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ORIGINAL RESEARCH

A Functional Polymorphism under Positive Evolutionary Selection in ADRB2 is Associated with Human Intelligence with Opposite Effects in the Young and the Elderly

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Abstract Comparative genomics offers a novel approach to unravel the genetic basis of complex traits. We performed a two stage analysis where genes ascertained for enhanced protein evolution in primates are subsequently searched for the presence of non-synonymous coding SNPs in the current human population at amino acid sites that differ between humans and chimpanzee. Positively selected genes among primates are generally presumed to determine phenotypic differences between humans and chimpanzee, such as the enhanced cognitive ability of our species. Amino acid substitutions segregating in humans at positively selected amino acid sites are expected to affect phenotypic differences among humans. Therefore we conducted an association study in two family based cohorts and one population based cohort between cognitive ability and the most likely candidate gene among the five that harbored more than one such polymorphism. The derived, human-specific allele of the beta-2 adrenergic receptor Arg16Gly polymorphism was found to be the increaser allele for performance IQ in the young, family based cohort but the decreaser allele for two different measures of cognition in the large Scottish cohort of unrelated individuals. The polymorphism is known to affect signaling activity and modulation of beta-2 adrenergic signaling has been shown to adjust memory consolidation, a trait related

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to cognition. The opposite effect of the polymorphism on cognition in the two age classes observed in the different cohorts resembles the effect of ADRB2 on hypertension, which also has been reported to be age dependent. This result illustrates the relevance of comparative genomics to detect genes that are involved in human behavior.

Keywords Comparative genomics · Human evolution · IQ · Genetic association · Functional polymorphism

Introduction

Understanding the heritability of complex traits has proven more difficult compared to Mendelian diseases and is faced with the problem of ascertaining the functional variant following the commonly used linkage disequilibrium based mapping approaches (Cardon and Bell 2001). Given the need for novel methods to identify functional genetic variants on a genome-wide scale, we focused on a recent and interesting addition to the field of human genetics and performed a two-stage approach to ascertain amino acid substitutions that affect a brain related complex trait in humans based on a comparative genomic analysis.

Comparative genomics studies the macroevolution of our species searching for the genetic basis of the adaptive phenotypic divergence that uniquely identifies *Homo sapiens*. The basic approach is to ascertain genes that exhibit faster than random sequence divergence between species. Genes under positive selection in the lineage leading to humans have been identified this way (Clark et al. 2003; Nielsen et al. 2005). Although brain related genes show little evidence of positive selection in the lineage leading to humans they have been shown to have a higher rate of divergence within primates compared to rodents (Dorus et al. 2004), confirming the general belief that becoming human has involved phenotypic changes to our brain. Indeed, two genes involved in brain development, *MCPH1* (Evans et al. 2005) and *ASPM* (Mekel-Bobrov et al. 2005) were both shown to continue to evolve adaptively in the human race. However, as yet none of the fast evolving genes could be linked directly to an adaptive phenotype, such as cognitive ability (e.g., Mekel-Bobrov et al. 2007).

Several studies have provided statistical evidence for a link between evolutionary selection in primates and functional variation within humans. Deleterious mutations tend to be localized at conserved protein residues (Bustamante et al. 2005; Arbiza et al. 2006; Miller and Kumar 2001). More interestingly, a comparison of genes involved in "normal" genetic variation, Mendelian disease and complex disorders showed that genes involved in complex traits exhibit a higher rate of protein evolution (Thomas and Kejariwal 2004). Apparently, genes involved in the adaptive evolution of our species are more likely to harbor functional polymorphisms that affect variation in complex traits between humans.

Given the latter result, it is tempting to assume that ascertaining genes under recent positive selection would identify plausible candidate genes for complex traits (but see Mekel-Bobrov et al. 2007). Here we present the results of a genome-wide search for genes that exhibit enhanced rate of protein evolution in primates. Following the identification of positively selected genes that are expressed in the central nervous system (CNS) we conducted a second step of the analysis where non-synonymous coding SNPs in these genes were ascertained at positions that differ between the human and chimpanzee reference sequences. This approach differs from other approaches (Clark et al. 2003; Nielsen et al. 2005; Dorus et al. 2004) and is based on the assumption that genes involved in recent adaptive evolution of our species are more likely to harbor functional variants in the extant human population (as suggested by Bustamante et al. 2005; Arbiza et al. 2006; Miller and Kumar 2001; Thomas and Kejariwal 2004). We therefore identify amino acid changes specific to humans that are obvious candidates to have a functional effect on a brain related phenotype that has diverged during primate evolution. To confirm the prediction that such positively selected amino acid variants affect phenotypic differences among humans a genetic association study was performed with three measures of cognitive ability based on two independent family-based Dutch samples and in a large cohort of unrelated Scottish individuals with similar cognitive phenotypic measures at age 11 and at age 70, namely the Lothian Birth Cohort of 1936 (LBC1936) (Deary et al. 2007).

Materials and methods

Data and analysis

The NCBI Homologene database build 39.1 was downloaded and the relevant information on the rate of nucleotide substitutions between humans and chimpanzee and mouse and rat was extracted using custom made scripts in a Linux environment. The ratio between the non-synonymous vs. synonymous substitution rate was calculated using Jukes and Cantor's method (Weir 1983). As the distribution of Ka/Ks values was different between primates and rodents, the data were standard normalized (i.e., mean and SD set to 0 and 1, respectively) before comparing the ratio between lineages. Genes were selected if the Ka/Ks ratio in primates was higher than one and was more than two standard deviations lower in rodents. All statistical analyses were performed in SPSS 11.5.

Simulation study

The evolver and codeml programs from the PAML package were used. Evolver can simulate the evolution of coding sequences in the four species conditioned on the known topology of the phylogenetic tree connecting the species and the observed average Ka/Ks values. Codeml subsequently analyses the data to generate the ratio of non-synonymous vs. synonymous substitution rate. Based on these values the false discovery rate of the procedure was estimated.

Genetic association

The ABI Taqman genotyping assay was used to genotype all individuals from the Dutch cohorts for SNPs rs1042713 and rs1042714. LBC1936 was genotyped for SNPs rs1042713 and rs1042714 using KASPar, by Kbiosciences (Herts, UK).

The QTDT software package was used to perform a family-based association analysis in two Dutch cohorts phenotyped for several measures of cognitive ability (for details see below and Gosso et al. 2006). IQ data were corrected for age and sex, and all analyses were performed while modeling the environmental and (poly-) genetic components of variance. For LBC1936, linear regression and conditional haplotype-based association analysis was performed using the PLINK application (Purcell et al. 2007). Gender and age in days at testing were used as covariates in the analysis.

Cohorts and cognitive tests

The two independent family-based Dutch samples of 391 (mean age 12.4 years, 161 families) and 409 (mean age 36.7 years, 113 families) subjects respectively were phenotyped for three measures of cognitive ability: full scale, verbal and performance IQ. The Dutch adaptations of the child and adult versions of the Wechsler Intelligence Scale were used. A detailed description of the cohorts and the measurement instruments used are given elsewhere (Gosso et al. 2006; Polderman et al. 2006), barring the inclusion of 133 additional adult samples ascertained under the same method. The LBC1936 population-based cohort of 1,063 subjects (mean age 69.53 years, minimum 67.17, maximum 71.3, SD 0.94 years) was phenotyped for a variety of cognitive tests described elsewhere (Deary et al. 2007). For this study, the Moray House test (a general measure of cognitive ability with an emphasis on verbal reasoning) and Matrix Reasoning from the Wechsler Adult Intelligence Scale III^{UK} (assessing non-verbal reasoning) were analyzed in subjects who did not show signs of possible dementia according to the Mini-Mental State Examination (all participants had scores >23). Additionally, Moray House test scores are available for this cohort at age 11.

Results

Simulation study to estimate false positive rate

To estimate the false positive rate from our comparative genomic analysis, the evolution of 25,000 protein coding DNA sequences each 1800 bps long was simulated using the PAML software package (Yang 1997). The simulations were conditioned on the known topology of the phylogenetic tree connecting the four species (human, chimp, rat, and mouse) and on the observed average Ka/Ks ratio in the real dataset. The accuracy of the simulation for recreating realistic DNA sequence evolution was confirmed by considering the average nucleotide divergence between the four simulated species. The "human"–"chimp", "rat"–"mouse", and "human"–"mouse" divergence was about 96, 93 and 85%, respectively, closely matching the real data. The simulated dataset was subsequently used to estimate the false positive rate, as the percentage of the 25,000 "genes" that would have been called positive in the real dataset. The variability in the estimated false positive rate was very small based on 15 replicates, with an average of $1.5 \pm 0.1\%$.

Positively selected genes in primates

The NCBI Homologene database (build 39.1) contained information on the number of synonymous and non-synonymous substitutions between humans and chimpanzee for more than 13,000 genes. Six hundred and twenty six genes had a Ka/Ks ratio higher than 1. For 311 of these genes, data were also available from the mouse–rat comparison as well. Because the aim of this analysis was to identify enhanced protein evolution specific to the primate lineage, genes were considered only if the normalized Ka/Ks ratio in mouse–rat was more than two standard deviations lower than the human–chimp comparison. Two hundred and two of the 311 genes that fulfilled this criterion are listed in Supplementary Table 1. Because data on all four species was available for only 7,080 genes, the percentage of positive genes is 2.9%. This is twice the expected false positive rate ($P = 2.57 \times 10^{-19}$, one-sample *t*-test).

Positively selected genes are predominantly expressed in the human brain

The number of genes found to have an enhanced rate of protein evolution specifically among primates exceeds what could be expected by chance, and this set of genes can

be expected to contain genes that have contributed to the phenotypic divergence between the two species. This divergence is highly likely to have involved brain related phenotypes. Consequently we performed a search with this gene list in the NCBI UniGene database for genes expressed in the CNS. According to this database 32% (28,149 out of 86,810 entries) of the human genome is expressed in the brain. Among the 334 genes with only human vs. chimpanzee data available this percentage was 60%, but among the 202 genes with evidence of primate specific positive selection, 79% was expressed in brain ($P = 2 \times 10^{-40}$, one sample *t*-test). This result confirms that the criteria we applied for the comparative genomic analysis indeed significantly enriches the sample for brain-related genes and is consistent with previous findings (Dorus et al. 2004) and shows that becoming human has significantly coincided with the phenotypic evolution of our brain.

Non-synonymous coding SNPs at amino acids that differ between humans and chimpanzee

Given that the genes identified in this analysis are predominantly brain related, we hypothesized that among these genes there are candidate genes for variation in behavioral traits. A wide range of phenotypes are potential "candidate traits": here we consider the trait of human cognitive ability since this is likely to have been subject to adaptive evolution. Hence positively selected human proteins with expression in the CNS can be expected to affect cognitive abilities. Among the 202 "confirmed" positively selected genes, 160 (79%) are expressed in the CNS and among these 22 (14%) contain non-synonymous coding SNPs, i.e., amino acid changing mutations, at amino acid positions that differ between the human and chimpanzee reference sequences. Effectively, the current human population segregates "derived", "human specific" and "ancestral", "chimpanzee specific" variants of these proteins (Table 1). We consider that these fast-evolving brain-expressed genes have indeed contributed to the phenotypic divergence between the two species, and thus hypothesize that the polymorphic alleles still present in the human population contribute to phenotypic differences among modern humans for the same traits that have diverged during our speciation. From the 22 genes identified here and listed in Table 1, five harbor more than one amino acid changing polymorphisms. One out of these five, ADRB2 is a priority candidate gene to investigate cognitive ability for biological and functional reasons (see "Discussion"). To address this issue, we performed a genetic association study for IQ using the two non-synonymous coding SNPs (rs1042713 and rs1042714) in the beta-2 adrenergic receptor as markers in two independent family-based Dutch samples and in a large cohort of over 1,000 Scottish individuals. These two SNPs are within 27 bp from each other but are in relatively low LD ($r^2 = 0.375$ in the CEU Hapmap population).

Genetic association between the beta-2 adrenergic receptor and cognition

For the Dutch family based cohorts, the QTDT software package (Abecasis et al. 2000) was used to first check for inconsistencies in the genotypes and test for Hardy-Weinberg equilibrium (HWE). No inconsistencies were found which suggests that no systematic genotyping error has occurred, but ADRB2 rs1042714 was not in HWE. The basic assumption of the current approach is that the polymorphisms under study have been subject to natural selection, and this might be a reason for failure to be in HWE equilibrium. However, to avoid ambiguity in the interpretation of the results of the association study this marker was not followed up. Furthermore a test for population stratification was carried out with the remaining SNP. This tests involves a comparison of the between and within family genetic variation and is implemented in QTDT. ADRB2 rs1042713 showed significant stratification (PIQ: $P = 0.012$), consequently only the family-based evidence for association will be interpreted. ADRB2 rs1042713 showed evidence for family-based association with performance IQ in the young cohort ($P = 0.012$) (Table 2), but not in the adult cohort.

In the Scottish cohort, both SNPs were in HWE, thus both single SNP and a two SNP haplotype analysis has been performed using PLINK. rs1042713 and rs1042714 were both found to be associated with Matrix Reasoning ($\beta = 0.072$, $P = 0.02$ and $\beta = 0.076$, $P = 0.014$, respectively) in the increasing direction of the ancestral alleles. In addition, the ancestral alleles were associated with increasing Moray House Test scores; rs1042713 at age 70 ($\beta = 0.07$, $P = 0.025$), and rs1042714 at age 11 ($\beta = 0.074$, $P = 0.023$). The haplotype based analysis confirmed that the GG haplotype is the increaser haplotype for Matrix Reasoning ($P = 0.021$), but as such the effect is opposite to what was found in the young, family-based cohort. In both the family and population cohort, the polymorphism explained $\sim 1\%$ of the phenotypic variation in cognitive ability. It should be noted, however, that effect sizes are usually overestimated in sample sizes of this order.

Discussion

Ascertaining the genetic variants that affect complex traits has proven to be more difficult compared to detecting mutations for monogenetic disorders. Currently, genome-

Table 1 Twenty-two fast evolving, brain expressed genes that segregate "derived", "human specific" and "ancestral", "chimpanzee specific" variants of these proteins in the extant human population

Gene	rs #	SNP status	Allele	Allele frequency CEU	Protein residue	Amino acid position
HEXB	rs11556045	Derived	A	0.788	Lys [K]	121
		Ancestral	G	0.212	Arg [R]	
KAZALD1	rs807037	Derived	G	0.65	Gly [G]	255
		Ancestral	C	0.35	Ala [A]	
TMEM86A	rs7945285	Derived	T	1	Val [V]	215
		Ancestral	C	0	Ala [A]	
FLJ38725	rs3764147	Derived	G	0.246	Val [V]	254
		Ancestral	A	0.754	Ile [I]	
PLA2G4B	rs3816533	Derived	C	0.839	Arg [R]	422
		Ancestral	T	0.161	Cys [C]	
USP8	rs11638390	Derived	A	0.825	Thr [T]	739
		Ancestral	G	0.175	Ala [A]	
MGC14151	rs8522	Derived	T	Unknown	Leu [L]	13
		Ancestral	C	Unknown	Pro [P]	
SCRN2	rs17856536	Derived	G	Unknown	Arg [R]	103
		Ancestral	A	Unknown	Lys [K	
	rs17856535	Derived	G	Unknown	Gly [G]	411
		Ancestral	A	Unknown	Ser [S]	
STXBP4	rs1156287	Derived	G	Unknown	Gly [G]	92
		Ancestral	A	Unknown	Arg [R]	
ZNRF4	rs17304380	Derived	A	0.142	His [H]	163
		Ancestral	G	0.858	Arg [R]	
	rs8103406	Derived	T	0.34	Ser [S]	157
		Ancestral	G	0.66	Ala [A]	
	rs2240744	Derived	A	0.15	Gln [Q]	78
		Ancestral	G	0.85	Arg [R]	
ARTN	rs2242637	Derived	A	Unknown	Gln [Q]	19
		Ancestral	G	Unknown	Arg [R]	
SH2D2A	rs926103	Derived	G	0.258	Ser [S]	52
		Ancestral	A	0.742	Asn [N]	
ETNK2	rs3737655	Derived	A	Unknown	Gln [Q]	10
		Ancestral	C	Unknown	Pro [P]	
GTSE1	rs6008600	Derived	A	Unknown	Thr [T]	181
		Ancestral	G	Unknown	Ala [A]	
Il8RA	rs16858811	Derived	T	Unknown	Met [M]	31
		Ancestral	G	Unknown	Arg [R]	
DKFZP564J102	rs4862653	Derived	A	0.083	Lys [K]	146
		Ancestral	G	0.917	Glu [E]	
	rs4862650	Derived	A	0.083	Lys [K]	41
		Ancestral	G	0.917	Glu [E]	
FLJ23577	rs6897513	Derived	A	0.517	Asn [N]	71
		Ancestral	C	0.483	His [H]	
CAST	rs754615	Derived	G	0.583	Cys [C]	408
		Ancestral	C	0.417	Ser [S]	
ADRB2	rs1042714	Derived	C	0.533	Gln [Q]	27
		Ancestral	G	0.467	Glu [E]	
	rs1042713	Derived	A	0.325	Arg [R]	16
		Ancestral	G	0.675	Gly [G]	

Table 1 continued

Gene	rs #	SNP status	Allele	Allele frequency CEU	Protein residue	Amino acid position
UBD	rs2076487	Derived	C	1	Ala [A]	99
		Ancestral	G	0	Gly [G]	
	rs2076484	Derived	T	1	Leu [L]	51
		Ancestral	C	0	Ser [S]	
LOC441376	rs16889283	Derived	G	Unknown	Gly [G]	96
		Ancestral	C	Unknown	Arg [R]	
ZFP37	rs2282076	Derived	T	0.417	Val [V]	7
		Ancestral	A	0.583	Asp [D]	

Table 2 Association study of rs1042713 in two independent family-based Dutch cohorts between three measures of cognitive ability and the ancestral vs. derived allele of a protein that undergone recent positive selection in primates

Cohort	Phenotype	*N*	Stratification		Family-based		Population-based	
			$\chi 2$	*P*-value	$\chi 2$	*P*-value	$\chi 2$	*P*-value
Young	FSIQ	359	2.31	0.13	3.20	0.07	0.94	0.33
	VIQ	360	0.00	0.99	0.12	0.73	0.35	0.55
	PIQ	359	6.37	0.01	6.42	0.01	0.75	0.39
Adult	FSIQ	350	0.60	0.44	1.20	0.27	0.62	0.43
	VIQ	350	0.10	0.76	0.28	0.60	0.21	0.65
	PIQ	350	1.05	0.31	1.35	0.25	0.33	0.57

Full scale, verbal and performance IQ are abbreviated as FSIQ, VIQ and PIQ

wide association studies are often considered the most promising search strategy, but require genotyping a very large number of neutral polymorphisms on very large sample sets. The cost of such an enterprise and the collection of a sufficiently large sample can still be prohibitive while the functional variant may still remain undetected. Alternative strategies to identify potentially functional genetic variants are therefore clearly needed. Because speciation involves phenotypic divergence in adaptive, usually complex, traits it is tempting to hypothesize that the genetic changes during speciation should involve loci that remain to control phenotypic variation in the relevant phenotypes within the newly emerged species (Thomas and Kejariwal 2004). Next to the obvious implications for evolutionary genetics, this assumption opens up the intriguing possibility of ascertaining the genetic basis of phenotypic variation in humans from a comparative genomic analysis.

Our cognitive ability exceeds that of our closest primate relatives and some of the genetic changes during human speciation have affected our intelligence. At the same time IQ is a highly heritable trait in humans with estimates of broad-sense heritability up to 80% (McGue et al. 1993; Deary et al. 2006). Genetic linkage and association studies have shown before that segregating variants influence individual differences in cognitive ability (Buyske et al. 2006; Luciano et al. 2006; Posthuma et al. 2005). As such, human intelligence is a good "candidate trait" to be investigated in the context of a comparative genomic approach. Focusing on a brain-related phenotype also seems to be warranted by the vast overrepresentation of genes expressed in the CNS among those that exhibit accelerated protein evolution specific to the primate linage. This result on its own confirms the common assumption that the phenotypic evolution of our brain has significantly coincided with human speciation (Dorus et al. 2004). 14% of the fast evolving, brain-related human genes harbored potentially functional polymorphisms suggesting that on a genome-wide scale at least several hundred such "derived" vs. "ancestral" variants should be present in the human genome at positively selected amino acid sites. All of these can be expected to affect phenotypic variation in an adaptive, complex trait and because this approach identifies candidate mutations rather than candidate genes any significant association directly links the phenotype to a plausible causative variant.

The beta adrenergic receptors belong to the G-protein-coupled receptor superfamily and mediate some of the physiological actions of catecholamines (noradrenaline and adrenaline) in a variety of tissues (Liggett 2000). Different beta-receptor subtypes have been characterized (Liggett 2000). The beta-2 receptor is expressed in the smooth

muscle of both the airways and blood vessels (to a lesser extent), and also can be found in the CNS (Hillman et al. 2005b). There is evidence that the beta adrenergic receptors might have a role in memory and learning formation. Noradrenalin (NA) which is the ligand for the adrenergic receptors exerts an ample range of functions in brain affecting cognition, behavior and emotion (Kobayashi and Kobayashi 2001). NA projections have been shown to extend to brain regions including hippocampus, amygdala, cerebral cortex and thalamus (Kobayashi and Kobayashi 2001). In particular, the hippocampus has been implicated in certain aspects of memory and in learning (Kobayashi and Kobayashi 2001; Bliss and Collingridge 1993). Furthermore, neurons from the hippocampus have been shown to exhibit activity dependent synaptic enhancement. This long-term potentiation (LTP) is the proposed model for memory and learning processes (Bliss and Collingridge 1993). These studies are supported by a few functional animal studies, in which deficits in memory and learning have been related to impaired NA synthesis (Kobayashi and Kobayashi 2001). In addition, animal studies, show that the activation of the beta-2 receptors induce LTP in neurons in hippocampus (Hillman et al. 2005a) and increase performance in tasks evaluating long-term memory and learning with the infusion of specific β2-agonists (Gibbs and Summers 2000), while β2-antagonists impaired memory consolidation (Gibbs and Summers 2005). More specifically, it has been shown that noradrenergic activation of the basolateral complex of the amygdala, involving β-adrenoreceptors, selectively enhances memory consolidation for emotionally arousing experiences (Roozendaal et al. 2006). The consensus of these animal studies is that higher beta-2 adrenergic receptor activity enhances memory/learning.

Genetic variation in the human beta-2 adrenergic receptor includes non-synonymous polymorphisms and the molecular function of the two missense mutations evaluated here has been studied before (Green et al. 1994). The substitutions of Gly for Arg at amino acid 16 (Arg16Gly; A → G in base pairs, rs1042713), Glu for Gln at amino acid 27 (Gln27Glu; C → G in base pairs, rs1042714), and a combination of both substitutions were considered. All three receptors variants displayed normal agonist binding and signaling activity. However, the two mutations differed markedly in the degree of agonist-promoted down regulation of receptor expression. The A → G, but not the C → G polymorphism enhanced the baseline level of isoproterenol and induced down regulation of receptor density from 26 to 41%. Consequently, the Gly16 protein (the G allele) has lower receptor density (Snyder et al. 2006) and presumably lower total signaling activity. The other (Gln27Glu; C → G) mutation did not enhance receptor down regulation.

The present analysis was based on the plausible assumption that loci involved in the adaptive phenotypic divergence during speciation would be the same genes that affect variation in the phenotype within the new species (Thomas and Kejariwal 2004). Focusing on non-synonymous variation at amino acid sites that differ between species in genes previously ascertained for increased rate of molecular divergence may potentially unravel a class of functional mutations that is bound to affect highly important traits in humans. The reproductive and immune systems as well as a broad range of behavioral phenotypes are thought to be under strong selection during speciation (Torgerson et al. 2002; Hughes 1997; Sterck et al. 1997). Any human (disease) phenotype related to these processes is likely to be controlled by genes that have been subject of recent selection. In this study we focused on an obvious "human specific" trait that has undergone strong phenotypic divergence during human speciation and is heritable in our species. The comparative genomic analysis resulted in a set of candidate genes with an acceptable false discovery rate. Subsequent ascertainment of "ancestral" vs. "human specific" protein variants in genes expressed in the CNS resulted in only one gene to be followed up. The derived, human specific allele in the beta-2 adrenergic receptor (rs1042713) is known to reduce agonist induced down-regulation of receptor density and was found to increase cognitive ability in the young Dutch cohort but was associated with a decrease in the Scottish cohort at age 70. The contrasting results for the age groups ~12, ~37 and 70 years for this functional variant are similar to the effect of a mutation in the brain-derived neurotrophic factor shown to be associated with age-related change in reasoning skills (Harris et al. 2006) and might be explained by the observation that different age classes are known to differ in the genetic architecture of cognitive ability reflected in different heritability estimates (see Gosso et al. 2006). A similar shift in monoaminergic neurotransmission with ageing is seen for dopaminergic systems regulated by catechol-*O*-methyltransferase. In younger cohorts the Val/Val isoform of the Val158Met amino acid substitution is associated with impaired cognition (Starr et al. 2007) whilst in older adults the Val/Met form is associated with relatively impaired cognitive test performance compared to Val/Val and Val/Met (Harris et al. 2005), an example of the 'Goldilocks effect'. It is plausible that the degree of beta-2 adrenergic receptor down regulation differs across the life course at the same local agonist concentrations and this explains the different cognitive outcomes for younger and older cohorts. Previous functional studies have shown that rs1042713 and not rs1042714 affects beta-2 adrenergic receptor down regulation (Green et al. 1994; Snyder et al. 2006). These results suggest that the associations observed with rs1042713 may indeed be biologically relevant,

however, our own results for rs1042714 (which is in albeit low LD with rs1042713; $r^2 = 0.375$) and the Moray House Test scores at age 11 in the LBC1936 sample do not clearly support this.

Interestingly, it has recently been shown that the most common haplotype of ADRB2, which carries the G allele of rs1042713, has an age-dependent effect on hypertension in a European American population, similar to the pattern observed here. The G allele carrying haplotype decreases risk for hypertension in the young but increases it in the elderly (Bao et al. 2005). Assuming that low risk for hypertension and high IQ are the beneficial phenotypic states, the G allele is associated with a deleterious and a beneficial phenotype in both age classes. It seems that in both age classes the genotype that conveys higher cognitive ability is associated with elevated risk for hypertension.

Although further investigation is needed to unravel how this functional variant exerts opposite effects on cognition in different age classes, our study supports the use of interspecies comparisons to ascertain functional mutations that may affect complex human traits. Given the increasing availability of full genome sequences of phylogenetically related species, we expect similar approaches to significantly contribute to the understanding of the genetic basis of complex traits in general and of human behavior in particular.

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