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Educational attainment-related loci identified by GWAS are associated with select personality traits and mathematics and language abilities

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ABSTRACT

A recent genome-wide association study of educational attainment identified three significant single nucleotide polymorphisms (SNPs) (rs9320913, rs11584700, and rs4851266). In this study, we expanded this previous work by investigating behavioral correlates of these SNPs in a Han Chinese sample (rs9320913 was not available in our data and was thus replaced by rs12202969, which is in high linkage disequilibrium [i.e., correlations of alleles] with the former, $r^2 = 0.96$ in Han Chinese population based on the 1000 Genomes Project). Association analysis for individual SNPs showed significant associations between rs4851266 and a measure of language ability (Chinese word recognition), and between rs12202969 and a personality trait (fear of negative evaluation) and a measure of mathematical ability (number paired-associates learning). A polygenic score based on these three SNPs was also significantly associated with the measures of mathematical and language abilities. Specifically, educationally advantaged alleles identified in the previous study were associated with less fear of negative evaluation and higher mathematical and language abilities in the current study. This exploratory study provides evidence of psychological mechanisms for the association between genes and educational attainment.

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1. Introduction

Educational attainment usually refers to the highest degree of education that an individual has completed. Twin studies showed that educational attainment had moderate heritability, ranging from 18% to 77% across different studies (Branigan, McCallum, & Freese, 2013).

A recent genome-wide association study, using a large Caucasian sample, showed that three independent single nucleotide polymorphisms (SNPs, rs9320913, rs11584700, and rs4851266) were associated with educational attainment (Rietveld et al., 2013). They found positive associations between years of schooling and allele A of rs9320913 (located on Chromosome 6 at position 98,691,454 bp near the gene *LOC100129158*), and positive associations between college completion and allele A of rs11584700 (located on Chromosome 1 at position 202,843,606 bp near the gene *LRRN2* [leucine rich repeat neuronal 2]) and allele T of

rs4851266 (located on Chromosome 2 at position 100,184,911 bp near the gene *LOC150577*). These researchers suggested that follow-up studies should use the candidate gene approach to explore the potential associations between these genetic variants and well-measured endophenotypes, such as personality and cognition (Flint & Munafò, 2013; Rietveld et al., 2013).

Indeed, Ward et al. (2014) recently reported a significant association between the composite score of the three educational attainment-related SNPs (i.e., rs9320913, rs11584700, and rs4851266) and children's school performance as measured by the Standard Assessment Tests (SATs) at age 13–14 years. More specifically, rs9320913 was found to be associated with both the English ($p = 0.002$) and mathematics scores ($p = 0.015$), but there were no significant associations between the other two SNPs (i.e., rs11584700 and rs4851266) and the test scores ($p > 0.05$).

The current study aimed to expand on the work by Ward et al. (2014) by examining possible associations between genetic variants linked to educational attainments and various endophenotypes, including an extensive set of measures of personality, mental health, cognition, and mathematical and language abilities (see Methods for a brief description and see Chen et al., 2013 Table S2 for details). Using data from an existing project (e.g.,

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Chen et al., 2013), we analyzed the associations between these behavioral measures and three SNPs (i.e., rs12202969, rs11584700, and rs4851266). SNP rs12202969 was used instead of rs9320913 because the latter is not available in our data and these two SNPs are 8.5 kb apart and have extremely high linkage disequilibrium (LD), $r^2 = .98$ and $.96$ in European and Han Chinese populations, respectively, based on the data of the 1000 Genomes Project (<http://browser.1000genomes.org>). Allele A of rs9320913 corresponds to allele A of rs12202969. Results should help elucidate the underlying mechanisms for the genetic basis of educational attainment.

2. Material and methods

2.1. Participants

Of the original sample of 480 subjects (Chen et al., 2013), genetic data for the three SNPs were available for 342, who were the subjects of the current study. All subjects were healthy Han Chinese undergraduates (sophomores, mean age = 20.42 years, $SD = .89$, range 18–22 years old; 55% female) from Beijing Normal University in China. All subjects had normal or corrected-to-normal vision and had no neurological or psychiatric history based on their self-report. They all signed written informed consent. This study was approved by the Institutional Review Board (IRB) of Beijing Normal University, China.

Individuals in this sample were all unrelated to one another. To ensure that there was no stratification effect in our sample, we conducted the following analyses using PLINK software (Purcell et al., 2007). First, we used PLINK to calculate the genomic inflation factor (lambda values) based on the genome-wide data of our subjects. In the current study, lambda values were all near 1 indicating no stratification effect on the association results. Second, we assessed the stratification effects by PLINK via clustering individuals into homogenous subsets based the genome-wide average proportion of alleles shared identity by state (IBS) between any two individuals. At the first level, we constrained the cluster solution to two classes, and the results showed that only one subject belonged to the second cluster, suggesting little systematic stratification. Deleting one subject made little difference to the results, so data for all 342 subjects were analyzed.

2.2. Behavioral assessment

To explore the potential behavioral correlates of educational attainment-related SNPs, we analyzed the data from a battery of behavioral measures including 17 personality and mental health self-reported measures, 18 cognitive tasks, 9 mathematical tasks, and 5 language tasks (see Chen et al. (2013) Table S2, for details). These measures have been widely used in previous research and proved to have good psychometric properties. Preliminary analyses resulted in three measures with significant ($p < 0.01$) associations with the targeted SNPs (see Table S1 for complete results on all measures). Therefore, we describe the three measures in greater detail below.

2.2.1. Personality: Fear of negative evaluation

Fear of negative evaluation was measured by a personality questionnaire called Brief Fear of Negative Evaluation (BFNE). It includes 12 items measuring the degree to which people experience apprehension at the prospect of being evaluated negatively (Leary, 1983). For example, a sample item is "I often worry that I will say or do the wrong things." Each item is answered using a five-point Likert scale, ranging from 1 (not at all characteristic of me) to 5 (extremely characteristic of me). Previous research has

shown that the scale has satisfactory reliability and construct validity (Leary, 1983). Similar to previous studies, the Cronbach alpha value was 0.90 in the current study.

2.2.2. Mathematical ability: Number paired-associates learning

This test was based on a study by Delazer et al. (2005) and a previous study for measuring mathematical ability among Chinese college students (Wei, Yuan, Chen, & Zhou, 2012). An artificial operation ' \S ' was defined as ' $12b - 9a + 70$ '. That is, $a \S b = 12b - 9a + 70$. For example, $5 \S 3 = 61$ (because $5 \S 1 = 12 \times 3 - 9 \times 5 + 70$). Two other examples of the equations were $2 \S 1 = 64$, $4 \S 2 = 58$. Fifteen equations were created based on the artificial operation. The participants, however, were not given the above definition of the operation. Instead, they were briefly presented an expanded form of the operation (i.e., ' $a \S b = (10 - a) \times b - (b - a) \times (b - a) - (a - 3) \times (b - 1) + a \times a + b \times b + (100 - 10 \times a - b) - 27$ '). Because of its complexity, the participants were not able to memorize the definition of the artificial operation. Instead, they were asked to memorize the associations between pairs of operands and their answer for the 15 equations. During the learning stage, an equation was presented in the middle of the screen for 10 s. After subjects memorized all equations, they were tested. During the test stage, participants needed to judge whether a given equation (e.g., ' $5 \S 3 = 61$ ', ' $5 \S 3 = 64$ ') was correct or not. Half of the trials in the test stage were correct equations, and the other half were incorrect equations. Each trial was presented for 9 s. After the test stage, participants learned the equations again and were tested again. The percentage of correct answers on the second test was analyzed. The split-half reliability of this test was 0.69.

2.2.3. Language ability: Chinese word recognition

Participants were asked to read aloud very low-frequency Chinese characters (50 characters in total). These characters were selected from a Chinese character psycholinguistic norm (Liu, Shu, & Li, 2007). This task was used in a previous study that measured Chinese reading ability (Mei et al., 2013). The subjects were told that they would see some Chinese characters and have 5 s to read each character aloud before they were prompted to move on to the next character. The experimenter pointed to each Chinese character one by one from left to right, asked the subject to read each character aloud within 5 s, and recorded the performance of the subject on the answer sheet. The number of characters recognized correctly was used as the index for this task.

2.3. Genotyping

A 4 ml venous blood sample was collected from each subject. Genomic DNA was extracted according to the standard method within 2 weeks after the blood sample was collected. All samples were genotyped using the standard Affymetrix genotyping protocol (Affymetrix, Inc.). As described in Table 1, the allele frequencies of genotyped SNPs (rs11584700, rs4851266, and rs12202969) in our sample were very similar to those of the Asians (e.g., Chinese and Japanese) in the HapMap dataset (www.hapmap.org [phase 3]). All SNPs met the criteria of a call rate of >95%, Minor Allele Frequency (MAF) of >0.05, and Hardy–Weinberg equilibrium (HWE) of $p > 0.05$ in the current study.

2.4. Polygenic score

Following the procedure used by Rietveld et al. (2013), we created a polygenic score based on the selected SNPs for each individual. The polygenic score for the i th individual was calculated as

Table 1Allele frequencies of candidate SNPs shown by ethnic groups. Data were from the present study and the HapMap dataset (www.hapmap.org [phase 3]).

SNP	Chr	Position (bp)	Nearest gene	Ref. allele	Reference allele frequency				
					Chinese Current study	Chinese CHB HapMap	Japanese JPT HapMap	Europeans CEU HapMap	African ASW HapMap
rs12202969	6	98,682,944	LOC100129158	A	0.38	0.42	0.33	0.49	0.22
rs4851266	2	100,184,911	LOC15050577	T	0.59	0.54	0.51	0.41	0.08
rs11584700	1	202,843,606	LRRN2	A	0.68	0.68	0.61	0.79	0.92

Note: Chr: chromosome; bp: base pair; Ref allele: reference allele. Population descriptors: CHB: Han Chinese in Beijing, China; JPT, Japanese in Tokyo, Japan; CEU: Utah residents with Northern and Western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection; ASW: African ancestry in Southwest USA.

Table 2Significant associations between SNPs and behavioral phenotypes after controlling for age and sex ($p < 0.01$).

SNP	Ref allele	Freq	(Personality) Fear of negative evaluation ($M \pm SD = 42.64 \pm 9.48$)			(Mathematics) Number paired-associates learning ($M \pm SD = 0.73 \pm 0.11$)			(Language) Chinese word recognition ($M \pm SD = 31.58 \pm 7.77$)		
			β	T	p	β	T	p	β	T	p
			rs12202969	A	0.38	-0.15	-2.69	<u>0.0075</u>	0.19	3.23	<u>0.0014</u>
rs4851266	T	0.59	0.10	1.86	0.0640	0.03	0.58	0.5636	0.21	3.59	<u>0.0004</u>
rs11584700	A	0.68	-0.01	-0.10	0.9233	-0.07	-1.21	0.2273	0.00	0.02	0.9810

Note: Ref allele: references allele; Freq: frequency in the current data; significant associations are underlined and in bold.

$$\hat{g}_i = \sum x_{ij} \hat{b}_j$$

x_{ij} is the number of copies of the effect allele for SNP j , and \hat{b}_j is the estimated SNP effect from the multiple-SNP analysis. Following their method, we created a polygenic score for each individual based on three SNPs (i.e., rs12202969, rs11584700, and rs4851266). Specifically, the polygenic score for each subject = (the number of copies of the A allele for rs12202969) * (0.101) + (the number of copies of the A allele for rs11584700) * (-0.095) + (the number of copies of the T allele for rs4851266) * (0.082). The \hat{b}_j estimates were based on the genetic association of EduYears data as reported in Table 1 and Table S9 of Rietveld et al. (2013).

2.5. Data analyses

Quantitative trait genetic association analysis was carried out by using Plink v1.07 (Purcell et al., 2007), including allelic association tests between individual SNPs and behavioral measures after controlling for sex and age. Because this was an exploratory study, we set the significance threshold at $p < 0.01$ without further correction for multiple comparisons. Multiple regression analysis was used to examine the associations between a polygenic score based on the three SNPs and behavioral measures after controlling for sex and age.

3. Results

Table 2 shows the significant ($p < 0.01$) associations between three candidate SNPs (i.e., rs11584700, rs4851266, and rs12202969) and behavioral phenotypes after controlling for sex and age. Allele A of rs12202969 was associated with lower scores of fear of negative evaluation ($\beta = -0.15$, $t = -2.69$, $p = 0.0075$, Cohen's $d = -0.31$) and higher scores of number paired-associates learning ($\beta = 0.19$, $t = 3.23$, $p = 0.0014$, Cohen's $d = 0.39$). Allele T of rs4851266 was associated with higher scores of Chinese word recognition ($\beta = 0.21$, $t = 3.59$, $p = 0.0004$, Cohen's $d = 0.44$). However, there was no association between rs11584700 and the three behavioral indices ($p > 0.05$). In order to see whether these associations were robust, we conducted additional analyses that included additional covariates. Results showed that the associations between rs12202969 and number paired-associates learning, between

rs12202969 and fear of negative evaluation, and between rs4851266 and Chinese word recognition remained significant ($p < 0.05$) after controlling for age, sex, intelligence [measured with Raven's Advanced Progressive Matrices (Raven, Raven, & Court, 1998)], and parental education attainment [the higher of the two parents' educational attainment, (Santelli, Lowry, Brener, & Robin, 2000)].

Similar to the findings using individual SNPs, after controlling for age and sex, the polygenic score based on these three SNPs was associated with higher scores of number paired-associates learning ($\beta = 0.18$, $t = 2.93$, $p = 0.0037$, Cohen's $d = 0.35$) and higher scores of Chinese word recognition ($\beta = 0.13$, $t = 1.98$, $p = 0.0490$, Cohen's $d = 0.25$). However, the association between the polygenic score and fear of negative evaluation was not significant ($\beta = -0.03$, $t = -0.51$, $p = 0.6125$, Cohen's $d = -0.06$).

We also conducted the post hoc power analysis. In the current study, SNP rs12202969 accounted for 2.2% of the variance of fear of negative evaluation; SNP rs12202969 accounted for 3.8% of the variance of number paired-associates learning; SNP rs4851266 accounted for 5.3% of the variance of Chinese word recognition. Using the software Quanto (version 1.2.4) (i.e., a power calculator for genetic association studies developed by Drs. Gauderman and Morrison, <http://biostats.usc.edu/Quanto.html>), we calculated post hoc the number of subjects required when the desired power was set at 80% at a significance level of 0.05 (2-sided), and the power we had given our sample size. For SNP rs12202969 and fear of negative evaluation, a sample size of 353 would yield 80% power; but with our 311 subjects, the power was 75%. For SNP rs12202969 and number paired-associates learning, a sample size of 203 would yield 80% power; with our 277 subjects, the power was 91%. For SNP rs4851266 and Chinese word recognition, a sample size of 144 would yield 80% power; with our 265 subjects, the power was 96%. Therefore, the statistical power was adequate for exploring the associations between the individual SNPs and three phenotypes above.

Finally, the polygenic score accounted for 3.5% of the variance of number paired-associates learning, 2.4% of the variance of Chinese word recognition, and 0.2% of the variance of fear of negative evaluation. For the polygenic score and number paired-associates learning, a sample size of 213 would yield 80% power; but with our 277 subjects, the power was 89%. For the polygenic score and Chinese word recognition, a sample size of 313 would yield

80% power; but with our 265 subjects, the power was 73%. For the small association between the polygenic score and fear of negative evaluation, it would require a sample size of 3822 to yield 80% power, so our 311 subjects was underpowered at 12%. In sum, our sample size was adequate to detect modest effects but not small effects.

4. Discussion

In the present study, we explored behavioral mechanisms that might have explained the role of three SNPs in educational attainment as found in a previous GWAS (Rietveld et al., 2013). Results showed significant associations of rs12202969 with a personality trait (fear of negative evaluation) and a measure of mathematical ability (number paired-associates learning), and of rs4851266 with a measure of language ability (Chinese word recognition). Educationally advantaged alleles identified in the previous study were associated with less fear of negative evaluation, better number paired-associates learning, and better Chinese word recognition in the current study. Furthermore, the polygenic score based on the three SNPs was associated with the mathematical and language scores. These results suggested that these specific skills might be the behavioral mechanisms underlying the link between the three SNPs and educational attainment. In the following paragraphs, we discuss these behavioral mechanisms in the context of relevant research literature.

First, fear of negative evaluation, as measured by the Brief Fear of Negative Evaluation (BFNE) scale, reflects people's worries about failing in the eyes of other people (Leary, 1983). Consequently, individuals scoring high on this scale would have fear of failure, characterized by habitual feelings of worry, unpleasant tension, and lack of confidence about future performance. Individuals with such fears would have difficulty functioning during an important test. Indeed a recent study showed that subjects with greater fear of failure had lower educational attainment (Kuyper, Van der Werf, & Lubbers, 2000). Supporting the role of genes in such personality traits, a twin study showed that the personality trait of fear of negative evaluation had a heritability of about 42% (Stein, Jang, & Livesley, 2002). Taken together the results of our study and those of Rietveld et al. (2013), it appears that individuals with allele A of rs12202969 were more likely to have lower fear of negative evaluation and consequently higher levels of eventual educational attainment.

Second, mathematical skills are certainly critical for educational attainment (Kaufman, Kaufman, Liu, & Johnson, 2009). We found that number paired-associates learning was a significant mechanism. Because our sample was college students, it made sense that basic number cognition might not have played an important role. Indeed, a recent study that investigated the correlates of college students' performance on a test of advanced mathematics showed that number paired-associates learning was positively correlated with advanced mathematics even after controlling for general cognitive processing, basic numerical processing, spatial processing, and language processing (Wei et al., 2012). Our finding that individuals with allele A of rs12202969 were better at number paired-associates learning than other genotypes was consistent with the results found by Ward et al. (2014). They could explain the finding of Rietveld et al. (2013) about this allele's role in educational attainment.

Third, language ability is also an important predictor of educational attainment (Deary, Strand, Smith, & Fernandes, 2007). Interestingly, a recent twin study of a Chinese sample suggested that Chinese word recognition (i.e., the ability to read Chinese words aloud correctly) had the highest heritability (73%) as compared to many other Chinese language abilities (Chow, Ho, Wong, Waye, & Bishop, 2011). We found that subjects with allele T of rs4851266

were more likely than those with other genotypes to score higher on the Chinese word recognition test. Therefore, this allele may have resulted in an advantage in native language learning and hence in educational attainment as found by Rietveld et al. (2013).

Finally, taken together the contributions of all three SNPs, the results seemed consistent across all three studies (ours as well as those of Rietveld et al., 2013, and Ward et al., 2014): The polygenic score of the three SNPs was associated with academic performance and hence educational attainment. To further support this academic skills-specific explanation of the links between the three SNPs and educational attainment as proposed by Ward et al. (2014), we had null findings for almost all other general measures of personality and cognitive abilities.

Several limitations of this study need to be noted. First, the physiological functions of the identified SNPs linked to educational attainment were not directly explored in the present study or two previous studies (Rietveld et al., 2013; Ward et al., 2014). Additional molecular functional studies are needed to investigate the biochemical mechanisms underlying this association. Second, it should be noted that the current study was based on a sample of healthy Han Chinese college students. The use of a normal healthy college sample to investigate the gene-behavioral associations has both strengths and weaknesses. With a homogeneous sample (in terms of age range, ethnicity, physical and mental health status, cognitive abilities, etc.) like Chinese college students, our results were less likely than those from heterogeneous samples to be confounded by group differences (or population stratification). Moreover, with the limited variance in our dependent variables such as cognitive abilities, the significant effects we found probably represented conservative estimates of the true effects. Nevertheless, given the particular nature of our sample, our results may or may not be generalizable to other populations. Future research should replicate our findings with various samples such as community samples of different ethnicities. Third, this was an exploratory study to identify potential behavioral mechanisms underlying the previously documented gene-behavior associations, so we used an arbitrary and lenient threshold of $p < 0.01$ without using more stringent corrections for multiple comparisons. These results are thus preliminary and their value is to provide some bases for future hypothesis-testing research. Finally, we used the candidate gene approach to examine the associations between three educational attainment-related SNPs and behavioral phenotypes in the current study. However, both education attainment and its endophenotypes are influenced by multiple genetic variants (Bae et al., 2013; Docherty et al., 2010). For example, a previous study showed that the personality trait of novel seeking is a mediator in the relationship between the dopamine D4 receptor (DRD4) gene and predisposition to higher education (Keltikangas-Järvinen, Elovainio, Kivimäki, Ekelund, & Peltonen, 2002). Future studies should examine other genetic variants that contribute to both educational attainment and its endophenotypes.

In conclusion, this study provides evidence for behavioral mechanisms involved in the association between genes and educational attainment. Different SNPs seemed to be mediated by different behaviors such as personality traits and mathematical and language abilities in their effects on educational attainment.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.paid.2014.08.028>.

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