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The PCSK6 gene is associated with handedness, the autism spectrum, and magical ideation in a non-clinical population

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ABSTRACT

Common polymorphisms in the gene PCSK6, whose protein product mediates the development of brain and body asymmetry through the NODAL pathway, have recently been associated with handedness in three studies, making it a key candidate gene for understanding the developmental and expression of human lateralization. We tested the hypothesis that the PCSK6 VNTR polymorphism rs1053972 influences the expression of handedness and aspects of dimensional schizotypy and autism. For a sample of 709 healthy individuals, rs1053972 genotype was significantly associated with categorical measures of handedness, and with dimensional handedness in subsets of the population with high schizotypy and magical ideation or a lack of strong right-handedness. Both findings showed evidence of stronger or exclusive effects among females, compared to males. Genotypes of PCSK6 also showed significant sex-limited associations with magical ideation, a component of positive schizotypal cognition measured using the Schizotypal Personality Questionnaire, and total autism score, measured using the Autism Spectrum Quotient. These results partially replicate previous studies on effects of PCSK6 rs1053972 genetic variation on handedness phenotypes, link the PCSK6 gene with the dimensional expression of neurodevelopmental conditions in healthy individuals, and show that associations of this gene with handedness and psychological phenotypes exhibit evidence of sex-limited effects.

1. Introduction

Handedness is a fundamental physical representation of lateralization, given that about 95% of right-handed individuals are left lateralized for language, compared to about 70% of left-handers (Knecht et al., 2000). Handedness is usually considered as dichotomous, but it also varies continuously in magnitude, from strong right-handedness, to mixed handedness, to strong left-handedness, with absolute handedness largely independent of handedness direction (Ocklenburg et al., 2014). Handedness, directional or absolute, has been associated with a remarkable range of cognitive and psychological phenotypes (Corballis, 2012), as well as with the expression of neurodevelopmental and psychiatric conditions such as dyslexia and schizophrenia (Annett, 2011; Brandler and Paracchini, 2014; Hirnstein and Hugdahl, 2014). As such, it represents one of the primary correlates of variation in human brain structural and functional organization (Corballis, 2009).

The genes involved in brain symmetry overlap to some unknown degree with those that underlie handedness (Ocklenburg et al., 2014; Somers et al., 2015), although few such genes have yet been characterized (McManus et al., 2013). Heritabilities for measures of handedness are substantial, ranging from about 25–65% across different studies (Medland et al., 2009; Yin-Ju et al., 2015). Heritability of structural brain asymmetry is comparable in magnitude to that of handedness (Herve et al., 2013), with estimates ranging from 20 to 37% for various neuroanatomical regions (Jahanshad et al., 2010).

What genes underlie this heritability? Recent candidate-gene studies have reported evidence for the involvement of several genes in handedness, including PCSK6 (proprotein convertase subtilisin/kexin type 6), AR (androgen receptor), and LRRTM1 (leucine rich repeat transmembrane neuronal 1), each of which also demonstrates notable functional links with the development of this phenotype (Corballis, 2009, 2012). These studies are important because they are providing the first clear and replicated evidence for the genetic basis of human handedness, with important implications for understanding brain development, functional and structural brain lateralization, and neurodevelopmental conditions, such as schizophrenia, dyslexia and autism, that involve alterations in lateralized cognitive functions (Annett, 2011; Lindell and Hudry, 2013; Hirnstein and Hugdahl, 2014). Such

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candidate genes should also be useful in evaluating genetic models of handedness, especially models that postulate alleles at one or more loci mediating either a right-shift in handedness, or a lack of right shift with handedness strength and direction due to non-deterministic factors (McManus et al., 2013).

PCSK6, also referred to as PACE-4, is a gene at chromosomal location 15q26.3 whose enzyme product is involved in a suite of developmental pathways including left-right asymmetry (Scerri et al., 2011; Watanabe et al., 2015). Genetic variation in this gene has recently been associated with handedness in three studies.

First, a GWAS by Scerri et al. (2011) found an association between relative hand skill at a pegboard task and rs11855415, a single nucleotide polymorphism (SNP) within the gene PCSK6, in three samples of individuals with reading disability, and by meta-analysis of these cohorts. This association was not detected in a non-clinical sample, although carriers of the minor, handedness-associated allele showed evidence of reduced variability in relative hand skill compared to non-carriers.

Second, a GWAS by Brandler et al. (2013) demonstrated that rs7182874, an intronic SNP within PCSK6, was associated with pegboard relative hand skill, by meta-analysis of cohorts of individuals with dyslexia. This marker showed a lack of association with handedness in the same non-clinical population analyzed by Scerri et al. (2011), suggesting that effects of the SNP are mediated by gene by environment interaction (the presence or absence of reading disability), as also reported for SNPs in the gene LRRTM1 (Francks et al., 2007; Ludwig et al., 2009).

Third, an intronic 33 base pair variable-number tandem repeat (VNTR) polymorphism in PCSK6 (rs10523972) was found to have significant associations with directional handedness consistency and degree of handedness, as quantified using the Edinburgh Handedness Inventory, in a non-clinical population (Arning et al., 2013). Four to 11 copies of this VNTR repeat are present in humans, with six (20.4%) and nine (70.2%) copies being most common, and the distribution strongly bimodal. Arning et al. (2013) reported that heterozygotes for short (six or fewer repeats) and long (nine or more repeats) alleles showed reduced consistency of right-handedness compared to those who were homozygous for the long allele, and that heterozygotes also showed more inconsistency in hand preference. By their analysis, genotypic variation for this PCSK6 VNTR was thus strongly associated with absolute handedness ($p=0.001$), and marginally non-significantly associated with handedness direction ($p=0.07$).

These links between PCSK6 and handedness appear to be attributable to its role in the NODAL pathway, which organizes the development of body and brain asymmetry (Beddington and Robertson, 1999; Roussigné et al., 2009; Brandler et al., 2013; Blum et al., 2014). This developmental process involves rotational movement of cilia that causes nodal flow (movement of fluid at the node), which is detected by non-motile cilia. These non-motile cilia transduce the signal as an increase in calcium levels in the endodermal cells on the left side of the node, which determines left-right asymmetric gene expression (Takao et al., 2013). NODAL, a secretory protein in the TGF-B superfamily, is expressed on one side of the node, and through a positive feedback loop, it induces further expression of itself as well as other genes involved in left-right asymmetry development (Babu and Roy, 2013). The PCSK6 gene product PACE4 cleaves NODAL into its active form, thus triggering left-right axis development. Although specific links of PCSK6 expression patterns with handedness development have yet to be elucidated, these findings demonstrate that PCSK6 exhibits strong functional connections with developmental asymmetries of the body and brain, which supports findings from the three studies of PCSK6-associated handedness phenotypes conducted thus far.

To the extent that variation in the PCSK6 gene modulates the expression of handedness, it might also be expected to influence aspects of cognition and psychological traits that are associated with handedness and lateralization of the brain. The apparent restriction of some PCSK6 effects to individuals with dyslexia, but not non-clinical individuals (Scerri et al., 2011; Brandler et al., 2013), indeed suggests effects of this gene on lateralized cognitive abilities, especially given evidence of higher levels of mixed handedness and altered brain lateralization in dyslexia (Peters et al., 2006; Annett, 2011), and associations of dyslexia with schizotypal cognition and schizophrenia (Richardson, 1994; Bersani et al., 2006; Condray, 2005; Edgar et al. 2006). Does genetic variation in PCSK6 influence the expression of cognitive and psychological phenotypes that have been linked with alterations in brain lateralization, as expected given its associations with handedness?.

In this study, we first tested for association of the PCSK6 VNTR rs10523972 with categorical and dimensional handedness phenotypes in a non-clinical population, as an attempt to reproduce the main findings of Arning et al. (2013). Second, we tested the hypothesis that rs10523972 genotypic variation is associated with dimensional variation in measures of schizotypy and autism.

Schizotypy and autism were analyzed here because each has been associated with variation in handedness, brain lateralization, or both phenotypes in previous studies. Schizotypy and schizophrenia have been associated with increased levels of mixed handedness across many studies and by meta-analysis (Sommer et al., 2001; Preti et al., 2007; Somers et al., 2009; Tsuang et al. 2013; Barrantes-Vidal et al., 2013), with especially strong links of mixed handedness with magical ideation, a positive phenotype of schizotypal cognition that involves increased fantasy-based and hyper-imaginative cognition (Barnett and Corballis, 2002; Badzakova-Trajkov et al., 2011; Chapman et al., 2011). These findings are notably concordant with the reduced cerebral lateralization found in schizophrenia and schizotypy (Leonhard and Brugger, 1998; Oertel-Knochel et al., 2010; Crow et al., 2013), which is associated with increased expression of positive schizophrenia phenotypes (Mitchell and Crow, 2005). Some schizophrenia risk genes and alleles also influence cerebral lateralization or handedness (e. g., Ocklenburg et al., 2014), indicating that pleiotropic effects modulate the expression of these three phenotypes.

Autism has been linked with alterations to brain lateralization and atypical patterns of handedness (e.g., Escalante-Mead et al., 2003; Floris et al., 2013), although clear consensus on the patterns of alteration has not yet been reached. Thus, to the extent that variation in the PCSK6 VNTR rs10523972 mediates lateralized brain development and function as well as handedness in non-clinical populations, it is expected to affect variation in these psychological traits.

Based on the considerations above, we tested several *a priori* predictions (in addition to the other analyses conducted): (1) reduced right handedness among heterozygotes for the PCSK6 VNTR rs10523972, and associations of genotype with dimensional handedness (Arning et al. 2013); (2) associations of higher magical ideation and total schizotypy with mixed handedness, and with any PCSK6 genotype that is found to be associated with mixed handedness; and (3) associations of autism score with atypical handedness (with a precise prediction not justifiable), and with any PCSK6 genotype that is found to be associated with this atypicality. These latter predictions are predicated on the existence of pleiotropy between handedness and psychiatric phenotypes, for effects of PCSK6 genotype.

2. Methods

2.1. Participants and ethics approval

Data was collected from 709 undergraduate students (476 females and 233 males, mean ages: males 19.5, SD=2.5; females 19.2, SD=2.4) at the University of Alberta and Simon Fraser University. The work was approved by Human Research Ethics at University of Alberta and by the Simon Fraser University Research Ethics Board, and all subjects provided written informed consent prior to participation in the study.

2.2. Psychological measures

The Waterloo Handedness Questionnaire (WHQ) was used to quantify handedness. The WHQ is a 32-item self-report questionnaire that asks participants to identify which hand they use for a variety of tasks (Steenhuis et al., 1990). Response choices and resulting scores range from always left (−2), usually left (−1), to either hand (0), usually right (+1), to always right (+2), with overall scores ranging from −64 to +64. For directional, categorical handedness coded as left/right, 'right' was considered as > 0 and 'left' as < 0, and for left/mixed/right coding, 'left' was −64 to −20, 'mixed' was −19 to +19, and 'right' was +20 to +64. Absolute handedness, which represents handedness strength independent of direction, was quantified by taking the absolute value of handedness scores. Direction and strength of handedness combined was quantified using overall WHQ score (called 'total WHQ'). This measure of handedness allows direct evaluation of right or leftward shifts in handedness, as done by Francks et al. (2007) for effects of genotypic variation in the gene LRRTM1.

The Autism Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) was used to quantify the extent to which participants of normal intelligence endorsed traits consistent with the autism spectrum. The AQ is a 50-item self-report questionnaire that assess autistic traits across five domains (with 10 questions per domain) including: (1) social skills; (2) communication; (3) attention to detail; (4) attention switching; and (5) imagination. Responses range from 'definitely agree' to 'definitely disagree' on a 4-point Likert scale and participants mildly or strongly endorse a trait in the 'autistic' direction, they score one point for that question. Scores range up to 10 for each subscale, and up to 50 overall. Given that previous studies of handedness in autism have been restricted to the diagnosis itself (rather than subscales of autism, for example), only Total Autism score was analyzed here.

Schizotypy was assessed using the Schizotypal Personality Questionnaire-Brief Revised (SPQ-BR) (Callaway et al., 2014). This questionnaire includes 32 items using a 5-point Likert-scale format, with response choices ranging from 'strongly disagree' to 'strongly agree'. The questionnaire includes seven subscales, constricted affect and social anxiety (which make up the 'Interpersonal' scale), magical thinking; unusual perceptions, and ideas of reference (which make up the 'Cognitive-Perceptual scale), and eccentric behavior and odd speech (which make up the 'Disorganized' scale); total schizotypy is the sum of all three scales. Scores can range from 0 to 160 with increasing scores reflecting higher levels of schizotypy. Given that previous studies of handedness in relation to schizophrenia and schizotypy have predominantly reported effects for schizophrenia, total schizotypy, and/or magical ideation subscale, only Total Schizotypy and Magical Ideation were analyzed here.

2.3. DNA data collection

DNA was collected through saliva samples from Caucasian individuals over two years from the University of Alberta and Simon Fraser University. DNA samples were amplified with the same primers as used by Arning et al. (2013) (Forward: 5′ CATCGCTGATTCGCACATCACCAGGCTTGTTTTCTGCA 3′, Reverse: 5′ CAGCCTACGTGTGTGGTGACA 3′), which amplify across the 33-base pair VNTR within PCSK6. PCR was conducted as follows: 3 μL of DNA was added to the following PCR recipe: 11.1 μL water, 1.9 μL forward primer, 0.3 μL labelled forward primer, 2.2 μL reverse primer, and 3.4 μL ProMega Master Mix and the 25 μL was run in the thermocycler (Stage 1: 95 C for 5 min, Stage 2: 35 cycles of 95C for 30 s, 58C for 30 s, 72C for 40 s, Stage 3: 72C for 10 min). The PCR amplified samples were genotyped at Simon Fraser University using a LI-COR 4300 Genetic Analyzer apparatus. The genotypes were analyzed using GeneImagIR software (Version 3.52, Scanalytics, LI-COR, Inc., Lincoln, NE).

2.4. Analyses

Contingency table data were analyzed using Fisher's Exact tests (using R version 3.1.0 for 3 by 3 tables, and http://www.quantitativeskills.com/sisa/statistics/fiveby2.htm), for 2 by 3 tables. We measured absolute handedness (the absolute value of WHQ score, between 0 and 64) to assess handedness strength independent of direction, and 'total handedness' (WHQ scores from −64 to +64) as a measure of strength and direction of handedness combined. These handedness measures were analyzed with non-parametric tests due to strong skewness that was not amenable to adequate transformation; autism quotient scores, magical ideation scores, and total schizotypy scores were analyzed using parametric tests.

Given substantiated sex differences in handedness phenotypes (Papadatou-Pastou et al., 2008), autism quotient scores (Baron-Cohen et al., 2001), and aspects of schizotypy (Dinsdale et al., 2013), analyses were conducted on both the complete data set, and on females and males separately. Significant values were Bonferroni-adjusted for multiple comparisons for the psychometric data testing for associations of these traits with PSCK6 genotypes (three tests: total autism score, magical ideation score, total schizotypy score; threshold of $P < 0.0166$ for sex-pooled tests) and the twofold, sex-specific tests (six tests total; significance threshold of $p < 0.00833$). Tests for associations of total autism score, magical ideation score, and total schizotypy score with dimensional handedness were also subject to appropriate levels of Bonferroni adjustment (threefold, $p < 0.0166$, for samples pooled by sex, and sixfold, $p < 0.00833$, for males or females considered separately). Confidence intervals (95%) are presented for effect size measures (Cohen's d′ for two group comparisons, Spearman's rho for correlations, η^2 for ANOVA and Kruskal-Wallis tests – the latter was calculated as $\eta^2 = \chi^2/(N-1)$) using non-parametric bootstrap resampling (1000 resamplings) of the data (Efron and Tibshirani, 1994). For η^2, 0.01 is a small effect size and 0.06 is a medium effect size.

3. Results

The distribution of PCSK6 VNTR alleles ranged from four to twelve repeats, with a strong binomial pattern distribution (Fig. 1). Six and nine 33-bp repeats were most common (20.8% and 70.1% respectively). This distribution was virtually identical to that of Arning et al. (2013), who reported frequencies of 20.4% and 70.2% for the six and nine repeat groups. Following Arning et al. (2013), VNTR genotypes were categorized into 'short' (S, six or fewer repeats) and 'long' (L, nine or more repeats), with 7- and 8-repeats excluded. The distributions for males and females were virtually indistinguishable, as were the genotype distributions (SS, SL, LL) between males and females (3×2 contingency table, $\chi^2 = 0.17$, $p = 0.92$).

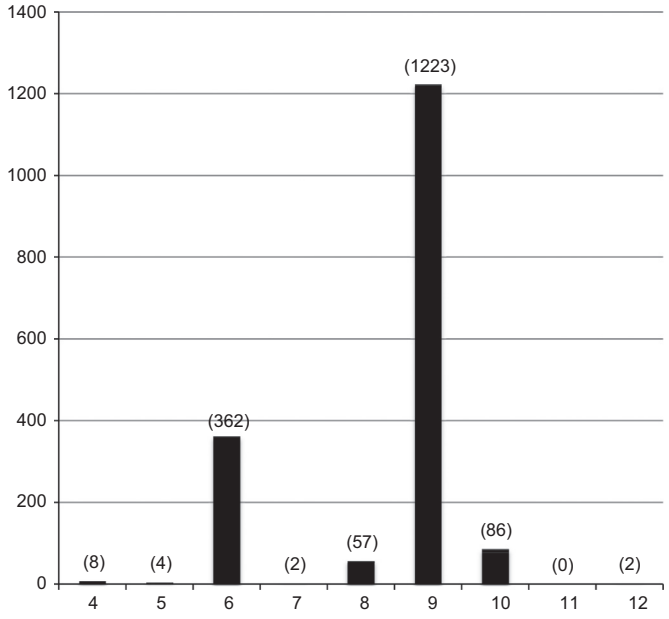

Fig. 1. Frequency distribution of alleles for 33 base-pair PCSK6 VNTR repeat.

Males exhibited lower levels of absolute handedness than females (i.e., more-mixed handedness) (Mann-Whitney test, males: mean 38.45, SD=14.5; females: mean 42.35, SD=15; p=0.00016; Cohen's d′=−0.265, 95% c.i. −0.43 to −0.12), as found in previous studies (Papadatou-Pastou et al., 2008), and total handedness was also lower in males than females (Mann-Whitney test, males: mean 33.28, SD=24.1; females: mean 37.03, SD=25.5, p=0.00097, d′=−0.15. 95% c.i −0.30 to 0.00). Females also exhibited higher average scores for magical ideation (males: mean 7.03, SD=3.32; females: mean 8.14, SD=3.64; t-test, t=4.05, p=0.0001; d′=−0.32, 95% c.i. −0.47 to −0.16), whereas males had higher scores for AQ (males: mean 17.76, SD=5.67; females: mean 16.74, SD=5.07; *t*-test, t=2.3, p=0.0207; d′=0.19, 95% c.i. 0.03–0.34); there was no sex difference, however, in total schizotypy (males: mean 82.38, SD=14.8; females: mean 80.44, SD=15.9, *t*-test, t=1.6, p > 0.10, d′=0.13, 95% c.i. −0.02 to 0.28). Overall means for both sexes are 81.08 (SD=15.6) for total schizotypy, 7.78 (SD=3.6) for magical ideation and 17.1 (SD=5.3) for autism (total AQ score).

3.1. Categorical handedness

To test for effects of PCSK6 genotype on handedness, we first compared genotype frequencies between individuals divided into handedness categories (Table 1). By Fisher's exact test, the three genotype groups SS, SL and LL differed significantly in frequencies of left versus right handedness. This difference was primarily the result of variation among females, who showed a lower incidence of right-handedness among SL heterozygotes (Table 1). These results are concordant with those of Arning et al. (2013), who also demonstrated reduced right-handedness among SL individuals.

Fisher's exact tests were also performed using 3 by 3 contingency tables, comparing the genotypes strong left, mixed, and strong right (Table 1). By these analyses, the three genotype groups differed significantly in handedness category for both sexes pooled (Table 1). The highest levels of strong right-handedness were found among individuals with the LL genotype (87.6%), with intermediate levels for the SL genotype (83.5%), and the lowest levels for the genotype SS (76.3%), which also showed the highest frequency of individuals with mixed handedness. As for the left versus right analysis, these differences appeared to be due predominantly to effects in females.

3.2. Dimensional handedness

We next tested for associations of PCSK6 genotype with absolute handedness (handedness strength, regardless of direction) and total handedness (WHQ score between −64 and +64). By Kruskal-Wallis tests, there were no associations of absolute or total handedness with genotype (P > 0.05 for all analyses)(Table 2).

Given that effects of PCSK6 on handedness in some previous studies have been restricted to populations with dyslexia or reading disability (Scerri et al., 2011; Brandler et al., 2013), and that dyslexia involves high levels of mixed and left handedness and schizotypy (Richardson, 1994; Peters et al., 2006; Annett, 2011), we next conducted analyses of PCSK6 genotype effects on absolute and total handedness after restricting the sample to: (1) individuals with WHQ scores between −64 and +40, which excludes strong right-handers, or (2) individuals with values above the average for total schizotypy, and above the (sex-specific) average for magical ideation. By the former analysis, females differed (nominally) significantly in total handedness, but not absolute handedness, by PCSK6 genotype (Table 2). By the latter analysis, total handedness showed significant variation by PCSK6

Table 1
Analyses of categorical handedness using fisher's exact tests.

	Gender	Handedness categories	SS	SL	LL	Exact P-value, cohen's d
2 × 3 tables	All	Left	3 (7.9%)	34 (14.0%)	34 (7.9%)	
		Right	35 (92.1%)	209 (86.0%)	394 (92.1%)	**0.043**
	Males	Left	1 (8.3%)	8 (10.3%)	15 (10.6%)	
		Right	11 (91.7%)	70 (89.7%)	127 (89.4%)	0.96
	Females	Left	2 (7.7%)	26 (15.8%)	19 (6.6%)	
		Right	24 (92.3%)	139 (84.2%)	267 (93.4%)	**0.0070**
3 × 3 tables	All	Strong left	2 (5.3%)	24 (9.9%)	17 (4%)	
		Mixed	7 (18.4%)	16 (6.6%)	36 (8.4%)	**0.0054**
		Strong right	29 (76.3%)	203 (83.5%)	374 (87.6%)	
	Males	Strong left	1 (8.3%)	6 (7.7%)	9 (6.3%)	
		Mixed	2 (16.7%)	5 (6.4%)	13 (9.1%)	0.60
		Strong right	9 (75%)	67 (85.9%)	121 (84.6%)	
	Females	Strong left	1 (3.9%)	18 (10.9%)	8 (2.8%)	
		Mixed	5 (19.2%)	11 (6.7%)	23 (8.1%)	**0.0021**
		Strong right	20 (75%)	136 (82.4%)	253 (89.1%)	

Note: One male had WHQ score of zero and thus could not be categorized as to right or left.

Table 2
Variation among individuals with different PCSK6 genotypes in dimensional handedness.

		SS mean (SD) (N)	SL mean (SD) (N)	LL mean (SD) (N)	χ^2	Kruskal-Wallis P value
All data	WHQ total, all	33.92 (24.55)(38)	32.95 (29.33)(243)	37.58 (22.23)(427)	1.89	0.389
	WHQ total, males	29.75 (26.05)(12)	33.18 (25.34)(78)	33.63 (23.42)(143)	0.717	0.699
	WHQ total, females	35.85 (24.10)(26)	32.84 (31.11)(165)	39.56 (21.38)(284)	1.63	0.443
All data	WHQ absolute, all	37.60 (18.22)(38)	41.56 (14.71)(243)	41.10 (14.74)(427)	1.344	0.511
	WHQ absolute, males	35.25 (16.99)(12)	39.23 (14.08)(78)	38.29 (14.53)(143)	0.710	0.710
	WHQ absolute, females	38.69 (18.98)(26)	42.65 (14.91)(165)	42.51 (14.67)(284)	0.642	0.642
WHQ between −64 and +40	WHQ total, all	17.52 (20.84)(21)	13.32 (30.00)(121)	21.15 (22.41)(199)	4.82	0.090
	WHQ total, males	20.33 (22.69)(9)	19.89 (24.77)(46)	19.22 (22.67)(78)	0.074	0.964
	WHQ total, females	15.42 (20.10)(12)	9.29 (32.27)(75)	22.40 (22.24)(121)	8.44	0.0147*
WHQ between −64 and +40	WHQ absolute, all	24.19 (11.94)(21)	30.61 (11.54)(121)	28.71 (11.10)(199)	4.23	0.121
	WHQ absolute, males	27.67 (10.91)(9)	30.15 (9.42)(46)	27.76 (10.37)(78)	0.70	0.706
	WHQ absolute, females	21.58 (12.46)(12)	30.89 (12.72)(75)	29.32 (11.55)(121)	5.10	0.078
Schizotypy and magical ideation above mean	WHQ total, all	38.45(19.23)(11)	23.18 (30.30)(60)	34.37 (25.67)(121)	8.11	0.017*
	WHQ total, males	31.33 (20.49)(6)	26.29 (24.08)(17)	23.80 (27.30)(36)	0.027	0.99
	WHQ total, females	47.00 (15.23)(5)	21.95 (32.61)(43)	38.85 (23.72)(85)	11.48	**0.0032***
Schizotypy and magical ideation above mean	WHQ absolute, all	38.45 (19.23)(11)	35.25 (14.18)(60)	40.12 (15.06)(121)	5.63	0.071
	WHQ absolute, males	31.33 (20.49)(6)	31.59 (15.94)(17)	32.53 (15.49)(36)	0.193	0.91
	WHQ absolute, females	47.00 (15.23)(5)	36.70 (13.34)(43)	43.34 (13.74)(85)	7.59	0.022*

*$p < 0.05$.
*Bold: $p < 0.05$ after Bonferroni adjustment.

Table 3
Variation among individuals with different PCSK6 genotypes in AQ-autism score, SPQ-magical ideation, and SPQ total Schizotypy.

Psychological phenotype (test, scale)		Gender (476 females, 233 males for all tests)	SS mean (SD)	SL mean (SD)	LL mean (SD)	ANOVA F value	P value
AQ	Autism	All	19.08 (5.975)	17.25 (5.169)	16.80 (5.265)	3.45	0.032*
	Autism	Males	16.92 (4.963)	18.94 (5.728)	17.19 (5.634)	2.57	0.079
	Autism	Females	20.08 (6.222)	16.45 (4.692)	16.61 (5.069)	6.15	**0.0023***
SPQ	Magical ideation	All	8.211 (4.288)	7.650 (3.497)	7.810 (3.553)	0.45	0.638
	Magical ideation	Males	9.917 (4.795)	6.500 (2.800)	7.080 (3.333)	5.78	**0.0036***
	Magical ideation	Females	7.423 (3.880)	8.194 (3.666)	8.175 (3.609)	0.53	0.586
	Total schizotypy	All	84.95 (15.94)	81.10 (15.74)	80.73 (15.50)	1.28	0.279
	Total schizotypy	Males	81.33 (17.75)	84.06 (15.05)	81.56 (14.49)	0.746	0.475
	Total schizotypy	Females	86.62 (15.11)	79.71 (15.91)	80.31 (15.99)	2.14	0.119

*$p < 0.05$.
*Bold: $p < 0.05$ after Bonferroni adjustment.

genotype, nominally for both sexes combined and among females after Bonferroni adjustment, with medium effect sizes ($\eta^2 = 0.04$, c.i. = 0.008 − 0.12 for WHQ total, both sexes; $\eta^2 = 0.086$, c.i. = 0.020-0.204 for WHQ total, females); absolute handedness showed nominal significance for females, with a medium effect size; $\eta^2 = 0.057$, c.i. = 0.01 − 0.17 (Table 2). These results suggest that PCSK6 VNTR genotype exhibits effects on dimensional handedness, as demonstrated in Arning et al. (2013), but only among individuals who are relatively high in schizotypy and magical ideation.

3.3. Handedness and PCSK6 genotype in relation to schizotypy

Diagnoses of schizophrenia, and high levels of schizotypy (especially magical ideation), represent among the strongest and most consistent correlates of mixed handedness and reduced strength of handedness, as described above. We investigated such effects in our data by testing for correlations of absolute handedness with magical ideation score and total schizotypy, and by using *t*-tests to compare individuals with mixed handedness to individuals with strong right or strong left handedness, for total schizotypy and magical ideation.

Absolute handedness was significantly, inversely associated with magical ideation score (Spearmann's rho = −0.129, p = 0.00059, both sexes, 95% c.i. −0.20 to −0.06; males: rho = −0.202, p = 0.002, 95% c.i. −0.32 to −0.08; females: rho = −0.132, p = 0.0039, 95% c.i. −0.22 to −0.05), and with total schizotypy score (Spearmann's rho = −0.139, p = 0.00021, both sexes, 95% c.i. −0.21 to −0.07; males: rho = −0.150, p = 0.022, 95% c.i. −0.28 to −0.02; females: rho = −0.130, p = 0.0045, 95% c.i. −0.22 to −0.04)(233 males, 476 females for all tests).

Total schizotypy score was significantly higher among mixed-handed (mean 85.83, SD = 18.28, N = 59) than among right and left handed individuals combined (mean 80.65, SD = 15.29, N = 650; t-test, t = 2.11, p = 0.039, both sexes; d′ = 0.31, 95% c.i. 0.01–0.61), with the difference due mainly to effects in males (mixed-handed mean = 93.45, SD = 17.76; right plus left handed mean = 81.35, SD = 14.14, t = 2.96, p = 0.0074, d′ = 0.75, 95% c.i. 0.25–1.32; females: mixed-handed mean = 81.92, SD = 17.5; right plus left handed mean = 80.31, SD = 15.98, t = 0.55, p = 0.58, d′ = 0.10, 95% c.i. −0.39 to 0.46). Scores for magical ideation were non-significantly higher among mixed-handed individuals (mixed-handed mean = 8.61, SD = 3.85; right plus left handed mean = 7.7, SD = 3.54, t = 1.75, p = 0.0847, d' = 0.25, 95% c.i. = −0.01 to 0.51, both sexes). These findings indicate that more-mixed handedness is associated with higher total schizotypy, and possibly with higher magical ideation, in this population.

If PCSK6 VNTR genotype mediates aspects of handedness, cerebral lateralization, or both phenotypes, then schizotypy and

magical ideation may be expected to differ between the three genotype groups. Levels of total schizotypy did not differ between SS, SL and LL individuals overall (Table 3). Levels of magical ideation likewise did not differ across genotype groups overall, for both sexes combined (Table 3). However, among males, magical ideation showed substantial, Bonferroni-adjusted significant variation among genotypes, whereby, in particular, males with the SS genotypes showed considerably higher scores on magical ideation than did SL and LL individuals, with a medium effect size (Table 3) ($\eta^2=0.05$, c.i.=0.007–0.014 for Magical Ideation, males).

3.4. Handedness and PCSK6 genotype in relation to autism spectrum traits

Like schizophrenia, autism has been associated with atypical handedness and lateralization, although for autism much less research has been conducted on this question and patterns of association remain unclear.

Absolute handedness was significantly, inversely associated with total AQ score (Spearmann's rho= −0.085, p=0.0235, both sexes, 95% c.i. −0.16 to −0.01), and this effect was due predominantly or exclusively to effects from females (rho= −0.11, p=0.0211, 95% c.i. −0.20 to −0.02; males: rho=0.01, p=0.87, 95% c.i. −0.14 to 0.11)(233 males, 476 females for these tests). Total AQ score, for the sexes combined, was nominally associated with PCSK6 genotype, but this association did not withstand Bonferroni adjustment (Table 3). By contrast, among females, total AQ score was significantly associated with genotype after Bonferroni adjustment (Table 3); in particular, females with the SS genotype exhibited higher scores than did females with the SL and LL genotypes, with a small effect size (Table 3) ($\eta^2=0.02$, c.i.=0.003 − 0.007 for Total AQ score, females).

4. Discussion

Genetic variation in the gene PCSK6 has been associated with human handedness in three recent studies, making it one of the strongest candidate genes for effects on this phenotype. We genotyped the VNTR locus rs10523972 in PCSK6 in a large non-clinical population, to attempt replication of a previous study of this locus and expand tests for PCSK6 genotypic effects to include two psychological phenotypes, dimensional schizotypy and autism, that have been linked in previous studies with handedness variation and atypical lateralization.

Our analyses yield two primary results. First, PCSK6 VNTR genotype was significantly associated with categorical handedness (right versus left, and right versus mixed versus left), and with dimensional handedness in a subset of our population (individuals with high schizotypy and magical ideation, and individuals who are not strongly right handed). These findings provide a partial replication of Arning et al. (2013), especially with regard to reduced right-handedness among SL heterozygotes. We also found effects of VNTR genotype on handedness predominantly or exclusively among females, suggesting sex-specific influences comparable to those reported in analyses of androgen receptor microsatellite polymorphisms effects on handedness variation (Medland et al., 2005; Hampson and Sankar, 2012).

The finding that effects of PCSK6 genotype on dimensional handedness were found only among individuals who were not strongly right-handed, or exhibited high scores for total schizotypy and magical ideation, may help to explain previous reports on links of PCSK6 genotype with handedness among individuals with dyslexia (who show high schizotypy as well as mixed and left handedness; Richardson, 1994; Bersani et al., 2006; Condray, 2005; Edgar et al., 2006; Peters et al., 2006; Annett, 2011), but not among non-clinical individuals (Scerri et al., 2011; Brandler et al., 2013). The causes of such effects remain unclear, but may involve reduced ability to detect genotypically-mediated handedness shifts among individuals with strong right-handedness, or intrinsically stronger effects of PCSK6 genotype among mixed and left handed, or more-schizotypal, individuals. The generality of such effects is also suggested by convergent findings for LRRTM1, where a schizophrenia-linked haplotype has likewise been associated with handedness among individuals with dyslexia but not among non-clinical individuals, in replicated studies (Francks et al., 2007; Ludwig et al., 2009; Leach et al., 2014).

Second, we found that PCSK6 VNTR genotypes were associated with dimensional schizotypy and autism scores. In particular, individuals with the SS genotype exhibited markedly higher levels of magical ideation (among males) and higher AQ scores (among females) than did individuals with SL or LL genotypes. These results indicate, most generally, that PCSK6 VNTR genotypes mediate the expression of psychological phenotypes that involve atypical cerebral lateralization, such that this locus apparently exerts pleiotropic effects on both handedness and psychological-cognitive phenotypes. These results should not be unexpected given that, as noted above, some associations of the PCSK6 gene (and the handedness-associated gene LRRTM1) with handedness appear to be specific to populations with dyslexia. The main psychological findings reported here, that the SS genotype engenders higher magical ideation in males and higher AQ scores in females, are also concordant with data showing that in this population mixed-handedness and reduced strength of handedness are associated with higher levels of magical ideation and schizotypy (most notably in males), and with higher Autism Quotient scores (most notably in females).

Considered together, these findings suggest the hypothesis that PCSK6 SS genotypes exert effects on neurodevelopment that mediate the expression of higher levels of mixed handedness, that in males involves higher magical ideation and schizotypy, and in females involves higher levels of dimensional autism spectrum psychological traits. These contrasting patterns in the two sexes are intriguing in that males with the SS genotype exhibit higher, more female-typical levels of magical ideation, whereas females with this genotype exhibit higher, more male-typical AQ scores, suggesting specific effects of this genotype on sexually-dimorphic psychological phenotypes. Sex specific effects comparable to those reported here have also been reported by Medland et al. (2005) in their study of androgen receptor gene microsatellite variation and handedness, and Hampson and Sankar (2012) reported, also for this AR locus, that mixed-handed males showed longer repeat length, which functionally represents a more 'feminized' pattern of androgen sensitivity. Further studies that jointly collect data on candidate genes for handedness, and dimensional schizotypy and autism, will be necessary for further evaluation of such hypotheses and effects.

4.1. Limitations

The primary limitations of this study are its reliance on a single measure of self-report handedness, relatively small sample sizes of individuals homozygous for the SS PCSK6 allele, and relatively small sample sizes for males compared to females. We have also genotyped only a single marker in the PCSK6 gene, such that the differences that we observed, and the observed developmental-genetic effects on handedness, may be attributable to closely-linked loci rather than the microsatellite variation itself.

4.2. Conclusions

This study has partially replicated previous evidence (Arning et al. 2013) for effects of the PCSK6 microsatellite locus rs1053972 on human handedness, and thus further establishes this gene as a primary candidate for mediation of this fundamental human phenotype. The results described here also help to resolve an intriguing pattern in the literature, discovery of gene-handedness associations in clinical populations (e. g., with dyslexia) but not non-clinical ones, by showing stronger effects of PCSK6 genotype on handedness among individuals with higher scores on schizotypy and magical ideation. Finally, effects of PCSK6 rs1053972 genotype have been extended to psychiatric phenotypes (autism spectrum and magical ideation scores) in a non-clinical population, which demonstrates sex-specific pleiotropy between handedness and psychiatric traits. These results thus implicate PCSK6 in mediation of brain developmental pathways that jointly impact upon handedness, autism and aspects of schizotypy. Considered together, these findings motivate studies of PCSK6 focused on brain imaging genetics, task-based handeness, and effects of gender on gene-phenotype associations, which should provide further insights into the roles of this gene in neurodevelopment, cognition and behavior.

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