PCSK6 is associated with handedness in individuals with dyslexia

Thomas S. Scerri[1,†], William M. Brandler[1,†], Silvia Paracchini[1,†], Andrew P. Morris[1], Susan M. Ring[2], Alex J. Richardson[5], Joel B. Talcott[3], John Stein[4] and Anthony P. Monaco[1,*]

[1]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK, [2]Department of Social Medicine, University of Bristol, Bristol BS8 2BN, UK, [3]School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK, [4]Department of Physiology, University of Oxford, Parks Road, Oxford, OX1 3PT, UK and [5]Department of Social Policy and Social Work, University of Oxford, OX1 2ER, UK

Approximately 90% of humans are right-handed. Handedness is a heritable trait, yet the genetic basis is not well understood. Here we report a genome-wide association study for a quantitative measure of relative hand skill in individuals with dyslexia [reading disability (RD)]. The most highly associated marker, rs11855415 ($P = 4.7 \times 10^{-7}$), is located within *PCSK6*. Two independent cohorts with RD show the same trend, with the minor allele conferring greater relative right-hand skill. Meta-analysis of all three RD samples is genome-wide significant ($n = 744$, $P = 2.0 \times 10^{-8}$). Conversely, in the general population ($n = 2666$), we observe a trend towards reduced laterality of hand skill for the minor allele ($P = 0.0020$). These results provide molecular evidence that cerebral asymmetry and dyslexia are linked. Furthermore, PCSK6 is a protease that cleaves the left–right axis determining protein NODAL. Functional studies of *PCSK6* promise insights into mechanisms underlying cerebral lateralization and dyslexia.

INTRODUCTION

The population bias in right-handedness is a characteristic feature of humans (1). There is some variance across cultures (2,3); however, there are no known populations in the world in which left-handers are the majority.

Handedness is correlated with cerebral asymmetries and right-handedness implies a dominance of the left hemisphere for motor function. Since Paul Broca reported in 1861 the case of a patient who had aphasia caused by a lesion in the left hemisphere (4), there has been significant interest in the idea that language laterality and handedness are linked. It has been proposed that handedness emerged as a consequence of the evolution of language (5). Moreover, theories dating back almost a century posit a connection between handedness and neurodevelopmental disorders such as specific language impairment (SLI) and dyslexia (6), two disorders affecting language and reading skills, respectively, with a prevalence between 5 and 10% (7).

However, no convincing association has been found between either hand preference or hand skill and neurodevelopmental disorders (8,9). Functional brain imaging studies have shown a weak correlation between handedness and cerebral dominance for language; with 96% of strong right-handers, compared with 73% of strong left-handers, showing left-hemisphere dominance for language (10).

There is suggestive, but mixed, evidence indicating that there may be atypical cerebral asymmetry in patients with dyslexia or SLI (reviewed in 11,12). With respect to reading disability (RD), Galaburda *et al.* (13,14) reported eight consecutive post-mortem specimens with RD that had reduced planum temporale asymmetry. Subsequent structural MRI studies have been inconsistent in replicating this finding (reviewed in 11). However, a meta-analysis of 17 functional neuroimaging studies comparing RD individuals to controls during reading tasks suggests that underactivation of the left hemisphere is found in the inferior parietal, superior temporal, middle and inferior temporal and

*To whom correspondence should be addressed at: Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK. Tel: +44 1865287503; Fax: +44 1865287650; Email: anthony.monaco@well.ox.ac.uk
†These authors contributed equally.

fusiform regions (15). Conversely, there were no abnormalities of activation observed in the right hemisphere or cerebellum (15). One study included in this meta-analysis demonstrated that adults with RD have reduced activation of the left middle and inferior temporal regions during reading tasks (16). The same authors subsequently showed using voxel-based morphometry that these same regions show cortical structural disorganization (17), providing a link between functional and structural differences of the left hemisphere in individuals with RD.

Understanding the genetic basis of handedness can help define the relationships between handedness, language, cerebral asymmetry and neurodevelopmental disorders. Handedness is a heritable trait, with additive genetic effects accounting for about a quarter of the variance (9,18). Molecular studies are consistent with a polygenic model of handedness. Linkage analyses have identified several loci including 2p12-q11 (19,20), 10q26 (21) and 12q21-23 (22). To date, only two specific genes have been suggested as candidates for handedness. The imprinted gene *LRRTM1* in 2p12-q11 has been associated with handedness and schizophrenia (23), and a candidate gene approach identified the X-linked androgen receptor (24). However, a recent genome-wide association study (GWAS) (25) found no single nucleotide polymorphisms (SNPs) were associated with handedness at *P*-values below 5×10^{-8}, the standard genome-wide threshold for significance.

Here we report a GWAS for a quantitative measure of relative hand skill (peg-board task). The most highly associated SNP is in an intron of PCSK6, a gene that encodes a protein involved in left-right axis determination (26). We then replicate this association in two other independent samples with RD reaching overall genome-wide significance.

RESULTS

We genotyped 197 unrelated individuals with RD (stage 1) on the Illumina 550k SNP array and used imputation to test over 2 million SNPs for association with a quantitative measure of relative hand skill (peg-board task) (27). The samples were selected as part of an ongoing study of dyslexia from a large sample of families, each with at least two siblings who show symptoms of dyslexia (28,29). The peg-board task measures the time taken by the subjects to move a row of 10 pegs from one location to another with the left hand (L) and right hand (R) separately (30). From these data, we derived the measure PegQ $[2(L - R)/(L + R)]$, which adjusts for overall differences in hand skill between subjects (19). This task produces an approximately normally distributed variable with a positive mean (Supplementary Material, Fig. S1); a positive PegQ indicates superior relative right-hand skill, and a negative PegQ indicates superior relative left-hand skill.

After the initial filtering and quality control stages, we tested 192 individuals for association with PegQ. There was no evidence of population structure and the genomic control statistic (λ) was 1.0031. No SNPs gave *P*-values below 5×10^{-8} in stage 1 (Supplementary Material, Table S1, Figs S2 and S3). The strongest association signal in the genome for directly genotyped SNPs was with rs9806256 ($P = 1.1 \times 10^{-6}$), while for imputed SNPs peak association was observed with rs11855415 ($P = 4.7 \times 10^{-7}$). Both SNPs are located within a cluster of five highly correlated SNPs, spanning 12 kb from introns 14–18 of *PCSK6* (proprotein convertase subtilisin/kexin type 6, also known as *PACE4*, Fig. 1) on chromosome 15q26. Individuals with the minor (derived) allele have significantly greater relative right-hand skill compared with those carrying the major (ancestral) allele. The mean effect size of each copy of the minor allele is 0.60 standard deviations (SD) to the positive (right-handed) end of the PegQ distribution (see 'β' in Table 1).

PCSK6 is known to play a key role in regulating left-right axis specification (26), making it a highly attractive candidate gene for involvement in handedness. We therefore followed up the initial association by genotyping rs11855415 and rs9806256 in an independent sample of individuals with RD who had also performed the same peg-board task (stage 2, $n = 376$). The results showed the same trend for increased right-hand skill in carriers of the minor allele; the association was nominally significant with rs11855415 ($P = 0.033$, β = 0.19, Table 1), but did not meet significance for rs9806256. Next, we genotyped these same two SNPs in individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort who have performed a similar peg-board task, and have been characterized by similar reading measures to the two previous stages. We selected individuals (stage 3, $n = 197$) to closely match the ascertainment criteria for RD used in stages 1 and 2. The analyses showed a significant increase in relative right-hand skill associated with the minor allele of both rs11855415 ($P = 0.0025$, β = 0.36, Table 1) and rs9806256 ($P = 0.00067$, β = 0.40, Table 1). Meta-analysis of all three stages yielded a *P*-value of 2.0×10^{-8} (Table 1, Supplementary Material, Fig. S4) for rs11855415, exceeding accepted genome-wide thresholds for significance. The data indicated that in the total sample of RD individuals, the average effect size of the minor allele is 0.35 SD towards the positive end of the PegQ distribution.

Then we investigated whether the effect of rs11855415 that we had observed in people with RD could be detected in the general population. We analyzed all the available ALSPAC children with a PegQ score ($n = 2666$), excluding those with neurodevelopmental disorders and performance IQ < 85 (see Supplementary Methods). The PegQ distribution for this sample compared with the ALSPAC children with RD in stage 3 shows neither a significant difference in the mean ($P = 0.54$), consistent with a previous study (9), nor a difference in the likelihood of being left- or right-handed ($P = 0.69$). In this general population sample, we did not detect significant association between rs11855415 and relative hand skill ($P = 0.25$, β = −0.038). However, the distribution of PegQ for each genotype in this general population sample shows that the PegQ scores for carriers of the minor allele appear clustered tightly around the mean (Supplementary Material, Fig. S5). This suggests that carriers of the minor allele in the general population cohort show reduced variability in relative hand skill, centered on the mean, in contrast to the RD sample which shows an increase in relative right-hand skill. To test this, we ran the association analysis for rs11855415 using the absolute value of the standardized PegQ score as a quantitative trait (Supplementary Material, Fig. S6). This compared scores closer to the mean against scores at both tails of the PegQ distribution.
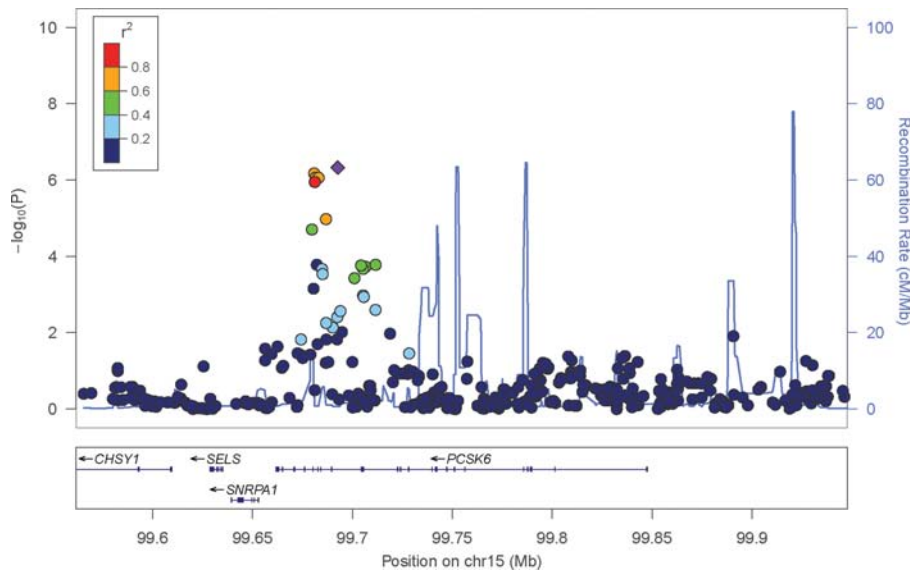
Figure 1. Visualization of *P*-values at the *PCSK6* locus. Negative $\log_{10}$ of the *P*-values for all genotyped and imputed SNPs around *PCSK6* plotted in Locus-Zoom (53). Linkage disequilibrium (r^2) with the most highly associated SNP, rs11855415 (purple diamond), is calculated based on the HapMap phase II CEU population and is shown by the colour of the SNPs. The recombination rate is shown by the blue line and the locations of genes in this locus are shown in the panel below the plot.

This analysis showed a nominally significant trend towards the mean of the PegQ distribution for carriers of the minor allele ($P = 0.0020$). Future studies will establish whether a similar pattern of findings is seen in other population cohorts.

DISCUSSION

We have performed the first GWAS for a quantitative measure of human handedness, as assessed by the peg-board task (30). The most highly associated SNP, rs11855415, is within an intron of *PCSK6*. The minor allele of this SNP confers increased relative right-hand skill. This association replicated in two independent groups with RD and is the first SNP for handedness to be identified at a genome-wide significant level. This effect was specific for people with RD, while the minor allele of rs11855415 showed a significant trend towards reduced laterality of hand skill in the general population.

PCSK6 encodes a proprotein convertase that processes proteins such as NODAL from their latent precursors into biologically active products (31). NODAL is a TGFβ-related protein that specifies both the anteroposterior and left–right axes (32). In a proportion of mouse embryos null for *Pcsk6*, the normally asymmetrically expressed *Nodal*, *Lefty* and *Pitx2* mRNAs are bilaterally expressed (26). Some embryos subsequently display laterality defects such as situs ambiguous (26). *PCSK6* is widely expressed in humans, with particularly high expression in the liver, corpus callosum (the band of axon fibers that connect the left and right hemispheres) and spinal cord (33). Mutations in other *NODAL* pathway members have been linked to lateralization defects such as holoprosencephaly, which is an incomplete separation of the forebrain into discrete hemispheres (34,35).

The observation that the increase in relative right-hand skill associated with rs11855415 in *PSCK6* is specific to RD suggests there may be epistatic interaction between *PCSK6* and RD susceptibility genes. There have been a number of candidate genes proposed for RD, including *DCDC2* (36,37), *DYX1C1* (38), *KIAA0319* (39,40) and *ROBO1* (41). These genes are all involved in neuronal migration or axon guidance (42). It may be that the functional change in *PCSK6* subtly alters the initial left–right patterning of the early embryo, and this has a downstream effect during neuronal migration on the development of cerebral asymmetry. Future studies will seek to explain the difference between RD and general population subjects.

The genome-wide significance of SNP associations in *PCSK6*, coupled with the role of this gene in a known biological pathway for left–right asymmetry, make it a strong candidate for involvement in cerebral asymmetry and thus handedness. Further work will seek to identify the functional genetic change in *PCSK6*, along with its mechanism of action. Our results are consistent with the emerging view that handedness is determined by multiple interacting genetic and environmental factors. Interestingly, *PCSK6* has been found to be a direct target of FOXP2 (43), while PCSK6 may interact with SRPX2 (44). Mutation in either *FOXP2* or *SRPX2* may lead to severe forms of language or speech impairment. It would therefore be interesting to investigate the effect of *PCSK6* on handedness in individuals with other neurodevelopmental disorders such as SLI. Understanding the genes that modify the effects of *PCSK6* in RD individuals will offer us a significant insight into the etiology of both cerebral asymmetry and dyslexia.

MATERIALS AND METHODS

Study participants

Individuals were initially selected from our collection of families with RD (45) for severity of phenotype to undergo

Table 1. Association analysis results for the two markers in *PCSK6* in individuals with RD

Marker	Stage 1				Stage 2				Stage 3				Meta-analysis				
	n	βᵃ	SEᵇ	P-value	n	βᵃ	SE	P-value	n	βᵃ	SE	P-value	n	βᵃ	P-value	β 95Lᶜ	β 95Uᶜ
rs11855415	191	0.60	0.12	4.7×10^{-7}	368	0.19	0.09	0.033	185	0.36	0.12	0.0025	744	0.35	1.99×10^{-8}	0.23	0.47
rs9806256	192	0.56	0.12	1.1×10^{-6}	364	0.12	0.09	0.18	182	0.40	0.12	0.00067	738	0.31	2.34×10^{-7}	0.19	0.43

ᵃThe mean effect size of each copy of the minor allele measured in standard deviations.
ᵇStandard error.
ᶜ95% confidence intervals for β (L, lower, U, upper).

a GWAS for RD. Genotype data were generated for 197 individuals (stage 1). Families were recruited from the Dyslexia Clinic of the Royal Berkshire Hospital and range in age from 6 to 25 years. A second sample of 376 unrelated individuals with RD (stage 2) was recruited from either the Dyslexia Research Centre clinics in Oxford and Reading or the Aston Dyslexia and Development Clinic in Birmingham. The majority of these individuals are between 8 and 18 years old. The third sample of unrelated individuals came from the Avon Longitudinal Study of Parents and Children (ALSPAC). For ALSPAC, all pregnant women in the Bristol, UK, area with a then expected delivery date between 1 April 1991 and 31 December 1992 were approached for participation in the study that has today resulted in a general population cohort of about 14 000 children (46). For the present study, we assigned individuals from the ALSPAC cohort into a sub-group with RD ($n =$ 197, stage 3), and a sub-group representing the general population without a neurodevelopmental disorder ($n =$ 2667). Complete details of the ascertainment criteria for the RD and general population sub-groups are given as Supplementary Material.

Relative hand skill phenotype

The Pegboard test (30) was administered to the individuals who were analyzed in stages 1 and 2. The test involved the measurement of the time taken by the subjects to move, with each hand, a row of 10 pegs on a board from one location to another. This test was repeated five times for each hand in all the subjects. From these data, we derived the measure of relative hand skill (PegQ) for each subject, which was calculated as the difference between the average times for the left hand (L) and the right hand (R), L − R, divided by the average time for both hands combined, (L + R)/2, to correct for the overall difference in hand skill between the subjects (19). It has previously been shown that although overall hand skill improves with age (i.e. the peg test is performed in a shorter time with both hands), relative hand proficiency measured in this way does not change significantly between the ages of 3 and 15 (47). Therefore, although there is a large range of ages in our study (6–25 years), we do not expect this to significantly affect our results. The distribution of PegQ in stage 1 had a mean of 0.0796 (SD = 0.099), and in stage 2 it was 0.061 (SD = 0.091). The mean PegQ score is similar to Francks' (19) sample, which had a mean of 0.072. The PegQ distribution is unimodal, continuous and approximately normal, making it suitable as a quantitative phenotype for a GWAS (Supplementary Material, Fig. S1).

A peg test similar to that used in stages 1 and 2 was performed in the ALSPAC sample as part of a battery of manual dexterity tests known as Movement ABC (48). In this case, there were 12 pegs on the table that the child picked up one-at-a-time and placed in a peg board. After initial practice, children performed the test once (due to time constraints) with each hand. The mean PegQ was slightly different in stage 3 at 0.113 (SD = 0.199), while the distribution remains unimodal, continuous and approximately normal. One outlier was removed from further analysis (PegQ = 1.50).

Genotyping and quality control

The 197 individuals (stage 1) were genotyped on the Illumina® 550k Platform according to the manufacturer's instructions. One individual was excluded based on having a call rate <95%. We filtered out all SNPs with either minor allele frequency (MAF) < 0.05 (due to the small sample size), or successful genotype call rate < 0.95, or violation of Hardy–Weinberg equilibrium ($P < 5.7 \times 10^{-7}$; in autosomes). In total, 477 347 SNPs passed quality control. Principal component analysis (PCA) to test for individuals with divergent ancestry compared with CEU HapMap3 samples was performed as detailed previously (49), and as a result four further individuals were excluded (Supplementary Material, Fig. S7). In order to investigate population structure within our sample, we repeated our PCA without the HapMap3 samples. We tested for association between PegQ and the first two principal components (PC, adjusting for age and sex) in a linear regression model. There was no evidence of association with either component ($P = 0.244$ for PC1 and $P = 0.184$ for PC2). We thus did not adjust for these components in our subsequent GWAS association analysis.

The 376 unrelated cases (stage 2) were genotyped for the two SNPs, rs11855415 and rs9806256, as part of a larger study using iPLEX assays from Sequenom® (San Diego, CA, USA), according to the manufacturer's instructions. We also re-genotyped the original 197 individuals (stage 1) with these assays, and found 99% concordance for the directly genotyped rs9806256 and 98% concordance for the imputed SNP rs11855415. The Hardy–Weinberg equilibrium P-values for the SNPs were rs11855415 $P = 0.243$ and rs9806256 $P = 0.820$. The integrated genotyping system (50) was used to store genotypes.

The same two SNPs were genotyped in the ALSPAC children cohort by KBiosciences, Hertfordshire, UK (www.kbioscience.co.uk date last accessed 12/11/10), using a fluorescence-based competitive allele-specific PCR (KASPar) assay. The Hardy–Weinberg P-values for the two SNPs were rs11855415 $P = 0.018$ and rs9806256 $P = 0.248$.

Imputation

We used IMPUTE [v2.0.5 for autosomes, (27) and v1.0.0 for the X chromosome, (51)] to estimate the genotypes of SNPs not directly genotyped on the Illumina 550k platform. This involved combining information from (i) the directly genotyped SNPs, (ii) a densely genotyped reference panel (60 CEPH founders from HapMap Phase II, build 35) and (iii) a fine-scale recombination map, to infer the missing genotypes. Imputed SNPs were filtered out if they had an MAF < 0.05 or proper info <0.5, allowing for us to test a further 1 709 209 SNPs for association.

Statistical analysis

Directly genotyped and imputed SNPs were tested for association with the quantitative measure PegQ under an additive model using SNPTEST (v1.1.4, www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html date last accessed 12/11/10) simultaneously specifying sex and age as covariates. For the X chromosome, analysis in stage 1 was conducted

by running the association test separately for the two sexes in SNPTEST and combined the results using GWAMA [v1.4, (52)], which performs fixed effects meta-analysis using inverse-variance weighting of the parameter estimates. The results for the three stages were then meta-analyzed using GWAMA (52). The values of PegQ were standardized in all stages separately to give a mean of 0, and a SD of 1. For the two SNPs genotyped in ALSPAC in the general population, the absolute value of the standardized PegQ score was also run as a quantitative phenotype.

To rule out any underlying confounding effects, we tested rs11855415 and rs9806256 for association with measures of hand motor skill, IQ and reading ability (CCI, CCN, OLSON, READ, SPELL, SPOON, defined in Supplementary Material), in each of the three stages as well as in the general population sample whenever data were available, and found no significant association (Supplementary Material, Table S2).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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