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Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: A study in monozygotic and dizygotic twins☆

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***Background/Aims*: This study aimed to determine the heritability of serum alanine aminotransferase (S-ALT) and fasting serum insulin (fS-insulin) concentration as well as determine the association of these measures with liver fat content in young adult monozygotic (MZ) and dizygotic (DZ) twins.**

***Methods*: Three hundred and thirteen individual twins were recruited from a population-based cohort ($n = 4929$). The study subjects represented a wide range of body mass indexes (BMI), were free of any diseases or regular medications and had an intake of less than two drinks of alcohol/day. To verify that S-ALT is a marker of liver fat, it was measured by proton magnetic resonance spectroscopy (^{1}H MRS) in 66 subjects. Heritability estimations were performed using BMI- and gender-adjusted values.**

***Results*: Intra-pair correlations were significantly higher in the MZ twins than the DZ twins for both S-ALT (0.65 for MZ and 0.04 for DZ) and fS-insulin (0.58 and 0.34, respectively). Heritability of S-ALT was 55% and that of fS-insulin 61%. In the 66 subjects S-ALT ($r = 0.70$ for women and $r = 0.50$ for men, $p \leqslant 0.01$ for both) and fS-insulin ($r = 0.58$ and $r = 0.59$, respectively, $p \leqslant 0.01$ for both) concentrations correlated significantly with liver fat content.**

***Conclusions*: These twin data suggest that approximately 60% of the variation in S-ALT, a marker of liver fat content, is genetically determined.**

***Keywords*: Heritability; ALT; Insulin; Liver fat; NAFLD; Twins**

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Abbreviations: AIC, Akaike Information Criterion; BMI, body mass index; CI, confidence interval; DEXA, dual-energy X-ray absorptiometry; d.f., degrees of freedom; DZ, dizygotic; fS-, fasting serum; fP-, fasting plasma; ^{1}H MRS, proton magnetic resonance spectroscopy; MRI, magnetic resonance imaging; MZ, monozygotic; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; S-ALT, serum alanine aminotransferase; S-AST, serum aspartate aminotransferase; S-GGT, serum gamma glutamyl transferase; SNP, single nucleotide polymorphism.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of steatosis and elevated liver enzymes in the US [1]. NAFLD is defined as fat accumulation in the liver exceeding 5–10% by weight in subjects who do not consume significant amounts of alcohol and who do not have clinical or laboratory evidence of autoimmune, viral or toxin-induced liver disease, or of inborn errors of metabolism [2]. An increase in liver fat due to NAFLD is an integral feature of the metabolic syndrome and closely correlated with all its components; central obesity, insulin resistance, dyslipidemia and hypertension [3].

Liver fat and serum alanine aminotransferase (S-ALT) correlate with body mass index (BMI), but the relationship is weak [4]. Thus, at any given BMI, liver fat content and S-ALT vary considerably. In lipodystrophic patients, liver fat is increased despite an almost complete lack of subcutaneous fat [5]. The causes of interindividual variation in liver fat content independent of BMI and obesity are unclear. There are almost no data regarding the possible contribution of genetic factors to variation in liver fat content. Family clustering [6,7], interethnic variation [8,9] and single nucleotide polymorphism (SNP) studies (e.g. genes involved in lipid metabolism, inflammation, oxidative stress and iron metabolism) suggest that genetic factors may be important in determining steatosis [10–16] and non-alcoholic steatohepatitis (NASH) [17–26].

In this study, we estimated the relative roles of genetic and environmental influences on inter-individual variation in S-ALT and fasting serum insulin (fS-insulin) concentrations in young adult monozygotic (MZ) and dizygotic (DZ) twins. The relationship between S-ALT and fS-insulin and directly measured (proton magnetic resonance spectroscopy, ^{1}H MRS) liver fat content was determined in a subset of 66 subjects.

2. Patients and methods

2.1. Subjects and study design

The participants were recruited from a population-based longitudinal study (FinnTwin16) of five consecutive birth cohorts (1975–1979) of twins ($n = 4929$ individuals), their siblings and parents, identified through the national population registry of Finland [27]. The twins had been studied by questionnaires at 16, 17, 18.5 and 23–27 years of age. The present study subjects were same-sex pairs enrolled based on their responses to questions on weight and height at the last follow-up, with the aim to cover the full BMI range of both normal-weight and obese subjects. The present sample comprised of MZ ($n = 120$ twins, 57 full pairs, 36 female and 21 male pairs) and same-sex DZ ($n = 193$ twins, 88 pairs, 50 female and 38 male pairs) twins. The mean ± SE BMI of the study sample, based on self-reported data was 24.1 ± 0.2 kg/m^2, range 17.6–42.9 kg/m^2, which is comparable with the whole cohort (22.9 ± 0.1 kg/m^2, range 14.0–44.2 kg/m^2). Exclusion criteria included (1) any known acute or chronic disease other than obesity based on medical history and physical examination, electrocardiogram and standard laboratory tests (blood counts, serum creatinine, serum thyroid stimulating hormone (S-TSH) and electrolyte concentrations), (2) pregnancy and lactation (in women), (3) clinical signs or symptoms of hepatitis or inborn errors of metabolism, (4) a history of use of toxins or drugs associated with liver steatosis, (5) regular medications other than oral contraceptives, (6) self-reported alcohol consumption more than 2 drinks (24 g of alcohol) per day [28] (daily average of a four-week period by questionnaire, 39 individuals were excluded because of excess drinking) and (7) unstable weight (±5 kg) for the past three months. Among female subjects, 12 women had given birth to one child, five to two children and two to three children. Zygosity was confirmed by genotyping of ten informative genetic markers [29]. All pairs were Caucasian, and their mean age was 27.3 ± 0.2 years (range 23.2–32.2).

Each subject was studied after an overnight fast of 10–12 h. Blood samples were taken for screening purposes as detailed above and for measurements of S-ALT, serum aspartate aminotransferase activity (S-AST), serum gamma glutamyl transferase activity (S-GGT) and fS-insulin and fasting plasma glucose (fP-glucose) concentrations. Medical history was reviewed and physical examination was performed, and weight and height were measured barefoot in light clothing to calculate BMI (kg/m^2). Percent body fat was measured by using dual-energy X-ray absorptiometry (DEXA) (Lunar Prodigy, software version 2.15, Madison, WI) [30]. To validate the use of S-ALT as a marker of liver fat content in the present study, liver fat was measured by ^{1}H MRS in a subgroup of 66 subjects (46 MZ and 20 DZ subjects) with BMIs ranging from 19.4 kg/m^2 to 45.8 kg/m^2. We also analysed how liver fat content differs between MZ twins discordant for obesity ($n = 13$ twin pairs, 6 female and 7 male pairs) using a previously characterized subgroup [29,31–35], whose intra-pair BMI differences were on the average 5.0 ± 0.5 kg/m^2. Informed consent was obtained from each patient included in the study. The protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the Ethical Committee of the Helsinki University Central Hospital.

2.2. Liver fat content (^{1}H MRS)

The liver fat content was determined using proton magnetic resonance spectroscopy as previously described [4].

2.3. Analytical procedures

fP-Glucose concentrations were measured in duplicate with the glucose oxidase method using Glucose Analyzer II (Beckman Instruments, Fullerton, CA) [36], fS-insulin with radioimmunoassay (Phadeseph Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden), and S-ALT, S-AST and S-GGT activities as recommended by the European Committee for Clinical Laboratory Standards.

2.4. Statistical analyses

Non-normally distributed data were used after logarithmic transformation. For individual twins, the statistical analyses, significance tests and 95% confidence intervals (95% CI) were corrected for clustered sampling of co-twins within pairs by using survey methods [37]. Pearson's correlation coefficients were calculated to determine associations between liver fat, liver enzymes, fS-insulin and BMI. Analysis of covariance was used to compare slopes and intercepts of regression lines between liver fat vs. S-ALT and liver fat vs. fS-insulin for men and women. If neither slopes nor intercepts differed between women and men, a common regression equation was calculated for all data. Wald test (T-test adapted for clustered twin data) for independent samples was used to compare males and females, and MZ and DZ twins. Twin similarity within each zygosity group was assessed using intra-pair correlations to provide initial evidence for familial aggregation and the presence of genetic effects. These statistical analyses were performed using the Stata statistical software (release 9.0; Stata Corporation, College Station, TX) and GraphPad Prism (version 4.00 for