

Studies in 3,523 Norwegians (HUNT2) and Meta-Analysis in 11,571 Subjects Indicate that Variants in the *HNF4A* P2 Region are Associated with Type 2 Diabetes in Scandinavians

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Received for publication 12 April 2007 and accepted in revised form 4 September 2007.

Additional information for this article can be found in an online appendix at <http://diabetes.diabetesjournals.org>.

ABSTRACT

OBJECTIVE: Recent publications have found an association between common variants near the *HNF4A* P2 promoter and type 2 diabetes in some populations but not in others, and the role for *HNF4A* in type 2 diabetes has remained unclear. In an attempt to address these inconsistencies, we investigated *HNF4A* SNPs in a large population-based sample and included a meta-analysis of published studies.

RESEARCH DESIGN AND METHODS: We genotyped twelve SNPs in the *HNF4A* region in a Norwegian population-based sample of 1,644 individuals with type 2 diabetes and 1,879 controls (The HUNT2 Study). We combined our data with all previously published case/control studies and performed a meta-analysis.

RESULTS: Consistent with initial studies, we found a trend towards association for the SNPs rs1884613 (OR = 1.17 <1.03-1.35>) and rs2144908 (OR = 1.21 <1.05-1.38>) in the P2 region and for rs4812831 (OR = 1.21 <1.02-1.44>) located 34 kb downstream of the P2 promoter. Meta-analysis, comprising 12,292 type 2 diabetes cases and 15,519 controls, revealed a non-significant OR of 1.05 <0.98-1.12> but with significant heterogeneity between the populations. We therefore performed a sub-analysis including only the data for subjects from Scandinavia. Among the 4,000 Scandinavian cases and 7,571 controls, a pooled OR of 1.14 <1.06-1.23> (p = 0.0004) was found for the SNP rs1884613.

CONCLUSIONS: Our results suggest that variation in the *HNF4A* region is associated with type 2 diabetes in Scandinavians, highlighting the importance of exploring small genetic effects in large, homogenous populations.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; HNF, hepatocyte nuclear factor; HUNT, The Nord-Trøndelag Health Study; LD, linkage disequilibrium; MODY, maturity-onset diabetes of the young; SNP, single nucleotide polymorphism.

INTRODUCTION

Genetic variants in the hepatocyte nuclear factor 4 α (*HNF4A*) gene may be involved in type 2 diabetes development. *HNF4A* encodes a transcription factor with an important role in hepatocyte and pancreatic transcriptional regulation. The two promoters, P1 and P2 are located 45.5 kb apart on chromosome 20q. While *HNF4A* transcripts in the liver are primarily of P1 origin, the P2 promoter drives expression in the pancreas where it regulates genes involved in insulin secretion and glucose homeostasis (1-3). The importance of *HNF4A* in glucose metabolism is further reflected by maturity-onset diabetes of the young 1 (MODY1), which is caused by mutations in *HNF4A* and characterized by impaired insulin secretion and monogenic inheritance (4-7). Several type 2 diabetes studies have shown linkage to the 20q region and two initial reports found evidence for association between *HNF4A* and type 2 diabetes, highlighting the genetic segment surrounding the P2 promoter as the prime candidate region (8; 9). Subsequent studies have shown conflicting results between different populations (8-21). Thus, it is still not established whether the *HNF4A* locus harbours heritable variation involved in development of type 2 diabetes. Meta-analyses may prove useful to evaluate such conflicting results. Recently, such analyses have revealed that common genetic variations in *TCF7L2*, *PPARG*, and *KCNJ11* explain part of the heritable fraction of type 2 diabetes (22-25). Extensive meta-analysis for *HNF4A* P2 has not been undertaken. We therefore decided to genotype a Norwegian case/cohort sample of 3,827 individuals for twelve *HNF4A* SNPs, and to combine the results with all previously published studies in a meta-analysis of P2 promoter SNPs as risk factors for type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. The study subjects were participants \geq 20 years of age in an

extensive population-based study (HUNT; The Nord-Trøndelag Health Study) from 1995-1997 (HUNT2) (26).

Cases that either were GAD antibody-positive and had age of diabetes onset before 40 years, or had age at onset less than 30 years with insulin treatment initiated during the first year of diagnosis, or had continuously been on insulin treatment since the year of diagnosis, were excluded due to suspected type 1 diabetes. For 459 diabetic subjects, with no GAD antibody measurements, subjects with diabetes onset before 40 years were excluded. The study groups finally consisted of 1,644 subjects with type 2 diabetes and 1,879 non-diabetic (self-reported) controls.

The study was approved by the regional committee for research ethics and the Norwegian Data Inspectorate, and performed according to the Helsinki Declaration.

Genotyping. The SNPs were genotyped according to the manufacturers' instructions using Sequenom's MassARRAY iPLEX System. Markers genotyped are depicted in Figure 1.

Statistical methods. To examine the allelic association of each particular SNP with diabetes, we used a logistic regression model with age, gender and BMI as cofactors, as implemented in the PLINK software (<http://pngu.mgh.harvard.edu/purcell/plink/>). Supplementary Table 1 (available at <http://diabetes.diabetesjournals.org>) presents the data without adjustment for the cofactors mentioned above. All SNPs examined were consistent with Hardy-Weinberg equilibrium in the control samples. Haplotype frequency estimates and haplotype comparisons were performed using the Unphased software (27).

Considering a risk allele frequency of 18% and a multiplicative model we had approximately 61% power to detect an OR of 1.14 at the 0.05-level (Genetic Power

Calculator, <http://pngu.mgh.harvard.edu/~purcell/gpc/>). For the meta-analysis, we searched Medline to collect all published literature on *HNF4A* P2 polymorphisms and checked the reference lists of the retrieved articles. We found twelve type 2 diabetes case/control studies on the three *HNF4A* P2 polymorphisms rs1884613, rs1884614 and rs2144908. When needed, we contacted the corresponding authors to obtain exact genotype counts for all studies. We did not include the Japanese samples from Hara et al. (17) because detailed genotype counts for that study were unavailable. The Silander et al. study (9) was excluded from the meta-analysis since the same individuals was included in an extended study published recently by the same group (19). For the meta-analysis, we applied a random effect model as implemented by the “metan” command in Stata 8.0, with the estimate of heterogeneity being taken from the Mantel-Haenszel model and analyzed all data using Stata 8.0 (Stata Statistical Software, Stata Corp., College Station, Texas). Family studies were not included in the over-all meta-analysis but results are presented in Supplementary Table 2 for comparison (as presented in publications or via personal communications). P-values presented were not corrected for the number of tests performed. All confidence intervals (CI) are presented as 95% CIs.

RESULTS

Trend towards an association between SNPs near the *HNF4A* P2 promoter and type 2 diabetes. The demographic characteristics of the study subjects are described in Table 1. Based on reported associations in previous studies, we focused our study on the most commonly studied P2 promoter region SNPs and the region upstream of *HNF4A* (altogether ten SNPs). Two commonly studied SNPs within the gene were also included (Figure 1). Genotype analysis confirmed an LD-pattern in the Norwegian population similar to that of other populations (including HapMap

data), with a block of tight LD covering the P2 promoter region and relatively weak LD with and within the *HNF4A* gene (13).

The allelic association results from the Norwegian case/cohort data are summarized in Table 2 (adjusted for age, sex and BMI), and in further detail in Supplementary Table 1 (available at <http://diabetes.diabetesjournals.org>). There was a trend towards increased OR for the rare alleles at markers rs1884613 (OR = 1.17 <1.03-1.35>) and rs2144908 (OR = 1.21 <1.05-1.38>) genotyped in most of the previously reported studies, and rs4812831 located 34 kb downstream the P2 promoter (OR = 1.21 <1.02-1.44>).

Haplotypes were estimated for the three associated markers; rs1884613, rs2144908 (both near the P2 promoter) and rs4812831 (Supplementary Table 3). The results suggest that the “at-risk” P2 alleles are associated only when combined on the same haplotype with the A-allele at rs4812831 ($p = 0.02$). Similar haplotype frequency estimates were found with the Haploview and PLINK software (data not shown).

Meta-analysis shows that variation in the *HNF4A* P2 region is associated with type 2 diabetes in Scandinavians. Although the p-values presented would not remain significant after correction for multiple testing, our study of a Norwegian population-based sample suggests an association between SNPs near the *HNF4A* P2 promoter and diabetes. To assess the role of variation in the *HNF4A* P2 region more extensively, we therefore performed a meta-analysis on all published studies including the present data.

We found 14 PubMed-listed studies testing for association between common *HNF4A* variation and type 2 diabetes (8-21). The case/control studies covered 15 different samples, consisting of 27,811 subjects from various ethnic groups. Four family-based samples were also found. No common single SNP had been genotyped across all studies, but all studies included at

least one out of three SNPs surrounding the *HNF4A* P2 promoter (rs1884613, rs1884614 or rs2144908). From our own data (Figure 1), the HapMap data and previous publications it is evident that rs1884613, rs1884614 and rs2144908 are virtually interchangeable ($r^2 \sim 1.0$) and therefore it is valid to use any of the three SNPs in an over-all meta-analysis. We chose to use allele counts for the most commonly used marker, rs1884613, in all studies where it was genotyped, and markers rs1884614 or rs2144908 in consecutive order for studies lacking rs1884613.

Figure 2 presents the meta-analysis plot of the common allelic OR from 12,292 type 2 diabetes cases and 15,519 controls from all 15 samples. Nine samples showed a trend of elevated OR for the minor allele, two showed no trend and four revealed a trend in the opposite direction. The combined OR estimate for the minor allele among cases compared to controls was not significant ($p = 0.17$, $OR = 1.05 < 0.98-1.12 >$). However, a statistical test for homogeneity of the odds revealed evidence for heterogeneity between the studies ($p = 0.009$). We therefore restricted the analysis to Scandinavian samples only, since they are considered to share similar genetic background. In this sample of 4,000 cases and 7,571 controls we did not find any evidence for heterogeneity of the odds ($p = 0.85$). The results showed a significant association between type 2 diabetes and the minor allele at rs1884613 ($OR = 1.14 < 1.06-1.23 >$, $z = 3.56$, $p = 0.0004$). Furthermore, all family-based samples trended towards increased risk associated with the minor alleles (Supplementary Table 2).

We also performed a Scandinavian meta-analysis on rs3818247 (located in the 3' part of the gene) for which all Scandinavian samples were genotyped. This result was not significant ($p = 0.13$). Influence plot and funnel plot analyses did not show evidence for publication bias in any test strata (data not shown).

DISCUSSION

Here, we present data from a combined sample of 11,571 Scandinavian individuals that support a role in type 2 diabetes for common variants near the *HNF4a* gene. Our results highlight the power and limitations of meta-analyses in genetic association studies. On its own, the results from the Norwegian cohort were suggestive but inconclusive. By combining results from several populations with similar genetic background we gained power to detect an association with a relatively small effect size ($OR = 1.14$, $p = 0.0004$) which current genome-wide association studies are not powered to detect. However, in contrast to the two most consistently replicated type 2 diabetes polymorphisms of similar size effects, *KCNJ11*(E23K) and *PPARG* (P12A) (22; 23), we failed to identify an over-all association for the *HNF4A* P2 promoter SNPs in the total pooled sample. Whereas the former SNPs probably are the etiological variants themselves, there is limited evidence to suggest that the tested *HNF4A* P2 promoter region SNPs have a direct biological role. Hence, if the SNPs tested are markers for an as yet not genotyped causal variant, varying patterns of LD in different populations could mirror the apparently conflicting results. Other reasons for the detected heterogeneity could possibly be different clinical ascertainment criteria between studies. However, apart from a younger mean age of diabetes onset in the French study and a higher average BMI in the US GCI sample compared to other samples included in the meta-analysis, we do not find a consistent explanation.

Our study was not designed to cover all the variation in the region since we did not have sufficient power to fine-map a locus with an effect in the range estimated from previous studies. The Winckler et al. report (13), along with recent HapMap data, clearly illustrate that no study has managed to capture most of the extensive variation in the region. We therefore focused our tagging efforts on the region between the P2 and P1 promoters not extensively covered by

previous studies. Interestingly, apart from the P2 region SNPs, we also found a similar sized association for rs4812831 located 34 kb downstream of the P2 promoter.

Analyses of three-marker haplotypes made up of the two P2 promoter SNPs and rs3818247 suggest that the P2 promoter SNP association might be secondary to LD with a variant on the “at risk haplotype” in our Norwegian sample. Although these results remain speculative until they are robustly replicated in other populations, such haplotype effects could potentially explain the conflicting results between populations. Very recently, the group reporting the Finnish association (9), published a combined analysis of their original sample and an extended sample suggesting that the most interesting region might be somewhere downstream of the P2 promoter (19). Furthermore, in the recent French whole-genome association study (28), a weak association for rs2425637, located only 5 kb downstream rs4812831 in the promoter region, was reported. Hence, there is some evidence to support a role for *HNF4A* promoter region variation in type 2 diabetes, but more comprehensive genotyping both upstream and downstream of the P2 promoter in homogeneous populations is warranted.

We believe that our post hoc Scandinavian stratified meta-analysis is rather conservative since the Scandinavian population is historically and genetically homogenous (29). The allele frequencies are similar within the population (minor allele frequency range: cases 0.203 - 0.185 and controls 0.187 - 0.154). Nevertheless, we cannot rule out selection bias until our results have been confirmed in other samples of Scandinavian ancestry. Interestingly, support for our conclusion is found in a recent Scandinavian study of gestational diabetes (30), a phenotype which is likely to share some genetic components with type 2 diabetes. The Scandinavian study reported an equally sized non-

significant trend towards increased risk (OR=1.14 <0.96-1.37>) associated with the minor allele at marker rs2144908. Importantly, the HUNT samples were validated by genotyping known type 2 diabetes risk variants in *TCF7L2* and *KCNJ11*. Results from both loci were very similar to estimates from other Caucasian populations (manuscript in preparation).

In conclusion, there seems to be a modest but consistent association between SNPs in a haplotype block covering the P2 promoter in all Scandinavian samples tested. Our data is in agreement with results from other homogeneous populations such as the Amish and Finnish populations, but different from other populations. This suggests that the SNPs genotyped are not the functional variants and addresses the need for further extensive studies of the *HNF4A* region.

ACKNOWLEDGEMENTS

We thank S. Erdal and A. Badiee for technical assistance. We also thank Drs A. Bagwell, L. Baier, K. Wanic, L. Love-Gregory, A. Permutt, M. Vaxillaire, C. M. Damcott, A. Shuldiner, M. Itakura, J. Holmkvist and L. Groop for providing genotype counts for the meta-analysis. This study was supported by the University of Bergen, Haukeland University Hospital, Helse Vest, Innovest and Norwegian Research Council. Genotyping was in part provided by CIGENE at the national technology platform, and supported by the Functional Genomics Programme (FUGE) in the Research Council of Norway. The Nord-Trøndelag Health Study (HUNT) is a collaboration between the HUNT Research Center, the Norwegian University of Science and Technology, Verdal, the Norwegian Institute for Public Health and Nord-Trøndelag County Council. The diabetes part of HUNT was supported by funds from GlaxoSmithKline Norway and the Norwegian Diabetes Association.

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TABLE 1
Clinical characteristics of the study samples

	Type 2 diabetes	
	cases	Controls
Samples (<i>n</i>)	1,644	1,879
Gender (males/females)	776/877	886/993
Age at diagnosis (years)	58.4 ±15.1	-
Age at examination (years)	68.4 ±12.1	56.3 ±18.4
BMI (kg/m ²)	29.2 ±4.8	26.5 ±4.2
Serum cholesterol (mmol/L)	6.2 ±1.3	6.1 ±1.3
Serum triglycerides (mmol/L)	2.5 ±1.6	1.9 ±1.2
Serum HDL (mmol/L)	1.2 ±0.4	1.4 ±0.4
Random serum glucose (mmol/L)	9.6 ±4.2	5.4 ±1.4

TABLE 2

Comparisons of allele frequencies in the Norwegian sample of 1,644 type 2 diabetes patients and 1,879 controls

Marker	Position (bp)*	Minor/ major allele	Minor allele frequency		Allelic**		
			Cases	Controls	OR	95% CI	P-value
rs1884613	-3925	G/C	0.198	0.181	1.17	1.03-1.35	0.02
rs2144908	1377	A/G	0.197	0.178	1.21	1.05-1.38	0.007
rs6103716	15290	C/A	0.339	0.338	1.03	0.92-1.16	0.57
rs6065725	20675	A/G	0.314	0.316	0.99	0.88-1.11	0.89
rs6017335	26485	A/G	0.486	0.467	1.06	0.96-1.18	0.26
rs6031580	30941	A/G	0.272	0.280	0.96	0.85-1.08	0.46
rs4812831	33920	A/G	0.113	0.097	1.21	1.02-1.44	0.03
rs3092370	35763	A/G	0.469	0.482	0.93	0.83-1.03	0.16
rs717248	41265	G/A	0.030	0.032	0.96	0.70-1.32	0.81
rs717247	41444	G/A	0.301	0.313	0.93	0.83-1.05	0.24
rs1028583	66421	T/G	0.354	0.358	0.98	0.88-1.10	0.76
rs3818247	73140	T/G	0.330	0.347	0.92	0.82-1.03	0.15

* Position relative to start of the *HNF4A* P2-promotor (according to Goldenpath: hg17, dbSP: build 123).

** Calculations based on logistic regression with age, BMI and sex as cofactors

FIGURE LEGENDS

FIG. 1. LD plot across the *HNF4A* locus in the Norwegian population. The *HNF4A* coding exons and the P2 promoter are illustrated with black crossbars on the top line and the location of the P1 promoter with a grey crossbar. The twelve successfully genotyped SNPs are listed below the line. The bottom part of the figure presents an LD plot based on the r^2 -measure. Each diamond represents the strength of pairwise r^2 , with black indicating strong LD and logarithm of odds score. The pairwise r^2 -values are written in the boxes. There are three two-marker haplotype blocks as suggested by the Gabriel et al. method. The leftmost block contains the P2 promoter. The two rightmost SNPs located within the coding region of the gene show very little LD with the promoter region SNPs. The LD-plot was produced by the Haploview version 3.2 software.

FIG. 2. Meta-analysis plot of *HNF4A* case/control studies. A: Meta-analysis plot of all case/control populations comprising 12,292 type 2 diabetes cases and 15,519 controls. Allelic OR = 1.05 <0.98-1.12>, $p = 0.17$. Evidence of heterogeneity was detected between samples ($p=0.009$). B: Meta-analysis plot among the 4,000 Scandinavian cases and 7,571 controls. OR = 1.14 <1.06-1.23>, $p=0.0004$ was found for the SNP rs1884613. The ORs for the pooled analyses were calculated using a random effect model.

Figure 1

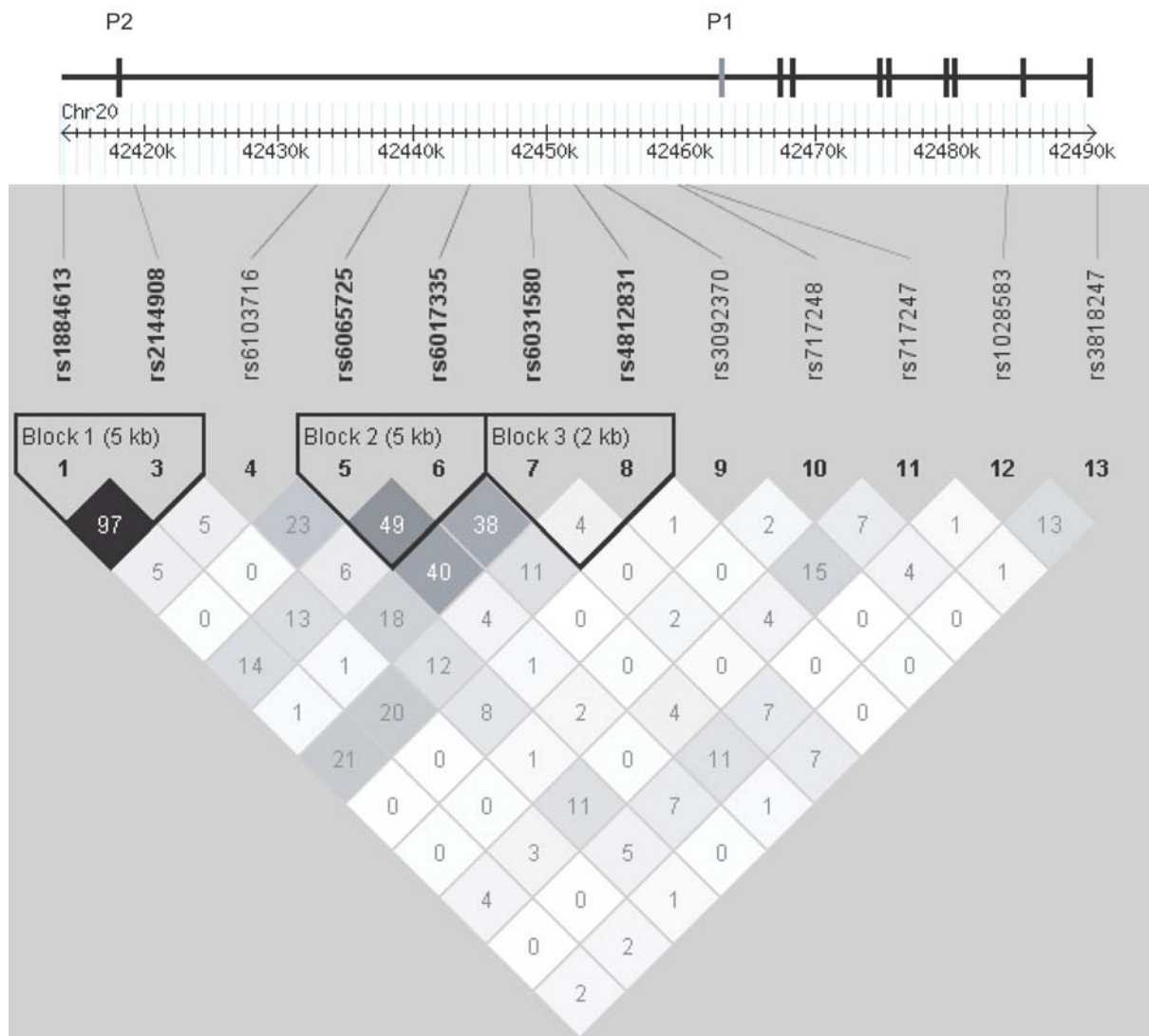
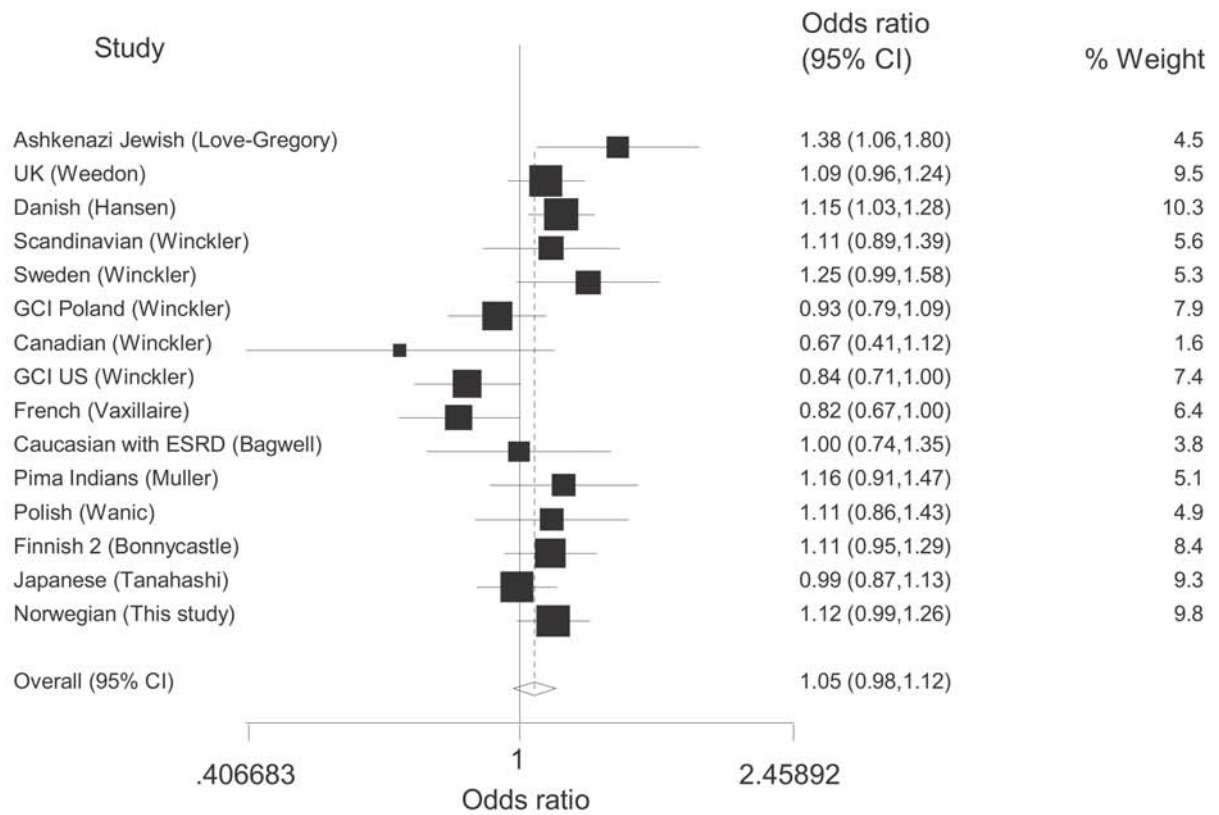


Figure 2

A. All studies



B. Scandinavian studies

