A Rare Variant in *COL11A1* Is Strongly Associated with Adult Height in Chinese Han Population

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ABSTRACT

Human height is a highly heritable trait in which multiple genes are involved. Recent genome-wide association studies have identified that *COL11A1* is an important susceptibility gene for human height. To determine whether the variants of *COL11A1* associate with adult and children height, we analyzed splicing and coding single-nucleotide variants across *COL11A1* through exome-targeted sequencing and two validation stages with a total 20,426 Chinese Han samples. A total of 105 variants were identified by exome-targeted sequencing, of which 30 SNPs were located in coding region. The strongest association signal between Chr1_103380393 and height was P -value of 4.8×10^{-7} . Chr1_103380393 also showed nominal significance in the validation stage ($P = 1.21 \times 10^{-6}$). Combined analysis of 16738 samples strengthened the original association of chr1_103380393 with adult height ($P_{combined} = 3.1 \times 10^{-8}$), with an increased height of 0.292sd (standard deviation) per G allele (95% CI: 0.19–0.40). There was no evidence ($P = 0.843$) showing that chr1_103380393 altered child height in 3688 child samples. Only the group of 12–15 years showed slight significance with P -value of 0.0258. This study firstly shows that genetic variants of *COL11A1* contribute to adult height in Chinese Han population but not to children height, which expand our knowledge of the genetic factors underlying height variation and the biological regulation of human height.

KEY WORDS: Rare variant; *COL11A1*; Adult height; Chinese Han population

INTRODUCTION

Human height, a classic polygenic trait that involves multiple genetic loci, is the result of various growth and development processes and also associated with various human diseases under strong genetic influence with an estimated heritability of up to 80%–90% (Macgregor et al., 2006). Since 2008, multiple genome-wide association studies (GWASs) have identified about 200 susceptibility genes or loci associated with height with a genome-wide significance (Weedon et al., 2007; Kimura et al., 2008; Sanna et al., 2008; Liu et al., 2010; Perola, 2011; Hao et al., 2013; Tetens et al., 2013). Genetic variants affecting height variation might provide new insight into human growth and development, as well as the genetic architecture of common human traits and diseases (Weedon and Frayling, 2008; Wood et al., 2014).

Evidence has suggested that *COL11A1* may influence human height by interacting with pathways involved in bone development (Lanktree et al., 2011). A series of studies have reported that genetic defects of *COL11A1* are accompanied by bone related diseases (Richards et al., 2012; Raine et al., 2013). Mutations in *COL11A1* cause various types of chondrodysplasias in humans, such as Stickler syndrome type II and Marshall syndrome (Annunen et al., 1999). For genetically modified *COL11A1* gene, limb bones of newborn *cho/cho* mice are wider at the metaphysics than normal bones and only about half the normal length (Li et al., 1995). Recently, GWASs revealed SNPs (rs4338381 and rs7517682) of *COL11A1* that have significantly associated with height in individuals derived from European ancestry and other ethnicities (Lanktree et al., 2011; Wood et al., 2014). The GWAS

implications couple with the established notion that coding variants profoundly influence various diseases and quantitative phenotypes, raising the assumption that coding SNPs of *COL11A1* may contribute to the development of human height.

Although numerous genetic loci associated with human adult height have been identified by GWASs, most of them are common variants located in the non-coding regions, emphasizing the necessity of identifying rare variants with lower frequency in exonic regions, which might reveal biological functional insights for genetic effects on height susceptibility. A recent study performed the whole-genome exome sequencing in a relatively small number of samples, and successfully unraveled the contribution of coding variants with minor allele frequency (MAF) <5% to height variation (Du et al., 2014). In this study, we attempted to detect and verify the genetic association between susceptible coding SNPs of *COL11A1* and adult or children height in Chinese Han population.

RESULTS

Demographic characteristics of the study subjects

We selected the data information of 20,426 individuals from our previous study. For the discovery stage, 8190 subjects (4657 males and 3533 females) come from mid-east China, mainly Anhui, Shandong, Henan and Jiangsu provinces. In the validation stage, 8548 healthy adults (4358 males and 4190 females) were included. All the adult subjects were over 20 years old at the time of examination. In addition, 3688 children subjects, including 2377 boys and 1311 girls, were also recruited from

Anhui province and Shanghai, under the age of 15 years old, with 53.3% (1966/3688) younger than 3 years old. There were no significant differences of gender, height, or BMI distributions among female *versus* male. The characteristics of the samples were sufficient and available for analysis (All data collected are shown in Table S1).

Variants of *COL11A1* associate with adult height

The coding and exon–intron boundary (± 50 bp) regions of *COL11A1* were sequenced in 8190 adult samples. A total of 105 variants were identified, including three common variants with $MAF > 5\%$, eight rare variants with $0.5\% < MAF < 5\%$ and 94 low frequency variants with $MAF < 0.5\%$. Approximately 82.9% (87/105) of the variants was previously unreported in the human genetic database (dbSNP, build 130). In addition, 30 SNPs were located in coding regions, including 26 missense and 4 synonymous variants. There has not been a nonsense or frame shift variant observed in this study. (All data are shown in Table S2)

Genetic association analysis in adult height

To increase the statistical power, we mainly focused on the variants with $MAF > 0.5\%$. We first performed an association analysis for the 11 SNPs with $MAF > 0.5\%$ in 8190 adult samples. We identified three SNPs (chr1_103380393, rs992100, and rs111980222) that reached the significant threshold 4.5×10^{-3} (multiple corrected $P = 0.05/11$). The SNP strongest association with height was chr1_103380393 with P value of 4.80×10^{-7} (Table 1). The other two SNPs, rs992100 and rs111980222, were in strong linkage disequilibrium (LD) with chr1_103380393 ($r^2 = 0.809$ and $r^2 = 0.541$, respectively) (Fig. 1). We thus genotyped chr1_103380393 in the validation stage (Fig.

S1), and SNP chr1_103380393 also showed nominal significance ($P = 1.21 \times 10^{-6}$). Combined analysis of 16738 samples strengthened the original associations of chr1_103380393 with adult height ($P_{\text{combined}} = 3.10 \times 10^{-8}$), with an increased height of 0.292sd (standard deviation) per G allele (95% CI: 0.19–0.40) (Table 1).

Genetic association analysis in children height

To determine the association between chr1_103380393 and children height, we stratified the children samples ($n = 3688$) into five groups depending on the age. The results showed only 12–15 years age group with slightly statistical significance ($P = 0.0258$). There was no evidence ($P = 0.843$) showing that chr1_103380393 affects children height in total (Table 1).

Verification of rs4338381 and rs7517682 in Chinese Han population

Previous GWASs have identified two height-related markers rs4338381 and rs7517682 in *COL11A1* region in individuals of non-Asian ancestry. To assess whether the two reported SNPs are also associated with Chinese adult height, we genotyped rs4338381 and rs7517682 in the validation stage (Fig. S1). The genotype distributions and allele frequencies of chr1_103380393, rs4338381 and rs7517682 in target sequencing and validation stages are shown in Table 2. We found that SNPs rs4338381 and rs7517682 did not show strong evidence for association with adult height in our study ($P = 0.45$ and 0.017 , respectively).

DISCUSSION

Bone and cartilage mostly consist of extra-cellular matrix (ECM). Collagens are

major functional components of ECM, which perform multiple fundamental functions in human body especially in regulating bone longitudinal growth. Collagens can act as signal transducers and regulate cellular proliferation, migration, ECM adaption and degradation, with a key role in the maintenance of the physiological structure and function of the bone. The structural and functional abnormality of ECM will lead to various clinical phenotypes including fractures, lumbar disc herniation, osteoporosis and severe height related syndrome. Type XI collagen (COL11), a quantitatively minor component of ECM, is important for cartilage collagen fibril formation and ECM organization. COL11 is composed of $\alpha 1$ (XI), $\alpha 2$ (XI) and $\alpha 3$ (XI) chains, which are encoded by genes *COL11A1* (1p21.1), *COL11A2* (6p21.3) and *COL2A1* (12q13.11-q13.2) respectively (Keene et al., 1995). Evidence suggests that *COL11A1* influences human height by interacting with pathways involved in bone development (Li et al., 1995).

Human height is a heritable quantitative trait influenced by complex interplay between genetic variants, environmental and nutrient factors. Although the recent GWASs data enable us to identify hundreds of genetic loci that are associated with height, the identified SNPs to date only explain approximately 10% of the variation to height, indicating more susceptibility loci influencing height (Lango Allen et al., 2010). Moreover, most of the implicated SNPs are common variants with small effect size lying on intergenic and/or intronic regions, emphasizing the need to explore potentially causal elements on exonic or splicing regions that may functionally relate to human height (Perola, 2011). The *COL11A1* gene have been linked with normal

adult height in GWAS studies but the coding or causal variants have not yet been extensively studied, which might be due to small sample size or limited available sequencing data.

In this study, our primary aim is to identify the potentially causal variants in *COL11A1* that affects human height. We thus evaluated the exonic and intragenic variants of *COL11A1* in 8190 Chinese adults by using exome sequencing, and identified three SNPs (chr1_103380393, rs992100, and rs111980222) that are significantly associated with adult height. The most significant association signal of chr1_103380393 was further confirmed in additional 8548 adult subjects. The variant chr1_103380393 is located between the 50th and 51st exons of *COL11A1* gene, suggesting intragenic noncoding polymorphism might execute an important role in regulating gene function, and further supporting the notion that Type XI collagen is critical for human height development. After retrieving the public ChIP-seq data of the chr1_103380393 region from GM12878 cell line, we found that the region is not enriched for chromatin structure marked by H3K4me3, H3K27Ac and DnaseI hypersensitivity sites (DHS) (Fig. S2), indicating that this variation may not be directly involved in active regulation of this gene. The regulatory function of this variation may be through some undetermined mechanism. *COL11A1* encodes one of the two α chains of type XI collagen, a minor fibrillar collagen, which is essential for skeletal morphogenesis (Li et al., 1995). Type XI collagen is a heterotrimer but the third α chain is a post-translationally modified $\alpha 1$ type II chain. Mutations in *COL11A1* are associated with type II Stickler syndrome and Marshall syndrome

(Annunen et al., 1999). Type II Stickler syndrome is usually a dominant disorder due to mutations in the collagen gene *COL11A1*, and is characterized by unusually severe/profound hearing loss and a series of damage in eye, bone and joint, and blood vessels (Snead and Yates, 1999). We proposed that *COL11A1* might be a key regulatory gene affecting the bone growth, and acts as an important factor in human height.

We firstly identified that chr1_103380393 in *COL11A1* was associated with adult height in the two-stage study, but this relationship was not associated with children height. However, we discovered the group (12–15 years) with slightly statistical significance by stratified analysis. There was no evidence showing that the chr1_103380393 altered children height in total from 0 to 15 years old. Due to the shortage of children samples in this experiment, we do not know whether there is a relationship between chr1_103380393 and children height among 15- to 18-year- old group. Further studies need much more samples to identify the association between chr1_103380393 and children height. Despite the association with height for individuals of European and Africa ancestry (Lanktree et al., 2011; Wood et al., 2014), the reported top hits mapped to this region (rs4338381 and rs7517682) did not show strong evidence for association with adult height in our validation samples. The reason for this phenomenon might be due to the limitation of the modest sample size in our validation stage, which probably influences the result of the association test with height. Furthermore, the MAF for both SNPs varies widely across Chinese (CHB), European (CEU) and Africa (RYI) populations in the Hapmap project

(www.hapmap.org). For example, the G allele of rs4338381 is substantially lower in CHB when compared with CEU and RYI populations (10.5%, 38.9% and 40.3% for CHB, CEU and RYI respectively). There might be different peak signal associated with height in different ethnicity, as indicated in current study. We observed weak LD among chr1_103380393, rs992100 and rs111980222, suggesting the newly identified SNP represents a new signal and might not be covered by previous hits. Since the different distributions of height between populations suggest different genetic backgrounds, analysis in different populations would be useful.

In summary, our study confirmed that *COL11A1* is a candidate gene associated with adult height. Further, we firstly identified an intragenic variant chr1_103380393 in *COL11A1* contributing to adult height in Chinese Han population. Our results extend our knowledge of the genetic factors underlying height variation and the biological regulation of human height, which will help to identify the true causative variants and provide a better understanding of the mechanisms underlying human growth and development in the future.

MATERIALS AND METHODS

Study subjects

All the subjects were enrolled for this study from Institute of Dermatology, Anhui Medical University, China. This institute mainly focuses on genetic research of common dermatosis, such as psoriasis, atopic dermatitis, vitiligo, systemic lupus erythematosus and leprosy. Up to date, more than 100,000 persons completed informed consent and accepted the survey and clinical examinations. There is no reported correlation between height and above dermatosis, which allows us to carry out height genetic research using dataset of these diseases. In total, 20,426 individuals were collected in this study. All samples with medical conditions affecting adult height, such as dwarfism, gigantism and acromegaly, were excluded. All the participants provided written informed consent. This study was approved by Ethic Committee of Faculty of Medicine at Anhui Medical University.

Target capture and exome sequencing

Our group performed target sequencing for 1364 genes, including *COL11A1*, in 20005 samples with 64× average coverage for the target regions, and the method for targeting, sequencing, alignment, variant calling and annotation have been described previously (Tang et al. 2014). In brief, the selected target exonic regions were enriched to construct a SeqCapEZ library by Roche NimbleGen. Exome sequencing was performed by HiSeq2000 platform and generated 90-bp paired end reads. Raw sequencing data were aligned to NCBI human genome reference assembly (build 36.3) using BWA (Burrows-Wheeler Aligner) and recalibrated by TableRecalibration in

Genome Analysis Toolkit (GATK v1.6). Genotype information was called by SOAsnp (v1.05) in targeted and flanking 50-bp regions for each sample. The software ANNOVAR was used to categorize SNPs into different groups based on their genic location and expected function on encoded gene products. The SNPs were also classified into “known” and “unknown” categories according to whether they present in dbSNP (version 135). The quality control for SNPs or samples also includes: call rate $\geq 90\%$ for each probes on all samples, samples with individual call rate $\geq 90\%$, and Hardy-Weinberg test P -value $\geq 1.0 \times 10^{-4}$. In order to explore what kind of variants of *COL11A1* contributes to adult height, we extracted the target sequencing data of our psoriasis project, including 8190 adult subjects, and tested whether these variants are associated with height.

SNPs selection and genotyping

The SNPs showing statistical significance after adjusting for multiple comparisons in the discovery stage were selected for further validation. There were 11 SNPs with $MAF > 0.5\%$ and three SNPs (chr1_103380393, rs992100 and rs111980222) reached the statistically significant threshold 4.5×10^{-3} ($0.05/11$). For SNPs located in blocks with strong LD ($r^2 > 0.8$), the one with the most significant signal entered into the validation stage. Genotyping of chr1_103152981 was conducted in additional 8548 adults and 3688 children. We also genotyped the two reported SNPs rs4338381 and rs7517682 (Lanktree et al. 2011; Wood et al. 2014) in the validation stage of 8548 adults. Genotyping for the validation stage was conducted using MALDI-TOF (matrix-assisted laser desorption time of flight) mass spectrometry (Sequenom, USA).

A total of 20ng genomic DNA was used for PCR amplification, and then products were treated by shrimp alkaline phosphatase (SAP) and single base extension reaction. After cleaning with resin, nanoliter volumes of samples were transferred to Spectrochip. Genotype raw data preparation and cluster visualization were performed by Typer 4.1 Analyzer software.

Statistics analysis

The Hardy-Weinberg equilibrium (HWE) was calculated by using Pearson's χ^2 test to determine whether individual variations were in HWE at each locus in the cohort. In the association analysis, linear regression and additive genetic model were applied for genetic association test including age and gender as covariates. All the association tests were done with Plink 1.06. Linear regression coefficients and standard error of mean (s.e.m) were used to measure the strength of the association. To avoid false-positive findings due to multiple comparisons, we applied the stringent Bonferroni correction method that can robustly control the false discovery rate. Association analysis in replication stage was performed and meta-analysis of the two stages was further proceeded by GWAS meta-analysis (GWA-MA) using an inverse-variance weighted meta-analysis on the regression coefficients. The structure of the LD block was examined by Haploview 3.02. $P_{\text{Bonferroni}} < 0.05$ was considered to be significant.

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FIGURE TITLE

Fig. 1. Linkage disequilibrium analysis of variants of *COL11A1* with MAF > 0.5%

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Table 1. The association of adult height with chr1_103380393 genotype in participants used for target sequencing and replication samples.

Study group	Gender	Number of samples	Mean height, in cm (95% c.i.) by genotype			Per-G allele	<i>P</i> value
			AA	AG	GG	effect size (s.e.m.)	
Target sequencing							
	Male	4657	/	170.3 (169.6, 171.0)	171.8 (171.1, 172.5)	−0.268 (0.053)	4.80E-07
	Female	3533	/	160.4 (159.7,161.1)	161.8 (161.1, 162.5)		
Validation in adults							
	Male	4358	169.0 (168.0, 169.9)	169.1 (168.1, 170.1)	171.0 (170.0, 171.9)	−0.286 (0.059)	1.21E-06
	Female	4190	/	161.3 (160.3, 162.3)	162.8 (161.8, 163.8)		
Meta-analysis in two studies		16738				−0.292 (0.053)	3.10E-08
Validation in children							
0–3 yrs	Male	1348	/	73.7 (69.8, 77.6)	74.4 (70.5, 78.2)	0.069 (0.128)	0.590
	Female	618	/	79.9 (74.6, 85.4)	75.8 (70.5, 81.2)		
3–6yrs	Male	338	/	109.8 (103.9, 115.6)	107.4 (101.5, 113.2)	0.093 (0.233)	0.689
	Female	274	/	102.6 (92.8, 112.3)	106.9 (97.2, 116.6)		
6–9yrs	Male	269	/	119.2 (106.9, 131.5)	129.8 (117.5, 142.1)	−0.379 (0.357)	0.288
	Female	158	/	121.5 (102.6, 140.4)	125.1 (106.2, 144.0)		
9–12yrs	Male	238	/	148.9 (143.3, 154.5)	146.1 (140.5, 151.7)	0.198 (0.272)	0.467
	Female	135	/	149.0 (136.9, 161.1)	145.3 (133.2, 157.4)		
12–15yrs	Male	184	145.0 (138.0, 151.9)	156.6 (149.6, 163.6)	162.1 (155.1, 169.1)	−0.651 (0.291)	0.0258
	Female	126	/	154.8(144.2, 156.4)	158.5 (147.9, 169.1)		
Meta-analysis in children cohorts		3688				−0.018 (0.09)	0.843

Target sequencing was performed for samples from our previous psoriasis project. Individuals <20 years of age were excluded in target sequencing and validation stages. Children with atopic dermatitis were <15 years. *P* values are calculated using linear regression under an additive model, corrected for age and sex. Effect size (regression coefficient) and standard error of mean (s.e.m.) are expressed in standard deviation units. Meta-analysis results for adult and child cohorts are shown separately.

Table 2. Genotype distributions and allele frequencies of chr1_103380393, rs4338381 and rs7517682 in target sequencing and validation stages

Study group	SNP	Number of samples	Genotype frequency		Allele frequency			HWE <i>P</i> -value
			A	B	AA	AB	BB	
Exonic target sequencing	chr1_103380393							
Discovery set		8190	370 (2.4%)	15142 (97.6%)	0 (0%)	370 (4.8%)	7386 (95.2%)	0.024
Validation	chr1_103380393							
Adult		8548	293 (1.7%)	16795 (98.3%)	2 (0%)	289 (3.4%)	8253 (96.6%)	1
Children		3688	114 (1.6%)	7250 (98.4%)	1 (0%)	112 (3%)	3569 (96.9%)	0.590
	rs7517682							
Adult		8548	1580 (10.2%)	15502 (89.8%)	76 (0.9%)	1428 (16.7%)	7037 (82.4%)	0.690
Children		3688	697 (10.4%)	6679 (89.6%)	26 (0.7%)	645 (17.5%)	3017 (81.8%)	0.210
	rs4338381							
Adult		8548	448 (2.7%)	16224 (97.3%)	0 (0%)	448 (5.4%)	7888 (94.6%)	0.048
Children		3688	178 (3.2%)	6678 (96.8%)	0 (0%)	178 (5.2%)	3250 (94.8%)	0.170