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Nicastrin gene polymorphisms, cognitive ability level and cognitive ageing

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Abstract

The hypothesis that polymorphisms in the gene for nicastrin (*NCSTN*) are associated with differences in cognitive level and ageing was tested in 462 relatively healthy surviving participants of the Scottish Mental Survey 1932. None had a history of dementia. They were tested on the Moray House Test of verbal reasoning at age 11 in 1932 and at age 79 between 1999 and 2001. At age 79 they also took tests of non-verbal reasoning, short- and long-term verbal declarative memory, Verbal Fluency, and a short screening test for dementia. Subjects who possessed at least one copy of the *NCSTN* B haplotype (Hap B) had higher scores on the Moray House Test (a test principally of verbal reasoning) at age 11 ($p = 0.036$) and age 79 ($p = 0.027$). The effect of Hap B on cognition at age 79 was non-significant after adjusting for the effect at age 11. Therefore, the effect of Hap B in this sample is on the life-long stable trait of cognitive ability, and not on age-related cognitive change. The possibility that this result might be a selection effect was not supported by the samples being in Hardy–Weinberg equilibrium with respect to the distribution of *NCSTN* genotypes.

Keywords: Nicastrin; IQ; Polymorphism; Intelligence; Cognition; Ageing; Scottish Mental Survey; Dementia

Age-related cognitive decline in humans is associated with lower quality of life and increased dependency [9,17]. The extent of cognitive decline in old age ranges from mild or normative changes to greater or non-normative changes, typically associated with recognisable neuropathology. Finding the determinants of these differences is now a research priority, pursued with a view to understanding and then ameliorating cognitive deterioration and susceptibility to dementia [12]. Among the determinants are genetic differences [8,14]. The best known of these are variations in the gene for apolipoprotein E, which are related to pathological (Alzheimer dementia) and non-pathological age-associated cognitive decline in humans [5,6,21]. Genetic differences associated with Alzheimer's disease provide a promising starting point for seeking influences on normative cognitive ageing [8], because the neuropathology of Alzheimer's disease occurs extensively in the absence of clinical dementia and may explain some of the differences in cognitive ageing [3].

Mutations in the presenilin genes are the most frequent known causes of early onset Alzheimer's disease. Nicastrin is a Type I transmembrane glycoprotein [27], part of the γ-secretase multi-protein complex involved in cleaving the Notch receptor, and more generally in the intramembrane cleavage of a number of substrates, releasing extracellular and cytoplasmic protein fragments [26]. The complex's other components are presenilin, Aph-1 and Pen-2 [10,22]. Importantly, γ-secretase cleaves amyloid precursor protein within the plane of the cell membrane, releasing a toxic amyloid beta

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protein 38–43 (mostly 42) amino acids in length [22,26]. This is the main component of the plaques in Alzheimer disease. The nicastrin gene (*NCSTN*) maps to chromosome 1q22-23 in humans, and the region shows suggestive linkage to [1] and association with [15] late-onset Alzheimer disease. *NCSTN* has four haplotypes defined by four single nucleotide polymorphisms, one of which is common (A) and three are rarer (B–D). The B haplotype of *NCSTN* (Hap B) was associated with familial early onset Alzheimer disease, especially among people who lack the $\varepsilon 4$ allele of the *APOE* gene [11], but this was not found in a subsequent study [4]. On this basis we proposed that polymorphisms in the nicastrin gene would be associated with normative cognitive ageing in the absence of dementia.

Here we examine whether the Hap B and *NCSTN* genotype are associated with normal cognitive ageing in a group of people whose cognitive functions were assessed at age 11 in 1932 and again at age 79 between 1999 and 2001. We also examined whether variability in the *NCSTN* gene were associated with the life-long trait of cognitive ability.

The subjects of the present study were surviving participants from the Scottish Mental Survey of 1932 (SMS1932) [7,20]. They were recruited in the Edinburgh area via letters from their general practitioners and from media calls. They form the Lothian Birth Cohort 1921 (LBC1921). SMS1932 took place on June 1st, 1932 and was a nationwide survey that tested the mental ability of almost all eligible children born in 1921 and attending school in Scotland ($N = 87{,}498$). The SMS1932 was conducted by the Scottish Council for Research in Education who retained the data and gave permission for the present study. The LBC1921 comprises 550 subjects. Their mean age at re-testing was 79.1 (S.D. = 0.6). They were relatively healthy, all lived independently in the community, and were able to travel to the test centre. Fuller descriptions of the recruitments and medical and other assessments are given elsewhere [7]. For the present report, 535 were successfully genotyped for the four SNPs used to derive *NCSTN* haplotypes. For the main analyses we applied the following criteria. Subjects must have: MHT results from age 11 and age 79; no history of dementia; Mini-Mental State Examination Score of 24 or greater; successful *NCSTN* genotyping. This yielded 462 subjects. Of these, 458 (269 women, 189 men) also had full data on the cognitive tests described below.

The mental test used in the SMS1932 was a version of the Moray House Test No. 12 (MHT) [7,20]. It is a group-administered test with a time limit of 45 min. The items are predominantly of a verbal reasoning type, but there are also numerical, spatial and some other types of question. The MHT has concurrent validity at age 11 and in old age. Here, we re-administered the MHT at age about 79. MHT scores at age 11 and age 79 were adjusted for age at testing and converted to standard IQ-type scores, with mean = 100 and S.D. = 15. Subjects also undertook tests of non-verbal reasoning (Raven's Standard Progressive Matrices: Raven) [19], short- and longer-term verbal declarative memory (Wechsler Logical Memory Test: Logical Memory) [23], Verbal Fluency [16], and a short screening test for cognitive pathology (Mini-Mental State Examination: MMSE) [13].

Table 1
Results of pair-wise LD statistics between all pairs of markers

	1	2	3	4
Cramer's V (p-value)				
1		0.072	0.985	0.923
2	0.011		0.071	0.075
3	<0.0001	0.013		0.937
4	<0.0001	0.013	<0.0001	
Absolute value of D' (p-value)				
1		1.00	1.00	0.97
2			0.99	1.00
3				1.00

DNA samples were examined for four informative polymorphisms in the *NCSTN* gene (rs12239747/c.636A→G, rs2274185/IVS6+18C→G, rs7528638/IVS10-5C→G, and IVS16-119G→C) that allow the definition of four major haplotypes [11]. The haplotypes are referred to as Hap A (ACCG), Hap B (GCGC), Hap C (AGCG), Hap D (ACCC). An additional very rare haplotype termed Hap E (GCCG) was also observed. Genotyping was carried out by KBiosciences (UK) using the Amplifluor chemistry. Pair-wise LD statistics were generated for each polymorphism using the Genecounting program [28]. There is strong linkage disequilibrium between the four SNPs (Table 1).

The frequency (%) of *NCSTN* haplotypes among the 535 LBC1921 subjects with successful *NCSTN* genotyping was: A/A = 385 (72.0), A/B = 67 (12.5), A/C = 61 (11.4), A/D = 10 (1.9), A/E = 2 (0.4), B/B = 4 (0.7), B/C = 3 (0.6), C/C = 3 (0.6). This distribution did not differ from Hardy–Weinberg equilibrium ($\chi^2 = 3.64$, d.f. = 4, $p > 0.05$). The frequency (%) of *NCSTN* haplotypes among the 462 LBC1921 subjects who in addition met the inclusion criteria described above was: A/A = 325 (70.3), A/B = 61 (13.2), A/C = 57 (12.3), A/D = 8 (1.7), A/E = 2 (0.4), B/B = 4 (0.9), B/C = 2 (0.4), C/C = 3 (0.6). This distribution did not differ from Hardy–Weinberg equilibrium ($\chi^2 = 2.92$, d.f. = 4, $p > 0.05$).

The first analysis investigated whether there was an association between possession of Hap B and cognitive test scores. Subjects were divided into those with ($N = 66$) and without ($N = 392$) at least one copy of Hap B. General linear modelling (multivariate analysis of variance) was used. The outcome variables were MHT age 11, MHT age 79, Raven, Logical Memory, Verbal Fluency, and MMSE. Sex and the presence or absence of Hap B were fixed effects. There were significant effects of Hap B on MHT age 11 ($F_{1,454} = 4.4$, $p = 0.036$, $\eta^2 = .010$), MHT age 79 ($F_{1,454} = 4.9$, $p = 0.027$, $\eta^2 = .011$), and a trend toward an effect on Raven ($F_{1,454} = 2.9$, $p = 0.090$, $\eta^2 = .006$). Possession of Hap B had no significant effect on Logical Memory, Verbal Fluency or MMSE. There were no significant sex × Hap B interactions on any of the cognitive tests. Table 2 shows the estimated marginal means (and their 95% confidence intervals) from this analysis. Sub-

Table 2
Estimated marginal means (95% CI) for the effect of Nicastrin gene polymorphisms on cognitive test scores

	Presence or absence of Nicastrin haplotype B		Nicastrin genotypes (based on haplotypes A–D)			
	B+	B−	A/A	A/B	A/C	A/D
Age 11						
Moray House Test	104.0 (100.4, 107.7)	99.8 (98.3, 101.3)	100.0 (98.4, 101.7)	103.6 (99.8, 107.5)	98.7 (94.8, 102.6)	99.5 (89.2, 109.7)
Age 79						
Moray House Test	104.3 (100.8, 107.9)	100.0 (98.6, 101.4)	99.9 (98.3, 101.5)	104.5 (100.8, 108.2)	99.9 (96.1, 103.6)	104.5 (94.6, 114.4)
Raven's Progressive Matrices	33.2 (31.1, 35.3)	31.2 (30.4, 32.1)	31.3 (30.3, 32.2)	33.3 (31.1, 35.5)	30.8 (28.5, 33.0)	33.0 (27.1, 38.9)
Wechsler Logical Memory	32.4 (29.3, 35.6)	31.7 (30.5, 33.0)	31.7 (30.3, 33.1)	32.3 (29.0, 35.6)	31.6 (28.2, 34.9)	32.6 (23.8, 41.4)
Verbal Fluency	40.5 (37.5, 43.6)	39.9 (38.7, 41.1)	39.7 (38.4, 41.1)	40.9 (37.7, 44.1)	41.5 (38.2, 44.7)	37.9 (29.3, 46.4)
Mini-Mental State Examination	28.2 (27.8, 28.6)	28.3 (28.1, 28.4)	28.3 (28.1, 28.4)	28.2 (27.8, 28.5)	28.4 (28.0, 28.7)	28.6 (27.6, 29.6)

jects who possessed Hap B had higher MHT age 11 and 79 scores than those who did not.

The significant effects of Hap B on MHT age 79 and a trend toward an effect on Raven could suggest that the genetic effect was on general mental ability rather than a specific verbal reasoning ability assessed by MHT. The correlations among the mental tests at age 79 suggested such a general factor. MHT age 79 correlated with Raven, Logical Memory, and Verbal Fluency at 0.69, 0.39, and 0.37, respectively. Raven correlated with Logical Memory and Verbal Fluency at 0.34 and 0.24, respectively. All of these correlations were significant at $p < 0.01$. Logical Memory and Verbal Fluency correlated 0.10 ($p = 0.027$). Principal components analysis on these four tests also suggested a general factor. By scree slope analysis, and by the eigenvalues greater than 1.0 criterion, there was a single component accounting for 53.4% of the variance. Scores on this general ability factor (the first unrotated principal component) were saved for each individual and used as the outcome variable in a further general linear model with Hap B and sex as fixed effects. The effects of Hap B on the general ability factor tended toward significance ($F_{1,454} = 3.2$, $p = 0.074$, $\eta^2 = .007$).

Other studies have examined whether possession of the ε4 allele of the gene for apolipoprotein E (*APOE*) interacts with *NCSTN* genotype to affect cognition in old age. We previously reported that possession of the *APOE* ε4 allele and *APOE* genotype influence MHT at age 79 but not at age 11 [5], and also influence Logical Memory [6]. Of the 458 subjects whose data were analysed in the previous paragraphs, 454 also had successful *APOE* genotyping. We re-ran the analysis with *APOE* ε4 status as an additional fixed effect. There were no significant HapB × *APOE* ε4 allele status interactions on any cognitive test.

The second analysis examined whether Hap B was associated with cognitive change between age 11 and age 79. General linear modelling (multivariate analysis of covariance) was used. MHT age 79 was the dependent variable. Sex and Hap B were fixed effects. MHT age 11 was a covariate. There were significant effects of MHT age 11 ($F_{1,457} = 321.2$, $p < 0.001$, $\eta^2 = .413$) and sex ($F_{1,457} = 7.4$, $p = 0.007$, $\eta^2 = .016$), but no significant effect of Hap B ($F_{1,457} = 1.1$, $p = 0.30$, $\eta^2 = .002$).

The third analysis examined the association between Hap B and the life-long, stable trait of cognitive ability as measured using the MHT. A repeated measures general linear modelling analysis was used. MHT was the repeated measure (at age 11 and age 79), and Hap B and sex were fixed effects. The effect of Hap B was significant ($F_{1,458} = 5.3$, $p = 0.022$, $\eta^2 = .011$). There was no significant main effect of sex and no significant sex × Hap B interaction. The sex × MHT interaction was significant, as reported previously [7].

The fourth analysis examined the association between *NCSTN* genotypes and cognitive functions. For this analysis, subjects with the four commonest genotypes and full data were included: A/A ($N = 325$), A/B ($N = 61$), A/C ($N = 57$), and A/D ($N = 8$). General linear modelling (multivariate analysis of variance) was used. The outcome variables were MHT age 11, MHT age 79, Raven, Logical Memory, Verbal Fluency, and MMSE. Sex and *NCSTN* genotype were fixed effects. There were no significant effects of *NCSTN* genotype on any of the cognitive test scores. The estimated marginal means from this analysis are shown in Table 2. Post-hoc comparisons showed that the A/B genotype had a higher mean MHT age 79 score than A/A genotype ($p = 0.024$).

The main positive result is the finding of higher mean MHT scores at age 11 and age 79 among people who possessed a copy of Hap B. In a typical study, in which mental test scores are available only from old age, this might be construed as there being a possible association with cognitive ability in old age. However, there was an association with MHT at age 11 and, when this was taken into account in the analysis of covariance, there was no additional association with MHT at age 79. This and the repeated measured analysis shows that the association of Hap B is with the stable trait of MHT score across the lifespan: it is a genetic association with cognitive *level* rather than cognitive *change*. This is the first report of an association between *NCSTN* polymorphisms and normal cognitive differences and requires to be replicated. The effect size was small, at just over 1% of the variance in MHT scores. Overall, general

intelligence differences in humans show a heritability of about 0.5 [2,18]. Therefore, if replicated, the contribution of *NCSTN* would be about 2% of the genetic effect. The data here are valuable as the sample has an unusually informative phenotype concerning cognitive ability at both ends of the human lifespan. It is possible that the effect is a type 1 error; strict adjustment for multiple testing would reduce the effect to non-significance. The present data are equivocal on whether the effect of Hap B was on general mental ability (the first unrotated principal component from the four tests taken at age 79) or on a more specific verbal reasoning ability (the MHT). The effect on the former tended towards significance whereas the latter was significant. This issue requires further investigation with further samples, which have batteries that include mental tests assessing a variety of cognitive domains.

Though the possibility that *NCSTN* B haplotype might be associated with a greater risk of Alzheimer's disease has not been replicated, it means that the present results are in the opposite to the expected direction. One possibility examined was that, because MHT age 11 is associated with a greater risk of late-onset dementia [25] and earlier death [24], then those survivors at age 79 who carried another putative risk factor, such as Hap B, might be expected to be higher in trait intelligence. This selective survival idea would predict departure from Hardy–Weinberg equilibrium, which was not found in this sample. On the other hand, if Hap B is truly associated with higher MHT age 11 scores, any effect of lower childhood IQ to increase risk of early onset Alzheimer's disease would be attenuated. This would help to explain why lower childhood IQ appears to be a risk factor for late-onset rather than early-onset dementia in the Scottish population [25].

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