by a 2-cM deletion on the mouse homolog of chromosome 2, in a region containing the mouse *SNAP-25* gene. Mice hemizygous for this deletion exhibit a wide spectrum of phenotypic and neurological abnormalities such as ophthalmic deformation, head bobbing, circling, hyperactivity and small body size.[45,48,49] Because of the observed increased hyperactivity of hemizygous *Coloboma* mice, the role of *SNAP-25* in attention deficit hyperactivity disorder (ADHD) has been tested in several studies.[50–54] All, except one (Xu *et al.*[54]), report a significant association of *SNAP-25* with ADHD in humans. The exact role of *SNAP-25* in ADHD, however, remains unknown. ADHD is a neuropsychiatric condition characterized by hyperactive behavior and impaired attentive ability, resulting in both social and academic dysfunction. The present study suggests that involvement of *SNAP-25* may not be specific to the hyperactivity component of ADHD, but plays a more general role in learning and memory, through its effect on LTP and synaptic plasticity.

Both individual and haplotype analyses were conducted with two SNPs that showed significant association with intelligence in our study, tagging the 5′UTR region of *SNAP-25* gene. Genetic (non)coding variants lying within this non-coding region might be regulating this protein expression. These variants may influence regulatory binding sites, which in turn may modify gene expression and consequently neurotransmitter release regulation. Subtle changes in the fine-tuning at the neurotransmitter release machinery level, as well as in the interaction between neurotransmitter receptor subtypes, might be manifest in substantial differences when LTP is being achieved. This complex fine-tuning may be reflected as individual differences in memory and learning, two fundamental aspects of human intelligence. Future functional studies will provide the insight needed in order to disentangle the complex interplay among *SNAP-25* gene (non)coding variants and cognitive ability.

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