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ARTICLE

Variations in the four and a half LIM domains 1 gene (*FHL1*) are associated with fasting insulin and insulin sensitivity responses to regular exercise

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Abstract
Aims/hypothesis The expression of the four and a half LIM domains 1 gene (*FHL1*) is increased in the muscle of individuals who show an improvement in insulin sensitivity index (S_I) after 20 weeks of exercise training. The aim of the present study was to investigate associations between three *FHL1* single nucleotide polymorphisms (SNPs) and variables derived from an IVGTT, both in the sedentary state and in response to exercise training, in participants in the HERITAGE Family Study.

Materials and methods SNPs were typed using fluorescence polarisation methodology. Analyses were performed separately by sex and in black and white individuals.
Results In black participants, no associations were found with any of the SNPs. In white women (n=207), SNP rs9018 was associated with the disposition index (D_I), which is calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$) multiplied by acute insulin response to glucose (AIR_g; pmol/l×10 min), and the glucose disappearance index (K_g) training responses (p=0.016 and p=0.008, respectively). In white men (n=222), all SNPs were associated with fasting glucose levels ($p \leq 0.05$) and SNP rs2180062 with the insulin sensitivity index (S_I) (p=0.04) in the sedentary state. Two SNPs were associated with fasting insulin training response. Fasting insulin decreased to a greater extent in carriers of the rs2180062 C allele (p=0.01) and rs9018 T allele (p=0.04). With exercise training, S_I ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$: 0.68±0.20 vs −0.77±0.44, p=0.046), D_I (319±123 vs –528±260, p=0.006) and K_g (per 100 min: 0.09±0.04 vs −0.14±0.8, p=0.03) improved more in the C allele carriers at rs2180062 than in the T allele carriers.
Conclusions/interpretation Fasting insulin and S_I responses to exercise training were associated with DNA sequence variation in *FHL1* in white men. Whether these associations exist only in white men remains to be investigated.

Keywords *FHL1* · Gene–exercise interaction · MINMOD · Polymorphism · Risk factors · SLIM1 · SLIMMER · KYO–T · Single nucleotide polymorphism · SNP

Abbreviations

AIR_g	acute insulin response to glucose
D_I	disposition index
K_g	glucose disappearance index

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S_G	glucose effectiveness
S_I	insulin sensitivity index
SNP	single nucleotide polymorphism
VO_{2max}	maximum volume of oxygen utilisation

Introduction

The four and a half LIM domains 1 gene (*FHL1*, found on chromosome Xp27.2) is a member of the gene family encoding the LIM domain-containing proteins and is translated into three protein isoforms that are mainly produced in skeletal muscle [1–3]. LIM domains are cysteine-rich zinc finger motifs that provide an interface for protein–protein interactions found in nuclear and cytoplasmic proteins including homeodomain transcription factors and kinases [4]. LIM domain proteins play important biological roles in cytoskeleton organisation, cell fate determination and organ development [5]. Interestingly, one LIM domain gene (*ISL1*) has been identified as a positional candidate gene for obesity and leptin levels, and is suggested to be involved in body weight regulation and glucose homeostasis [6]. *FHL1* mRNA is more abundant in oxidative fibres of skeletal muscle and its expression increases with passive stretch-induced hypertrophy [3].

Physical activity and diet play a role in the prevention of type 2 diabetes [7–10]. However, there is considerable heterogeneity in the metabolic responses to regular exercise. The HERITAGE Family Study has provided evidence for the genetic and non-genetic determinants of many phenotypes in the sedentary state and in response to training. We have reported on the familial resemblance for traits related to insulin and glucose metabolism both in the sedentary state and in response to exercise training [11, 12]. Among participants in HERITAGE, there was a 10% mean increase in the insulin sensitivity index (S_I) in response to the standardised 20 week training programme. The changes in the S_I were not correlated with the changes in body weight, waist circumference or cardiorespiratory endurance [13].

We recently reported differences in skeletal muscle gene expression patterns between HERITAGE participants who increased their S_I with exercise training vs carefully matched subjects who showed no improvement [14]. Using microarray and RT-PCR methodologies, we identified the *FHL1* gene as a novel candidate for the S_I training response. We found that *FHL1* mRNA expression was increased by 70% in those who improved their S_I with training [14].

Here we have expanded our study of the *FHL1* gene as a novel candidate for the S_I training response by investigating the associations between DNA sequence variations in the gene and insulin-related phenotypes in all subjects of HERITAGE. The associations were investigated in the sedentary state and in response to 20 weeks of an endurance training programme.

Materials and methods

The HERITAGE Family Study design and methods have been described previously [15]. Briefly, the study was designed to investigate the role of genetic factors in the cardiovascular and metabolic adaptations to 20 weeks of endurance training in white and in black families. All subjects were required to be sedentary and in good health and to meet a series of inclusion criteria [15]. The offspring were required to be aged ≥17 years and the parents ≤65 years. All participants were sedentary at baseline, defined as not having engaged in regular physical activity more than once a week over the previous 6 months. Participants were excluded if they had a BMI >40 kg/m^2 (unless they were able to meet the demands of the exercise tests and exercise training programme), hypertension or were using lipid-lowering, hypoglycaemic or anti-hypertensive medication. For this report, we analysed data from 429 white adults (222 men, 207 women) and 173 black adults (70 men, 103 women). Of the white participants, 162 were parents (88 men, 74 women) and 267 were adult offspring (134 men, 133 women). Among the black participants, 42 were parents (15 men, 27 women) and 131 were offspring (55 men, 76 women). The Institutional Review Boards at the five participating centres of HERITAGE approved the study protocol. Written informed consent was obtained from each participant.

The programme has been described elsewhere [15, 16]. Briefly, the exercise intensity of the 20 week programme was customised for each participant based on the heart rate to oxygen uptake relationship measured at baseline. During the first 2 weeks, the subjects trained at a heart rate corresponding to 55% of maximum volume of oxygen utilisation (VO_{2max}) for 30 min per session. Duration was gradually increased to 50 min per session and intensity to the heart rate associated with 75% of the baseline VO_{2max} and then sustained for the last 6 weeks. Training frequency was three times per week, with all training sessions performed under supervision on cycle ergometers in the laboratory at the participating clinical centres.

Measurement of glucose, insulin and IVGTT-derived variables Fasting plasma glucose and insulin levels were analysed at baseline and after the 20 week training programme, 1 day after the last training session. Glucose was measured enzymatically using a reagent kit (Diagnostic

Chemicals, Oxford, CT, USA). Plasma insulin was determined using radioimmunoassay after polyethylene glycol separation [17]. Polyclonal antibodies (with a cross-reactivity >90% with pro-insulin and presumably its conversion intermediates) were used [18]. Therefore, insulin refers to immunoreactive insulin defined as the sum of insulin, pro-insulin and split-proinsulin. The intra- and inter-assay coefficients of variation for baseline insulin were 7.7 and 10.3%, respectively. During the IVGTT, no insulin or secretagogues were used. Details of these analyses have been reported previously [13]. A frequently sampled IVGTT was administered in the morning after an overnight fast of 12 h. From the IVGTT data, S_I, acute insulin response to glucose (AIR_g), disposition index (D_I) and glucose effectiveness (S_G) were derived from the Minimal Model (MINMOD) Millennium software [19]. The S_I represents the increase in net fractional glucose clearance rate per unit change in serum insulin concentration after the intravenous glucose load. The AIR_g was derived as the integrated area under the insulin curve between 0 and 10 min of the IVGTT and is used as a measure of insulin secretion [19]. The D_I, calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$) multiplied by AIR_g (pmol/l×10 min), is a measure of the ability of the pancreatic beta cells to compensate for changes in insulin sensitivity. S_G measures the ability of glucose as such, independently of changes in plasma insulin, to increase glucose disposal and to suppress endogenous glucose output. Glucose disappearance index (K_g) estimates glucose disappearance based on the slope of the line derived from least-squares regression of the natural logarithm of plasma glucose from 10 to 60 min during the IVGTT and was used as a measure of overall glucose tolerance.

Molecular studies DNA was extracted from lymphoblastoid cell lines [20]. Genotyping for the *FHL1* polymorphisms was performed with template-directed dye-terminator incorporation assay with fluorescence polarisation detection [21]. Briefly, genomic DNA containing the rs2180062 C>T, rs2236003 C>T and rs9018 T>C single nucleotide polymorphisms (SNPs) located at chromosomal positions 134956908, 135015770 and 135018602 (dbSNP database, http://ncbi.nlm.nih.gov/projects/SNP, last accessed in May 2007) was amplified using PCR primers flanking the variant site. Unincorporated nucleotides and remaining unused primers were degraded by exonuclease I and shrimp alkaline phosphatase. Single base primer extension reactions were carried out with dye-terminators specific for the alleles present on the template. The genotype call rate was over 98% for all markers. A Mendelian check among the HERITAGE Families revealed no errors, and all SNPs were in Hardy–Weinberg equilibrium.

FHL1 haplotypes were constructed manually. In men, the phase of the haplotypes could be deducted directly from the SNP data. In women, the phases of the haplotypes in those who were heterozygotes at two or three SNP loci were confirmed using haplotype information from other family members: parents' haplotypes in daughters and sons' haplotype in mothers. There was only one double heterozygote in a white family, whose haplotype could not be confirmed. Results for the haplotypes in white individuals are presented in Electronic supplementary material (ESM) Tables 1 and 2.

Statistical analyses All statistical analyses were performed using the SAS Statistical Software package. In women, a χ^2 test was performed to assess whether the observed genotype frequencies were in Hardy–Weinberg equilibrium. Sex-by-race subgroup association analyses were performed for the following reasons: (1) significant sex-related differences in fasting insulin and IVGTT-derived variables; (2) location of the gene on the X chromosome; (3) racial differences for the baseline IVGTT phenotypes; and (4) significant racial differences in allele and genotype frequencies. The associations between *FHL1* genotypes and phenotypes were analysed using a MIXED procedure. The non-independence among family members was adjusted using a 'sandwich estimator,' which asymptotically yields the same parameter estimates as ordinary least-squares or regression methods, but the standard errors and consequently hypothesis tests are adjusted for the familial dependencies. Age and BMI were included as covariates in the model. Because parents are unrelated individuals, they were analysed with a MIXED model, without non-independence adjustment. Fasting insulin and fasting glucose were logarithmically transformed, and the S_I, AIR_g and D_I variables were square root transformed to normalise their distributions before analysis. All training responses were adjusted for their respective baseline values.

Results

Frequencies of the rs2180062 C, rs2236003 C and rs9018 T alleles in white individuals were 0.797, 0.594 and 0.522, respectively; in black individuals, these frequencies were 0.378, 0.793 and 0.354. Allele frequencies were comparable to those reported in the HAPMAP database (http://www.HapMap.org, last accessed in May 2007). In women, genotype frequencies were in Hardy–Weinberg equilibrium. The markers were not in complete linkage disequilibrium, with r^2=0.139, 0.092 and 0.288 in white participants, and 0.255, 0.132 and 0.235 in black participants, for the

combinations rs2180062*rs2236003, rs2180062*rs9018 and rs2236003*rs9018, respectively.

No significant associations with any of the SNPs were found in black individuals (see ESM Tables 3, 4, 5 for details).

Subject characteristics are presented in Table 1.

In the sedentary state, all three *FHL1* polymorphisms were associated with fasting glucose levels in white men. The S_I was associated with the rs2180062 SNP in men. No significant associations were found in women. Associations with fasting insulin, glucose and IVGTT-derived variables were seen in men in response to training.

In men, the rs2180062 C>T SNP, located within the 5′ UTR of the *FHL1* gene, was associated with higher baseline glucose and exercise-induced changes in various measures of glucose homeostasis (Table 2). Men with the C allele had higher baseline glucose (5.20±0.04 mmol/l [mean±SE]) than those with the T allele (5.06±0.07 mmol/l, p=0.02) and their fasting insulin levels decreased more with exercise training (–9.1±2.0 vs +6.0±3.3 pmol/l, p=0.012). This reduction in fasting insulin with regular exercise was concordant with improvements in S_I, D_I and K_g. Men with the C allele had greater improvements in S_I ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$: 0.68±0.20 vs –0.77±0.44, p=0.046), D_I (+319±123 vs –528±260, p=0.006) and K_g (per 100 min: 0.09±0.04 vs –0.14±0.8, p=0.03) compared with the T allele carriers. To represent the differences in the S_I and D_I response according to the rs2180062 C>T SNP in men, we plotted the pre- and post-training values for S_I and AIR_g (Fig. 1). It can be seen that the improvements in S_I and D_I with exercise training in subjects with the C genotype were not at the expense of increased AIR_g.

The rs2236003 C>T polymorphism, located in intron 3, was associated with fasting glucose at baseline in men (Table 3). Those with the C genotype had lower fasting glucose (p=0.015). There were no associations in women.

In men, the rs9018 T>C polymorphism, located at the 3′ UTR of the *FHL1* gene, was associated with fasting glucose before training and with fasting glucose and insulin training responses; in women it was associated with the D_I response

Table 1 Characteristics of subjects

Variable	Black individuals		White individuals	
	Men (n=70)	Women (n=103)	Men (n=222)	Women (n=207)
Age (years)	33.1±11.1	32.9±10.9	36.5±14.9	35.2±14.1
BMI (kg/m^2)				
Pre-training	27.4±5.2	28.2±6.0	26.7±4.8	25.2±5.0
Training response	−0.13±0.7	−0.09±1.1	−0.14±0.7	−0.07±0.8
Fasting plasma glucose (mmol/l)				
Pre-training	5.27±0.63	5.00±0.60	5.20±0.60	4.95±0.55
Training response	0.14±0.53[a]	0.19±0.48[a]	0.01±0.42	0.04±0.37
Fasting plasma insulin (pmol/l)				
Pre-training	72.3±43.9	75.6±46.5	71.1±44.8	60.0±26.8
Training response	−4.9±27.2	−5.4±21.9[a]	−6.1±25.8[a]	−3.7±21.1[a]
S_I ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$)[b]				
Pre-training	2.56±2.19	2.80±2.19	3.93±2.84	4.75±2.99
Training response	0.49±1.94[a]	0.33±2.16[a]	0.46±2.70[c]	0.00±2.66
AIR_g (pmol/l×10 min)				
Pre-training	1,510±1,362	1,607±1,252	739±614	585±395
Training response	−85±575	−46±495	−64±341[c]	−20±213[c]
D_I[d]				
Pre-training	3,041±2,516	3,528±2,897	2,274±1,747	2,394±1,672
Training response	480±2,457	472±2,272	185±1,663	−54±1,542
S_G (per 100 min)				
Pre-training	1.9±1.1	2.0±1.2	1.6±0.8[a]	1.7±0.9
Training response	0.2±1.5	0.3±1.5	0.2±1.1[a]	0.1±1.1
K_g (per 100 min)				
Pre-training	1.70±0.68	1.91±0.67	1.54±0.57	1.71±0.56
Training response	0.06±0.65	0.13±0.64[a]	0.06±0.53	0.001±0.52

Data are means±SD

[a] Statistically significant response (p<0.05) to exercise training

[b] Units are taken from the MINMOD program. To convert values to SI units ($\times 10^{-4}$ min^{-1} $[pmol/ml]^{-1}$) multiply by 0.167

[c] Statistically significant response (p<0.001) to exercise training

[d] D_I is calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$) multiplied by AIR_g (pmol/l×10 min)

Table 2 Associations between the rs2180062 C>T *FHL1* SNP and fasting glucose, insulin and IVGTT-derived variables in white individuals

Variable	Women							Men				
	CC, n=125		CT, n=70		TT, n=6			CC, n=176		TT, n=40		
	Mean	SE	Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
Fasting plasma glucose (mmol/l)												
Pre-training[a]	4.89	0.04	5.01	0.07	4.77	0.22	0.28	5.20	0.04	5.06	0.07	0.02
Training response[b]	0.05	0.03	0.01	0.05	0.00	0.10	0.89	−0.03	0.03	0.18	0.06	0.054
Fasting plasma insulin (pmol/l)												
Pre-training[a]	55.6	2.2	55.9	3.0	51.5	7.3	0.70	62.3	2.7	55.5	4.3	0.14
Training response[b]	−5.3	1.8	−1.5	3.0	−7.3	4.4	0.53	−9.1	2.0	6.0	3.3	0.012
S_I ($\times 10^{-4}$ min^{-1}[μU/ml]$^{-1}$)[c]												
Pre-training[a]	4.28	0.23	4.33	0.33	6.05	1.63	0.76	3.35	0.18	3.96	0.52	0.044
Training response[b]	0.06	0.22	−0.11	0.32	−0.95	0.91	0.83	0.68	0.20	−0.77	0.44	0.046
AIR_g (pmol/l × 10 min)												
Pre-training[a]	592	31	563	45	517	46	0.63	679	39	723	84	0.35
Training response[b]	−26	18	−18	28	29	90	0.78	−62	26	−108	39	0.27
D_I[d]												
Pre-training[a]	2,300	125	2,219	187	2,942	713	0.77	2,048	112	2,398	282	0.08
Training response[b]	−31	132	−149	180	128	993	0.82	319	123	−528	260	0.006
S_G (per 100 min)												
Pre-training[a]	1.7	0.1	1.7	0.1	1.6	0.6	0.73	1.5	0.1	1.6	0.1	0.43
Training response[b]	0.1	0.1	0.0	0.1	0.2	0.9	0.50	0.2	0.1	0.1	0.2	0.75
K_g (per 100 min)												
Pre-training[a]	1.72	0.05	1.72	0.07	1.83	0.23	0.99	1.52	0.04	1.61	0.08	0.15
Training response[b]	0.00	0.04	−0.05	0.06	0.10	0.22	0.56	0.09	0.04	−0.14	0.08	0.03

Values are means±SE

[a] Adjusted for age and baseline BMI

[b] Adjusted for age and baseline BMI plus the respective baseline values

[c] Units are taken from the MINMOD program. To convert values to SI units ($\times 10^{-4}$ min^{-1} [pmol/ml]$^{-1}$) multiply by 0.167

[d] D_I is calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ min^{-1} [μU/ml]$^{-1}$) multiplied by AIR_g (pmol/l×10 min)

to exercise training (Table 4). In the sedentary state, men with the T allele have higher fasting glucose (p=0.05) and higher fasting insulin levels (p=0.06) with lower S_I (p=0.059) than individuals with the T allele. In response to training, fasting glucose and insulin decreased to a greater extent in the T allele carriers than in those with the C allele (p=0.04). In women, the TT genotype at the rs9018 SNP was associated with a significantly higher training-induced improvement in

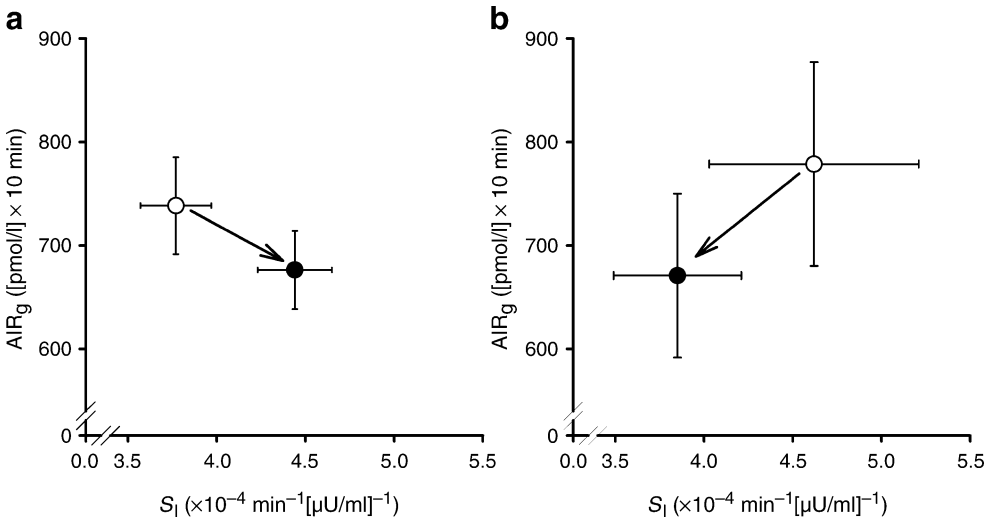

Fig. 1 Pre- and post-training effects on S_I and AIR_g for white men according to the C genotype (n=176) **a** and T genotype (n=40) **b** of the rs2180062 C>T *FHL1* SNP. *Open circles*, mean values pre-training; *closed circles*, mean values post-training. *Error bars* denote SE

Table 3 Associations between the rs2236003 C>T *FHL1* SNP and fasting glucose, insulin and IVGTT-derived variables in white individuals

Variable	Women							Men				
	CC, n=76		CT, n=85		TT, n=40			CC, n=123		TT, n=92		
	Mean	SE	Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
Fasting plasma glucose (mmol/l)												
Pre-training[a]	4.90	0.06	4.98	0.06	4.85	0.07	0.54	5.09	0.04	5.29	0.07	0.015
Training response[b]	0.01	0.05	0.04	0.04	0.08	0.05	0.56	0.08	0.04	−0.08	0.05	0.16
Fasting plasma insulin (pmol/l)												
Pre-training[a]	55.0	3.1	56.3	2.7	51.8	2.8	0.62	61.2	3.0	59.8	3.6	0.31
Training response	−6.9	2.5	−2.4	2.4	−1.8	3.0	0.57	−4.0	2.3	−10.3	2.8	0.059
S_I ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$)[c]												
Pre-training[a]	4.55	0.34	4.11	0.28	4.22	0.32	0.69	3.47	0.24	3.48	0.25	0.38
Training response	−0.42	0.31	0.10	0.27	0.79	0.35	0.16	0.26	0.25	0.62	0.25	0.28
AIR_g (pmol/l×10 min)												
Pre-training[a]	528	38	611	42	579	45	0.44	732	49	629	51	0.20
Training response	−19	24	−13	25	−48	33	0.40	−73	30	−78	34	0.27
D_I[d]												
Pre-training[a]	2,119	151	2,357	188	2,261	185	0.38	2,145	127	2,096	180	0.98
Training response	−104	154	−129	186	230	210	0.52	193	154	33	154	0.38
S_G (per 100 min)												
Pre-training[a]	1.5	0.1	1.8	0.1	1.8	0.2	0.13	1.5	0.1	1.6	0.1	0.26
Training response	0.3	0.2	0.0	0.1	−0.1	0.1	0.77	0.3	0.1	0.1	0.1	0.77
K_g (per 100 min)												
Pre-training[a]	1.63	0.06	1.74	0.07	1.77	0.08	0.21	1.53	0.04	1.56	0.07	0.60
Training response	0.01	0.05	−0.02	0.06	0.06	0.07	0.65	0.07	0.05	0.01	0.06	0.41

Values are means±SE

[a] Adjusted for age and baseline BMI

[b] Adjusted for age and baseline BMI plus the respective baseline values

[c] Units are taken from the MINMOD program. To convert values to SI units ($\times 10^{-4}$ min^{-1} $[pmol/ml]^{-1}$) multiply by 0.167

[d] D_I is calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$) multiplied by AIR_g (pmol/l×10 min)

D_I (p=0.016) and K_g (p=0.008). The association with the D_I response remained significant even after adjustments for BMI changes. In one individual with TT genotype we saw an extreme decrease of S_I (-12.2×10^{-4} $min^{-1}[\mu U/ml]^{-1}$), which was included for the analysis presented here. However, when the analysis was performed excluding this individual, the genotype associations with D_I and K_g remained, but the significance levels changed to p=0.004 and p=0.01, respectively. No other associations with IVGTT-derived variables were found in women.

In white men, the rs2180062 C>T SNP explained 2.4, 3.3 and 2.4% of the variance for the fasting insulin response, S_I response and D_I response respectively.

Discussion

The objective of this study was to expand on our observation of the *FHL1* gene as a novel candidate for the S_I response to exercise training in all subjects of the HERITAGE Family Study [14]. Therefore, we tested for associations between *FHL1* SNPs and insulin-related phenotypes at baseline and in response to regular exercise. All SNPs were associated with fasting glucose levels in men. In addition, the C allele carriers at the rs2180062 SNP had greater improvements in the S_I and D_I response to exercise training.

It is well known that regular exercise leads to favourable changes in insulin sensitivity in most people [13]. However, the extent of the changes is highly variable and, in the HERITAGE Family Study, men had larger improvements in their S_I than women [13]. Why some individuals under the same exercise training protocol increase their insulin sensitivity while others do not is not understood at this time. In this regard, we have recently reported data on differences in gene expression from individuals selected on the basis of their S_I response to exercise training [14]. High and low responders had similar ages (30±5 years), BMI, % body fat and baseline S_I but displayed marked differences in the S_I response to exercise training ($\times 10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$: 3.6±0.5 vs −1.2±0.5, n=8 per group). We identified *FHL1* gene expression as one of the candidates responsible for the divergent S_I response to exercise.

Table 4 Associations between the rs9018 T>C *FHL1* SNP and fasting glucose, insulin and IVGTT-derived variables in white individuals

Variable	Women							Men				
	TT, n=62		CT, n=92		CC, n=47			TT, n=110		CC, n=104		
	Mean	SE	Mean	SE	Mean	SE	p value	Mean	SE	Mean	SE	p value
Fasting plasma glucose (mmol/l)												
Pre-training[a]	4.93	0.06	4.95	0.06	4.89	0.07	0.98	5.25	0.06	5.10	0.05	0.05
Training response[b]	0.09	0.04	0.03	0.04	0.01	0.06	0.25	−0.07	0.04	0.09	0.04	0.04
Fasting plasma insulin (pmol/l)												
Pre-training[a]	53.7	3.0	57.7	2.4	52.8	3.9	0.35	65.3	3.6	57.2	2.9	0.06
Training response	−2.9	2.4	−3.6	2.4	−6.0	3.1	0.43	−11.6	2.9	−0.7	2.0	0.04
S_I ($\times 10^{-4}$ $min^{-1}[\mu U/ml]^{-1}$)[c]												
Pre-training[a]	4.19	0.29	4.15	0.28	5.08	0.47	0.12	3.22	0.22	3.74	0.27	0.059
Training response	0.48	0.31	−0.13	0.26	−0.56	0.45	0.11	0.50	0.20	0.22	0.31	0.90
AIR_g (pmol/l×10 min)												
Pre-training[a]	583	39	616	41	497	46	0.38	697	55	678	46	0.67
Training response	21	28	−47	22	−27	30	0.31	−88	32	−62	31	0.63
D_I[d]												
Pre-training[a]	2,243	155	2,340	173	2,271	227	0.87	2,067	160	2,165	136	0.88
Training response	329	192	−204	171	−365	189	0.016	19	129	245	186	0.30
S_G (per 100 min)												
Pre-training[a]	1.8	0.1	1.7	0.1	1.5	0.1	0.18	1.6	0.1	1.5	0.1	0.23
Training response	0.0	0.1	0.0	0.1	0.2	0.2	0.76	0.2	0.1	0.2	0.1	0.65
K_g (per 100 min)												
Pre-training[a]	1.80	0.07	1.74	0.06	1.63	0.08	0.39	1.56	0.06	1.51	0.05	0.33
Training response	0.09	0.07	−0.07	0.05	−0.04	0.07	0.008	0.01	0.05	0.06	0.05	0.75

Values are means±SE

[a] Adjusted for age and baseline BMI

[b] Adjusted for age and baseline BMI plus the respective baseline values

[c] Units are taken from the MINMOD program. To convert values to SI units ($\times 10^{-4}$ min^{-1} $[pmol/ml]^{-1}$) multiply by 0.167

[d] D_I is calculated as S_I generated from the MINMOD program ($\times 10^{-4}$ min^{-1} $[\mu U/ml]^{-1}$) multiplied by AIR_g (pmol/l×10 min)

Unfortunately, we do not have individual RNA samples to verify sex-related differences in *FHL1* expression levels.

FHL1 mRNA expression is increased in muscle with passive stretch-induced hypertrophy [22], is more abundant in skeletal muscle oxidative fibres [3] and increases in individuals who improve their S_I in response to exercise training [14]. FHL1 functions as a scaffold protein in the actin-based cytoskeleton, localises at focal adhesions and stress fibres and may be transferred to the nucleus by binding integrin proteins [4]. FHL1 plays an important role during the early stages of skeletal muscle differentiation, specifically in the α5-β1-integrin-mediated signalling pathway [23]. SLIMMER, an FHL1 isoform, is found in the nucleus of myoblasts and in the cytoplasm of myotubes, suggesting distinct roles in the cytoskeleton and in nuclear–cytoplasmic communication [3, 4].

The principal finding of our study is that regular physical activity decreased S_I and D_I, while increasing fasting insulin levels in men with the less frequent T allele at the rs2180062 SNP (Fig. 1). These results are relevant since individuals with the T genotype represent almost 20% of the white population. The increment in fasting insulin levels associated with the T genotype training response could echo the decreased insulin sensitivity. Consequently, we observed a large fall in D_I. Given the large variability in the response to exercise, those individuals with the T genotype seem to belong to a subgroup that does not benefit much in terms of IVGTT-derived variables, whereas those with the C genotype benefit substantially. This does not necessarily mean that men with the T genotype are at risk of developing diabetes, since other physiological benefits could be derived from an exercise training programme that could mitigate the T genotype effects.

Our data suggest that *FHL1* may play a role in the improvements in IVGTT-derived variables induced by exercise training. DNA sequence variations in the *FHL1* gene could possibly influence the capacity of skeletal muscle adaptation to the demands of exercise training. Probably *FHL1* sequence variants impair its function as a scaffold protein in the integrin-mediated or other kinase signalling pathways, which in turn may reduce the metabolic effects of exercise on insulin sensitivity. The DNA variants studied here are not associated with amino acid changes in the *FHLI* gene, but may be functional. The

SNP rs2180062, for example, localises at the 5′ UTR of one of the isoforms coded by this gene. A survey of this sequence for protein-binding regulatory elements using the AliBaba 2.1 search engine [24] reveals that this variant is located in a region with potential binding sites for the transcription factors CeMyoD (also known as HLH-1) and Sp1. In our in silico analysis, the C→T transition creates binding sites for the transcription factors NF-1 and AP-2, eliminating CeMyoD and Sp1 binding. The SNP rs9018 localises at the 3′ UTR region and could potentially affect stem–loop structure, processing and stability of mRNA [25]. To date, there are no experimental data to validate this concept.

The *FHL1* gene localises in a region (Xp27.2) that is susceptible to inactivation. The fact that most of the associations presented here were not found in women might be due to the effects of random X chromosome inactivation on FHL1 production [26]. Perhaps a mechanism overriding expression of an allele associated with poor response to exercise training could result in a skewed distribution of muscle FHL1 production. This would result in slight exercise training changes and thus attenuate the genotype–phenotype associations [27]. Testing this hypothesis with female homozygotes for all three SNPs, we found a borderline significance for the S_I response ($p=0.06$) at the rs2236003 SNP and confirmed the associations with D_I and K_g at the rs9018 SNP.

The differences in the regular exercise-induced changes in fasting insulin accounted for by the *FHL1* SNPs did not fully explain the heterogeneity in the responses. They account only for 2 to 3% of the variability in insulin sensitivity and fasting insulin responses. This should not be surprising, given the multifactorial and polygenic nature of the regulation of glucose–insulin metabolism. We did not find any significant associations in the black participants. While we have limited statistical power due to the small number of subjects for some genotypes, this ethnic difference remains to be fully investigated.

Caution is warranted when interpreting our findings. Although decreased S_I, D_I and K_g responses to exercise training were associated with the T allele at the rs2180062 SNP in male individuals, it could also be due to other reasons, such as a type I error (false-positive association). All the reported significance levels were nominal p values and were not adjusted for multiple comparisons. If we had considered that at least 14 tests were performed in this report for each SNP and if the commonly suggested Bonferroni correction had been used, none of the tests would have reached statistical significance. However, the Bonferroni correction is not optimal in this case, because not all of these tests were independent. Only the replication of this study in other cohorts would allow verification of the validity of this initial finding.

The HERITAGE Family Study has revealed that regular exercise improves insulin sensitivity and glucose disposal, although there is large inter-individual variability in the degree of improvement. DNA sequence variations in the *FHL1* gene could be related to these responses. Our data indicate that, in white men, the T allele at the rs2180062 SNP in the *FHL1* gene is associated with decreased S_I, D_I and K_g responses to exercise training.

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