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Common variation in the CD36 (fatty acid translocase) gene is associated with left-ventricular mass

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Abstract

Aims—Genetic variation in the fatty acid translocase (CD36) gene has been shown in animal models to affect several risk factors for the development of left-ventricular hypertrophy, but this phenotype has not, thus far, been investigated in humans. We examined the relationship between common genetic polymorphisms in the CD36 gene and left-ventricular mass.

Methods and results—We studied a cohort of 255 families comprising 1425 individuals ascertained via a hypertensive proband. Seven single-nucleotide polymorphisms which together tagged common genetic variation in the CD36 gene were genotyped using a SEQUENOM MALDI-TOF instrument. There was evidence of association between the rs1761663 polymorphism in intron 1 of the CD36 gene and left-ventricular mass determined either by echocardiography ($P = 0.003$, $N = 780$) or electrocardiography ($P = 0.001$, $N = 814$). There was also association between rs1761663 genotype and body mass index ($P < 0.001$, $N = 1354$). Genotype was associated with between 2 and 8% differences in these phenotypes per allele. After adjustment for the effect of body mass index, there remained significant associations between genotype and left ventricular mass measured either by echo ($P = 0.017$) or ECG ($P = 0.007$).

Conclusions—Genotype at the rs1761663 polymorphism has independent effects both on body mass index and left-ventricular mass. Genes with such pleiotropic effects may be particularly attractive therapeutic targets for interventions to modify multiple risk factors for cardiovascular events.

Keywords

CD36; genetics; left-ventricular mass; obesity

Introduction

Left-ventricular (LV) hypertrophy is a strong independent predictor of cardiovascular morbidity and mortality. Previous data indicate that LV mass (LVM) has substantial heritability whether measured by electrocardiography or echocardiography [1]. However, the genetic differences between individuals accounting for this heritability remain largely unknown. Hypertension and body mass index (BMI) are strongly associated with LVM. If pleiotropic genetic effects (that is, effects on more than one phenotype) on these strongly correlated cardiovascular risk factors exist in human populations, the responsible genes might be particularly attractive targets for therapeutic interventions [2].

The CD36 gene (also known as fatty acid translocase) is widely expressed, and encodes a membrane protein that facilitates fatty acid uptake and utilization by metabolic tissues. Genetic mapping studies in the spontaneously hypertensive rat showed that CD36 deficiency can be responsible for insulin resistance and hypertriglyceridemia, and more recently expression quantitative trait locus (QTL) studies in the same model showed that genetically mediated deficiency in the renal expression of CD36 can cause hypertension [3,4]. However, higher levels of CD36 expression in certain tissues may be adverse; for example, macrophage CD36 up-regulation contributes to foam cell formation in atheromatous plaques [5].

CD36 has myocardial effects in mouse models. CD36-deficient mice have a 10% higher heart weight-to-body weight ratio; [6] and CD36-deficient mouse hearts have a reduced tolerance of ischemia [6,7]. Contrasting evidence, however, suggests a deleterious role of CD36 expression in the myocardium. Myocardial CD36 expression is up-regulated in aged mice, and there is a concomitant increase in intramyocardial lipid content associated with energetic compromise; better cardiac function and a blunted hypertrophic response to ageing is observed in aged CD36-deficient mice [8].

Taken together, the animal model data strongly suggest that CD36 is a strong candidate gene for pleiotropic effects on LVM and factors related to the metabolic syndrome in humans. Although many previous genetic-epidemiological studies have investigated the role of CD36 mutations and polymorphisms in insulin resistance and dyslipidemia, none has as yet studied association between common variation in CD36 and LVM [9,10]. Previous studies in the Japanese population suggested a possible role for CD36 deficiency in the development of hypertrophic cardiomyopathy, though taken together these studies were inconclusive [11]. We previously presented evidence suggestive of genetic linkage of LVM to chromosome 7 [12], in a chromosomal region neighboring the CD36 gene. We now present an association study of SNPs tagging common genetic variation in CD36 in a cohort of families ascertained via a proband with hypertension, and phenotyped for LVM using echocardiography and electrocardiography (ECG).

Methods

The collection strategy of this family study has been described previously [13]. Briefly, families were ascertained in 1993–1996 through a proband diagnosed with essential

hypertension. The following criteria defined eligibility as a proband: a mean systolic blood pressure (SBP) over 140 mmHg and mean diastolic blood pressure (DBP) over 90 mmHg on daytime ambulatory blood pressure monitoring; or greater than three office blood pressure readings greater than 160 mmHg systolic and 95mmHg diastolic; or treatment with two or more anti-hypertensive drugs. These relatively stringent criteria were applied to provide maximum security that probands were indeed at the upper end of the population blood pressure distribution. Secondary hypertension was excluded using the standard screening protocol applied in the hypertension clinic.

In order to be suitable for the study, families were required to consist of at least three siblings clinically assessable for blood pressure if at least one parent of the sibship was available to give blood for DNA analysis, and to consist of at least four assessable siblings if no parent was available for DNA analysis. Quantitatively assessed sibships were recruited either in the generation of the proband or his/her offspring. When members of the sibship were found to be hypertensive, families were extended and the spouses and offspring of hypertensive sibs collected. The majority of the individuals in the family collection therefore have blood pressures within the conventionally accepted 'normal range', and the family collection includes some extended families, though most are nuclear families. The study received ethical clearance from the appropriate review committees, and corresponded with the principles of the Declaration of Helsinki. All participants gave informed consent to participate in the study.

Blood pressure was measured using ambulatory monitoring for a period of 24 h in all participants willing to undergo monitoring, using the A&D TM2421 monitor according to a previously described protocol [13]. A full medical and lifestyle history was taken. Anthropometric measurements of height, weight, waist and hip circumferences were carried out according to standard methods. DNA was extracted from blood samples using standard methods. Families were recalled for additional cardiovascular phenotyping in 1999–2001, at which time electrocardiographic and echocardiographic measurements were made using standard protocols previously described in this cohort, and outlined in detail in the Supplementary Information (<http://links.lww.com/HJH/A78>) [12].

Tag single-nucleotide polymorphisms (SNPs) within the CD36 gene and 15Kb in either direction (to incorporate close-range upstream and downstream regulatory sequences) were identified by reference to the SNP data from the HapMap CEU samples of northern and western European ancestry (<http://www.hapmap.org>). The region consists of two major haplotype blocks. The tagging strategy was implemented with the Tagger utility in the Haploview software package [14]. Seven tag SNPs were required. Selected SNPs, their location in the region, and the linkage disequilibrium pattern across the gene are shown in Fig. 1. Multiplex genotyping was carried out using a SEQUENOM MALDI-TOF instrument as previously described [13]. Control individuals of known genotype were included in every plate, and 100 randomly selected samples were genotyped twice for each polymorphism. The estimated genotype error rate was less than 1%. Genotyping was carried out blinded to phenotypic information. Sequenom primers and conditions are shown in Supplementary Table 1 (<http://links.lww.com/HJH/A78>).

Mendelian inheritance of all the genotypes, and Hardy-Weinberg equilibrium for each marker, were checked using PEDSTATS [15]. We examined the phenotypes for normality. All variables required log-transformation to adequately conform to a normal distribution. We adjusted the phenotypes for the significant covariates age, age-squared, sex, smoking, alcohol consumption, habitual physical activity, and each of the major classes of anti-hypertensive drug (beta-blockers, calcium antagonists, diuretics, and ACE inhibitors) using linear regression, as previously described [16]. The log-transformed, covariate-adjusted residuals were entered into the quantitative trait genetic association analyses, which were performed using a variance-components approach implemented in MERLIN [17]. This takes account of shared polygenic effects in members of the same pedigree, as previously described [18]. We used the bioinformatic functional analysis tool FASTSNP (<http://fastsnp.ibms.sinica.edu.tw>) to investigate the effect of intronic SNPs on predicted transcription factor binding sites or on splicing.

Results

A total of 1425 participants from 255 families were recruited to the study (of whom 52.4% were women, and 36.1% hypertensive). The median family size was five people; 60% of families comprised between four and six genotyped and phenotyped members. Seventy-one per cent of families were two-generation and 29% were three-generation. Eighty-four per cent of families had an assessable sibship in the generation of the proband, whereas 16% of families consisted of a proband and their nuclear family (spouse and children over 18 years) only. Electrocardiograms and echocardiograms were obtained from 955 family members (449 men and 506 women), representing a 67% response rate to the invitation for the second phase of phenotyping. After excluding patients with structural heart disease ($N = 69$), technically inadequate echocardiograms ($N = 60$), and electrocardiographic abnormalities ($N = 18$), 868 and 829 patients were eligible for the genetic analyses of electrocardiographic and echocardiographic phenotypes, respectively. Excluded individuals were older, were more often hypertensive, diabetic, and male. The electrocardiographic analyses included 224 families (395 men and 473 women), and the echocardiographic analyses included 222 families (362 men and 467 women). Characteristics of the population for the phenotypes of interest are represented in Table 1.

Genotyping was successful at all SNPs in at least 95% of the population. All markers were in Hardy-Weinberg equilibrium at the 5% significance level. Supplementary Table 2 (<http://links.lww.com/HJH/A78>) shows allele frequencies and marker heterozygosity. Allele frequencies were very similar to the HapMap data for the CEU population (www.hapmap.org) except for rs10499858, which failed Hardy-Weinberg equilibrium in the HapMap data, but was satisfactory in our population. As expected from the tagging strategy we employed, the correlation between SNPs was generally modest (Supplementary Figure 1, <http://links.lww.com/HJH/A78>), and corresponded well with previous publicly available data. The composition and frequency of the common haplotypes present at the locus were in close agreement with those described in the HapMap CEU population.

The SNP rs1761663 in the first intron of CD36 had a minor allele frequency of 0.371 in our total population, which was not significantly different when we considered only the

subpopulation that had had LVM measured. The major allele of this SNP is thymidine (T) and the minor allele is cytosine (C). Genotype at this SNP was strongly associated with the log-transformed, covariate-adjusted residuals of BMI, fat mass, and both echocardiographic and electrocardiographic LVM (Table 2). Values of all of these phenotypes were higher with the major T allele. The associations were significant under either an additive (linear trend) or recessive (CC + CT versus TT) model; the association with ECG LVM was stronger under an additive model, whereas the association with the other phenotypes was stronger under a recessive model (Table 2). The fitted model implies that the untransformed values were between 2% (for BMI) and 8% (for echocardiographic LVM) different between the lowest and highest value genotypes; that is, a difference of around 0.4 kg/m² in BMI and 16 g in echo LVM. However, genotype accounted for less than 1% of the total phenotypic variance of any of the traits of interest. Since BMI is a significant predictor of LVM, and BMI was associated with genotype at rs1761663, we analyzed the LVM phenotypes both with and without correction for BMI. As expected, when we additionally adjusted for the association of genotype with BMI, the strength of the associations between rs1761663 genotype and LVM became somewhat weaker, but they remained significant ($P = 0.017$ for association between genotype and fully adjusted echo LVM; $P = 0.007$ for association between genotype and fully adjusted ECG LVM). After full adjustment, there was a mean difference of 4–6% in echo or ECG LVM between the lowest and highest value genotypes at rs1761663 (Table 2). There was no heterogeneity between participants with and without hypertension with regard to the association between LVM and rs1761663 genotype. Certain of the other CD36 SNPs showed less strong, but still statistically significant, association with LVM than rs1761663; however, after adjustment for the effect of rs1761663, there was no residual association between genotype at any of the other SNPs and LVM measured either by ECG or echo. There was no association between genotype at any CD36 SNP and SBP, DBP or hypertension affection status.

Discussion

We have shown that genotype at the rs1761663 SNP in the CD36 gene has pleiotropic effects on LVM (measured either by ECG or echo) and BMI. The minor C allele at this SNP is associated with lower values of all these phenotypes, suggesting a potentially protective role against these cardiovascular risk factors. The association with LVM was of larger magnitude than the association with BMI, and it persisted (for both ECG and echo measurements) when LVM was additionally adjusted for BMI. The rs1761663 SNP is situated in intron 1 of the gene. It has no known function and FASTSNP analysis did not indicate any predicted effect on splicing or transcription factor binding. Functional studies will be required to clarify whether the effect we have observed is due to this SNP or through linkage disequilibrium with a neighboring SNP. The C allele, although the minor allele in our Caucasian population, is probably the ancestral allele; it is the major allele in the African origin Yoruba (Nigeria) and Luhya (Kenya) populations in which rs1761663 was typed in the HapMap.

This is the first study of which we are aware to demonstrate association between LVM and CD36 variants in humans. Differences of 5–8% per allele in both electrocardiographic and echocardiographic LVM were associated with rs1761663 genotype. Because of the

pleiotropic effect of the SNP on BMI and LVM that we observed, the association with LVM weakened after additional correction for BMI, but it remained significant for both ECG and echo determined LVM.

A number of previous studies have examined association between particular candidate genes and LVM; however, the most comprehensive evaluation of genetic effects on LVM to date has involved genome-wide association study (GWAS) of 12 612 individuals conducted by Vasani *et al.* [19]. In that study, no locus affecting LVM was identified at genome-wide significance, and indeed the region of chromosome 7 in which CD36 is located did not yield any SNP associations with LVM significant at P less than 10^{-5} . In understanding the discrepancy between our study and that much larger negative investigation, it should be recalled that we selected families via a hypertensive proband in the top 5% of the blood pressure distribution, and further selected for the inclusion of additional hypertensive patients in extending nuclear pedigrees. This resulted in a substantial genetic 'loading' of our cohort for higher blood pressure (with around one-third of participants having blood pressures in the upper 5% tail). Since higher BP is a key predisposing factor for higher LVM, our cohort also had significantly higher LVM (by about 30%) than the community-ascertained cohorts studied by Vasani *et al.* Such selection would be expected to substantially increase our power to detect genetic effects. Analysis of rs1761663 genotypes in additional cohorts enriched for high blood pressure (or otherwise selected for higher LVM) would be of considerable interest in future studies.

The present study employed detailed phenotyping methods, and its principal conclusion is supported by both electrocardiographic and echocardiographic data. Our statistical analyses took detailed account of potential confounding variables and differences in genetic model specification. However, certain limitations of the study should be acknowledged. Our selection criteria for hypertension increased our power to detect associations with LVM; also, those with higher blood pressures are at the greatest risk to develop LV hypertrophy and are thus a particularly clinically relevant group for study. However, it should be noted that our results may not be generalizable to families that are not genetically 'loaded' for hypertension by the stringent selection criteria we adopted. Echocardiographic phenotyping is less accurate for the determination of LVM than cardiac MR imaging; random error in the echo measurements could well have resulted in an underestimate of the size of the association in our study. Future studies of CD36 SNPs in patient groups ascertained or enriched for hypertension phenotyped using MR may quantify the association with greater precision.

Functional studies will be required in the future to ascertain the mechanism of the association we have described. In this regard, previous investigations in both animal models and human patients have illustrated the complex relationship between levels of CD36 expression in different tissues and a range of phenotypes, and indicated that regulation of CD36 expression is subject to significant tissue specificity. Investigation of the role of rs1761663 on CD36 expression in the tissue relevant to our principal finding, that is, myocardium, would be challenging to undertake in sufficient numbers of people to obtain robust results; it was not possible in our cohort of healthy volunteers.

In summary, we have shown a pleiotropic effect of the rs1761663 SNP in the CD36 gene on LVM and BMI; although the magnitude of the association with either phenotype is small, this finding extends the phenotypic range of the CD36 gene and suggests novel avenues for mechanistic investigation linking cardiac long-chain fatty acid uptake with myocardial hypertrophy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ACE	angiotensin-converting enzyme
CD36	the gene encoding fatty acid translocase
CEU	HapMap population of European Caucasian ancestry
GWAS	genome-wide association study
LDL	low-density lipoprotein
LV	left ventricle
LVM	left-ventricular mass
MR	magnetic resonance
QTL	quantitative trait locus
SNP	single-nucleotide polymorphism

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Fig. 1.

Single-nucleotide polymorphisms (SNPs) genotyped at the CD36 locus. The locations of the SNPs genotyped are indicated by arrows in the schematic figure of the CD36 gene (7q11.2/74.0 kb) in the upper panel. Rectangular blocks represent CD36 exons 1–14. Hapview output in the lower panel shows the linkage disequilibrium (D') relationships between HapMap phase 2 SNPs in the region, indicating the two principal haplotype blocks. Regions of strong LD are in dark gray.

Table 1
Characteristics of the study population

Variable	N	Minimum	LQ	Median	UQ	Maximum	R ² explained by covariates on logged data	Covariates used
Age	1425	18.7	35.7	50.9	60.9	90.7		
BMI (kg/m ²)	1402	16.7	23.1	25.4	28.2	51.8	15.2	Age, age ² , alcohol (females only), smoking ^a , exercise ^a
Fat mass (kg)	908	7.6	18.0	22.9	29.0	84.3	30.7	Sex, age, age ² , alcohol (females only), smoking, exercise
Echo LVM (g)	829	81.4	163.9	211.4	261.8	625.9	20.2	Age, sex, BMI,
ECG LVM (g)	868	93.7	135.0	153.7	176.5	254.1	34.4	Age, sex, BMI, exercise, smoking, calcium blocker

LVM, left ventricular mass.

^aThese variables were fitted as factors rather than regressor variables.

Table 2
Association between rs1761663 genotype and cardiovascular phenotypes

Variable	Mean (standard error, <i>n</i>)			<i>P</i> values for linear trend and for TT v (CT + CC)
	TT	CT	CC	
Adjusted loge (BMI)	0.018 (0.006, 539)	−0.015 (0.006, 631)	−0.001 (0.010, 184)	0.005, <0.001
Adjusted loge (fat mass)	0.037 (0.017, 321)	−0.021 (0.015, 438)	−0.032 (0.028, 122)	0.009, 0.006
Adjusted loge (Echo LVM)	0.045 (0.018, 288)	−0.027 (0.016, 376)	−0.036 (0.028, 116)	0.003, 0.001
Adjusted log (ECG LVM)	0.020 (0.009, 296)	−0.009 (0.008, 399)	−0.029 (0.014, 119)	0.001, 0.003
Adjusted loge (Echo LVM) additionally adjusted for BMI	0.035 (0.018, 282)	−0.018 (0.015, 373)	−0.030 (0.027, 116)	0.017, 0.012
Adjusted log (ECG LVM) additionally adjusted for BMI	0.013 (0.008, 293)	−0.002 (0.007, 396)	−0.029 (0.013, 119)	0.007, 0.040

LVM, left ventricular mass.