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Genome-wide Association Study of Anthropometric Traits in Korčula Island, Croatia

Aim To identify genetic variants underlying six anthropometric traits: body height, body weight, body mass index, brachial circumference, waist circumference, and hip circumference, using a genome-wide association study.

Methods The study was carried out in the isolated population of the island of Korčula, Croatia, with 898 adult examinees who participated in the larger DNA-based genetic epidemiological study in 2007. Anthropometric measurements followed standard internationally accepted procedures. Examinees were genotyped using HumanHap 370CNV chip by Illumina, with a genome-wide scan containing 316 730 single nucleotide polymorphisms (SNP).

Results A total of 11 SNPs were associated with the investigated traits at the level of $P < 10^{-5}$, with one SNP (rs7792939 in gene zinc finger protein 498, *ZNF498*) associated with body weight, hip circumference, and brachial circumference ($P = 3.59\text{--}5.73 \times 10^{-6}$), and another one (rs157350 in gene delta-sarcoglycan, *SGCD*) with both brachial and hip circumference ($P = 3.70\text{--}6.08 \times 10^{-6}$). Variants in *CRIM1*, a gene regulating delivery of bone morphogenetic proteins to the cell surface, and *ITGA1*, involved in the regulation of mesenchymal stem cell proliferation and cartilage production, were also associated with brachial circumference ($P = 7.82$ and 9.68×10^{-6} , respectively) and represent interesting functional candidates. Other associations involved those between genes *SEZ6L2* and *MAX* and waist circumference, *XTP6* and brachial circumference, and *AMPA1/GRIA1* and height.

Conclusion Although the study was underpowered for the reported associations to reach formal threshold of genome-wide significance under the assumption of independent multiple testing, the consistency of association between the 2 variants and a set of anthropometric traits makes *CRIM1* and *ITGA1* highly interesting for further replication and functional follow-up. Increased linkage disequilibrium between the used markers in an isolated population makes the formal significance threshold overly stringent, and changed allele frequencies in isolate population may contribute to identifying variants that would not be easily identified in large outbred populations.

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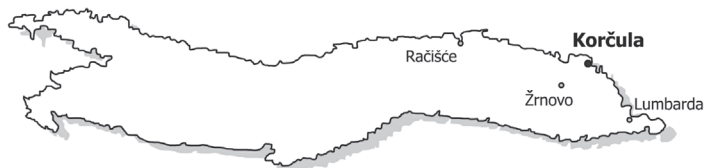
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Interest in anthropometric traits has a long history, ever since the ancient philosophical debates and pioneering attempts to understand human anatomy (1). Modern research on these traits has largely shifted from fundamental morphological anatomy toward the understanding of environmental and genetic factors which determine, affect, and modify these traits. While environmental factors are known to have an important effect on some of these traits, primarily weight and other weight-related traits (2), genetic factors remained elusive to researchers for a long time. Population genetics theory hypothesized, based on the pre-genomic knowledge and comparative studies in plants and animals (3), that majority of these traits will be complex and have highly polygenic background (2,4,5). Additionally, some of these traits have been reported to have very high heritability, with that of height being used as the example of an extremely heritable trait (generally in the range of 0.90 to 0.95) (2). However, the search for the genetic variants underlying height has long been futile, casting doubt on the fundamental assumption that highly heritable traits will be good candidates for gene mapping studies. It is only lately that very large consortia were formed and achieved the needed statistical power to identify the first candidate loci using genome-wide association studies and expanded our understanding of the determinants of human stature (6-11).

Figure 1.



Settlements on the island of Korčula, Croatia, included in the study.

The growing world-wide epidemic of obesity is one of the major issues in modern public health. Search for genes underlying obesity has also been rather unsuccessful, due to an even greater degree of complexity than for height, with large environmental effects and variation over time (12). The search for obesity genes is further complicated by the differences in the central and peripheral type of obesity (13,14). The genetic background of skinfolds has largely been under-investigated (15), with only a few genes implied in their regulation (15,16), some of which do seem to show strong interaction with the environment (17,18). Skinfold measurements may also show variation in time (19), making this research area highly complex and usually considered secondary to the more classical es-

timates of the amount of fat tissue, including body mass index and waist circumference or waist-to-hip ratio.

In this article, we report on a comprehensive genome-wide association study of height, weight, body mass index, and three circumference measurements – brachial, waist, and hip – in the isolated population of the island of Korčula in Croatia. The study is a part of a larger genetic epidemiology research program in Croatian island isolates, “10,001 Dalmatians.” The genetic epidemiology research program in Croatian island isolates began in 1999 (20,21), then expanded to study human genetic variation and effects of isolation and inbreeding (22-29), and finally entered the phase of focusing on diseases and gene mapping studies (8,30-35). By now, the research project has included more than 3000 examinees from isolated populations, and eventually it aims to reach 10 001 examinees.

SUBJECTS AND METHODS

This study was carried out in the adult population of the island of Korčula, Croatia. The field work was performed in 2007 in the eastern parts of the island, targeting healthy volunteers from the town of Korčula and villages Lumbarda, Žrnovo, and Račišće (Figure 1). Participants were invited by mail, posters, radio, and personal contacts. The sampling scheme for this study was convenient sampling, as the study aim was to include approximately 1000 of adult island inhabitants for the purpose of the genome-wide association analysis, regardless on the sample representativeness and demographic structure.

All examinees were aged 18 and over and had signed informed consent before entering the study, which was approved by the Ethical Committee of the Medical School, University of Zagreb. A total of 944 examinees were included in the study between March and December 2007. In all examinees, a large number of quantitative phenotypic traits were measured, including height, weight (and derived body mass index), and brachial, waist, and hip circumference. For these anthropometric measurements, standard methods were used (36).

DNA extraction was performed using Nucleon kits (Tepnel, Manchester, UK) and a total of 944 samples were genotyped in Institute of Human Genetics, Helmholtz Zentrum München, Germany. Genotyping was performed using Illumina HumanHap 370CNV (Illumina, San Diego, CA, USA), with a total of 346 027 single nucleotide polymorphism (SNP) markers. Quality control of the genotype

data, excluding markers with a call rate <98%, with minor allele frequency <2%, or out of Hardy-Weinberg equilibrium ($P < 10^{-10}$), left 316 730 SNPs in the analysis. Exclusion of individuals carrying markers with a call rate of <98% and those sampled twice in the study, left 898 people for inclusion in the analysis.

Relatedness between examinees was estimated from their whole genome data using the sharing of genome identical by descent (IBD) in PLINK (37). This method is robust to pedigree information errors, undeclared relationships, and samples swap, and gives realized sharing rather than expected one given by the pedigree information (for the same pedigree-based relationship, sharing varies within a range due to segregation and recombination stochastic events). Using this function, the sample could be grouped into 154 parent-child pairs, 107 sibling pairs, avuncular or equivalent 137, first cousins or equivalent 363, first cousins once removed or equivalent 1861, second cousins or equivalent 8962. The mean IBD sharing between all possible pairs of individuals was 0.003 (min = 0, max = 0.61).

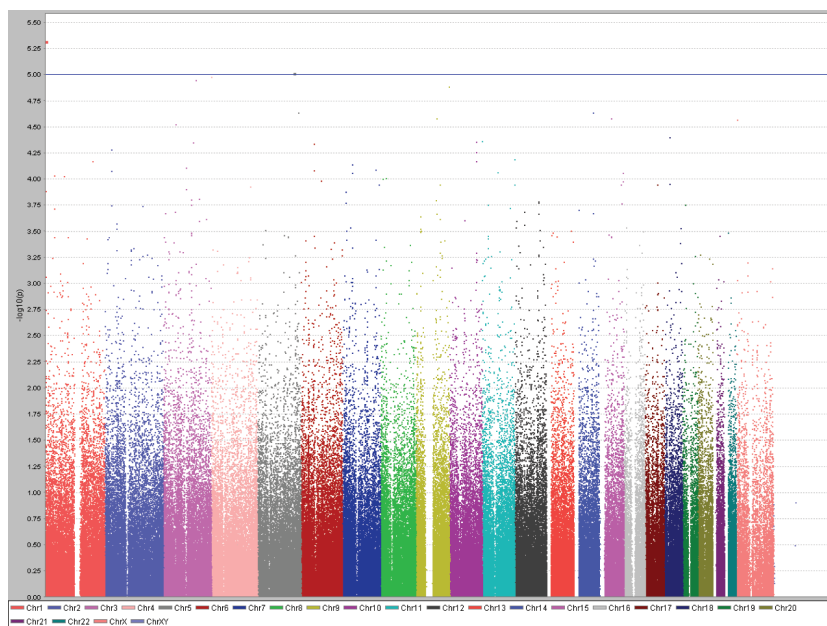
Genome-wide associations between anthropometric phenotypes (adjusted for age and sex) and SNP markers were analyzed using the “mmscore” function of the GenABEL R sta-

TABLE 1. Descriptive statistics (mean ± standard deviation, SD) of the investigated traits of the examinees (n = 898) from the Korčula island

Trait	Mean ± SD	Range
Height (mm)	1680.9 ± 90.8	1410.0-1970.0
Weight (kg)	79.1 ± 14.2	49.5-166.6
Body mass index (kg/m ²)	27.9 ± 4.1	16.6-53.8
Brachial circumference (mm)	333.4 ± 45.0	200.0-944.0
Hip circumference (mm)	1040.6 ± 78.6	748.0-1563.0
Waist circumference (mm)	941.4 ± 121.5	620.0-1385.0

tistical package (38), using an additive model. This score test for family-based association accounts for pedigree structure and allows unbiased estimations of SNP allelic effect (39). No correction for multiple testing was applied. The relationship matrix used in this analysis was generated by the identity-by-state (ibs) function of GenABEL, which used ibs genotype sharing to determine the realized pair-wise kinship coefficient similarly to the PLINK genome function. Minor allele frequencies were reported for the identified SNPs. Weighted genomic inbreeding coefficient was used in the inbreeding estimation (23). All identified SNPs that reached significance at the level of $P < 10^{-5}$ were visualized using HaploView software (MIT/Harvard Broad Institute, MA, USA).

Figure 2.



Genome-wide association study of height using Haploview software, showing peaks on chromosomes 1 and 5 reaching genome-wide significance level of $P < 10^{-5}$.

TABLE 2. A summary of single-nucleotide polymorphisms (SNP) that showed association with anthropometric traits that reached levels of genome-wide significance of $P < 10^{-5}$ *

Trait	SNP	Chromosome	Position	<i>P</i> value ($\times 10^{-6}$)	Minor allele frequencies	β	Standard error (β)	Position	Gene
Height	rs7513590	1	5070572	4.81	0.104	-0.316	0.069	no gene	no genes +/- 100kb
Height	rs12658202	5	152959747	9.77	0.453	0.110	0.025	intronic	glutamate receptor, ionotropic, AMPA 1 (<i>GRIA1</i>)
Weight	rs7792939	7	99045812	3.59	0.148	0.255	0.055	7kb 5'	zinc finger protein 498 (<i>ZNF498</i>)
Weight	rs7157940	14	93632946	3.13	0.458	0.118	0.025	5' UTR	hypothetical protein <i>LOC122509 (FAM14B)</i>
Body mass index	rs7590983	2	174070595	5.76	0.045	0.462	0.102	no gene	no genes +/- 100kb
Brachial circumference	rs1863080	2	36379940	7.82	0.102	0.307	0.069	56kb 5'	cysteine-rich motor neuron 1 (<i>CRIM1</i>)
Brachial circumference	rs7723398	5	52264862	9.68	0.222	0.191	0.043	intronic	integrin alpha 1 precursor (<i>ITGA1</i>)
Brachial circumference	rs32056	5	156006717	9.07	0.093	-0.325	0.073	intronic	delta-sarcoglycan (<i>SGCD</i>)
Brachial circumference	rs157350	5	156072147	3.70	0.105	-0.311	0.067	intronic	delta-sarcoglycan (<i>SGCD</i>)
Brachial circumference	rs7792939	7	99045812	4.90	0.148	0.255	0.056	7kb 5'	zinc finger protein 498 (<i>ZNF498</i>)
Brachial circumference	rs201789	13	49884119	6.20	0.279	0.170	0.038	intronic	<i>XTP6</i>
Hip circumference	rs157350	5	156072147	6.08	0.105	-0.306	0.068	intronic	delta-sarcoglycan (<i>SGCD</i>)
Hip circumference	rs7792939	7	99045812	5.73	0.148	0.256	0.056	7kb 5'	zinc finger protein 498 (<i>ZNF498</i>)
Waist circumference	rs7158173	14	64715003	3.93	0.471	-0.114	0.025	76kb 5'	<i>MAX</i>
Waist circumference	rs4787483	16	29792948	2.10	0.343	0.164	0.035	intronic	Seizure-related 6 homologue (mouse)-like 2 isoform (<i>SEZ6L2</i>)

*The table summarizes SNPs, their positions on the chromosomes, *P* values, minor allele frequencies, effect size and direction (expressed as β and standard error of β), effect allele, and implicated gene.

RESULTS

Genetic variants associated with height, weight, and body mass index

A total of 898 examinees were genotyped and included in the analysis. Descriptive statistics for the investigated traits are presented in Table 1. The average inbreeding coefficient was 0.013 ± 0.025 . Initial genome-wide association study revealed two SNPs associated with body height at the level of $P < 10^{-5}$ on chromosomes 1 and 5. The variant at chromosome 1 ($P = 4.81 \times 10^{-6}$) did not have a gene in proximity (± 100 kilobases). The variant on chromosome 5 ($P = 9.77 \times 10^{-6}$) implicated a gene ionotropic glutamate receptor *AMPA1 (GRIA1)* (Table 2, Figure 2).

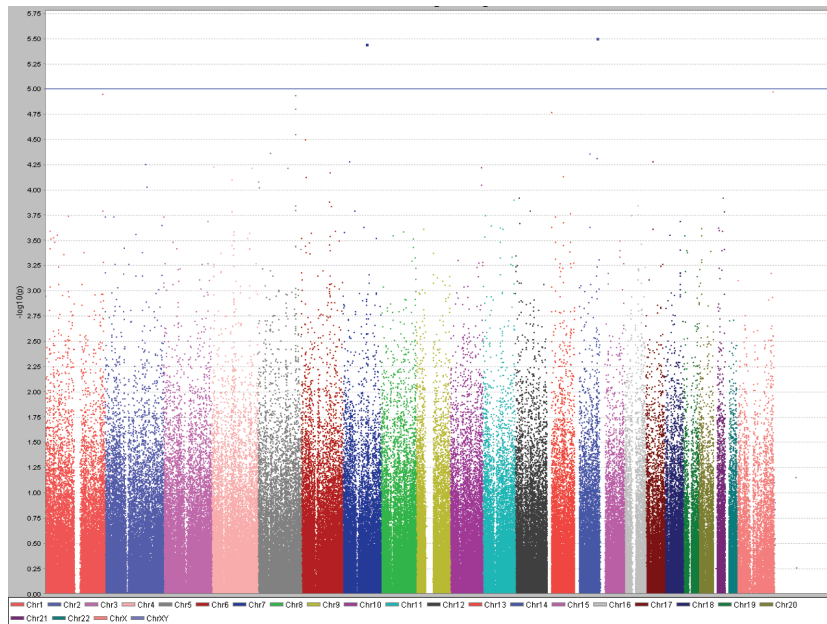
Two further SNPs were associated with weight, one belonging to hypothetical protein *LOC122509 (FAM14B)* gene on chromosome 14, while the other was within zinc finger protein 498 gene (*ZNF498*) on chromosome 7 (Table 2, Figure 3). The *P* value for the former variant was 3.13×10^{-6} and 3.59×10^{-6} for the latter.

Body mass index was associated with a single SNP on the chromosome 2 that was not associated with any gene (Table 2, Figure 4), with a *P* value of 5.76×10^{-6} .

Genetic variants associated with brachial, hip, and waist circumference

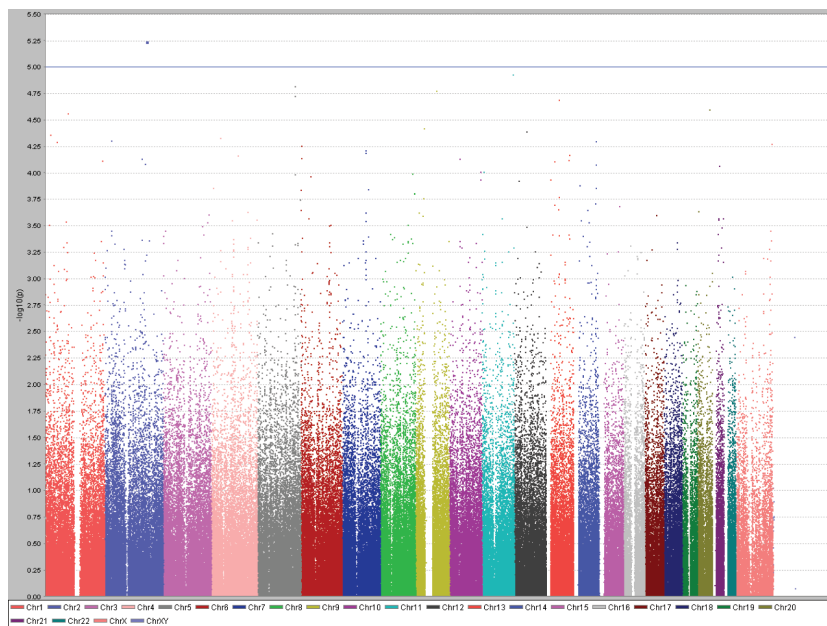
Brachial circumference showed association with 6 SNPs at chromosomes 2, 5, 7, and 13. Two of the SNPs (rs157350 and

Figure 3.



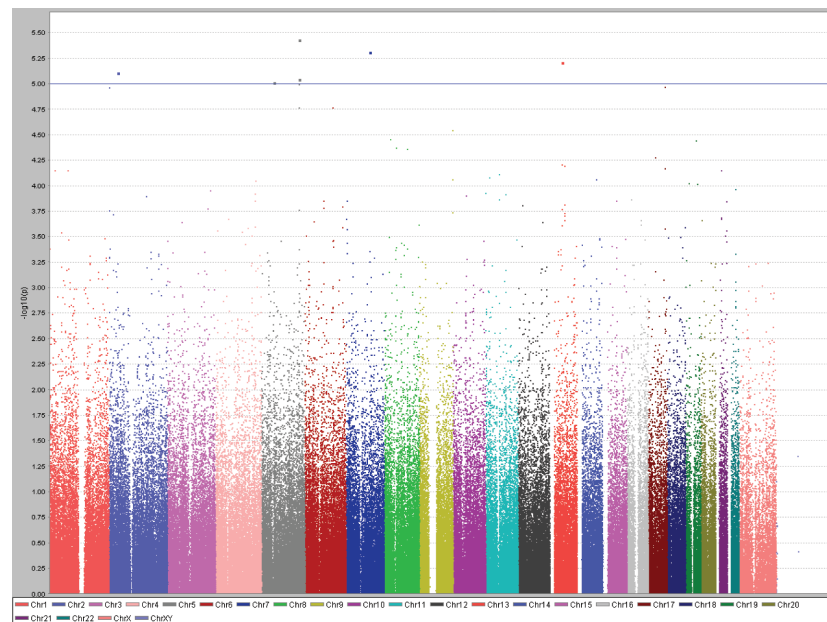
Genome-wide association study of weight using Haploview software, showing peaks on chromosomes 7 and 14 reaching genome-wide significance level of $P < 10^{-5}$.

Figure 4.



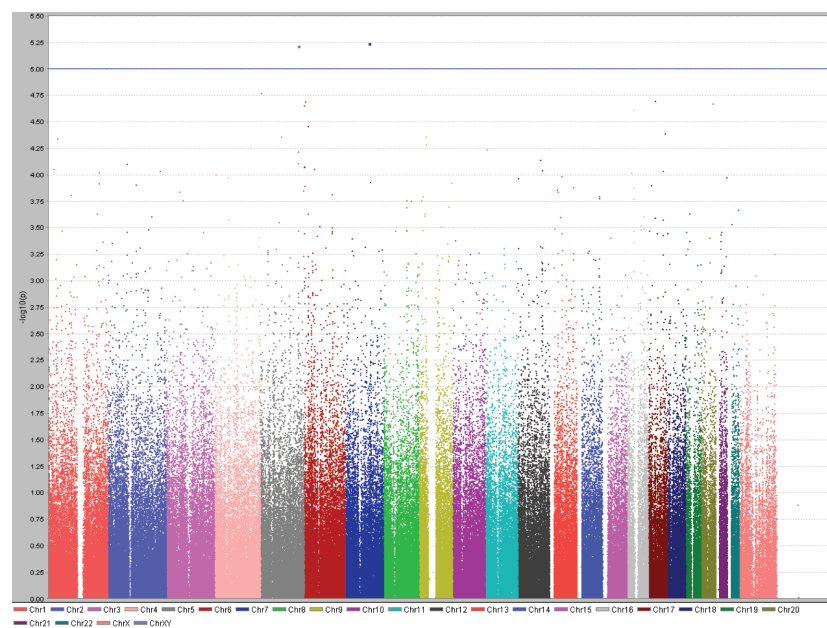
Genome-wide association study of body mass index using Haploview software, showing a peak on chromosome 2 reaching genome-wide significance level of $P < 10^{-5}$.

Figure 5.



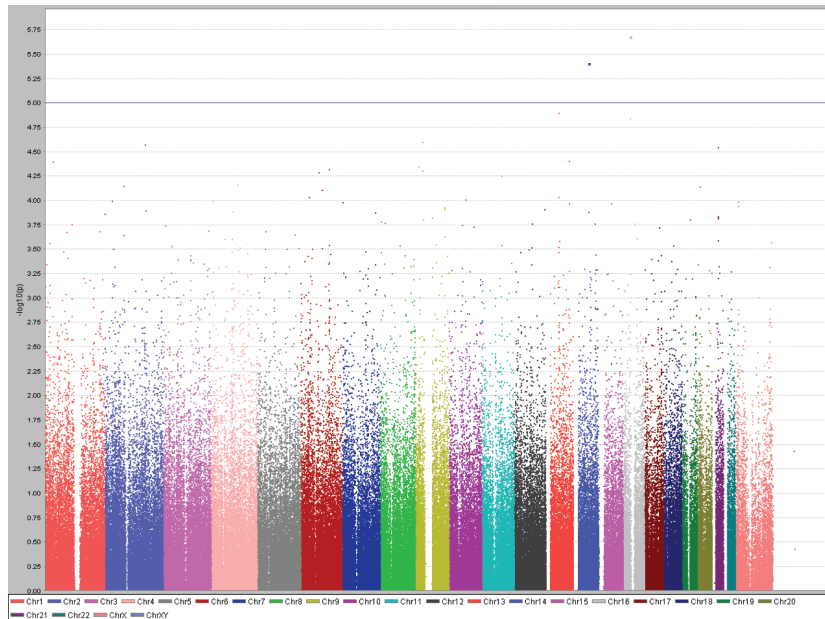
Genome-wide association study of brachial circumference using Haploview software, showing peaks on chromosomes 2, 5, 7, and 13 reaching genome-wide significance level of $P < 10^{-5}$.

Figure 6.



Genome-wide association study of hip circumference using Haploview software, showing peaks on chromosomes 5 and 7 reaching genome-wide significance level of $P < 10^{-5}$.

Figure 7.



Genome-wide association study of waist circumference using Haploview software, showing peaks on chromosomes 14 and 16 reaching genome-wide significance level of $P < 10^{-5}$.

rs7792939) implicated delta-sarcoglycan gene (*SGCD*). The SNP with the stronger effect (rs157350) of $P = 3.70 \times 10^{-6}$ was also associated with the hip circumference ($P = 6.08 \times 10^{-6}$). Both brachial and hip circumference showed associations with the SNP rs7792939 in zinc finger protein 498 (*ZNF498*) on chromosome 7 (Table 2, Figure 5 and 6), with significance of $P = 4.90 \times 10^{-6}$ and $P = 5.73 \times 10^{-6}$, respectively. The same SNP showed strong association with body weight. Other variants associated with brachial circumference included rs1863080 in cysteine-rich motor neuron 1 (*CRIM1*) gene on chromosome 2 ($P = 7.82 \times 10^{-6}$), rs7723398 in integrin alpha 1 precursor (*ITGA1*) gene on chromosome 5 ($P = 9.68 \times 10^{-6}$), and rs201789 in *XTP6* gene on chromosome 13 ($P = 6.20 \times 10^{-6}$).

Finally, waist circumference was associated with two SNPs, one on chromosome 14 (rs7158173) belonging to the *MAX* gene ($P = 3.83 \times 10^{-6}$) and the other on chromosome 16 (rs4787483) belonging to seizure-related 6 homologue (mouse)-like 2 isoform gene (*SEZ6L2*; $P = 2.10 \times 10^{-6}$) (Table 2, Figure 7).

DISCUSSION

The results of this study suggest several potential candidate genes involved in determining height, weight, and

body mass index, as well as the hip, waist, and brachial circumference. We identified novel genetic associations with 6 human anthropometric traits using an isolate population. In isolated populations, genome-wide association studies can be particularly useful because of several potential advantages. First, allele frequencies of some important variants may be skewed from those found in the general population due to a combination of population genetic effects such as founder effect, genetic drift, and inbreeding (2). This could dramatically increase the power of genome-wide association study to detect otherwise rare variants of reasonably large effects on phenotypes of interest, if their frequency is unusually increased in an isolate population, a suggestion that has been proposed for some isolated Croatian Adriatic islands (22). In addition, accepted methods of computation of formal threshold for genome-wide statistical significance that corrects for multiple testing performed for hundreds of thousands of genetic markers may be overly stringent in isolate populations because of their population genetic properties (22). Increased linkage disequilibrium in such populations makes many of the markers linked so that many of the performed tests are dependent on each other (linkage disequilibrium works against the assumption of the independence between all performed tests) (31), which in turn mathemati-

cally lowers the threshold for genome-wide significance for an unknown but substantial amount. Lowering the significance threshold means greater power for the studies performed in isolate populations. Due to the two mechanisms described above (2,22), it would not be unexpected that genetic variants underlying complex traits are identified in human isolate populations rather than in outbred population by using genome-wide scans of comparable density and much larger sample sizes (5,8).

In recent years, genome-wide association studies have proven their worth as an extremely powerful tool in identifying genetic variants underlying many human diseases and complex traits (40). Hundreds of genetic variants are presently being discovered by large consortia of scientists in pioneering efforts to use this reliable approach to provide hypothesis-free insights into the genetic architecture of complex human phenotypes (36). Our study succeeded in identifying 11 genetic loci that reached significance level of $P < 10^{-5}$. Although this does not make all of them necessarily genome-wide significant in formal turns, we believe that they should be carefully considered for replication in other populations and further functional studies due to combination of factors described above.

The gene *AMPA1* (*GRIA1*) implicated in height in our study has also been implicated in stimulus-reward learning in mice (41,42) and possibly in bipolar disorder and schizophrenia in humans (43,44), but possible mechanisms remain obscure, and the finding has yet to be replicated. It has not been associated with human height to date. However, human height has been strongly inversely associated with the risk of schizophrenia (45), making this finding potentially interesting for further replication and functional follow-up, as *GRIA1* gene could be a potential common factor contributing to both phenotypes.

It has recently been revealed that *SCGD* codes aminomutase catalyzing α -tyrosine to beta-tyrosine (46). The gene has been hypothesized to have a role in cardiomyopathy and muscular dystrophy (47). Furthermore, we have not been able to find information on the possible role and function of *ZNF498* gene. Thus, both genes represent good candidates for further functional studies to clarify their possible role in determining body shape and size.

CRIM1, implicated in brachial circumference, is an interesting candidate gene. It encodes a putative transmembrane protein with multiple cysteine-rich (CR) domains known to have bone morphogenetic proteins bind-

ing activity, and is usually associated with vertebrate central nervous system development and organogenesis (48). It has been suggested that it regulates the rate of processing and delivery of bone morphogenetic proteins to the cell surface (49). Interestingly, several reports linked it to control of body size (50,51), thus making it a promising candidate for further follow-up studies.

ITGA1, another gene implicated in brachial circumference, is an equally interesting candidate gene. It is a part of integrin collagen receptor locus on human chromosome 5q11.2 (52). Thus, it is involved in the early remodelling of osteoarthritic cartilage and plays an essential role in the regulation of mesenchymal stem cell proliferation and cartilage production (53). Further replication of our finding and follow-up functional studies will be necessary to confirm its regulation of brachial circumference and interactions with other genes.

Unlike the previous candidate genes, no plausible information is currently available on possible roles and functions of *XTP6*, *SEZ6L2*, and *MAX* genes that would help us understand their possible role in determining human body size and shape.

The results presented here suggest that some identified genes were implicated in more than one trait, suggesting that they might be responsible for the genetic action on the common underlying property for these traits. Other genes, such as *CRIA1* and *ITGA1*, represent very promising and biologically plausible functional candidates.

The shortcomings of this study primarily include the potential low statistical power, which is a consequence of the limited population size encountered in any genetic isolate. Studies performed in isolated populations should always seek replication to ensure that the findings are indeed representative of wider general human populations and not limited to specific circumstances of a particular isolate. Finally, variants identified in this study all require functional follow-up and replication in other populations in order to establish their true significance in determination of human body size and shape.

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reviewed according to best practice guidelines of international editorial organizations.

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