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ARTICLE

TCF7L2 is associated with high serum triacylglycerol and differentially expressed in adipose tissue in families with familial combined hyperlipidaemia

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Abstract
Aims/hypothesis Common DNA variants of the transcription factor 7-like 2 gene (*TCF7L2*) are associated with type 2 diabetes. Familial combined hyperlipidaemia (FCHL) is characterised by hypertriacylglycerolaemia, hypercholesterolaemia, or both. Additionally, disturbances in glucose metabolism are commonly seen in FCHL. Therefore, we hypothesised that *TCF7L2* may contribute to the genetic susceptibility for this common dyslipidaemia.
Methods We investigated the effect of the *TCF7L2* variants, rs7903146 and rs12255372, on FCHL and its component traits triacylglycerol (TG), total cholesterol (TC) and apolipoprotein B (ApoB) in 759 individuals from 55 Mexican families. As a replication sample, 719 individuals from 60 Finnish FCHL families were analysed. We also used quantitative RT-PCR to evaluate the transcript levels of *TCF7L2* in 47 subcutaneous fat biopsies from unrelated Mexican FCHL and normolipidaemic participants.
Results Significant evidence for association was observed for high TG for the T alleles of rs7903146 and rs12255372 (p=0.005 and p=0.01) in Mexican FCHL families. No evidence for association was observed for FCHL, TC, ApoB or glucose in Mexicans. When testing rs7903146 and rs12255372 for replication in Finnish FCHL families, these single nucleotide polymorphisms were associated with TG (p=0.01 and p=0.007). Furthermore, we observed statistically significant decreases in the mRNA levels (p=0.0002) of *TCF7L2* in FCHL- and TG-affected individuals. *TCF7L2* expression was not altered by the SNP genotypes.
Conclusions/interpretation These data show that rs7903146 and rs12255372 are significantly associated with high TG in FCHL families from two different populations. In addition, significantly decreased expression of *TCF7L2* was observed in TG- and FCHL-affected individuals.

Keywords Association · Familial combined hyperlipidaemia · Gene expression · *TCF7L2* · Triacylglycerol

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Abbreviations

ApoB	apolipoprotein B
FCHL	familial combined hyperlipidaemia
INCMNSZ	Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
qRT-PCR	quantitative RT-PCR
QTDT	quantitative transmission disequilibrium test
QTLD	quantitative trait linkage disequilibrium
SNP	single nucleotide polymorphism
TC	total cholesterol
TCF7L2	transcription factor 7-like 2
TG	triacylglycerol

Introduction

Recently intronic variants in the transcription factor 7-like 2 gene (*TCF7L2* [MIM602228]) were strongly associated with type 2 diabetes in samples from Iceland, the USA and Denmark [1]. Since the original report, these findings have been replicated in numerous type 2 diabetes studies [2–7]. The evidence of association with type 2 diabetes seems to be strongest in Europids [1–4]. TCF7L2 is a high mobility group box-containing transcription factor that has a role in the WNT signalling pathway. WNT proteins are powerful regulators of cell proliferation and differentiation, and their signalling pathway involves proteins that directly participate in gene transcription and cell adhesion [8]. Alterations of the WNT signalling pathway have been found in human diseases, including cancer and skeletal, neuronal and cardiovascular disorders [9, 10].

Familial combined hyperlipidaemia (FCHL) is a complex genetic disorder, associated with coronary artery disease. Affected individuals with FCHL may present with hypertriacylglycerolaemia, hypercholesterolaemia or mixed hyperlipidaemia [11]. FCHL profiles have also been associated with clinical features observed in type 2 diabetes, such as hypertriacylglycerolaemia, hyperinsulinaemia and impaired glucose tolerance [12, 13]. Considering this phenotypic overlap, we hypothesised that the *TCF7L2* variants previously associated with type 2 diabetes might also contribute to the FCHL phenotype. In this study, we investigated the role of the rs7903146 and rs12255372 variants, previously associated with type 2 diabetes [1], in FCHL and its component traits total cholesterol (TC), triacylglycerol (TG) and apolipoprotein B (ApoB) in multigenerational Mexican FCHL families. In addition, we tested fasting glucose for association in these Mexican FCHL families. The observed results for TG were tested for replication in Finnish FCHL families. We also examined *TCF7L2* transcript levels in fat biopsies of 47 Mexican unrelated FCHL cases and control individuals for differential gene expression.

Methods

Mexican FCHL families We investigated 55 extended Mexican FCHL families with a history of premature CHD, comprising 759 family members. All participants studied were Mexican Mestizos. These families were recruited in the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) in Mexico City, using the inclusion and exclusion criteria described previously [14, 15]. The Mexican age-specific and sex-specific (age/sex-specific) 90th percentiles for TC and TG [16] were used to classify the FCHL affection status. A total of 58 individuals were classified as type 2 diabetes-affected using the American Diabetes Association criteria [17]. Each participant provided written informed consent. The study design was approved by the ethics committees of the INCMNSZ and UCLA.

Finnish FCHL families We genotyped 719 Finnish individuals from 60 FCHL families. The families were recruited in the Helsinki and Turku University Central Hospitals, as described previously [18–20]. All study participants gave their informed consent before participating in the study and were collected in agreement with the Helsinki Declaration. The inclusion criteria for FCHL probands were: TC and/or TG levels ≥age/sex-specific 90th Finnish population percentile. To ensure that families were affected with the strict original FCHL criteria of Goldstein et al. [11], we also used premature CHD as an inclusion criterion for the probands, in accord with our earlier studies [19, 20]. CHD was confirmed either by angiography (>50% stenosis in one or more coronary arteries) or probands having suffered from myocardial infarction. In these families 40 individuals were classified as type 2 diabetes-affected. The study design was approved by the ethics committees of the participating centres.

The phenotypic characteristics of the FCHL-affected and unaffected individuals in the Mexican and Finnish FCHL families are presented in Table 1.

Biochemical analyses of Mexican and Finnish families In the Mexican FCHL families, all the measurements were performed by the Endocrinology and Metabolism Department of INCMNSZ with commercially available standardised methods, as previously described [14]. In the Finnish families, serum lipid and glucose parameters were measured as described earlier [18–20]. Patients who used lipid-lowering drugs were studied after their lipid-lowering treatment was withdrawn for 4 weeks, because based on the data of statin elimination rate and LDL kinetics, 1 month has been clinically recognised as a washout period for statins.

Table 1 Phenotypic characteristics of the 55 Mexican and 60 Finnish FCHL families included in the study

	Mexican		Finnish	
	FCHL-affected (M/F)	Unaffected (M/F)	FCHL-affected (M/F)	Unaffected (M/F)
n	261 (104/157)	337 (169/168)	322 (143/179)	460 (228/232)
Age (years)	41.0±12.3/44.0±11.7	41.9±15.8/41.4±13.9	42.6±11.7/47.1±5.3	45.0±14.3/42.4±14.2
BMI (kg/m^2)	26.9±4.5/26.6±4.3	26.4±4.2/26.0±3.5	27.7±3.6/27.7±4.6	26.0±4.6/24.7±4.4
TG (mmol/l)	4.0±2.5/3.1±1.6	2.2±0.9/1.5±0.8	3.8±2.5/3.3±1.3	1.5±0.6/0.8±0.6
TC (mmol/l)	6.3±1.2/6.5±1.0	4.9±0.8/4.9±0.8	7.4±1.0/7.1±1.2	5.6±0.9/5.4±0.8
ApoB (g/l)	1.2±0.3/1.3±0.3	1.0±0.2/0.9±0.2	1.5±0.4/1.3±0.3	1.0±0.2/0.9±0.2
Glucose (mmol/l)	5.3±1.6/4.8±0.9	4.9±1.3/4.6±0.7	4.9±0.8/5.2±1.8	4.7±0.8/4.8±0.8

Values are means ± SD
M/F, male/female
FCHL status: TG and/or TC 90th age/sex-specific Mexican and Finnish population percentiles

Single nucleotide polymorphism genotyping We genotyped 1,478 individuals from the 55 extended Mexican FCHL families and 60 Finnish FCHL families for the single nucleotide polymorphisms (SNPs) rs7903146 and rs12255372. Primers were designed for PCR, using the Primer3 program, and for detection, using the SNP Primer Design software (Pyrosequencing). The Genotyping was performed with the Pyrosequencing technique on the automated PSQ HS96A platform (Biotage, Uppsala, Sweden). The genotype call rate was 95% for both SNPs. For quality control, we replicated 3.5% of the genotyped samples. The percentage agreement between samples was >99%. The genotype error rate was 0.6% for both SNPs. The genotypes of the individuals with Mendelian errors were called as 0 in the analysis. Both SNPs were tested for a possible violation of Hardy–Weinberg equilibrium (HWE) in non-related groups of family members (founders, probands and spouses). Both SNPs were in HWE in all of these groups.

Statistical analysis Association analyses of qualitative and quantitative traits in Mexicans were performed using the quantitative transmission disequilibrium test (QTDT) [21] implemented in the genetic analysis package SOLAR [22–24]. The QTDT approach is robust to population stratification [23] and has been recognised as a powerful method that utilises data from all available relatives. In more homogeneous populations without population stratification such as the Finns, the quantitative trait linkage disequilibrium (QTLD) option is more powerful than the QTDT [23], because it utilises additional information by using also the founder genotypes. Stratification was tested as part of the QTLD procedure, which was further used in the analyses of the Finnish families. To evaluate the independent effect of the SNPs on TG, the analyses were performed using fasting glucose measurements as a covariate. For fasting glucose, the quantitative association analysis was performed by including age and sex as covariates. The glucose values were $\log_{10}$ transformed to approach a normal distribution. We also performed the association analysis for lipid traits by excluding the individuals with type 2 diabetes. Odds ratios and marginal effects, both adjusted for relatedness, were calculated for the qualitative lipid traits from the logistic regression coefficient obtained from SOLAR. For fasting glucose, analysed as a continuous trait, the effect size is provided as a β-coefficient obtained from SOLAR. Allele frequencies were estimated from all individuals using the DOWNFREQ program [25]. The PedCheck program was used to assess the genotype data for pedigree inconsistencies [26].

Real-time quantitative RT-PCR (qRT-PCR) Total RNA from 47 fat biopsies from umbilical subcutaneous adipose tissue under local anaesthesia from 31 Mexican unrelated cases (nine female, 22 male, aged 37.3±11.7 years, BMI 26.4±2.3 kg/m^2, TG 4.5±2.7 mmol/l, TC 7.0±1.6 mmol/l) and 16 unrelated control individuals (ten female, six male, aged 34.4±12.5 years, BMI 25.1±3.5 kg/m^2, TG 0.98±0.4 mmol/l, TC 4.3±0.6 mmol/l) was isolated. Of these individuals, seven had type 2 diabetes. The RNA extraction has been described in detail previously [27, 28]. The integrity of the RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent, Foster City, CA, USA). Synthesis of cDNA from 1 μg of Mexican FCHL fat biopsy total RNA was performed using the established methods for the SuperScript III First-Strand Synthesis System for RT-PCR (Qiagen, Valencia, CA, USA; P/N: 18080-051).

The real-time quantitative RT-PCR (qRT-PCR) was performed according to established methods from Qiagen as outlined in the manual for the QuantiTect SYBR Green PCR Handbook. The qRT-PCR was performed in 10 μl reactions using QuantiTect SYBR Green PCR Kit (Qiagen; P/N: 204125) on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, CA,

USA). Primer pairs were designed so that one primer was on an exon–exon junction. Primer sequences are listed in Electronic supplementary material Table 1. Two housekeeping genes were used to normalise the expression: *B2M* and *HPRT*. A Student's *t* test was applied to analyse for differential expression based on FCHL, TG, TC, ApoB, glucose and the SNP genotypes. The individuals defined as affected for FCHL were also affected for the TG trait. The real-time qRT-PCR experiments were performed twice, independently, using duplicates for each sample.

Results

We tested whether the *TCF7L2* variants, rs7903146 and rs12255372, previously associated with type 2 diabetes [1], also contribute to the FCHL component traits and glucose levels in Mexicans with FCHL. In addition we analysed an independent study sample of Finnish FCHL families for replication. We restricted the association analyses with the lipid traits to qualitative analyses using the Mexican and Finnish age/sex-specific 90th population percentiles as cutoffs, because these FCHL families were ascertained through a proband, and an additional first-degree relative with high TC or TG, resulting in reduced variance of the TC and TG levels. Therefore, in these families, the variance of the quantitative lipid traits may be limited for effective quantitative analysis.

The allele frequencies of the T allele in the Mexicans were 0.15 and 0.16 for the SNPs rs7903146 and rs12255372, respectively, and the linkage disequilibrium between these SNPs was D'=0.86, r^2=0.7 in 134 unrelated spouses. We utilised the QTDT analysis in the Mexican families to test for association, and results with a p value ≤0.05 were considered significant, because *TCF7L2* is a candidate gene and the lipid traits represent correlated traits. In these analyses, we corrected for glucose levels by including fasting glucose values as a covariate in the analysis in order to separate the known effects of *TCF7L2* on diabetes-related traits from its potential effects on lipids in FCHL. We observed significant evidence for association for high TG levels for the T alleles of both rs7903146 and rs12255372 SNPs (p=0.005 and p=0.01, respectively) in the Mexican FCHL families. The association results remained significant when we excluded the 58 Mexican participants with type 2 diabetes from the analysis (p=0.007 for rs7903146; and p= 0.01 for rs12255372; Table 2). The means by genotype of the analysed traits for SNPs rs7903146 and rs12255372 in the Mexicans are presented in Table 3. We also estimated the effect sizes for all tested traits (Table 4). For TG, the observed odds ratios were 1.83 (p=0.005) and 1.67 (p=0.01) for rs7903146 and rs12255372, respectively. Using the odds ratios we calculated that each copy of the T allele increased the probability of having high TG by 8 and 7% for SNPs rs7903146 and rs12255372, respectively, in these Mexican FCHL families. No consistent evidence of association was observed between the FCHL, TC or ApoB traits and either of the SNPs, and the p values remained non-significant (Table 4). Neither was the quantitative glucose trait associated with the *TCF7L2* variants (Table 4). The latter result may, however, reflect the small number of type 2 diabetes-affected participants (n=58) in these Mexican FCHL families.

To replicate this association with TG, we investigated the two SNPs for association with high TG in 60 extended Finnish FCHL families. In the Finns, the allele frequencies of the T allele were 0.19 and 0.17 for the SNPs rs7903146 and rs12255372, respectively, and the linkage disequilibrium between these SNPs was D'=0.89, r^2=0.7 in 144 unrelated spouses. Again utilising a qualitative approach where the TG trait was dichotomised based on the 90th age/sex-specific

Table 2 Association results of the *TCF7L2* variants for high TG adjusted for glucose levels in 55 Mexican and 60 Finnish FCHL families

Sample and SNP	Major/minor allele	MAF spouses	Associated allele	p value (including type 2 diabetes participants)	p value (excluding type 2 diabetes participants)
Mexicans					
rs7903146	C/T	0.15	T	0.005[a] (n=759)	0.007[a] (n=701)
rs12255372	G/T	0.16	T	0.01[a] (n=759)	0.01[a] (n=701)
Finns					
rs7903146	C/T	0.19	T	0.01[b] (n=719)	0.01[b] (n=679)
rs12255372	G/T	0.17	T	0.007[b] (n=719)	0.008[b] (n=679)

The TG status was defined using the Mexican and Finnish 90th age/sex-specific population percentiles, respectively. Fasting glucose levels were used as a covariate in these analyses

[a] p values were calculated using the QTDT test as implemented in the SOLAR genetic package

[b] p values were calculated using the QTLD test as implemented in the SOLAR genetic package

n indicates the number of individuals included in the analysis

MAF, minor allele frequency

Table 3 Mean by genotype of the analysed traits for SNPs rs7903146 and rs12255372 in the Mexican and Finnish FCHL families

	Male	Female	Male	Female	Male	Female
rs7903146						
Mexicans	C/C (n=514)		C/T (n=176)		T/T (n=25)	
TG (mmol/l)	1.9±0.59	1.6±0.49	2.2±0.58	1.8±0.59	2.5±0.70	1.9±0.56
TC (mmol/l)	5.1±1.09	5.4±1.16	5.2±1.08	5.6±1.37	5.6±1.46	5.5±1.19
ApoB (g/l)	1.0±0.28	1.0±0.29	1.0±0.30	1.1±0.32	1.2±0.27	1.1±0.27
Glucose (mmol/l)	4.8±0.42	4.6±0.37	5.0±0.51	4.9±0.50	4.5±0.39	5.0±0.97
Finns	C/C (n=457)		C/T (n=213)		T/T (n=22)	
TG (mmol/l)	1.7±0.54	1.5±0.39	2.0±0.57	1.4±0.30	2.0±0.60	1.9±0.49
rs12255372						
Mexicans	G/G (n=507)		G/T (n=190)		T/T (n=21)	
TG (mmol/l)	1.9±0.58	1.7±0.51	2.0±0.63	1.8±0.60	2.8±0.75	2.1±0.58
TC (mmol/l)	5.1±1.07	5.4±1.19	5.2±1.17	5.6±1.28	5.5±1.52	5.8±1.59
ApoB (g/l)	1.0±0.29	1.0±0.29	1.0±0.30	1.1±0.30	1.2±0.35	1.1±0.36
Glucose (mmol/l)	4.8±0.43	4.6±0.38	4.8±0.48	4.8±0.40	4.8±0.29	4.7±0.40
Finns	G/G (n=467)		G/T (n=195)		T/T (n=19)	
TG (mmol/l)	1.7±0.54	1.4±0.37	2.0±0.60	1.5±0.33	2.2±0.57	1.5±0.41

Values are means ± SD in the genotype groups of related family members. For traits not normally distributed, values were back-transformed from a $\log_{10}$ transformation
Glucose values are fasting glucose

Finnish population percentiles [16] and fasting glucose included as a covariate, we observed significant associations with the T alleles of rs7903146 and rs12255372 for high TG levels (p=0.01 and p=0.007) using the QTLD test. As in the Mexicans, we also observed significant evidence for association when we excluded the 40 Finnish participants with type 2 diabetes from the analyses (p=0.01 for rs7903146; and p=0.008 for rs12255372; Table 2). The means by genotype of the TG trait for SNPs rs7903146 and rs12255372 in the Finns are presented in Table 3.

Table 4 Effect sizes of the analysed traits in Mexican and Finnish FCHL families

	Affection status	rs7903146		rs12255372	
		p value	Effect size[a]	p value	Effect size[a]
Mexicans[b]	TG	0.005	1.83	0.01	1.67
	TC	0.63	1.09	0.90	0.98
	FCHL	0.11	1.33	0.30	1.19
	ApoB	0.09	1.34	0.39	1.16
	Glucose ($\log_{10}$)	0.58	0.005	0.35	0.008
Finns[c]	TG	0.01	1.45	0.007	1.51

[a] For TG, TC, FCHL and ApoB analysed as qualitative traits, the effect size is measured by odds ratio; for fasting glucose ($\log_{10}$ transformed) analysed as a continuous trait, the effect size is the regression coefficient
[b] p values were calculated using the QTDT test as implemented in the SOLAR genetic package
[c] p values were calculated using the QTLD test as implemented in the SOLAR genetic package

In the Finnish families the odds ratios for high TG were 1.51 (p=0.007) and 1.45 (p=0.01) for rs12255372 and rs7903146 (Table 4). Using the odds ratios we calculated that each copy of the T allele increased the probability of having high TG by 7 and 8% for SNPs rs7903146 and rs12255372, respectively, in these Finnish FCHL families.

To investigate transcript levels of *TCF7L2* for differential expression in FCHL, we performed real-time qRT-PCR analysis in 47 subcutaneous fat biopsies from Mexican FCHL cases and normolipidaemic control individuals. The clinical characteristics of these participants are shown in the Methods. We observed statistically highly significant decreases in the mRNA levels of *TCF7L2* in the FCHL- and TG-affected participants (p=0.0002; Fig. 1a,b). We also observed significant associations in the TC- (p=0.006) and ApoB- (p=0.003) affected participants (Fig. 1c,d), reflecting the fact that many of the FCHL cases also exhibit high TC and ApoB levels. *TCF7L2* expression was not altered by the SNP genotypes. No differences in the *TCF7L2* expression were observed between cases and control individuals for glucose levels or type 2 diabetes. The latter may be due to the small number of type 2 diabetes participants (n=7) among the Mexican individuals who underwent fat biopsy.

Discussion

Recent studies have consistently shown that *TCF7L2* is a strong susceptibility gene for type 2 diabetes [2–7].

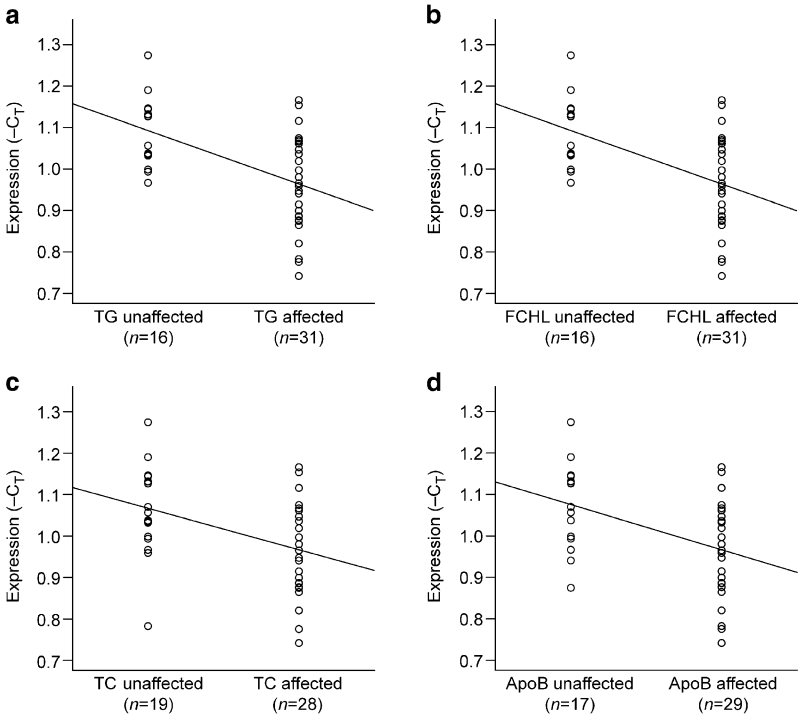

Fig. 1 Differential expression of *TCF7L2* between phenotypic groups stratified based on the 90th age/sex-specific percentiles. Expression is plotted for unaffected and affected individuals on the *y*-axis using negative cycle threshold (C_T), which is a relative measure of expression. A regression line has been added to the plots to show the trend of the difference in means. **a** Expression of *TCF7L2* for the TG trait; *t* statistic −4.2, $p=0.0002$ from Student's *t* test. **b** Expression of *TCF7L2* for the FCHL trait; *t* statistic −4.2, $p=0.0002$. **c** Expression of *TCF7L2* for the TC trait; *t* −2.9, $p=0.006$. **d** Expression of *TCF7L2* for the ApoB trait; *t* −3.2, $p=0.003$

Although several studies support the association between type 2 diabetes and *TCF7L2* variants [1–7], the underlying pathophysiological mechanisms of the associated TC7L2 variants are not known. In the present study, we investigated whether the *TCF7L2* variants, rs7903146 and rs12255372, previously strongly associated with type 2 diabetes, also contribute to the FCHL component traits and glucose levels in 55 Mexican FCHL families. Furthermore, the observed results were analysed for replication in 60 Finnish FCHL families. Our data indicate that rs7903146 and rs12255372 show significant evidence for association with high TG for the same risk allele in both the Mexican and Finnish FCHL study samples.

In the Mexican study sample, the allele frequencies of the T alleles and the linkage disequilibrium between the SNPs rs7903146 and rs12255372 were lower than the corresponding values reported in European and African-American controls for type 2 diabetes [1–5]. The allele frequencies for the T alleles of rs7903146 and rs12255372 observed in this study were, however, in a good agreement with a previous *TCF7L2* study for type 2 diabetes in Mexicans [29]. Regarding the Finns, the allele frequencies of this study correspond well with the allele frequencies reported previously in Finns for type 2 diabetes [30]. The data presented in this study provide strong evidence of an association between *TCF7L2* gene variants and high TG levels in FCHL families from two different populations. Although we observed differences in the allele frequencies for these variants between Mexican and Finnish populations, we detected significant evidence for association with high TG for the same SNPs. Importantly, the results were further supported by gene expression analysis of 47 fat biopsies from Mexican FCHL-affected individuals and controls.

We observed significantly lower gene expression of *TCF7L2* in unrelated TG- and FCHL-affected individuals than in unrelated normolipidaemic controls. In agreement with previous studies performed in subcutaneous fat biopsies from type 2 diabetes cases and controls [31–33], *TCF7L2* expression was not altered by the rs7903146 and rs12255372 genotypes. It is worth noting that in these previous studies the *TCF7L2* expression was, however, lower in type 2 diabetes-affected individuals. The function of *TCF7L2* in adipose tissue is not known. We hypothesise that *TCF7L2* and its as yet unknown variants may be involved in adipogenesis or adipose function by altering transcriptional regulation of genes leading to deposition TG.

We have previously reported that the upstream transcription factor 1 (USF1) and the hepatic nuclear factor-4 alpha (HNF4A) transcription factor are implicated in FCHL [14, 15, 18]. In the present study, we demonstrate for the first time that a third transcription factor, TCF7L2, previously associated with type 2 diabetes is also associated with a primary FCHL component trait, TG, in Mexican and Finnish FCHL families. As all of these transcription factors regulate a cascade of downstream genes, it is tempting to hypothesise that relatively minor changes in transregulation

of multiple target genes of USF1, HNF4A and TCF7L2 contribute to the complex FCHL phenotype. Further studies to explore the role of *TCF7L2* in FCHL could include resequencing of *TCF7L2* and regional linkage disequilibrium analysis in both Mexican and Finnish FCHL participants. This should help identify additional variants to be tested for differences in gene expression and functional significance in FCHL.

To the best of our knowledge, these data are the first to demonstrate that the T alleles in rs7903146 and rs12255372 are significantly associated with high TG levels in Mexican FCHL families, and a replication of this association in an independent study sample of Finnish FCHL families. The observed significant differential expression of *TCF7L2* between Mexican FCHL- and TG-affected participants and normolipidaemic control individuals provides functional evidence that *TCF7L2* is indeed involved in the pathogenesis of FCHL.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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