RESEARCH NOTE

Polymorphisms in *sh2b1* and *spns1* loci are associated with triglyceride levels in a healthy population in northern Sweden

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[Västermark Å., Jacobsson J. A., Johansson Å., Fredriksson R., Gyllensten U. and Schiöth H. B. 2012 Polymorphisms in *sh2b1* and *spns1* loci are associated with triglyceride levels in a healthy population in northern Sweden. *J. Genet.* **91**, 237–240]

Introduction

SH2B1, which encodes a signal transduction adaptor protein that interacts with multiple receptors including insulin and leptin receptors, has been established, together with other genes, as an obesity locus. Here, we studied the association of eight single nucleotide polymorphisms (SNPs) in four genes (*SH2B1*, *TMEM18*, *KCTD15*, and *NEGR1*) with obesity-related phenotypes in the North Swedish Population Health Study (NSPHS) cohort, consisting of 719 individuals from Karesuando parish in northern Sweden. We found that the known obesity SNPs in *SH2B1*, rs4788102 ($P = 0.0023$) and rs7498665 ($P = 0.0018$), were associated with triglyceride levels. To account for kinship, the *SH2B1* SNPs and four SNPs in the expanded region were analysed for association with triglyceride levels using SOLAR (sequential oligogenic linkage analysis routines). We found a stronger signal ($P = 0.0009$) for a SNP near *SH2B1*, rs8045689, located in an intron of *SPNS1* which encodes a protein that is structurally similar to a sphingolipid transporter. It is possible that disordered sphingolipid metabolism may influence triglyceride levels.

Genomewide association studies (GWAS) have recently identified many genes associated with obesity (Speliotes *et al.* 2010). In this study, we looked at eight SNPs in four relatively uncharacterized genes (Willer *et al.* 2009): sarcoma homology 2B1 (*SH2B1*), transmembrane protein 18 (*TMEM18*), potassium channel tetramerization domain (*KCTD15)*, and neuronal growth regulator 1 (*NEGR1*). We studied associations with 14 obesity-related phenotypes in NSPHS cohort (Igl *et al.* 2010), which differs in several respects from other as well as typical Swedish populations, including diet and exposure to cold climate, which may influence their obesity susceptibility caused by these genetic variants.

Replication of genetic variants identified through GWAS in independent cohorts is important as it solidifies the original associations. Smaller cohorts also allow the testing for relationship of genetic variants to other phenotypic variables, thus specifying the effect carried by the genetic variant. We chose to study eight SNPs based on the overlapping findings presented by Willer *et al.* (2009) and Thorleifsson *et al.* (2009). These SNPs are frequent in the population and relatively uncharacterized.

Of the study population, 15% follow a traditional lifestyle (TLS), including a three-fold higher consumption of game meat (Igl *et al.* 2010). The TLS individuals display higher total and low-density lipoprotein cholesterol, and the TLS women display a significantly higher body mass index, (BMI, 27.67 kg/m^2) compared to the modern-lifestyle women. It is known that prolonged exposure to Arctic climate can lower triglyceride levels, and elevate high-density lipoprotein (HDL) levels (Bojko and Larsen 1999). Inuits, another group exposed to the Arctic climate, rank highly in international comparisons of waist circumference, despite a high level of physical exercise (Hopping *et al.* 2010).

Methods

The NSPHS cohort consists of 719 individuals (339 men and 380 women), of average age 47 years, of which 391 individuals had at least one parent who was also a participant in this study.

Fourteen obesity-related phenotypes were studied: i) height (cm), ii) weight (kg), iii) hip size (cm), iv) BMI

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Keywords. Karesuando; NSPHS; *SH2B1* gene; SPNS1; triglycerides; SOLAR.

(kg/m^2), v) pulse (bpm), vi) systolic blood pressure (mm Hg), vii) diastolic blood pressure (mm Hg), viii) total cholesterol (TC, mg/dL), ix) LDL (mg/dL), x) HDL (mg/dL), xi) triglycerides (TG, mg/dL), xii) creatinine (mg/dL), xiii) glutamic pyruvic transaminase (units/litre), xiv) gamma-glutamyltransferase (units/litre). The phenotype data were collected as previously described (Igl *et al.* 2010).

Eight SNPs were selected from four obesity loci (Willer *et al.* 2009): rs3101336 and rs2815752 (*NEGR1*), rs6548238 and rs7561317 (*TMEM18*), rs4788102 and rs7498665 (*SH2B1*), rs29941 and rs11084753 (*KCTD15*). The genotyping was carried out using predesigned Taqman SNP genotyping assays (Applied Biosystems, Foster City, USA), and an ABI7900 genetic analyzer with SDS 2.2 software (Applied Biosystem) at the Uppsala Genome Center. Four additional SNPs in close proximity to the *SH2B1* locus were genotyped using a chip (Igl *et al.* 2010): rs9937676, rs8045689, rs8049837 and rs6565174.

To test for deviations from Hardy–Weinberg equilibrium, the Pearson's χ^2-test (1 d.f.) was applied. Quantitatively skewed variables were transformed using inverse hyperbolic functions to obtain normally distributed variables. Associations between genotypes and phenotypes were analysed using linear regression, assuming an additive model. The model was adjusted for age, sex and BMI. False discovery rate (FDR) (Benjamini and Hochberg 1995) was used to adjust for multiple testing, setting the 'q' parameter to 0.5 and $N = 112$ (14×8). All initial analyses were performed in PLINK 1.07 (Purcell *et al.* 2007). To adjust the models for relatedness among individuals, we used the 'polygenic-screen' command in SOLAR 6.3.0 (Almasy and Blangero 1998). Power calculations for quantitative traits were performed assuming an additive model using QUANTO software (v1.2.4, http://hydra.usc.edu/gxe/).

Results

We tested eight SNPs (table 1) for association with 14 obesity-related phenotypes using a linear regression model in PLINK (table 2). Two SNPs from *SH2B1* superseded the corresponding critical values; q-value is the FDR analogue of the p-value; qi is the q value of a hypotheis; m is the total number of hypotheses being tested (q_i/m), i.e. passed the FDR test. They showed association with triglycerides: rs4788102 ($P = 0.0023$) and rs7498665 ($P = 0.0018$). However, we were not able to replicate previous associations with BMI for the other six SNPs (Willer *et al.* 2009). We have between 5.4–19.7% power to detect the observed changes per allele, corresponding to a −0.056–0.344 change in beta value. We could be 80% sure to detect an effect size of 0.9. This means that, per allele, we have 80% power to detect a perallele change of 3.4% in BMI.

To account for the kinship in the population, the *SH2B1* SNPs previously associated with obesity and four additional SNPs in the same region were analysed for association with triglyceride levels using SOLAR (table 3). In all cases except one (rs9937676), the associations were slightly weakened when we adjusted for the pedigree. Of the six SNPs tested, rs8045689 (located in either intron 1 or 2, depending on splice variant of *spns1*, spinster homologue 1), displayed the most significant association: $P = 0.0009$. This SNP is in linkage disequilibrium with the *SH2B1* SNPs: $r^2 = 0.62$ (rs8045689 and rs4788102); $r^2 = 0.63$ (rs8045689 and rs7498665).

Discussion

In this study we evaluated eight SNPs that have previously been associated with obesity, also in northern European

Table 1. Properties and BMI association of eight obesity-related SNPs in the Karesuando population.

SNP	Chr: bp (locus)	A1	A2	MAF (A1)	HWE	Call rate (%)	BMI
rs3101336	1: 72751185 (*NEGR1*)	A	G	0.39	0.5769	0.971	β = −0.056 $P = 0.8335$
rs2815752	1: 72812440 (*NEGR1*)	C	T	0.39	0.7500	0.971	β = −0.07654 $P = 0.7743$
rs6548238	2: 634905 (*TMEM18*)	A	G	0.21	0.1127	0.971	β = −0.3145 $P = 0.3031$
rs7561317	2: 644953 (*TMEM18*)	A	G	0.21	0.0707	0.964	β = −0.3307 $P = 0.2782$
rs4788102	16: 28873398 (*SH2B1*)	A	G	0.49	0.0338	0.969	β = 0.3119 $P = 0.2109$
rs7498665	16: 28883241 (*SH2B1*)	G	A	0.49	0.0154	0.972	β = 0.3538 $P = 0.1531$
rs29941	19: 34309532 (*KCTD15*)	A	G	0.46	0.1709	0.969	β = −0.1712 $P = 0.5219$
rs11084753	19: 34322137 (*KCTD15*)	T	C	0.38	0.2615	0.969	β = 0.2555 $P = 0.327$

MAF, minor allele frequency; HWE, indicates *P*-values for deviation from Hardy–Weinberg equilibrium using Pearson's χ^2-test (1 d.f.), excluded if $P < 0.001$.

Table 2. Obesity-related phenotypes, average of variables in the Karesuando population.

Phenotype	Mean ± SD, in NSPHS dataset, ($N = 719$)
Height (cm)	164.46 ± 9.56
Weight (kg)	71.55 ± 15.31
Hip size (cm)	96.41 ± 13.81
BMI (kg/m^2)	26.41 ± 4.82
Pulse (bpm)	72.44 ± 5.75
Systolic blood pressure (mm Hg)	122.78 ± 18.64
Diastolic blood pressure (mm Hg)	74.16 ± 7.84
Total cholesterol (TC, mg/dL)	226.40 ± 51.78
Low-density lipoprotein (LDL, mg/dL)	136.51 ± 42.28
High-density lipoprotein (HDL, mg/dL)	61.67 ± 15.57
Triglycerides (TG, mg/dL)	196.72 ± 136.39
Creatinine (mg/dL)	0.84 ± 0.19
Glutamic pyruvic transaminase (units/litre)	25.24 ± 14.39
Gamma-glutamyltransferase (units/litre)	24.30 ± 35.47

The average triglyceride level for the minor allele homozygotes of rs8045689 is 228.4 mg/dL.

Table 3. Association with triglyceride levels and BMI in regions on chromosome 16.

SNP	Location	Allele	MAF (A1)	Triglyceride levels Pedigree+sex+age	Triglyceride levels Pedigree+sex+age+BMI	BMI Pedigree+sex+age
rs4788102	28873398, upstream *SH2B1*	A	0.49	β = +19.4 mg/dL $P = 0.008$	β = +18.7 mg/dL $P = 0.009$	β = +0.312 kg/m^2 $P = 0.6194$
rs7498665	28883241, *SH2B1*	G	0.49	β = +20.9 mg/dL $P = 0.004$	β = +19.7 mg/dL $P = 0.006$	β = +0.354 kg/m^2 $P = 0.3916$
rs9937676	28969480, *NFATC2IP*	G	0.39	β = −16.8 mg/dL $P = 0.0337$	β = −15.4 mg/dL $P = 0.0478$	β = −0.422 kg/m^2 $P = 0.4247$
rs8045689	28988269, intron of *SPNS1*	A	0.44	β = +25.5 mg/dL $P = 0.0009$	β = +24.8 mg/dL $P = 0.0009$	β = +0.203 kg/m^2 $P = 0.9984$
rs8049837	30089438, intron of *PPP4C*	A	0.025	β = +13.15 mg/dL $P = 0.7022$	β = +13.15 mg/dL $P = 0.6472$	β = +0.748 kg/m^2 $P = 0.8595$
rs6565174	30111904, intron of *GDPD3*	A	0.073	β = −10.97 mg/dL $P = 0.5761$	β = −10.97 mg/dL $P = 0.6606$	β = −0.598 kg/m^2 $P = 0.361$

The table presents beta values for the untransformed variable, and *P*-values. Location on Hsa XVI (bp), as from build 37.3 (GRCh37.p5).

cohorts. For example, rs7498665 was found to be significantly associated with obesity in a study comprising 18,014 middle-aged Danes (Sandholt *et al.* 2011), and in a study comprising 4923 adults from northern Sweden (Renstrom *et al.* 2009). We were not able to replicate these previous findings most likely due to the relatively small effect of these SNPs and our comparatively small sample size. However, the effect is in the same direction (allele G, β = +0.15) as previously reported for rs7498665 (Willer *et al.* 2009).

However, we discovered an association with triglyceride levels, not previously shown for rs4788102, which is located near *SH2B1*. The NSPHS cohort has been investigated as one of 45 cohorts in a meta-GWAS of blood lipid loci in more than 100,000 individuals (Teslovich *et al.* 2010), in which the *SH2B1* locus was not found to be associated with blood lipids. However, a study where *SH2B1* was knocked out in mice showed that this resulted in elevated triglyceide levels in plasma (Ren *et al.* 2007).

When accounting for kinship we found a stronger signal than that reported for the SNP near *SH2B1*, namely one SNP (rs8045689) located within *SPNS1*, which codes for a protein structurally similar to a sphingolipid transporter (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SPNS1). Sphingolipids are synthesized from fatty acids, and it is possible that disorders of sphingolipid metabolism (sphingolipidoses; Ohno 2011) may influence triglyceride levels indirectly. Sphingolipid synthesis requires palmitate and triglyceride availability (Belalcazar and Ballantyne 2011), and accumulation of ceramides (hydrogen-substituted sphingolipids) slows anabolism and is associated with insulin resistance (Bikman and Summers 2011). A formal fine-mapping approach would require more SNPs in the *SPNS1* region.

Acknowledgements

We thank Svea Hennix, District nurse, Karesuando parish and Charles Peterson, Texas Biomedical Research Institute, and Inger Jonasson for logistics and coordination of the health survey, for help with SOLAR. The studies were supported by the Swedish Research Council. The NSPHS as part of EUROSPAN (European Special Populations Research Network) was also supported by European Commission FP6 STRP grant number 01947 (LSHG-CT-2006-01947).

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Received 23 February 2012, in revised form 26 April 2012; accepted 10 May 2012
Published on the Web: 24 July 2012