Muscle Strength Is Associated With Vitamin D Receptor Gene Variants

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ABSTRACT: Vitamin D receptor (VDR) is an important candidate gene in muscle function. Scientific reports on the effect of its genetic variants on muscle strength are contradictory likely due to the inconsistent study designs. Hand grip strength (HGS) is a highly heritable phenotype of muscle strength but only limited studies are available on its genetic background. Association between VDR polymorphisms and HGS has been poorly investigated and previous reports are conflicting. We studied the effect of VDR gene variants on HGS in a sample of 706 schoolchildren. Genomic DNA was extracted from saliva samples and six candidate single nucleotide polymorphisms (SNPs) across the VDR gene were genotyped with Sequenom MassARRAY technique. HGS was measured with a digital dynamometer in both hands. Single marker and haplotype associations were adjusted for demographic parameters. Three SNPs, rs4516035 (*A1012G*; $p = 0.009$), rs1544410 (*BsmI*; $p = 0.010$), and rs731236 (*TaqI*; $p = 0.038$) and a 3′ UTR haploblock constructed by three SNPs (*Bsml-Taq1*-rs10783215; $p < 0.005$) showed significantly associations with HGS of the dominant hand. In the non-dominant hand, the effects of the *A1012G* ($p = 0.034$) and the 3′ UTR haploblock ($p < 0.01$) on HGS were also significant. Since the promoter SNP (*A10112G)* and the 3′ UTR haplotype were proved to be associated with the expression and the stability of the VDR mRNA in earlier studies, VDR variants can be supposed to have a direct effect on muscle strength. The individual genetic patterns can also explain the inconsistency of the previously published clinical results on the association between vitamin D and muscle function. © 2016 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:2031–2037, 2016.

Keywords: vitamin D receptor gene; hand grip strength; haplotype; genetic association; polymorphism

Vitamin D receptor (VDR) is a widely studied candidate gene for several musculoskeletal phenotypes. Polymorphisms of VDR have been showed to be associated with bone mineral density and fracture risk several times.[1–3] Osteoporotic fractures are related to the frequency of falls what was also described in association with VDR genotypes.[4,5] Falls are indirectly influenced by VDR polymorphisms via their effect on muscle function.[4] Therefore, VDR candidate polymorphisms have been also studied in association with different muscle strength-related phenotypes. However, results of these investigations are considerably inconsistent and hardly comparable because of the multiple types of muscle strength measurements and the stratified study populations.[4,6–11]

Hand grip strength (HGS) is an important muscle strength phenotype which can be accurately measured using simple, digitalized, cheap, and reliable methods both in child- and adulthood.[12] HGS is a typical multifactorial trait influenced by genetic and environmental factors. According to the concerning data, the heritability of HGS is accounted for at least 50–60%.[13,14] However, only few genes and gene variants have been reported in association with HGS so far and results have been explored mainly in adult and/or selected populations. Previously, an association with HGS and a polymorphism in uncoupling protein 3 gene (UCP3) had been reported in elderly.[15,16] A significant effect of angiotensin-converting enzyme (ACE) gene variants on HGS was revealed by Chiu et al.[17] in adolescent girls and by Yoshihara et al.[18] in elderly subjects. The combination of genomic variants in ACE, ACTN3 (α-actinin-3), and PPARA (peroxisome proliferator-activated receptor α) genes showed an association with HGS in schoolchildren in the recent study of Ahmetov et al.[19]

Contradictory results on the association of VDR variants and HGS are available in the literature. A positive association with HGS and VDR *BsmI* polymorphism was reported by Geusens et al.[7] in non-obese elderly Belgian women and the same associations with a trend were described in two other studies.[4,11] Another study failed to find any relationship between *BsmI* and HGS in Swedish women.[8] The *ApaI* polymorphism has been found to be associated with low HGS in elderly Chinese people in a recent study of Wu et al.[20] but Iki et al.[10] did not confirm these findings in Japanese women. The *TaqI* variant was found not to be related to HGS in the study of Iki et al.,[10] however, a positive association was demonstrated by Windelinckx et al.[11] These incongruent reports about the effect of VDR polymorphisms on HGS can be explained by the relative underpowered studies performed mainly on different subgroups of patients and the partial consideration of the confounding factors. Moreover, HGS was studied as one of the phenotypes or as an additional measurement in these studies.

The aim of the present study was to determine the effect of the VDR gene variants on HGS, a well-defined muscle strength phenotype. To avoid the biasing

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factors above, influence of VDR polymorphisms was analyzed on a large sample of young schoolchildren and the study was focused on HGS of both hands using an accurate and reliable phenotype measurement method.

MATERIALS AND METHODS

Subjects and Phenotypic Measurements

A total number of 706 children (360 male and 346 female pupils) were recruited from seven primary schools around the country (Table 1). The participating schools were selected from three different geographic regions of the country representing a general population sample. The research protocol was designed and implemented with regard to the Helsinki Declaration on human subjects testing and the study was approved by the Scientific and Research Ethics Committee of the Medical Research Council (431/PI/2007). Pupils from the 2nd to 5th grades were included into the study after their parents' informed consent. All the children were Caucasian. All subjects were healthy volunteers without any known disabling musculoskeletal or other chronic disease or functional limitation. Children with acute condition influencing the hand grip strength (e.g., hand trauma) were also excluded. Body height and weight were measured with calibrated tools. Maximum HGS in the dominant and non-dominant hands was determined using the recommended digital Jamar Plus+ hand dynamometer (Patterson Medical, Bolingbrook, IL).[21,22] The handle was adjusted so that the child's proximal interphalangeal joints lie exactly on the top of the adjustable handle. The measurement was made in sitting position with arm close to the body. The arm was flected to 90°, the forearm was in neutral position. The measurements were taken three times of the dominant hand and non-dominant hand as well. One minute resting time was applied between the measurements. The highest score of each hand was used in the further analyses. Reliability of the hand grip strength measurement was studied in a subsample of 105 pupils (Supplementary Table S1).

Genotyping

Saliva samples were collected from 674 children using the Oragene OG-500 kit (DNA Genotek Inc., Ontario, Canada). Genomic DNA was purified from the samples following the protocol of the manufacturer. DNA quantity and quality were checked using PicoGreen reagent (Molecular Probes, Inc., Eugene, OR). Six candidate SNPs (*A1012G*, *FokI*, *Ddel*, *BsmI*, *TaqI*, and rs10783215) across the VDR gene were selected for genotyping based on the previous literature reports (Table 2). Genotyping was performed by the Technology Centre, Institute for Molecular Medicine Finland (FIMM), University of Helsinki using a Sequenom MassArray technology and the iPLEX Gold reagents (Sequenom Inc., San Diego, CA). Allele discrimination is based on primer extension with single mass-modified nucleotides followed by MALDI-TOF mass spectrometry. Genotyping quality was examined by a detailed QC procedure consisting of success rate checks, duplicated samples, and positive and negative control samples. Genotyping was done blinded to the phenotypic data.

Table 1. Study Cohort ($n = 706$)

Variable	Mean (SD)
Age (y)	9.8 (1.2)
Gender (boy/girl %)	51%/49%
Weight (kg)	36.0 (9.1)
Height (cm)	141.2 (9.1)
BMI (kg/m^2)	17.8 (3.0)
Dominant hand (right/left %)	91%/9%
Hand grip strength dominant (N)	147.5 (42.1)
Hand grip strength non-dominant (N)	137.3 (38.3)

Statistical Analysis

Association between demographic variables and hand grip strength of dominant and non-dominant hands were analyzed in Pearson's correlation and backward linear regression models. In correlation analyses $r > 0.40$ was considered satisfactory ($r > 0.80$ as excellent, 0.61–0.80 very good, 0.41–0.60 good, 0.21–0.40 fair, 0–0.20 poor). Descriptive statistics (allelic and genotype distribution, Hardy–Weinberg equilibrium using the standard χ^2 goodness-of-fit test, minimal allele frequency (MAF)) and pairwise linkage disequilibrium (LD) between the SNPs were calculated in Haploview 4.2 software.[23] Haploblocks were determined by the D′-based default built-in algorithm originally published by Gabriel et al.[24] Associations between hand grip strength and genetic variants were analyzed using the "SNPassoc" R software package[25] and the THESIAS software.[26] Individual genotype–phenotype associations were studied in the codominant genetic model considering the distribution of HGS among the genotypes and the fact that this model is the most powerful one to detect associations in case of an unknown inheritance model.[27] In the applied generalized linear models (GLM), genetic information was coded according to the three genotypes of each SNP (e.g., AA, AG, GG groups). Haplotype frequencies were determined based on the stochastic version of the Expectation-Maximization (SEM) algorithm and the haplotype–phenotype associations were investigated applying log-likehood ratio tests (LRT) in THESIAS. Rare haplotypes less frequent than 1% were excluded from the analyses. All genetic analyses were adjusted for age, sex, weight, and height. p-Value less than 0.05 was considered significant. Power of the study calculation was performed in Quanto 1.2.4 software.[28]

RESULTS

Non-Genetic Determinants of Hand Grip Strength

Mean hand grip strength was $147.5 + 42.1$ Newton (N) and $137.3 + 38.3$ N at dominant and non-dominant hands in our cohort and the correlation between the strength of the two hands was excellent ($r = 0.83$, $p < 0.01$). HGS in both dominant and non-dominant hands were distributed normally in the sample population ($p > 0.05$ in Kolmogorov–Smirnov test). Age, weight, height, and gender correlated with both hands' grip strength (Fig. 1) and all four demographic parameters proved to be significant predictors in multivariate regression models (data not shown). The measurement method proved to be reliable characterized by a high ICC for both hands (0.98 and 0.95 for dominant and for non-dominant hands, respectively) (Supplementary Table S1).

Table 2. Studied VDR SNPs and Descriptive Statistics of Genotyping

No	rs Number	Traditional Name	Alleles	Region	Success Rate (%)	MAF	HWp
1	rs4516035	*A1012G*	C/T	Promoter	99.0	0.429	1.0
2	rs2228570	*FokI*	C/T	Exon 2	98.9	0.391	0.563
3	rs3782905	*DdeI*	C/G	Intron 2	98.3	0.295	0.402
4	rs1544410	*BsmI*	A/G	Intron 8	99.0	0.374	0.404
5	rs731236	*TaqI*	C/T	Exon 9	99.0	0.372	0.721
6	rs10783215	-	C/T	3′ UTR	99.5	0.474	0.419

MAF, minimal allele frequency; HWp, *p*-value of Hardy–Weinberg equilibrium.

Genetic Associations

Genotyping success rate was more than 98.5% for all SNPs. DNA quality was not enough good for further genotyping in six cases. The genetic association tests were conducted only on subjects who were successfully genotyped for all polymorphisms ($n = 643$). Minor allele frequencies of each SNP were more than 1% in the study population and all genotyped polymorphisms

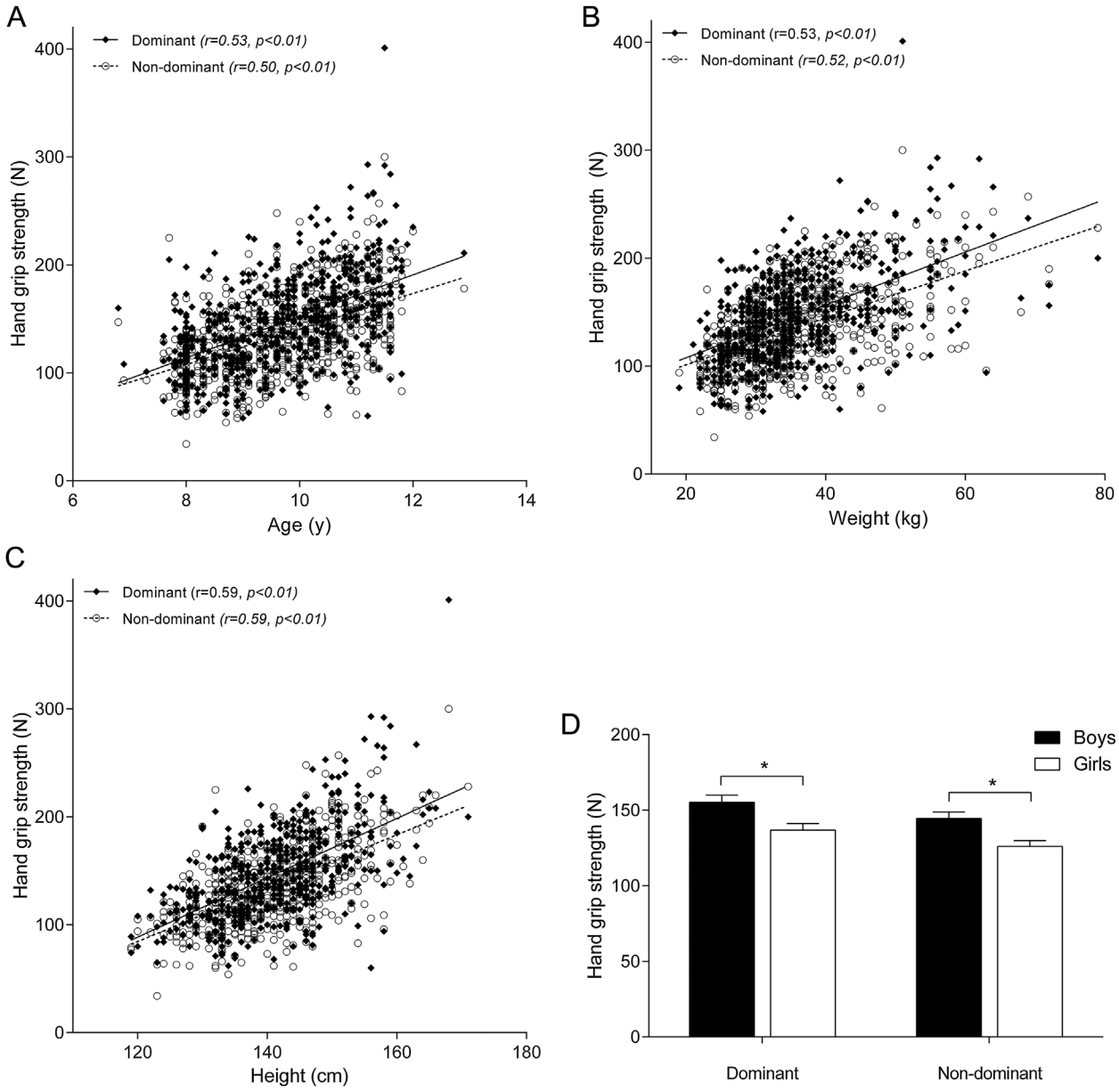

Figure 1. Association of demographic parameters with hand grip strength (A: age, B: weight, C: height, D: gender – mean, 95%CI, $^{*}p < 0.05$).

were in Hardy–Weinberg equilibrium (Table 2). A haploblock constructed by three SNPs in the 3′ part of the gene (rs1544410-rs731236-rs10783215) was identified (Supplementary Fig. S1).

Association analyses in codominant genetic models were adjusted for the non-genetic predictors as age, gender, weight, and height. Significant associations were found between three individual SNPs' and HGS (Table 3). The "TT" genotype of rs4516035 (A1012G) polymorphism was associated with the highest while the "CC" genotype was related to the lowest HGS in both hands ($p = 0.009$ and $p = 0.034$ for dominant and non-dominant hands, respectively). High dominant hand grip strength was found in children carrying the "AA" genotype of rs1544410 (*BsmI*, $p = 0.010$)) and in "CC" genotype carriers of rs731236 (*TaqI*, $p = 0.038$).

Three haplotypes with more than 1% frequency were identified inside the VDR haploblock located in the 3′ part of the gene. VDR haplotypes showed significant associations with the hand grip strength at both hands ($p < 0.005$ and $p < 0.01$ for dominant and non-dominant hands, respectively) (Fig. 2, Supplementary Table S2). The mean difference in hand grip strength between "ACT"' and "GTT" haplotypes was 12.1 N and 10.0 N in case of the dominant and the non-dominant hands, respectively.

Considering the mean of HGS in the dominant hand, our sample size provided 80% power to detect a 10 N difference in a covariate adjusted additive genetic model in case of 0.373 MAF.

DISCUSSION

Genetic associations with muscle strength-related phenotypes have been already reported for more than 40 candidate genes. This paper is the first report on the significant association between VDR gene variants and HGS—a well-defined muscle strength phenotype—in a general schoolchildren population. In single marker association tests "TT" genotype of rs4516035 (*A1012G*), "AA" genotype of rs1544410

Table 3. Hand Grip Strength Relation to VDR Genotypes ($N = 643$)

		Hand grip strength (N)							
		Dominant				Non-dominant			
SNP Genotypes	Frequency (n, %)	Mean	SD	95%CI	p	Mean	SD	95%CI	p
rs4516035									
CC	119 (18.5)	144.8	43.2	137.0–152.7		135.6	40.7	128.2–143.0	
CT	324 (50.3)	145.1	42.6	140.5–149.8	**0.009**	134.0	36.9	130.0–138.0	**0.034**
TT	200 (31.1)	148.9	40.8	143.2–154.6		137.6	38.3	132.2–142.9	
–									
rs2228570									
CC	241 (37.5)	146.2	43.8	140.7–151.8		134.6	40.1	129.5–139.7	
CT	300 (46.6)	145.2	42.2	140.4–150.0	0.534	135.7	37.7	131.4–140.0	0.520
TT	102 (15.8)	149.3	37.9	141.9–156.8		136.6	34.1	129.9–143.3	
–									
rs3782905									
CC	315 (48.9)	143.5	41.0	138.9–148.0		132.0	36.7	127.9–136.1	
CG	279 (43.3)	148.0	40.3	143.3–152.8	0.278	138.5	37.5	134.1–142.9	0.248
GG	49 (7.6)	154.0	56.4	137.8–170.2		140.1	47.5	126.4–153.7	
–									
rs1544410									
AA	87 (13.5)	151.7	41.7	142.8–160.6		138.0	38.7	129.8–146.3	
AG	310 (48.2)	148.2	44.7	143.2–153.1	**0.010**	136.5	38.7	132.2–140.9	0.169
GG	246 (38.2)	141.9	38.6	137.1–146.8		133.1	36.9	128.5–137.7	
–									
rs731236									
CC	87 (13.5)	151.9	41.9	143.0–160.9		138.5	39.3	130.1–146.8	
CT	304 (47.2)	147.9	44.2	142.9–152.9	**0.038**	136.4	38.4	132.1–140.8	0.116
TT	252 (39.1)	142.3	39.4	137.4–147.1		133.1	37.1	128.5–137.7	
–									
rs10783215									
CC	175 (27.2)	143.5	39.8	137.6–149.5		135.2	37.7	129.6–140.9	
CT	335 (52.1)	147.1	42.9	142.4–151.7	0.372	135.6	38.5	131.5–139.8	0.654
TT	133 (20.6)	147.8	43.2	140.4–155.2		135.1	37.6	128.7–141.6	

Mean, standard deviation (SD) and 95% confidence intervals (CI) of raw HGS corresponding to the different genotypes as well as the p-value of the associations in the covariate-adjusted codominant models (GLM) are represented. Bold indicates significant associations.

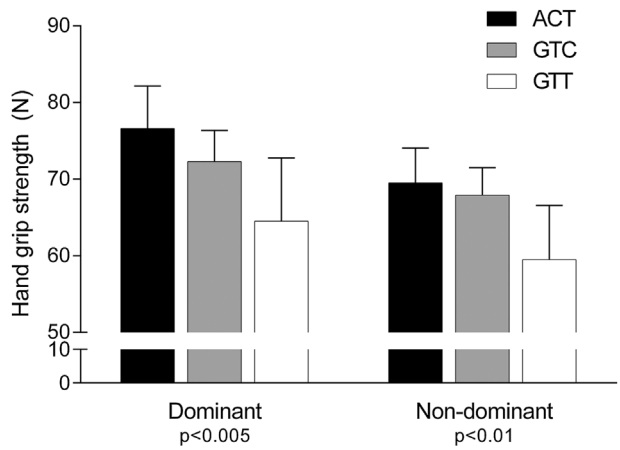

Figure 2. Estimated hand grip strength according to VDR haplotypes (mean, 95%CI and *p*-values of the global haplotype effect on HGS are represented).

(*BsmI*) and "CC" genotype of rs731236 (*TaqI*) were associated with stronger hand grip in the dominant and "TT" genotype of rs4516035 in the non-dominant hand in codominant genetic models. Consistently, the VDR haplotype constructed by the three SNPs (*Bsml*, *TaqI*, and rs10783215) located in the 3′ part of the gene showed a strong association with HGS in both hands. We found that the rarest "GTT" haplotype (traditionally named "*bTT*") was associated with the weakest HGS while a more common "ACT" (*BtT*) haplotype was related to the strongest HGS. VDR gene polymorphisms in relation to muscle function are less investigated and there is considerably few data about the correlation between the polymorphisms of VDR and muscle strength in a healthy young population. Only some studies in the literature analyzed the associations between the VDR polymorphisms and HGS—an important hereditary muscle phenotype—and, to our knowledge, association between the VDR genotypes and HGS in both (dominant and non-dominant) hands has not been published yet.

The *BsmI* polymorphism is located in the eighth intron in strong linkage disequilibrium with the two other genotyped SNPs from the 3′ part of the gene (*TaqI* and rs10783215). The traditionally named "*BB*" carrier women ("AA" genotype) were found to have higher fat-free mass and hamstring strength compared to "*bb*" ("GG") genotpye carriers.[8] Contradictory results were published by Geusens et al.[7] who first described the association between VDR polymorphisms and different muscle phenotypes and found that the "*B*" *BsmI* allele is associated with lower quadriceps muscle strength in non-obese women. This study reported the only significant association of *BsmI* with HGS so far, where the "*B*" allele was associated with lower HGS in non-obese women, however, this effect was inverse in obese subjects. The "C" allele of *TaqI* polymorphism (rs731236) is traditionally named as "*t*" in a number of studies. The *TaqI* is a non-synonymous SNP located in Exon 9. It was studied in relation with different muscle phenotypes by Iki et al.[10] and they did not find any association between the studied muscle parameters and the SNP. However, in another study, Windelinckx et al.[11] investigated the haplotype of *TaqI* with *BsmI* on the muscle phenotypes. In their study, a borderline non-significant ($p = 0.06$) trend was observed for HGS where "*Bt*" homozygotes had higher HGS than "*bT*" carriers. This association of the haplotype was significant for isometric and concentric knee extensor strength.

Our findings can provide some possible explanations for the discrepancies of earlier results. First, influence of the confounding environmental factors accumulating with aging was minimized in our study by analysis of a young children sample. Second, all genotype–phenotype associations were adjusted for the demographic variables which were proved to influence the HGS in both hands. Further, beside the single marker association tests, we also performed haplotype analyses for a deeper analysis of the possible genetic associations.

The previously published molecular studies may provide a reasonable explanation for the significant association between HGS and the genetic markers located in the two main regulatory regions of the VDR gene. The rs45160335 (*A1012G*) polymorphism is located in the 1e promoter region at the 5′ part of the gene. The "*A*" to "*G*" transition in this SNP negatively modifies the GATA-3 transcription factor binding ability of the VDR promoter.[29] "A" allele results in an increased promoter activity proved by Fang et al.[30] using luciferase activity measurements. The 3′ UTR haplotype seems to be associated with the VDR mRNA stability. In cell line studies, "*baT*" haplotype of *BsmI-ApaI-TaqI* construct was associated with a 15% higher mRNA level and a 30% slower mRNA decay compared to the "*BAt*" haplotype.[30] Based on these previous molecular results, the higher HGS associated with the "T" allele of the rs4516035 promoter SNP ("*A*" of *A1012G*) and the "ACT" 3′ UTR haplotype (*Bt*T) can be the direct genomic effects of the variants resulting in higher VDR expression and more stable VDR mRNA. However, the direct biological effect of the studied genomic variants has not been reported in muscle cells and further studies are needed to confirm the genetic associations above. Animal models have confirmed that vitamin D deficiency and congenital aberrations in the vitamin D endocrine system may result in muscle weakness.[31] VDR is a ligand-dependent transcription factor mediating the genomic effect of vitamin D on muscle cells. On the other hand, VDR can also act as the membrane receptor of vitamin D mediating its non-genomic actions.[31,32] Several RCTs investigated the influence of vitamin D deficiency and supplementation on muscle function and balance but the results have been contradictory and available evidence is limited by methodologically heterogeneous studies.[31,32] The controversial results can be related to

the possible modulating effect of the VDR gene variants. None of the published clinical studies considered the genetic background, however, it can modify the molecular effect of the vitamin D ligand at the cellular level.

Our study has got some important limitations. First, we used a cluster sampling technique by including different primary schools from different regions of the country and the whole healthy schoolchildren population in the studied age range was recruited from these schools into the study. However, the absent of the parents' consent and the exclusion of subjects with any missing genotype decreased the study population with 8.9%. Second, we did not apply any correction of the α-level during the genetic association testing process. We followed this method because we used a hypothesis driven approach where effect of candidate SNPs on a phenotype was calculated. Moreover, an important "internal" validation was used to confirm the associations of the study even if the Type I error rate was not conservatively reduced. The significant association between the VDR promoter SNP, 3′ UTR haplotype and HGS was found in case of both hands, underlying our working hypothesis that the muscle biology-related genetic background of HGS has to be uniform and independent of the hemispheric dominance. These limitations above can influence the reliability of our findings, therefore independent replications of the study on different populations are strongly recommended.

In this study, a positive association between muscle strength and some biologically significant candidate VDR gene variants was revealed. Based on these results, a direct effect of VDR polymorphisms on muscle function can be supposed, however, this hypothesis has to be further studied. The determination of the individual genetic background can be important not only in the estimation of the risk for a muscle function-related pathology, but it can also explain the discrepancy in the published clinical results on the association between vitamin D and muscloskeletal phenotypes. Our study need further replication on other cohorts, but based on the associations above, genotyping of candidate VDR variants in the future clinical studies focusing on vitamin D metabolism and muscle function can be proposed.

AUTHORS' CONTRIBUTIONS

Arpad Bozsodi helped in collecting and analyzing data, isolation of DNA and preparation of genotyping, and in writing of the article. Sara Boja and Agnes Szilagyi performed measurements and data collection. Annamaria Somhegyi performed measurement protocols and article correction. Peter Pal Varga performed measurement protocols, article correction, and funding. Aron Lazary helped in database building, statistical analysis, final editing of the article, and funding.

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