RESEARCH ARTICLE

Association of INSIG2 Polymorphism with Overweight and LDL in Children

Anne-Marie Kaulfers1*, Ranjan Deka2, Lawrence Dolan3, Lisa J. Martin4

1 Division of Pediatric Endocrinology, University of South Alabama, Mobile, Alabama, United States of America, 2 Department of Environmental Health, University of Cincinnati School of Medicine, Cincinnati, Ohio, United States of America, 3 Division of Endocrinology, Cincinnati Children’s Hospital Medical Center and University of Cincinnati School of Medicine, Cincinnati, Ohio, United States of America, 4 Divisions of Biostatistics and Epidemiology, Division of Human Genetics, Cincinnati Children’s Hospital Medical Center and University of Cincinnati School of Medicine, Cincinnati, Ohio, United States of America

* akaulfers@health.southalabama.edu

Abstract

Background

Dyslipidemia and overweight are common issues in children. Identifying genetic markers of risk could lead to targeted interventions. A polymorphism of SNP rs7566605 near insulin-induced gene 2 (INSIG2) has been identified as a strong candidate gene for obesity, through its feedback control of lipid synthesis.

Objective

To identify polymorphisms in INSIG2 which are associated with overweight (BMI ≥ 85% for age) and dyslipidemia in children. Hypothesis: The C allele of rs7566605 would be significantly associated with BMI and LDL.

Design/Methods

We genotyped 15 SNPs in/near INSIG2 in 1,058 healthy children (53% non-Hispanic white [NHW], 37% overweight) participating in a school based study. Genotype was compared with BMI and lipid markers, adjusting for age, gender, and puberty.

Results

We found a significant association between the SNP rs12464355 and LDL in NHW children, p < 0.001. The G allele is protective (lower LDL). A different SNP was associated with overweight in NHW: rs17047757. SNP rs7566605 was not associated with overweight or lipid levels.

Conclusions

We identified novel genetic associations between INSIG2 and both overweight and LDL in NHW children. Polymorphisms in INSIG2 may be important in the development of obesity through its effects on lipid regulation.
Introduction

Childhood obesity is associated with significant morbidity[1] and results in premature mortality[2]. The frequency of pediatric overweight, or body mass index (BMI) ≥ 85th percentile for age[3], is now at 32%, making it a major public health concern. Despite studies demonstrating that genetics plays a significant role in obesity (heritability estimates of 30–70%) [4], there are still many questions regarding which genes contribute to obesity. Understanding the genetics of obesity is a key step in establishing mechanisms for the development of obesity and targeted strategies for primary and secondary prevention of overweight and obesity in children.

Due to the ability of the Insulin-induced gene 2 (INSIG2) to regulate adipogenesis and lipid storage [5], INSIG2 is a strong candidate gene for obesity. INSIG2 is involved in feedback control of lipid synthesis. When sterols are present in the cell, INSIG2 blocks further cholesterol synthesis [6]. Engelking et al [7] showed that INSIG2 knockout mice weighed more than controls and the mice had a higher accumulation of cholesterol and triglycerides in the liver. Krapivner et al [8] showed that INSIG2 is also expressed in adipocytes, and this expression is enhanced during adipocyte regulation. These authors postulate that changes in adipocyte metabolism, due to functional polymorphisms in the INSIG2 gene, can lead to changes in BMI.

Several studies have shown a significant association between INSIG2 variant rs7566605 and obesity or BMI [4,9,10,11,12]. However, many other studies have failed to show association between rs7566605 and obesity phenotypes [8,13–29]. As obesity often co-occurs with dyslipidemia, other studies have examined the association with rs7566605 and one or more lipid markers (cholesterol, low-density lipoproteins (LDL), triglycerides, and/or high-density lipoproteins (HDL), with conflicting results [12,13,14,22,24,26,27,30,31]. This suggests that rs7566605 is not the causal variant; rather, another genetic variation in this region may be playing a role in development of obesity and/or dyslipidemia. However, few studies have examined genetic variation in the INSIG2 gene more thoroughly, except genome wide association studies which due to the large sample sizes require large heterogeneous cohorts[4]. Given the heterogeneity of the results thus far, it is important to thoroughly study INSIG2 genetic variation in homogenous cohorts.

We had the unique opportunity to analyze genetic variation in INSIG2 in a large school based cohort. By focusing on adolescents from a single school district in Ohio, the heterogeneity which many genetic studies face is minimized. We genotyped 15 single-nucleotide polymorphisms (SNPs): rs7566605, 13 tagging SNPs in INSIG2, and rs17047764, located downstream of INSIG2. Our hypothesis was that genetic variation in INSIG2 would be significantly associated with overweight and lipid measures.

Methods and Procedures

Population

In this study, we randomly selected 1,058 of the 2,501 students participating in the Princeton School District Study[32], a prospective school-based study of 5th through 12th graders on carbohydrate metabolism, in an urban-suburban school district. The children ranged in age from 10–18 years old and were unrelated. The students we selected (our cohort) had complete data and did not report mixed and/or Hispanic ethnicity. Race and ethnicity was self-reported. Population stratification was not done because this was a candidate gene study and we did not have access to ancestry informative markers. In the Cincinnati area, we have found that self-reported race and ethnicity aligns well with genetic continental ancestry (> 99% concordance). Children were excluded if they had a chronic disease, were taking medication known to affect carbohydrate metabolism, or were pregnant. At the study visit, parents completed a medical history.
including medication, chronic disease, and history of menarche for girls, and blood was taken by venipuncture after an overnight 10 hour fast. Written informed consent was obtained from all of the parents/guardians, with written assent obtained from all participants. This study was approved by the institutional review boards of Cincinnati Children’s Hospital Medical Center and the University of Cincinnati.

**Anthropometric measures/calculated variables**

Height and weight measurements, along with determination of pubertal status, were done with standard procedures and equipment as previously described [32]. BMI was calculated (weight (kg)/height (m)^2). BMI percentiles for age and sex were determined using the Centers for Disease Control and Prevention growth charts (www.cdc.gov/nccdphp/dnpa/growthcharts/sas.htm). Lean (< 85th BMI %) and overweight (≥ 85th BMI %) categories of adolescents were defined, consistent with the classification of overweight in children [3].

**Genotyping**

We genotyped 15 SNPs: 13 tagging SNPs within *INSIG2*, and SNP rs7566605 (located 10 kb upstream from the transcription start site of *INSIG2*), and rs17047764 (located downstream from *INSIG2*). These tagging SNPs were identified using the method of Carlson et al [33] and based on pair-wise r-square (>0.8). These SNPs covered both non-Hispanic white (NHW) and African American (AA) populations.

Blood samples were stored on wet ice immediately after collection, and buffy coats were stored at −80 degrees C until processing. DNA was extracted using Gentra Puregene kits. Genotyping was performed using the SNPlex TM platform (Applied Biosystems), which is based on multiple oligonucleotide ligation/polymerase chain reaction assay with a universal ZipChute TM probe detection from high-throughput multiplexed SNP genotyping. Details of the SNPlex protocol were described previously [34]. To assure genotypic quality, negative controls and blind duplicate samples were introduced in each batch of samples in the 96-well format.

**Statistical Analysis**

Analysis was conducted using JMP 7.0. Continuous variables were analyzed for normality. SNPs which deviated from Hardy-Weinberg equilibrium (HWE) were excluded from the analysis. All genetic analyses were conducted in non-Hispanic whites and African Americans separately. Linkage disequilibrium (LD) among SNPs was calculated using r^2 from JMP. SNP associations assumed an additive effect and were tested using regression (logistic for overweight, and linear for LDL, cholesterol, HDL, and triglycerides). Covariates included age, sex, and puberty stage. Lipid measures were ln transformed and back converted for figures.

To minimize false positive findings due to multiple testing, we accounted for four outcomes: LDL and cholesterol (considered as a single outcome), Overweight, HDL, and Triglycerides. We applied Bonferroni correction to the SNPs which entered the final analysis. Thus for whites, the significance threshold is 0.0013 (0.05/(4 phenotypes * 10 SNPs)); for blacks, the significance threshold is 0.001 (0.05/(4 phenotypes * 13 SNPs). However, as we sought to replicate previous reports, nominally significant associations (p ≤ 0.05) were also reported.

**Results**

**Characteristics of the Study Population**

Participant characteristics are listed in Table 1. Thirty-seven percent were overweight (≥ 85th BMI %) and sixty-three percent were lean. The African American and non-Hispanic
white populations were similar in age, sex, and pubertal status. Of the overweight cohort, forty-four percent were non-Hispanic white.

Genotype exclusions

Genotype calling failed in one SNP, rs10490624, and was excluded from the analysis. In whites, three SNPs, rs13003121, rs2161829, and rs11889497, were not in HWE ($p < 0.01$), and thus were excluded from the analysis. In African Americans, rs2161829 was not in HWE ($p < 0.01$), and thus was excluded from analysis. The SNPs included in the analysis, including minor allele frequency (MAF) for both non-Hispanic whites and African Americans, are listed in Tables 2 and 3. The average MAF was 0.22.

SNP of \textit{INSIG2} associated with overweight

Results of association testing between \textit{INSIG2} SNPs and overweight ($p$-values and beta estimates) are presented in Tables 2 and 3 for whites and blacks respectively. We did not find an association between overweight and rs7566605, but we did find a nominally significant association with overweight and rs17047757 in NHW children ($p = 0.043$). The G allele of rs17047757 has an odds ratio of 1.5 for overweight in children, (95% confidence interval 1.01–2.32). This SNP is in LD with rs7566605, $r^2$ of 0.20. LD plot for whites is shown in Fig. 1. No significant associations were identified in the African American cohort (Table 3) even though the LD structure (Fig. 2) was markedly similar to whites.

SNP of \textit{INSIG2} associated with LDL

We also found a strong significant association between rs12464355 and LDL in NHW children, $p < 0.001$ (Table 2). The minor allele, G, is protective, as shown in Fig. 3. Patients with AG or GG had lower LDL levels. We found no association with rs7566605 and cholesterol, LDL, triglycerides, or HDL ($p > 0.15$). In African Americans, no significant associations were detected (Table 3).

---

**Table 1. Characteristics of the study population.**

|                        | Non-Hispanic White | African American |
|------------------------|--------------------|------------------|
| n                      | 561                | 497              |
| Age (years)$^a$        | 14.3 ± 2.2         | 14.2 ± 2.2       |
| Sex (% male)           | 50                 | 50               |
| Puberty Stage (% pre/peri/post) | 12.3/42.1/45.6 | 11.5/37/51.5     |
| % Overweight (BMI $> 85\%$) | 30.1              | 44.7             |
| BMI (kg/m$^2$)$^a,b$   | 22.05 ± 4.91       | 24.02 ± 6.19     |
| Cholesterol (mg/dl)    | 151.54 ± 28.76     | 153.61 ± 27.00   |
| LDL (mg/dl)            | 88.63 ± 25.27      | 91.13 ± 23.74    |
| HDL (mg/dl)            | 45.25 ±10.21       | 48.80 ± 11.95    |
| Triglycerides (mg/dl)  | 88.20 ± 42.13      | 67.88 ± 30.32    |

$^a$Continuous variables presented as means ± s.d.

$^b$Significantly different ($p < 0.0001$).

BMI: Body Mass Index. LDL: low-density lipoprotein. HDL: High-density lipoprotein.

doi:10.1371/journal.pone.0116340.t001
In this study, we confirmed that genetic variation in INSIG2 is associated with both overweight and LDL in NHW children. Although we failed to find these associations with the SNP rs7566605, we identified a novel genetic association between SNP rs17047757 and overweight. In addition, we identified a novel, protective genetic association between SNP rs12464355 and LDL. These data support the concept that polymorphisms in INSIG2 appear to be important in the development of obesity through its effects on lipid regulation, but perhaps not through the previously associated variant.

Previous studies have found possible associations between INSIG2 polymorphisms and lipid synthesis. Tiwari et al observed a non-significant trend in the C allele of rs7566605 and antipsychotic medication induced weight gain. Similarly, Le Hellard et al reported a strong association of three SNPs located within or near the INSIG2 gene (rs17587100, rs10490624, and rs17047764) with antipsychotic medication-related weight gain. In contrast, Oki et al reported a positive association between the C allele of SNP rs7566605 and lower cholesterol in Japanese-American females, which suggests that this SNP may be protective when exposed to a high fat diet [22].

Our study also found a protective effect on LDL (those with the minor allele, G, of SNP rs12464355 had lower levels). This finding replicates a previous report of an association between rs12464355 and LDL [35]). Our failure to identify association between SNP rs7566605 and lipid levels is consistent with previous studies [12,13,14,24,26,27].

We also identified a polymorphism of the INSIG2 gene with overweight in children, but it is a different SNP than those identified by previous studies. Herbert et al [4] published the first Table 2. Associations of the variants of the INSIG2 gene with Overweight and LDL in Non-Hispanic White children.

| SNP        | Minor Allele | MAF | Overweight/Obese | LDL | HDL | Triglycerides |
|------------|--------------|-----|-------------------|-----|-----|--------------|
|            |              |     | p-value Beta ± se | p-value Beta ± se | p-value Beta ± se | p-value Beta ± se |
| rs7566605  | C            | 0.31| 0.30 −0.02±0.02   | 0.19| −0.03±0.02 | 0.82| −0.00±0.02 |
| rs1352083  | T            | 0.25| 0.27 −0.18±0.16   | 0.14| 0.03±0.02  | 0.31| −0.02±0.02 |
| rs13393332 | C            | 0.25| 0.22 −0.20±0.16   | 0.15| 0.03±0.02  | 0.34| −0.02±0.02 |
| rs12464355 | G            | 0.10| 0.92 0.02±0.22    | 2.7 × 10⁻⁵ | −0.12±0.03 | 0.60| −0.01±0.02 |
| rs2042492  | T            | 0.25| 0.31 −0.17±0.16   | 0.13| 0.03±0.02  | 0.24| −0.02±0.02 |
| rs9808111  | A            | 0.002| —                 | —   | —   | —            |
| rs10490625 | T            | 0.08| 0.50 −0.17±0.26   | 0.76| 0.01±0.03  | 0.52| 0.02±0.03 |
| rs889904   | A            | 0.49| 0.41 0.11±0.14    | 0.16| 0.03±0.02  | 0.27| 0.02±0.01 |
| rs17047757 | G            | 0.01| 0.043 0.43±0.21   | 0.39| 0.03±0.03  | 0.58| 0.01±0.02 |
| rs11889497 | G            | 0.07| —                 | —   | —   | —            |
| rs9308762  | C            | 0.17| 0.41 0.14±0.17    | 0.72| 0.01±0.02  | 0.73| 0.01±0.02 |
| rs13003121 | A            | 0.04| —                 | —   | —   | —            |
| rs17047764 | C            | 0.16| 0.49 −0.13±0.19   | 0.79| 0.06±0.02  | 0.55| −0.01±0.02 |

*Associated with overweight (BMI ≥ 85% for age) in Non-Hispanic Whites.
*Associated with LDL in Non-Hispanic Whites.
*Out of Hardy-Weinberg equilibrium in whites.

SNP: Single nucleotide polymorphism. MAF: minor allele frequency. INSIG2: Insulin-induced gene 2. LDL: low-density lipoproteins. HDL: high-density lipoproteins. SE: standard error.

Associations were made after adjusting for age, sex, and puberty stage using regression. To adjust for multiple testing, a p-value of 0.0013 was considered statistically significant, Bonferroni correction of (0.05/(4 phenotypes *10 SNPs).

doi:10.1371/journal.pone.0116340.t002

Discussion

In this study, we confirmed that genetic variation in INSIG2 is associated with both overweight and LDL in NHW children. Although we failed to find these associations with the SNP rs7566605, we identified a novel genetic association between SNP rs17047757 and overweight. In addition, we identified a novel, protective genetic association between SNP rs12464355 and LDL. These data support the concept that polymorphisms in INSIG2 appear to be important in the development of obesity through its effects on lipid regulation, but perhaps not through the previously associated variant.

Previous studies have found possible associations between INSIG2 polymorphisms and lipid synthesis. Tiwari et al observed a non-significant trend in the C allele of rs7566605 and antipsychotic medication induced weight gain. Similarly, Le Hellard et al reported a strong association of three SNPs located within or near the INSIG2 gene (rs17587100, rs10490624, and rs17047764) with antipsychotic medication-related weight gain. In contrast, Oki et al reported a positive association between the C allele of SNP rs7566605 and lower cholesterol in Japanese-American females, which suggests that this SNP may be protective when exposed to a high fat diet [22].

Our study also found a protective effect on LDL (those with the minor allele, G, of SNP rs12464355 had lower levels). This finding replicates a previous report of an association between rs12464355 and LDL [35]). Our failure to identify association between SNP rs7566605 and lipid levels is consistent with previous studies [12,13,14,24,26,27].

We also identified a polymorphism of the INSIG2 gene with overweight in children, but it is a different SNP than those identified by previous studies. Herbert et al [4] published the first
Table 3. Associations of the variants of the *INSIG2* gene with Overweight and LDL in African-American children.

| SNP       | Minor Allele | MAF   | Overweight/Obese | LDL       | HDL       | Triglycerides |
|-----------|--------------|-------|------------------|-----------|-----------|---------------|
|           |              |       | p-value | Beta ± se | p-value | Beta ± se | p-value | Beta ± se | p-value | Beta ± se |
| rs7566605 | C            | 0.26  | 0.84    | -0.03±0.02 | 0.89    | 0.00±0.02 | 0.82    | 0.00±0.02 | 0.29    | -0.03±0.03 |
| rs1352083 | T            | 0.27  | 0.45    | -0.11±0.15 | 0.05    | 0.04±0.19 | 0.97    | -0.00±0.02 | 0.45    | 0.02±0.03  |
| rs13393332| C            | 0.27  | 0.49    | -0.10±0.14 | 0.04    | 0.04±0.02 | 0.87    | 0.00±0.02 | 0.42    | 0.02±0.03  |
| rs12464355| G            | 0.02  | 0.87    | 0.09±0.53  | 0.79    | -0.02±0.07 | 0.93    | 0.00±0.07 | 0.34    | 0.10±0.10  |
| rs2042492 | T            | 0.27  | 0.48    | -0.10±0.15 | 0.04    | 0.04±0.02 | 0.96    | 0.00±0.02 | 0.47    | 0.02±0.03  |
| rs9808111 | A            | 0.06  | 0.57    | 0.15±0.27  | 0.27    | 0.04±0.04 | 0.21    | 0.04±0.03 | 0.63    | -0.03±0.05 |
| rs10490625| T            | 0.02  | 0.18    | 0.66±0.49  | 0.26    | 0.07±0.07 | 0.97    | 0.00±0.06 | 0.22    | 0.11±0.10  |
| rs889904  | A            | 0.58  | 0.27    | -0.15±0.14 | 0.17    | -0.03±0.02 | 0.84    | -0.00±0.02 | 0.014   | -0.06±0.03 |
| rs17047757| G            | 0.03  | 0.51    | -0.27±0.40 | 0.97    | -0.00±0.05 | 0.93    | -0.00±0.05 | 0.59    | -0.04±0.08 |
| rs11889497| G            | 0.24  | 0.61    | 0.08±0.15  | 0.39    | 0.02±0.02 | 0.32    | 0.02±0.02 | 0.23    | 0.03±0.03  |
| rs9308762 | C            | 0.17  | 0.92    | -0.02±0.18 | 0.07    | -0.05±0.03 | 0.12    | -0.04±0.02 | 0.39    | -0.03±0.04 |
| rs13003121| A            | 0.09  | 0.13    | -0.37±0.24 | 0.72    | -0.01±0.03 | 0.25    | 0.03±0.03 | 0.74    | 0.02±0.04  |
| rs17047764| C            | 0.38  | 0.45    | 0.10±0.14  | 0.67    | -0.01±0.02 | 0.67    | 0.01±0.02 | 0.12    | 0.04±0.03  |

SNP: Single nucleotide polymorphism. MAF: minor allele frequency. *INSIG2*: Insulin-induced gene 2. LDL: low-density lipoproteins. HDL: high-density lipoproteins. SE: Standard error.

Associations were made after adjusting for age, sex, and puberty stage using regression. To adjust for multiple testing, a p-value of 0.001 was considered statistically significant, Bonferroni correction of (0.05/(4 phenotypes * 13 SNPs).

doi:10.1371/journal.pone.0116340.t003

Figure 1. Linkage disequilibrium as measured by r² across *INSIG2* in Non-Hispanic Whites.

doi:10.1371/journal.pone.0116340.g001
study to implicate the C allele of the SNP rs7566605 in association with BMI. They found this strong association in a large genome-wide association study, and replicated the findings in 4 other separate populations. Since then, several other studies have also found a significant association with rs7566605 and obesity or BMI[9,10,11,12], but some did not[8,13–29], including this study.

The discordance between our data and previous studies suggest that another genetic variation of INSIG2 may be important in adiposity. The SNP rs7566605 may not be the causative SNP for adiposity, but may be in linkage disequilibrium with the causative SNP. Our study is

Figure 2. Linkage disequilibrium as measured by r^2 across INSIG2 in African-Americans.
doi:10.1371/journal.pone.0116340.g002

Figure 3. G allele of SNP rs12464355 in the INSIG2 gene is associated with lower LDL levels, p < 0.001 in Non-Hispanic Whites. Least Square Adjusted Mean of LDL (mg/dl) is shown. Predicted values are given from a multiple regression analysis, adjusting for age, gender, and pubertal stage. LDL levels were ln transformed and back converted.
doi:10.1371/journal.pone.0116340.g003
one of the first to look at other tagging SNPs, and our novel association is in LD with rs7566605.

Other studies support this idea that the causative SNP may be in LD with rs7566605. Krapivner et al[8] found that the G allele of the −102G/A promoter in INSIG2 is significantly associated with BMI, and may be the functional polymorphism. Ciullo et al [15] did not find an association between rs7566605 and BMI or obesity, but they detected a locus on chromosome 2q14.3 that displayed linkage with BMI and obesity in both populations. This locus contains the rs7566605 SNP.

A limitation to our study is that, although our sample size was large, it may not have been large enough to detect an influence of rs7566605 on overweight in children. However, our population is unique in that we were able to study children from one geographic region, who were all in the same school district, which reduces the degree of heterogeneity. One variable that limits our ability to compare our study to others is the variability in phenotypic definition. Some studies looked at overweight versus lean, and others did not.

Conclusion

We identified a novel SNP in the INSIG2 gene that is associated with overweight in NHW children, rs17047757, and one SNP that is associated with LDL in NHW children, rs12464355. We did not find an association with overweight or lipid profiles in children and rs7566605. These data suggest that polymorphisms in INSIG2 may be important in the development of obesity through its effects on lipid regulation.

Author Contributions

Conceived and designed the experiments: AK RD LD LM. Performed the experiments: AK RD. Analyzed the data: AK LD LM. Contributed reagents/materials/analysis tools: AK RD LM. Wrote the paper: AK RD LD LM.

References

1. Freedman DS, Katzmarzyk PT, Dietz WH, Srinivasan SR, Berenson GS (2009) Relation of body mass index and skinfold thicknesses to cardiovascular disease risk factors in children: the Bogalusa Heart Study. Am J Clin Nutr 90: 210–216. doi: 10.3945/ajcn.2009.27525 PMID: 19420092
2. Engeland A, Bjorge T, Sogaard AJ, Tverdal A (2003) Body mass index in adolescence in relation to total mortality: 32-year follow-up of 227,000 Norwegian boys and girls. Am J Epidemiol 157: 517–523. doi: 10.1093/aje/kwf219 PMID: 12631541
3. Barlow SE (2007) Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics 120 Suppl 4: S164–192. doi: 10.1542/peds.2007-2329C PMID: 18055651
4. Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, et al. (2006) A common genetic variant is associated with adult and childhood obesity. Science 312: 279–283. doi: 10.1126/science.1124779 PMID: 16614226
5. Dahlman I, Arner P (2007) Obesity and polymorphisms in genes regulating human adipose tissue. Int J Obes (Lond) 31: 1629–1641. doi: 10.1038/sj.ijo.0803657 PMID: 17563763
6. Yabe D, Brown MS, Goldstein JL (2002) Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. Proc Natl Acad Sci U S A 99: 12753–12758. doi: 10.1073/pnas.162488899 PMID: 12224332
7. Engelking LJ, Liang G, Hammer RE, Takaishi K, Kuriiyama H, et al. (2005) Schoenheimer effect explained—feedback regulation of cholesterol synthesis in mice mediated by Insig proteins. J Clin Invest 115: 2948–2958. doi: 10.1172/JCI25614 PMID: 16100574
8. Krapivner S, Popov S, Chernogubova E, Hellenius ML, Fisher RM, et al. (2008) Insulin-induced gene 2 involvement in human adipocyte metabolism and body weight regulation. J Clin Endocrinol Metab 93: 1995–2001. doi: 10.1210/jc.2007-1850 PMID: 18319320
9. Hotta K, Nakamura M, Nakata Y, Matsu T, Kamohara S, et al. (2008) INSIG2 gene rs7566605 polymorphism is associated with severe obesity in Japanese. J Hum Genet 53: 857–862. doi: 10.1007/s10038-008-0317-8 PMID: 18615239

10. Lyon HN, Emilsson V, Hinney A, Heid IM, Lasky-Su J, et al. (2007) The association of a SNP upstream of INSIG2 with body mass index is reproduced in several but not all cohorts. PLoS Genet 3: e61. doi: 10.1371/journal.pgen.0030061 PMID: 17456861

11. Rosskopf D, Bornhorst A, Rimbach C, Schwahn C, Kayser A, et al. (2007) Comment on “A common genetic variant is associated with adult and childhood obesity”. Science 315: 187; author reply 187. doi: 10.1126/science.1130571 PMID: 17218510

12. Zhang J, Lin R, Wang F, Lu M, Lin RY, et al. (2008) A common polymorphism is associated with body mass index in Uyghur population. Diabetes Res Clin Pract 81: e11–13. doi: 10.1016/j.diabres.2008.03.022 PMID: 18514965

13. Andreasen CH, Mogensen MS, Borch-Johnsen K, Sandbaek A, Lauritzen T, et al. (2008) Non-replication of genome-wide based associations between common variants in INSIG2 and PFKP and obesity in studies of 18,014 Danes. PLoS One 3: e2872. doi: 10.1371/journal.pone.0002872 PMID: 18682847

14. Rosskopf D, Bornhorst A, Rimbach C, Schwahn C, Kayser A, et al. (2007) Comment on “A common genetic variant is associated with adult and childhood obesity”. Science 315: 187; author reply 187. doi: 10.1126/science.1130571 PMID: 17218510

15. Ciullo M, Nutile T, Dalmasso C, Sorice R, Bellenguez C, et al. (2008) Identification and replication of a novel obesity locus on chromosome 1q24 in isolated populations of Cilento. Diabetes 57: 783–790. doi: 10.2337/db07-0970 PMID: 18162505

16. Dina C, Meyre D, Samson C, Tichet J, Marre M, et al. (2007) Comment on “A common genetic variant is associated with adult and childhood obesity”. Science 315: 187; author reply 187. doi: 10.1126/science.1129402 PMID: 17218508

17. Feng Y, Dong H, Xiang Q, Hong X, Wilker E, et al. (2007) Lack of association between rs7566605 and obesity in a Chinese population. Hum Genet 120: 743–745. doi: 10.1007/s00439-006-0258-2 PMID: 17024366

18. Hall DH, Rahman T, Avery PJ, Keavney B (2006) INSIG-2 promoter polymorphism and obesity related phenotypes: association study in 1428 members of 248 families. BMC Med Genet 7: 83. doi: 10.1186/1471-2350-7-83 PMID: 17137505

19. Kumar J, Sunkishala RR, Karthikeyan G, Sengupta S (2007) The common genetic variant upstream of INSIG2 gene is not associated with obesity in Indian population. Clin Genet 71: 415–418. doi: 10.1111/j.1399-0004.2007.00795.x PMID: 17489846

20. Kuzuya M, Ando F, Iguchi A, Shimokata H (2007) No association between rs7566605 variant and being overweight in Japanese. Obesity (Silver Spring) 15: 2531–2534. doi: 10.1038/oby.2007.301 PMID: 18070740

21. Loos RJ, Barroso I, O’Reilly S, Wareham NJ (2007) Comment on “A common genetic variant is associated with adult and childhood obesity”. Science 315: 187; author reply 187. doi: 10.1126/science.1130012 PMID: 17218509

22. Oki K, Yamane K, Kamei N, Asao T, Awaya T, et al. (2009) The single nucleotide polymorphism upstream of insulin-induced gene 2 (INSIG2) is not associated with hypercholesterolaemia, but not with obesity, in Japanese American women. Br J Nutr 101: 322–327. doi: 10.1017/S0007114508006557 PMID: 18570692

23. Skelly T, Pinheiro AP, Lange LA, Sullivan PF (2007) Is rs7566605, a SNP near INSIG2, associated with body mass in a randomized clinical trial of antipsychotics in schizophrenia? Mol Psychiatry 12: 321–322. doi: 10.1038/mp.4001956 PMID: 17389899

24. Smith AJ, Cooper JA, Li LK, Humphries SE (2007) INSIG2 gene polymorphism is not associated with obesity in Caucasian, Afro-Caribbean and Indian subjects. Int J Obes (Lond) 31: 1753–1755. doi: 10.1038/sj.ijo.0803645 PMID: 17471297

25. Tabara Y, Kawamoto R, Osawa H, Nakura J, Makino H, et al. (2008) No association between INSIG2 Gene rs7566605 polymorphism and being overweight in Japanese population. Obesity (Silver Spring) 16: 211–215. doi: 10.1038/oby.2007.25 PMID: 18223638

26. Vimalaswaran KS, Franks PW, Brage S, Sardinha LB, Andersen LB, et al. (2009) Absence of association between the INSIG2 gene polymorphism (rs7566605) and obesity in the European Youth Heart Study (EYHS). Obesity (Silver Spring) 17: 1453–1457. doi: 10.1038/oby.2008.650 PMID: 19197262

27. Wang HJ, Zhang H, Zhang SW, Pan YP, Ma J (2008) Association of the common genetic variant upstream of INSIG2 gene with obesity related phenotypes in Chinese children and adolescents. Biomed Environ Sci 21: 528–536. doi: 10.1016/S0895-3988(09)60013-1 PMID: 19263810
28. Wiedmann S, Neureuther K, Stark K, Reinhard W, Kallmunzer B, et al. (2009) Lack of association between a common polymorphism near the INSIG2 gene and BMI, myocardial infarction, and cardiovascular risk factors. Obesity (Silver Spring) 17: 1390–1395. doi: 10.1038/oby.2008.669 PMID: 19197259

29. Yang L, Wu Y, Li H, Yu Z, Li X, et al. (2008) Potential association of INSIG2 rs7566605 polymorphism with body weight in a Chinese subpopulation. Eur J Hum Genet 16: 759–761. doi: 10.1038/ejhg.2008.8 PMID: 18270535

30. Le Hellard S, Theisen FM, Haberhausen M, Raeder MB, Ferno J, et al. (2009) Association between the insulin-induced gene 2 (INSIG2) and weight gain in a German sample of antipsychotic-treated schizophrenic patients: perturbation of SREBP-controlled lipogenesis in drug-related metabolic adverse effects? Mol Psychiatry 14: 308–317. doi: 10.1038/mp.4002133 PMID: 18195716

31. Tiwari AK, Zai CC, Meltzer HY, Lieberman JA, Muller DJ, et al. Association study of polymorphisms in insulin induced gene 2 (INSIG2) with antipsychotic-induced weight gain in European and African-American schizophrenia patients. Hum Psychopharmacol 25: 253–259. doi: 10.1002/hup.1111 PMID: 20373477

32. Dolan LM, Bean J, D’Alessio D, Cohen RM, Morrison JA, et al. (2005) Frequency of abnormal carbohydrate metabolism and diabetes in a population-based screening of adolescents. J Pediatr 146: 751–758. doi: 10.1016/j.jpeds.2005.01.045 PMID: 15973311

33. Carlson CS, Eberle MA, Kruglyak L, Nickerson DA (2004) Mapping complex disease loci in whole-genome association studies. Nature 429: 446–452. doi: 10.1038/nature02623 PMID: 15164069

34. Pal P, Xi H, Sun G, Kaushal R, Meeks JJ, et al. (2007) Tagging SNPs in the kallikrein genes 3 and 2 on 19q13 and their associations with prostate cancer in men of European origin. Hum Genet 122: 251–259. doi: 10.1007/s00439-007-0394-3 PMID: 17593395

35. Do R, Bailey SD, Pare G, Montpetit A, Desbiens K, et al. (2010) Fine mapping of the insulin-induced gene 2 identifies a variant associated with LDL cholesterol and total apolipoprotein B levels. Circ Cardiovasc Genet 3: 454–461. doi: 10.1161/CIRCGENETICS.109.917039 PMID: 20858904