

**Identification of novel candidate genes for type 2 diabetes from a genome-wide association scan in the Old Order Amish: Evidence for replication from diabetes-related quantitative traits and from independent populations**

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Running title: A T2D GWAS in the Amish

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Received for publication 3 April 2007 and accepted in revised form 5 September 2007.

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

## ABSTRACT

**Objective.** Type 2 diabetes (T2D) is the result of complex interactions between genetic susceptibility and environmental factors.

**Research Design and Methods.** We performed a genome-wide association scan (GWAS) of T2D in the Amish; 93,087 SNPs were tested for association in 124 T2D cases and 295 normoglycemic controls. We tested the most strongly T2D-associated for association with oral glucose tolerance (OGTT) traits in 427 non-diabetic Amish individuals, and replication from GWAS from four independent populations (Caucasians from the Framingham Heart Study, Mexican Americans, Pima Indians, and Caucasians from Scandinavia).

**Results.** We identified 1,316 SNPs that were associated with T2D in the Amish ( $p < 0.01$ ). The strongest association ( $p = 1.07 \times 10^{-5}$ ) was for rs2237457, which is located in intron 4 of growth factor receptor-bound protein 10 (*GRB10*), an adaptor protein known to regulate signaling of insulin receptors. Of the T2D-associated SNPs in the Amish, 65 unique SNPs demonstrated internal associations ( $p < 0.01$ ) with at least one of five OGTT traits in non-diabetic individuals. Genes notable for internal associations include *ESRRG*, *FHIT*, *ADAM12*, and *C10orf59*. In external comparisons, 73 unique SNPs were associated with T2D in the Amish and at least one other population. Among these, SNPs in *SLC8A1* and *BCAT1* were associated with T2D in the Amish and two other populations. Other genes of potential interest include *GPC5*, *CSN3*, *INPP4B*, *LOC730727*, and *C1orf24*.

**Conclusions.** The results of our 100K GWAS of T2D in the Amish identified a tractable number of novel candidate genes that warrant further investigation.

Abbreviations: AFDS, Amish Family Diabetes Study; DGI, Broad-Lund-Novartis Diabetes Genome Initiative; FHS, Framingham Heart Study; GWAS, genome-wide association scan; HOMA-IR, homeostasis model assessment of insulin resistance; HWE; Hardy-Weinberg equilibrium; IGT, impaired glucose tolerance; ISI, insulinogenic index; LD, linkage disequilibrium; MAF, minor allele frequency; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; OR, odds ratio; SNP, single nucleotide polymorphism; T2D, type 2 diabetes mellitus

Type 2 diabetes (T2D), a complex disease that is characterized by insulin resistance and impaired beta-cell function, represents a serious global public health problem with over 100 million people worldwide who are affected. While the primary molecular defects in T2D remain largely unknown, it is clear that both genetic and environmental risk factors (including diet and physical inactivity) play critical roles. More than twenty genome-wide linkage scans of T2D have been published, with evidence for linkage reported to a number of loci, including regions on chromosomes 1, 3, 8, 10, 12, 14 and 20 (1-8). Of the numerous candidate genes studied for their functional role in pancreatic beta cell function, insulin action or energy metabolism, as well as positional candidate genes identified under linkage peaks, very few have variants that are consistently associated with T2D. Indeed, common variants in only a handful of genes (*PPAR $\gamma$* , *KCNJ11*, *CALPN10*, *TCF7L2*, *HNF4A*) have been replicated in multiple populations (9).

The Old Order Amish are a closed founder population who emigrated from Switzerland in the early 1700's. They are a well-suited population for carrying out genetic studies since they live a relatively homogeneous lifestyle and maintain extensive family history records. The Amish Family Diabetes Study (AFDS) was initiated in 1995 with the goal of identifying the genetic determinants of T2D (10). We found that the sibling relative risk ( $\lambda_s$ ) of T2D in the Amish is 3.28 (95% CI 1.58-6.80), similar to that observed in other Caucasian populations. Genome-wide linkage analysis of T2D and impaired glucose tolerance (IGT) conducted in AFDS pedigrees (6) revealed regions on chromosomes 1q and 14q, both of which have been implicated in linkage scans from other populations (1-5;7). Specific variants in several well-replicated T2D susceptibility genes are associated with T2D in the Amish,

including *TCF7L2* rs7903146 (p=0.008; OR = 1.53) (11) and *HNF4A* rs2425640 (p = 0.03; OR = 1.60) (12). These findings suggest that the common T2D gene variants in the Amish will likely be relevant to more outbred Caucasian populations.

Increased knowledge of common variation in the human genome learned as part of the HapMap initiative, coupled with advances in technologies that make possible the genotyping of hundreds of thousands of single nucleotide polymorphisms (SNPs) in many DNA samples from those with and without disease, have made possible genome-wide association scans (GWAS), a powerful approach to identify novel susceptibility genes for complex diseases (13;14). Recently, four GWAS studies of T2D have identified variants at several novel loci, including *SLC30A8*, *IGF2BP2*, *CKDAL1*, *CDKN2A/CKDN2B*, and *HHEX/IDE*, that show strong replicated association with T2D (15-18). In this paper, we report results from a GWAS of T2D in the Amish using the Affymetrix 100K SNP genotyping platform. We further characterize our findings using diabetes-related quantitative traits measured in nondiabetic Amish individuals. Lastly, we interpret the results of this scan in the context of three recently completed 100K GWAS studies for T2D, as part of the T2D 100K GWAS Consortium along with a publicly available 500K GWAS of T2D recently performed in a Scandinavian population.

## METHODS

### Study population and phenotype assessment

Individuals with T2D were identified from the Amish Family Diabetes Study (AFDS). Details of the AFDS have been previously described (10). Phenotypic characterization of participants included medical and family history, anthropometry, and a 3-hour, 75-gram oral glucose tolerance test (OGTT) with insulin levels. We based our

primary analyses on 124 T2D cases and 295 normal glucose tolerant (NGT) controls. T2D was defined by fasting plasma glucose level ( $\geq 7$  mmol/l), 2-h OGTT plasma glucose level ( $\geq 11.1$  mmol/l), random plasma glucose level ( $\geq 11.1$  mmol/l), the use of insulin or prescription oral glucose-lowering agents, or a diagnosis of diabetes documented by a physician. To minimize potentially misclassifying subjects with type 1 diabetes as having type 2 diabetes, cases with age of diagnosis  $<35$  years were excluded. NGT controls were  $>38$  years old at the time of study and were selected based on fasting plasma glucose level ( $<6.1$  mmol/l) and 2-h OGTT plasma glucose level ( $<7.8$  mmol/l).

We performed secondary quantitative analyses of our mostly highly associated signals ( $p < 0.01$ ) in a set of 427 nondiabetic Amish study participants, 132 of whom had impaired glucose tolerance (IGT) and 295 of whom were part of the NGT control group used in our primary analysis. We estimated the mean levels of two OGTT-derived quantitative glucose traits [fasting glucose (FASTG) and glucose area under the curve (GAUC)], and three insulin traits [insulinogenic index (ISI), insulin area under the curve (IAUC), and homeostasis model assessment of insulin resistance (HOMA-IR)] according to the SNP genotypes in these individuals. Total GAUC and IAUC were calculated based on measurements at 0, 30, 60, 90, 120, 150, and 180 minutes using the trapezoidal method. The ISI was calculated as  $[(\text{insulin at 30 min} - \text{fasting insulin}) / (\text{glucose at 30 min} - \text{fasting glucose})]$ . HOMA-IR was calculated as  $(\text{fasting insulin (mU/l)} \times \text{fasting glucose (mmol/l)}) / 22.5$ . Table 1 describes the characteristics of this sample. The study protocol was approved by the Institutional Review Board at the University Of Maryland School of Medicine, and informed consent was obtained from each study participant.

### Genotyping

Genomic DNA from leukocytes were genotyped using the Affymetrix GeneChip® Mapping 100K Array set which consists of two microarray chips (*XbaI* and *HindIII*) (Affymetrix, Santa Clara, CA). Total genomic DNA (250 ng) was digested with *XbaI* or *HindIII* restriction enzymes and processed according to the Affymetrix protocol. The GeneChip Genotyping Analysis Software (GTYPE 4.0) was used to generate DM-algorithm derived genotypes that were re-analyzed with the BRLMM genotype calling algorithm (confidence threshold of 0.33) to improve the proportion of heterozygote calls (19). As an initial quality control (QC) measure, BRLMM-generated chip files with call rates  $<90\%$  for both enzymes across all SNPs were excluded. The resulting median call rate across of the remaining 419 case-control samples was 97.5% (97.6% for *XbaI* and 97.4% for *HindIII*). We further removed individual SNPs with genotype call rates  $<90\%$ , monomorphic SNPs and SNPs with minor allele frequency (MAF)  $< 5\%$ , and those deviating from Hardy-Weinberg equilibrium (HWE) in controls ( $p < 0.001$ ). The number of monomorphic and low frequency SNPs ( $n = 26,816$ ) in the Amish was not appreciably different from that observed in more outbred Caucasians of the HapMap CEU sample. For this report, we focused our analysis on the 82,485 autosomal SNPs that passed our quality control standards.

The concordance rate for 11 QC samples that were run twice on the Affymetrix GeneChip® mapping panel was 97.5%. We also calculated a cross-platform concordance rate of 98% for 419 samples in which 61 SNPs were genotyped using the Affymetrix GeneChip® Mapping 100K panel and an independent Illumina 1536-plex GoldenGate assay. Online Supplementary Table 1 (available at <http://diabetes.diabetesjournals.org>)

summarizes the quality checks and informativeness of the data.

#### Association testing and SNP prioritization scheme

Our GWAS analysis and SNP prioritization scheme is shown in Figure 1. We selected the SNPs most highly associated with T2D in our Amish case-control dataset based on  $p$ -value rankings ( $p$ -value cutoff  $< 0.01$ ) and then used two complementary approaches to further prioritize them. In one approach, we evaluated the most highly T2D-associated SNPs for association with diabetes-related quantitative traits in an expanded set of 427 nondiabetic Amish subjects, 295 of whom were NGT controls from the primary T2D association analysis (internal consistency). In a parallel approach, we assessed replication of the most highly associated T2D-associated SNPs in the Amish in four independent GWAS from other populations (external replication).

#### T2D association analysis

We performed case-control association analysis using a variance component approach as implemented in the SOLAR software (20). Using a liability threshold model, we modeled the probability that the subject was a case or control as a function of the individual's age, sex, and genotype, conditional on the correlations in phenotype among relative pairs. Statistical testing was performed using a likelihood ratio test, in which we compared the likelihood of the data under a model in which the genotype effect was estimated against the likelihood of a nested model in which the genotype effect was constrained to be zero. Odds ratios (OR) were computed from variance components models. We chose to report the additive model as our primary analysis. Supplementary analyses using a dominant or recessive model did not yield any SNP showing genome-wide significance. Of the

82,485 SNPs, 611 had  $p < 0.01$  under a dominant model and 569 had  $p < 0.01$  under the recessive model. Our complete dataset with results from all models are available online

([http://www.medschool.umaryland.edu/amish\\_studies/index.asp](http://www.medschool.umaryland.edu/amish_studies/index.asp)). Pairwise linkage disequilibrium (LD) correlation statistics ( $r^2$ ) were computed using the HelixTree software version 5.0.2 (GoldenHelix, Bozeman, MT).

#### Quantitative trait analysis

For quantitative trait analyses performed in nondiabetic Amish subjects, we used the measured genotype approach in which we estimated the likelihood of an additive genetic model given the pedigree structure (21). Prior to analysis, all insulin traits (IAUC, ISI, HOMA-IR) were transformed by their natural logarithm to reduce skewness. Parameter estimates were obtained by maximum likelihood methods, and the significance of association was tested by the likelihood ratio test. Within each model, we simultaneously estimated the effects of age and sex. These analyses were performed using the SOLAR program (20).

#### Power calculations

Power calculations, based on the Genetic Power calculator of Purcell et al. (22) indicated that our sample would provide 80% power to detect a diabetes susceptibility allele having a genotype relative risk of 1.8 (for allele frequency of 30%, 124 cases and 295 controls, 8% population prevalence of diabetes, assuming a multiplicative model), and 80% power to detect a QTL accounting for 4% or higher of the trait variance for a continuously distributed phenotype (427 subjects).

#### In silico replication samples

We considered whether our best T2D association signals ( $p$ -value cutoff  $< 0.01$ ) replicated in at least one of three distinct populations (Framingham Caucasians, Mexican-

Americans, Pima), each with different study designs but performed using the same Affymetrix 100K genotyping platform. Descriptions of each corrected level. The strongest association ( $p = 1.07 \times 10^{-5}$ ) was for rs2237457 on chromosome 7, which is located in intron 4 of *GRB10*, an adaptor protein known to regulate signaling of insulin and insulin-like growth factor receptor-bound protein 10 (23-25) and in a Joint Summary Supplementary Table 1. We directly checked whether any of the 1,093 SNPs with the best T2D association signals ( $p < 0.01$ ) in the Amish was also associated with growth-factor receptors (26-28). In addition to T2D based on GEE (generalized estimating equations) and FBAT (family-based association tests) in the Framingham Heart Study (FHS), SNPs are located in or near *MSH6* (chr 2), Fisher's exact allelic association test in the Mexican-American study, and case-control and sib-based association tests in the Pima study. We also utilized publicly available pre-released data (March 2007) from a T2D GWAS carried out in a Scandinavian cohort of 1,464 T2D cases and these SNPs with T2D (Table 2).

1,467 matched controls and genotyped using the Affymetrix 500K platform by the Broad-Novartis Diabetes Genetics Initiative (DGI) (http://www.broad.mit.edu/diabetes/) (18). We specifically checked replication of 295 of 1,093 of our most highly T2D-associated SNPs that were present on both 100K and 500K Affymetrix genotyping arrays. Since LD structure may differ across populations and to limit multiple comparisons, we defined replication only if the same SNP was associated with T2D at  $p < 0.05$  with an odds ratios (ORs) in the same direction (i.e., reflective of the same allelic risk).

## RESULTS

Following quality-control and HWE checks, 82,485 informative SNPs were included in our analyses. The median physical inter-SNP distance was 11.3 kb and the average distance between SNPs was 29 kb. Under the additive model, a total of 1,093 SNPs, some of which were in linkage disequilibrium (LD), were associated ( $p < 0.01$ ) with T2D (Figure 2). The 50 most strongly T2D-associated SNPs (i.e., lowest  $p$ -values), are shown in Table 2. The complete dataset is available online at (http://www.medschool.umaryland.edu/amish

As a measure of internal consistency, we tested whether the 1,093 SNPs associated with T2D ( $p < 0.01$ ) were also associated with OGTT-derived quantitative traits in nondiabetic individuals. In these analyses, we considered two OGTT glucose traits (FASTG and GAUC) and three OGTT insulin traits (IAUC, HOMA-IR and ISI), and  $p < 0.01$  as our threshold for significance. Thirty-eight non-redundant ( $r^2 < 0.80$ ) T2D-associated SNPs were also associated with at least one glucose trait and showed the same allelic association as for diabetes (i.e., the diabetes risk allele was also associated with higher glucose levels), while 29 non-redundant T2D-associated SNPs were also associated with at least one insulin-related trait (Table 3). Of the top 16 SNPs associated with T2D at  $p < 1 \times 10^{-4}$ , rs2237457 in *GRB10* is the only one also associated with an OGTT trait ( $p = 0.001$  for GAUC). Two perfectly correlated ( $r^2 = 1$ ) T2D-associated SNPs in *ADAMTS1* (chromosome 5) ( $p = 0.004-0.005$ ) were associated with one glucose trait ( $p=0.006$  for GAUC) and one insulin trait ( $p=0.007$  for IAUC).

We next sought to determine which of our 1,093 most highly T2D-associated SNPs

were also associated with T2D in any of three independent populations for which the same 100K Affymetrix platform was used, or in the DGI Scandinavian population for which the 500K Affymetrix platform was used. We identified 80 non-redundant SNPs for which the same risk allele was also associated with T2D in one of the three studies from the T2D 100K GWAS Consortium ( $p < 0.05$ ) and 11 non-redundant SNPs that showed consistent association in the DGI sample ( $p < 0.05$ ) (Supplementary Table 2). In total, 3 SNPs demonstrated associations in the Amish as well as in two independent populations. The T allele for rs2540317 in *MFSD9* on chromosome 2 was associated with decreased risk of T2D in the Amish (OR 0.72,  $p = 0.007$ ), and showed nominal association in the Pima dataset (case-control OR 0.67,  $p = 0.042$ ; sib-based OR 0.50,  $p = 0.043$ ; summary OR 0.63,  $p = 0.016$ ) and also in the Mexican-American sample (case-control OR 0.75,  $p = 0.047$ ). The G allele in rs10515353 on chromosome 5 was associated with decreased risk of T2D in the Amish (OR 0.61,  $p = 0.005$ ) and also with decreased T2D risk in samples from Mexican-American (OR 0.69,  $p = 0.035$ ) and DGI (OR 0.79,  $p = 0.007$ ). The T allele in rs2242400 in *BCAT1* on chromosome 10 was associated with decreased risk of T2D in the Amish (OR 0.71,  $p = 0.004$ ), and also in the Pima dataset (Sib OR 0.66,  $p = 0.019$ ; summary OR 0.78,  $p = 0.034$ ) and the Mexican-American dataset (OR 0.67,  $p = 0.009$ ); borderline association was also seen in the DGI sample (OR 0.86,  $p = 0.051$ ). The direction of effect was the same for all studies.

Table 4 highlights our most consistent overall findings. We present 21 T2D-associated SNPs in the Amish ( $p < 0.005$ ) that also demonstrated either (a) association with a diabetes-related quantitative trait ( $p < 0.005$ ) in the Amish or *in-silico* replication of T2D association in one independent population ( $p < 0.005$ ). Of interest, the T allele in

rs3845971 in *FHIT* was associated with increased risk of T2D in the Amish (OR 1.42,  $p = 0.004$ ) and also in Mexican-Americans (OR 1.46,  $p = 0.004$ ) and with increased GAUC ( $p = 4.0 \times 10^{-4}$ ) in nondiabetic Amish subjects.

## DISCUSSION

In this report we described the results of a GWAS of T2D of 82,485 SNPs in the Old Order Amish, a genetically closed founder population with a homogeneous lifestyle. We reasoned that this population is likely to carry a subset of the same common T2D susceptibility variants as in the general population, and that these variants might be easier to identify.

GWAS studies are prone to false positives due to the very large number of statistical tests that must be performed. We were restricted by our relatively modest sample size, and also computationally in our attempts to define a genome-wide significance level for which follow-up was justified (ie. variance components tests were not feasible for the many replications needed for case-control permuted family datasets in the Amish). Thus we relied heavily on a prioritization of SNPs worthy of follow-up by testing for (a) internal consistency of T2D-associated SNPs with OGTT-derived quantitative traits in nondiabetic Amish individuals, (b) external replication of T2D-associations in three independent non-Amish 100K SNP GWAS studies, and (c) external replication in a 500K SNP GWAS of T2D in a large population of Scandinavians.

We found that no single SNP replicated consistently and in the same direction across all GWAS studies, nor were all SNPs associated with T2D also associated with quantitative traits in nondiabetic individuals. This is not particularly surprising since we expect that an appreciable number of T2D-associated SNPs will be false positives. Furthermore, a true susceptibility gene in one

population might not be readily discernible in other populations due to small sample sizes as well as differences in genetic background, linkage disequilibrium, and environmental exposures. Similarly, a true susceptibility gene for T2D might not show association with diabetes-related quantitative traits in nondiabetic individuals, especially since our OGTT-derived traits are only surrogates for gold-standard measures of insulin sensitivity and insulin secretion. Nevertheless, we were able to identify a number of candidate genes and loci that showed evidence for association with T2D in more than one population and/or were also associated with OGTT-derived quantitative traits. These results are intriguing but must be interpreted with caution. None of these loci fall within previously identified linkage regions for T2D (chromosomes 1 and 14) in the Amish.

Our strongest T2D association signal in the Amish was observed on chromosome 7 in a functionally relevant T2D candidate gene, *GRB10*. *GRB10* encodes several different isoforms of growth factor binding protein 10, and has been shown to bind to activated insulin receptor and act as a negative regulator of insulin action and glucose uptake (26-28). Overexpression of *GRB10* in mice causes postnatal growth retardation and insulin resistance (29). Our 100K GWAS contained a total of twelve SNPs in *GRB10*, six of which were associated with T2D ( $p < 0.05$ ) and were in partial LD with each other ( $r^2 = 0.16 - 0.78$ ). Rs2237457, located in intron 4, provided the lowest p-value for association (OR 0.61 for the G vs. A allele,  $p = 1.07 \times 10^{-5}$ ). This SNP was also strongly associated with OGTT glucose area under the curve in nondiabetic Amish individuals ( $p = 0.001$ ). Rs2237457 was not associated with T2D in the other three populations in which this SNP was genotyped or in the 500K SNP Scandinavian T2D GWAS; however, three SNPs (rs2190496, rs2237478, and rs7805310) in *GRB10* that were genotyped in the

Scandinavian cohort were associated with T2D ( $p = 0.029$ ,  $p = 0.01$ ,  $p = 0.004$ , respectively), and are in partial LD with rs2237457 ( $r^2 = 0.12-0.49$  in HapMap CEU). Lack of replication could suggest a false positive or that variation in *GRB10* is a true positive specific to the Amish due to a founder effect or context dependent phenotypic expression of the variant due to genetic background or environmental influences. Alternatively, this variant could be in LD with a functional variant and extended LD in the Amish enabled a T2D association to be detected in this population and not the others.

In a recent report by Di Paola et al. (30), the A allele of rs4947710, a synonymous coding SNP in *GRB10*, was associated with decreased risk of T2D in a relatively homogeneous population of Italian Caucasians ( $p = 0.0001$ ). This SNP was not part of the 100K SNP panel nor was our most highly T2D-associated SNP (rs2237457) genotyped in the Italian sample. We found that rs2237457 and rs4947710 are not in LD ( $r^2 = 0$ ) in HapMap CEU samples. However, rs10486757, another *GRB10* SNP associated with T2D in the Amish ( $p = 0.024$ ), is in LD with rs4947710 ( $r^2 = 0.64$  in HapMap CEU). Further investigation of *GRB10* is currently underway.

Our GWAS and replication strategy has several limitations. First, the relatively small sample size limits our ability to detect gene variants of modest effect size. Second, we recognize that the definition of external replication of our top SNPs across three independent 100K studies of T2D might represent a skewed distribution of the overall results since replication was limited to our ~1000 most highly T2D-associated SNPs. This approach was used to facilitate comparisons across populations and also to limit the number of false positive replications due to multiple comparisons. To the extent that we attempted to pursue signals that

represent the ‘lowest hanging fruit’, we believe that the approach we have taken is reasonable. A formal meta-analysis of the entire set of data from all four 100K studies is currently underway. Third, our replication approach was focused at the level of the SNP in order to avoid additional multiple comparisons. However, it is possible that we did not identify significantly-associated SNPs in other populations that were in LD with our top SNPs. This is particularly relevant for our comparisons with the Scandinavian 500K GWAS, for which we only assessed 27% of the SNPs identified in the Amish with  $p < 0.01$ .

The likelihood that we missed common variants important to T2D is high due to the relatively sparse density of the 100K SNP panel (mean intermarker distance = 29 kb) compared with other denser GWAS SNP panels. For example, SNPs in well-replicated genes *SLC30A8*, *IGF2BP2*, *CKDAL1*, *CDKN2A/CKDN2B*, *HHEX/IDE* found in four recently published T2D GWAS studies (15-18), and previously known T2D-associated variants in *TCF7L2*, *KCNJ11*, *HNF4A*, or *CAPN10* (9) were not adequately covered on the 100K genotyping panel, i.e.,  $r^2 < 0.8$  between the SNP of interest and SNPs on the 100K panel). As a positive control, we previously demonstrated *TCF7L2* SNP rs7903146 and the *HNF4A* promoter SNP rs2425640, neither of which is present on the 100K panel, were associated with T2D and IGT in the Amish Family Diabetes Study ( $p = 0.008$  OR = 1.57 and  $p = 0.04$ ; OR = 1.60, respectively) (12;28). Interestingly, rs10509645 in *HHEX* on the 100K panel ( $r^2 = 0.7$  with rs7923837 found previously to be strongly associated with T2D in other GWAS studies), was significantly associated with T2D in the Amish (OR = 1.30 for the G allele;  $p = 0.02$ ). Rs9300039 on chromosome 11, shown to be associated with T2D in the other

GWAS studies (17) was present on the 100K panel, but was not significantly associated with T2D in the Amish (OR = 1.09 for the C allele,  $p = 0.67$ ).

In summary, we presented results from our initial examination of a GWAS of T2D in the Amish. Although we did not identify any genes associated with T2D that reached genome-wide significance, we report a number of genes and loci that are worthy of further study based on replication in other studies or on QTL consistency. This report (and the three companion manuscripts) provides a valuable resource for other investigators to utilize in the search for the pathogenic variants for T2D.

## ACKNOWLEDGMENTS

This work was supported by NIH research grants T32 HL072751 Training Grant in Cardiac and Vascular Cell Biology; R01 DK54261; the University of Maryland General Clinical Research Center (M01 RR16500); Hopkins Bayview General Clinical Research Center (M01 RR02719); the Maryland Clinical Nutrition Research Unit (P30 DK072488); and the Baltimore Veterans Administration Geriatric Research and Education Clinical Center. We extend our thanks to our collaborators in the Type 2 Diabetes 100K GWAS Consortium for sharing pre-publication results from the Starr County Health Studies, Framingham Heart Study, and the Pima Indians, and to the Broad-Lund-Novartis Diabetes Genomic Initiative for access to results from their GWAS. We also thank Soren Snitker for helpful comments and Adam Naj for help with formatting figures for this manuscript. Lastly, we gratefully acknowledge our Amish liaisons and fieldworkers and the extraordinary cooperation and support of the Amish community, without whom these studies would not be possible.

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**Table 1.** Description of sample characteristics for T2D GWAS in the Amish.**T2D case - NGT control dataset**

<b>Characteristic</b>	<b>T2D cases (n=124)</b>	<b>NGT controls (n=295)</b>
Males (%)	33	52
Age (yrs)	51.3 ± 10.5	64.4 ± 12.9
BMI (kg/m <sup>2</sup> )	29.3 ± 5.8	27.4 ± 4.7

**Oral glucose tolerance test derived quantitative trait dataset**

<b>Characteristic</b>	<b>All (n=427)</b>	<b>Men (n=200 )</b>	<b>Women (n=227)</b>
Age (yrs)	51.9 ± 11.9	52.2 ± 11.9	51.7 ± 11.9
BMI (kg/m <sup>2</sup> )	27.7 ± 5.0	26.4 ± 4.0	28.9 ± 5.5
Fasting glucose (mmol/l)	5.09 ± 0.45	5.12 ± 0.47	5.07 ± 0.43
Glucose AUC (mmol/l·hr)	379.2 ± 68.0	362.6 ± 65.3	393.9 ± 67.1
Insulin AUC (mU/l·hr)	137.9 ± 88.9	108.5 ± 60.6	164.5 ± 101.3
HOMA-IR (mU·mmol/l <sup>2</sup> )	2.6 ± 1.7	2.6 ± 2.2	2.6 ± 1.1
ISI (U/g)	0.9 ± 1.5	0.85 ± 1.9	0.93 ± 1.0

All subjects were nondiabetic. Values are mean ± SD. Abbreviations: AUC, area under oral glucose tolerance test (OGTT) curve; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; ISI, insulinogenic index

**Table 2. Fifty SNPs most highly associated with T2D from Amish GWAS.**

SNP	Chr	Position*	Gene**	Alleles		Strand	N		Allele 2 Cases	Allele 2		T2D p-value†	OR***	T2D p-value‡ (BMI)
				1/2	2		Cases	Controls		Controls	p-value†			
rs2237457	7	50693638	<i>GRB10</i>	A/G	A/G	-	124	293	0.53	0.68	1.07 x 10 <sup>-5</sup>	0.61	1.46 x 10 <sup>-5</sup>	
rs980720	4	82272087	<i>PRKG2</i>	A/G	A/G	-	122	287	0.80	0.90	1.25 x 10 <sup>-5</sup>	0.52	4.87 x 10 <sup>-5</sup>	
rs10509199	10	65295820		G/T	G/T	-	124	295	0.31	0.46	1.68 x 10 <sup>-5</sup>	0.62	4.46 x 10 <sup>-5</sup>	
rs1373147	17	20147237	<i>SPECCI</i>	A/T	A/T	-	124	293	0.47	0.60	2.79 x 10 <sup>-5</sup>	0.63	5.26 x 10 <sup>-6</sup>	
rs4082516	10	71374662	<i>COL13A1</i>	C/G	C/G	-	122	293	0.77	0.88	3.19 x 10 <sup>-5</sup>	0.53	1.27 x 10 <sup>-5</sup>	
rs10509195	10	65193372		A/C	A/C	-	118	281	0.42	0.28	3.30 x 10 <sup>-5</sup>	1.62	7.1E-05	
rs1395931	2	123535443		A/G	A/G	+	124	293	0.68	0.58	6.36 x 10 <sup>-5</sup>	1.57	2.90 x 10 <sup>-5</sup>	
rs3136279	2	47871272	<i>MSH6</i>	G/T	G/T	-	124	294	0.20	0.11	6.81 x 10 <sup>-5</sup>	1.80	9.2E-05	
rs1446732	2	134374210		G/T	G/T	-	121	295	0.51	0.37	7.38 x 10 <sup>-5</sup>	1.55	0.0001	
rs10485249	6	70161908		C/G	C/G	+	124	295	0.82	0.92	7.96 x 10 <sup>-5</sup>	0.54	0.0004	
rs2703813	17	20055907	<i>SPECCI</i>	A/G	A/G	-	123	285	0.51	0.64	8.12 x 10 <sup>-5</sup>	0.64	2.36 x 10 <sup>-5</sup>	
rs1916412	10	65340801		C/T	C/T	-	124	293	0.62	0.49	8.35 x 10 <sup>-5</sup>	1.54	0.0002	
rs1916411	10	65340839		C/G	C/G	-	123	292	0.38	0.51	8.48 x 10 <sup>-5</sup>	0.65	0.0002	
rs3751797	16	85124993	<i>MTHFSD</i>	A/T	A/T	-	123	285	0.76	0.64	8.74 x 10 <sup>-5</sup>	1.60	0.0005	
rs930621	2	134418548		C/T	C/T	+	122	290	0.54	0.41	8.74 x 10 <sup>-5</sup>	1.55	0.0001	
rs10509201	10	65342248		A/G	A/G	-	123	293	0.62	0.49	8.80 x 10 <sup>-5</sup>	1.53	0.0002	
rs2158473	17	20079682	<i>SPECCI</i>	C/T	C/T	+	119	292	0.51	0.39	0.0001	1.54	3.00 x 10 <sup>-5</sup>	
rs2502497	6	75399083		G/T	G/T	+	116	279	0.05	0.10	0.0001	0.43	0.0002	
rs430123	5	106109632		A/G	A/G	-	114	272	0.16	0.27	0.0002	0.61	0.0002	
rs10504553	8	75038957	<i>TCEB1</i>	A/G	A/G	-	115	275	0.77	0.89	0.0002	0.56	0.0003	
rs9287428	2	133912538	<i>FLJ34870</i>	C/T	C/T	+	124	287	0.83	0.71	0.0002	1.62	0.0002	
rs10507601	13	55047379		A/G	A/G	+	123	294	0.04	0.12	0.0002	0.44	0.0006	
rs994952	6	78319674		A/G	A/G	+	120	287	0.52	0.42	0.0002	1.52	0.0002	
rs7604549	2	133923233	<i>FLJ34870</i>	A/G	A/G	-	123	294	0.26	0.40	0.0002	0.65	0.0002	
rs2737245	8	116727757	<i>TRPS1</i>	A/C	A/C	-	113	276	0.58	0.71	0.0002	0.67	0.0003	
rs1513287	3	114754898	<i>SIDT1</i>	A/G	A/G	+	120	289	0.58	0.44	0.0002	1.49	0.0003	
rs7817780	8	119486147	<i>SAMD12</i>	C/T	C/T	-	120	294	0.91	0.80	0.0002	1.85	0.0002	
rs297765	20	4435111		A/G	A/G	+	124	295	0.75	0.63	0.0002	1.55	0.0004	

rs4410442	3	112434500	A/G	-	123	294	0.48	0.34	0.0002	1.52	0.0008
rs1351916	2	139479918	A/T	-	122	288	0.37	0.56	0.0002	0.67	0.0006
rs1507666	2	123532455	A/T	-	119	285	0.44	0.56	0.0002	0.66	0.0002
rs10498934	6	83516566	C/T	-	124	293	0.57	0.69	0.0002	0.66	0.0004
rs721729	3	174671967	A/G	-	119	285	0.43	0.56	0.0002	0.65	0.0002
rs1492908	3	159869201	A/G	+	122	291	0.20	0.29	0.0002	0.62	0.0004
rs10484725	6	78325880	A/G	+	124	294	0.19	0.11	0.0002	1.75	0.0002
rs10487440	7	125763796	G/T	-	121	284	0.17	0.23	0.0002	0.59	0.0002
rs10498881	6	72999327	C/G	-	121	289	0.88	0.95	0.0002	0.48	0.0019
rs9317821	13	69008405	C/T	-	123	290	0.52	0.64	0.0002	0.67	0.0008
rs10487442	7	125850492	A/G	+	124	295	0.83	0.76	0.0003	1.68	0.0003
rs723397	5	107375942	C/T	+	123	295	0.24	0.36	0.0003	0.65	0.0014
rs6897150	5	165833376	A/G	-	123	294	0.26	0.14	0.0003	1.60	0.0003
rs1458405	6	78321198	G/T	-	112	270	0.51	0.40	0.0003	1.54	0.0002
rs10521205	17	12809867	A/G	-	122	292	0.16	0.26	0.0003	0.60	0.0007
rs1023738	3	174685924	C/T	-	123	294	0.44	0.55	0.0003	0.67	0.0002
rs1367313	3	159848232	C/T	-	124	295	0.79	0.71	0.0003	1.57	0.0005
rs10521204	17	12809385	A/C	+	124	293	0.83	0.74	0.0003	1.65	0.0007
rs2034531	4	137364060	A/G	-	123	289	0.80	0.88	0.0003	0.59	0.0006
rs1282090	3	112779804	A/G	+	124	295	0.82	0.68	0.0004	1.58	0.0011
rs9328099	6	2382544	A/G	-	122	294	0.74	0.84	0.0004	0.63	0.0004
rs10497567	2	181111709	A/G	+	124	295	0.93	0.85	0.0004	1.98	0.0005

\* Build 36.1. \*\* SNPs falling within gene boundaries. \*\*\*OR calculated from a liability threshold model in SOLAR and estimated as allele 2 vs allele 1. † *P*-values derived using variance components analysis under an additive genetic model, adjusted for age, sex and family structure. ‡ *P*-values derived using variance components analysis under an additive genetic model, adjusted for age, sex, BMI and family structure. Our complete dataset with results from all models are available online (<http://www.medschool.umaryland.edu/amishstudies/index.asp>). Abbreviations: BMI, body mass index; MAF, minor allele frequency; OR, odds ratio; T2D, type 2 diabetes.

**Table 3.** SNPs associated with T2D ( $p < 0.01$ ) and at least one OGTT-derived trait ( $p < 0.01$ ) in nondiabetic Amish subjects.

										OGTT trait analysis					
										T2D					
SNP	Chr	Position*	Gene**	Strand	Alleles 1/2	Freq Allele 2	$P$ -value <sup>†</sup>	OR <sup>‡</sup>	Trait	Mean 11 <sup>§</sup>	Mean 12 <sup>  </sup>	Mean 22 <sup>¶</sup>	$P$ -value <sup>†</sup>		
rs667222	1	55090696	<i>DHCR24</i>	-	A/G	0.75	0.005	0.71	GAUC	212.21	199.85	192.58	0.008		
rs6588186	1	66319597	<i>PDE4B</i>	-	A/G	0.78	0.008	1.45	GAUC	192.09	185.94	201.07	0.002		
rs570021	1	71217978	<i>PTGER3</i>	+	A/T	0.27	0.007	0.70	IAUC	550.64	613.59	690.13	0.007		
rs6424414	1	71228058	<i>PTGER3</i>	+	C/T	0.27	0.007	0.70	IAUC	548.85	614.86	683.35	0.007		
rs211706	1	75802445	<i>SLC44A5</i>	-	A/G	0.08	0.002	0.52	FASTG	50.90	49.36	48.19	0.006		
rs10493580	1	76277500	<i>LOC729766</i>	+	C/G	0.15	0.003	0.60	GAUC	199.25	186.85	189.20	0.005		
rs1030414	1	81241030		-	C/T	0.07	0.005	0.51	GAUC	197.22	182.88	154.78	0.003		
rs2257963	1	105672501		-	C/T	0.61	0.009	0.74	GAUC	208.52	195.36	190.49	0.009		
rs1516150	1	105758114		+	A/G	0.39	0.010	1.36	GAUC	190.76	195.70	208.75	0.009		
rs2576216	1	215202099	<i>ESRRG</i>	+	A/G	0.16	0.006	0.64	GAUC	200.81	187.83	190.21	0.002		
rs2818781	1	215203396	<i>ESRRG</i>	+	C/T	0.84	0.003	1.61	GAUC	168.17	189.21	201.24	$2.2 \times 10^{-4}$		
rs2576212	1	215204637	<i>ESRRG</i>	-	A/G	0.84	0.005	1.58	GAUC	178.70	188.25	200.71	0.001		
rs1343747	1	240794106		+	A/C	0.23	0.002	1.43	HOMA-IR	2.22	2.53	2.72	0.002		
rs10495824	2	34604049		-	C/T	0.76	0.008	1.41	IAUC	555.29	646.59	694.29	0.002		
rs10490049	2	40426854	<i>SLC8A1</i>	-	A/C	0.17	0.002	1.54	FASTG	677.25	635.10	545.83	0.002		
rs897097	2	55539162		+	A/G	0.61	0.001	1.46	GAUC	192.09	204.49	216.33	0.001		
rs2540317	2	102716239	<i>MFSB9</i>	-	C/T	0.78	0.007	0.72	HOMA-IR	2.09	2.37	2.49	0.010		
rs4324336	2	105593460		-	A/T	0.78	0.005	1.44	GAUC	216.62	199.07	189.80	$3.4 \times 10^{-4}$		
rs2321201	2	134136406		-	C/T	0.37	0.006	1.35	FASTG	50.05	49.75	51.32	0.002		
rs10510530	3	22459609		+	A/T	0.86	0.003	0.65	IAUC	539.03	627.77	628.08	0.006		
rs3845971	3	59975712	<i>FHIT</i>	+	C/T	0.72	0.004	1.42	FASTG	52.25	51.56	50.20	0.007		
rs1373340	3	66984977		-	A/C	0.86	0.007	0.67	GAUC	185.46	188.48	203.71	$1.0 \times 10^{-4}$		
rs2587015	3	147740690	<i>PLSCR1</i>	-	C/T	0.76	0.007	1.40	HOMA-IR	1.89	2.14	2.43	0.006		
rs2587014	3	147741153	<i>PLSCR1</i>	+	A/G	0.24	0.009	0.72	IAUC	529.61	519.62	610.68	0.009		
rs2587012	3	147741360	<i>PLSCR1</i>	-	G/T	0.24	0.009	0.72	IAUC	751.72	609.81	547.07	0.001		
									IAUC	548.12	610.88	743.82	0.001		
									IAUC	549.20	618.96	758.40	$4.0 \times 10^{-4}$		

rs837678	3	191168575	<i>LEPRELI</i>	-	C/T	0.23	0.007	1.39	FASTG	50.27	50.77	54.28	0.008
rs10517351	4	353522269		-	A/G	0.83	0.010	0.71	ISI	0.41	0.47	0.59	0.010
rs4128879	4	162098442		-	A/G	0.85	0.007	0.67	ISI	1.46	0.62	0.52	0.009
rs10521005	5	33638471	<i>AD4AMTSI</i>	-	C/T	0.46	0.004	0.74	IAUC	659.01	568.57	550.30	0.007
rs9292501	5	33639167	<i>AD4AMTSI</i>	+	A/G	0.45	0.005	0.75	GAUC	201.46	197.18	186.88	0.006
rs9292502	5	33639516	<i>AD4AMTSI</i>	+	A/G	0.46	0.003	0.74	IAUC	655.02	570.18	550.12	0.008
rs709668	5	96199942		-	C/T	0.28	0.009	0.73	GAUC	201.78	197.24	186.94	0.006
rs950664	5	103381835		+	C/T	0.62	0.009	1.35	IAUC	661.14	571.39	551.03	0.006
rs990133	5	108073268		+	C/T	0.86	0.004	1.58	GAUC	201.47	191.03	183.01	0.002
rs7720835	5	117269389		+	C/T	0.20	0.006	1.41	GAUC	188.26	190.43	205.91	1.4 x 10 <sup>-4</sup>
rs4365869	5	117282887		+	C/T	0.79	0.009	0.72	GAUC	169.38	189.40	197.90	0.006
rs1423003	5	147427736	<i>SPINK5</i>	-	A/T	0.45	0.004	1.38	HOMA-IR	2.26	2.48	3.25	0.002
rs1862446	5	147460749	<i>SPINK5</i>	+	A/G	0.45	0.006	1.37	HOMA-IR	3.24	2.46	2.27	0.003
rs1422930	5	167330171	<i>ODZ2</i>	+	A/C	0.09	0.003	0.52	HOMA-IR	2.51	2.32	2.12	0.008
rs7770797	6	6566961	<i>LY86</i>	-	C/T	0.43	0.005	0.73	HOMA-IR	2.50	2.32	2.12	0.009
rs10484908	6	108410287		+	C/G	0.11	0.009	1.53	IAUC	2.27	2.72	1.45	0.002
rs10237701	7	8860569		+	C/T	0.45	0.009	1.33	IAUC	536.16	579.90	649.95	0.010
rs10487563	7	48905713		-	C/T	0.23	0.002	0.68	GAUC	192.64	207.68	212.03	0.001
rs2237457	7	50693638	<i>GRB10</i>	-	A/G	0.63	1.1 x 10 <sup>-5</sup>	0.61	ISI	0.67	0.49	0.51	0.008
rs10499761	7	56161137	<i>LOC650200</i>	-	A/G	0.75	0.007	1.42	ISI	0.49	0.60	0.80	1.4 x 10 <sup>-4</sup>
rs3753107	7	91467087	<i>AKAP9</i>	-	C/T	0.77	0.006	0.72	GAUC	214.90	197.81	191.93	0.001
rs10488510	7	91498161	<i>AKAP9</i>	+	G/T	0.23	0.006	1.39	GAUC	178.50	192.32	201.72	0.001
rs647055	7	114159262		+	A/G	0.25	0.007	1.41	HOMA-IR	2.88	2.50	2.27	0.007
rs10488284	7	119742580	<i>KCNM2</i>	-	A/G	0.15	0.001	0.60	HOMA-IR	2.29	2.54	2.76	0.010
rs192392	8	53094411		+	A/C	0.54	0.004	0.74	IAUC	551.83	620.21	780.97	3.8 x 10 <sup>-4</sup>
rs2450148	8	53112873		-	C/T	0.47	0.010	1.31	ISI	0.49	0.65	0.68	0.001
rs10504133	8	53152586		+	C/T	0.51	0.002	0.72	HOMA-IR	2.49	2.14	2.53	0.003
rs7001645	8	105192526	<i>RIMS2</i>	+	C/G	0.44	0.007	1.34	IAUC	650.81	600.14	540.14	0.007
rs9297357	8	106211509		-	A/G	0.23	0.006	0.68	IAUC	537.54	580.77	669.94	0.002
rs10505229	8	115908561		+	C/T	0.18	0.002	1.50	IAUC	659.06	595.05	525.29	0.001
rs10511574	9	11941204		+	A/C	0.09	0.002	1.82	ISI	0.63	0.53	0.46	0.008
rs10511777	9	26773463		-	A/G	0.94	0.006	0.53	GAUC	201.81	185.25	199.13	0.001
rs10491665	9	29401568		+	A/C	0.09	0.006	0.58	GAUC	193.84	200.43	230.74	0.004
									GAUC	193.05	209.84	170.35	0.007
									FASTG	57.50	52.50	50.41	0.002
									GAUC	198.29	185.73	171.34	0.007

rs2804498	10	33660713	<i>NRP1</i>	-	A/G	0.40	0.001	1.42	FASTG	49.96	50.69	52.10	0.003
rs768676	10	44022687		+	A/T	0.89	0.002	0.61	FASTG	53.94	52.09	50.31	3.9 x 10 <sup>-4</sup>
rs1111803	10	72426094		+	C/T	0.55	0.008	0.75	GAUC	208.11	193.50	191.12	0.004
rs2437871	10	90268360	<i>C10orf5</i>	+	A/C	0.54	0.003	0.72	ISI	0.47	0.51	0.68	0.001
rs10509589	10	91804145		-	A/C	0.11	0.004	1.61	HOMA-IR	2.34	2.74	2.51	0.010
rs1887979	10	110133240		+	A/T	0.41	0.009	0.74	GAUC	201.35	193.00	187.75	0.010
rs10500651	11	5530156		+	G/T	0.73	0.006	1.42	HOMA-IR	2.88	2.46	2.27	0.005
rs7119814	11	107941856	<i>EXPH5</i>	+	C/T	0.14	0.007	1.50	ISI	0.58	0.46	0.29	0.008
rs10506173	12	39431860	<i>CNTN1</i>	+	A/G	0.95	0.007	0.55	FASTG	53.53	52.44	50.41	0.007
rs312272	12	39534200	<i>CNTN1</i>	+	C/T	0.69	0.007	1.37	FASTG	49.93	49.95	51.54	0.002
rs192852	12	39537359	<i>CNTN1</i>	-	C/T	0.32	0.009	0.74	FASTG	51.50	49.91	49.87	0.001
rs2289522	12	39616798	<i>CNTN1</i>	+	C/T	0.55	0.001	1.46	FASTG	49.75	50.55	51.55	0.004
rs3794247	12	39646028	<i>CNTN1</i>	+	A/C	0.57	0.002	1.42	FASTG	49.66	50.59	51.55	0.003
rs10521210	17	12966928		+	C/T	0.65	0.009	1.34	GAUC	184.59	193.63	199.65	0.009
rs9915220	17	13630703		+	A/G	0.95	0.001	2.72	FASTG	48.47	49.05	50.87	0.009
rs530205	18	42639148		+	C/T	0.61	0.009	0.76	IAUC	642.24	605.90	538.52	0.008
rs2953271	18	47696031		-	A/T	0.38	0.002	0.69	GAUC	200.87	195.67	183.77	0.004
rs10502971	18	49141959	<i>DCC</i>	+	A/G	0.59	0.009	1.34	GAUC	186.36	193.47	203.52	0.001
rs615696	18	49946910	<i>MBD2</i>	+	C/T	0.79	0.006	0.69	FASTG	51.99	51.10	49.99	0.010
rs739453	19	40771776		+	A/G	0.60	0.005	0.73	IAUC	685.23	586.92	536.51	0.002
rs3745718	19	53406965	<i>CARD8</i>	-	A/G	0.40	0.010	0.75	GAUC	202.60	193.05	186.48	0.003
rs297765	20	4435111		+	A/G	0.67	2.1 x 10 <sup>-4</sup>	1.55	FASTG	49.29	50.36	51.35	0.004
rs2255140	21	31975858	<i>SFRS15</i>	+	C/T	0.87	0.009	1.57	GAUC	186.37	191.30	203.12	0.001
									ISI	0.37	0.48	0.60	0.004

SNPs with  $p < 0.01$  for T2D associations were tested for consistency in a sample of nondiabetic individuals (295 of whom overlapped with the T2D association dataset). Direction of association was checked for glucose traits. Underlined SNPs are in high linkage disequilibrium ( $r^2 > 0.80$ ) \* Genome Build 36.1. \*\* SNPs fall within gene boundaries. †  $P$ -values derived using the additive genetic model, adjusted for age, sex and family structure. The complete dataset including results for dominant and recessive models are available at (<http://www.medschool.umaryland.edu/amishstudies/index.asp>). ‡ OR calculated from a liability threshold model for allele 2 vs. allele 1. † Mean values for traits are presented by genotype, with alleles shown in alphabetical order as "1/2". All Insulin traits (IAUC, HOMA-IR, ISI) were natural log-transformed prior to analysis. Abbreviations: FASTG, fasting glucose; GAUC, glucose area under the OGTT curve; HOMA-IR, homeostasis model assessment of insulin resistance; IAUC, insulin area under the OGTT curve; ISI, insulinogenic index.

**Table 4.** SNPs associated with T2D in the Amish ( $p < 0.005$ ) and providing evidence for either internal consistency (association with an OGTT derived quantitative trait in the Amish) or external replication (associated with T2D in an independent population).

SNP	Chr	Position *	Gene**	Strand	Associated with T2D in Amish ( $p < 0.005$ )			Internal consistency with OGTT derived quantitative traits in Amish ( $p < 0.005$ )			External replication with T2D in independent populations ( $p < 0.005$ )			
					Allele 1/2	Allele 2 Cases	Allele 2 Controls	p-value	OR***	Trait	p-value	Population	p-value†	OR***
rs689157	1	183074135	<i>C1orf24</i>	+	A/T	0.69	0.80	0.001	0.67	GAUC	0.002	Pima	0.002	0.65
rs2818781	1	215203396	<i>ESRRG</i>	+	C/T	0.89	0.81	0.003	1.61	HOMA-IR	0.002			
rs1343747	1	240794106	--	+	A/C	0.30	0.21	0.002	1.43	IAUC	0.002			
rs10490049	2	40426854	<i>SLC8A1</i>	-	A/C	0.23	0.14	0.002	1.54	FASTG	0.001			
rs3845971	3	59975712	<i>FHIT</i>	+	C/T	0.79	0.68	0.004	1.42	GAUC	4.0 x 10 <sup>-4</sup>	Mex-Am	0.004	1.46
rs9312113	4	63643792	--	+	C/G	0.75	0.64	0.004	1.41			FHS	0.013,	1.66
rs3775745	4	71147663	<i>CSN3</i>	-	A/C	0.26	0.39	0.002	0.70			Mex-Am	0.003	0.68
rs1422930	5	167330171	<i>ODZ2</i>	+	C/T	0.04	0.11	0.003	0.52	HOMA-IR	0.002			
rs9321743	6	140005650	--	-	A/C	0.42	0.32	0.003	1.42			FHS	0.002,	1.60
rs10487563	7	48905713	--	-	C/T	0.15	0.27	0.002	0.68	ISI	0.001			
rs2237457	7	50693638	<i>GRB10</i>	-	A/G	0.53	0.68	1.1 x 10 <sup>-5</sup>	0.61	GAUC	0.001			
rs10488284	7	119742580	<i>KCND2</i>	-	A/G	0.12	0.17	0.001	0.60	HOMA-IR	0.003			
rs10504133	8	53152586	--	+	C/T	0.43	0.54	0.002	0.72	IAUC	0.001			
rs10505229	8	115908561	--	+	C/T	0.27	0.15	0.002	1.50	GAUC	0.004			
rs2804498	10	33660713	<i>NRP1</i>	-	A/G	0.49	0.36	0.001	1.42	FASTG	0.003			
rs768676	10	44022687	--	+	A/T	0.85	0.91	0.002	0.61	FASTG	0.004			
rs2437871	10	90268360	<i>C10orf59</i>	+	A/C	0.47	0.57	0.003	0.72	ISI	0.001			
rs2289522	12	39616798	<i>CNTN1</i>	+	C/T	0.64	0.52	0.001	1.46	FASTG	0.004			
rs7986010	13	91517260	<i>GPC5</i>	+	A/G	0.44	0.34	0.002	1.41			FHS	0.004,	1.59

rs2953271	18	47696031	--	-	A/T	0.27	0.43	0.002	0.69	GAUC	0.004	0.042
rs297765	20	4435111	--	+	A/G	0.75	0.63	$2.1 \times 10^{-4}$	1.55	FASTG	0.004	

\* Genome Build 36.1. \*\* SNPs falling within gene boundaries. \*\*\* OR calculated from a liability threshold model in SOLAR and estimated as allele 2 vs allele 1. † Case-control general estimating equation (GEE) and family-based association test (FBAT) *p*-values given for Framingham Heart Study data; summary *p*-value given for Pimas. All Insulin traits (HOMA-IR, IAUC, ISI) were natural log-transformed prior to analysis. Abbreviations: FASTG, fasting glucose; FHS, Framingham Heart Study, GAUC, glucose area under the oral glucose tolerance (OGTT) curve; HOMA-IR, homeostatic model assessment of insulin resistance; IAUC, insulin area under the OGTT curve; ISI, insulinogenic index; MAF, minor allele frequency; Mex Am, Mexican American; OR, odds ratio.

## **FIGURE LEGENDS**

**Figure 1.** Schematic diagram of analysis and SNP prioritization approach for a 100K T2D genome-wide association scan in the Amish. Abbreviations: DGI, Broad-Lund-Novartis Diabetes Genome Initiative; FASG, fasting glucose during an oral glucose tolerance tests (OGTT); FHS, Framingham Heart Study; GAUC, OGTT glucose area under the curve; GWAS, genome-wide association scan; HOMA-IR, homeostatic model of insulin resistance; IAUC, OGTT insulin area under the curve; ISI, insulinogenic index; Mex-Am, Mexican Americans of the Starr County Health Study; NGT, normal glucose tolerant; OGTT, oral glucose tolerance test; SNPs, single nucleotide polymorphisms; T2D, type 2 diabetes

**Figure 2.** SNP association  $p$ -values ( $<0.01$ ) across all autosomal chromosomes.

Figure 1

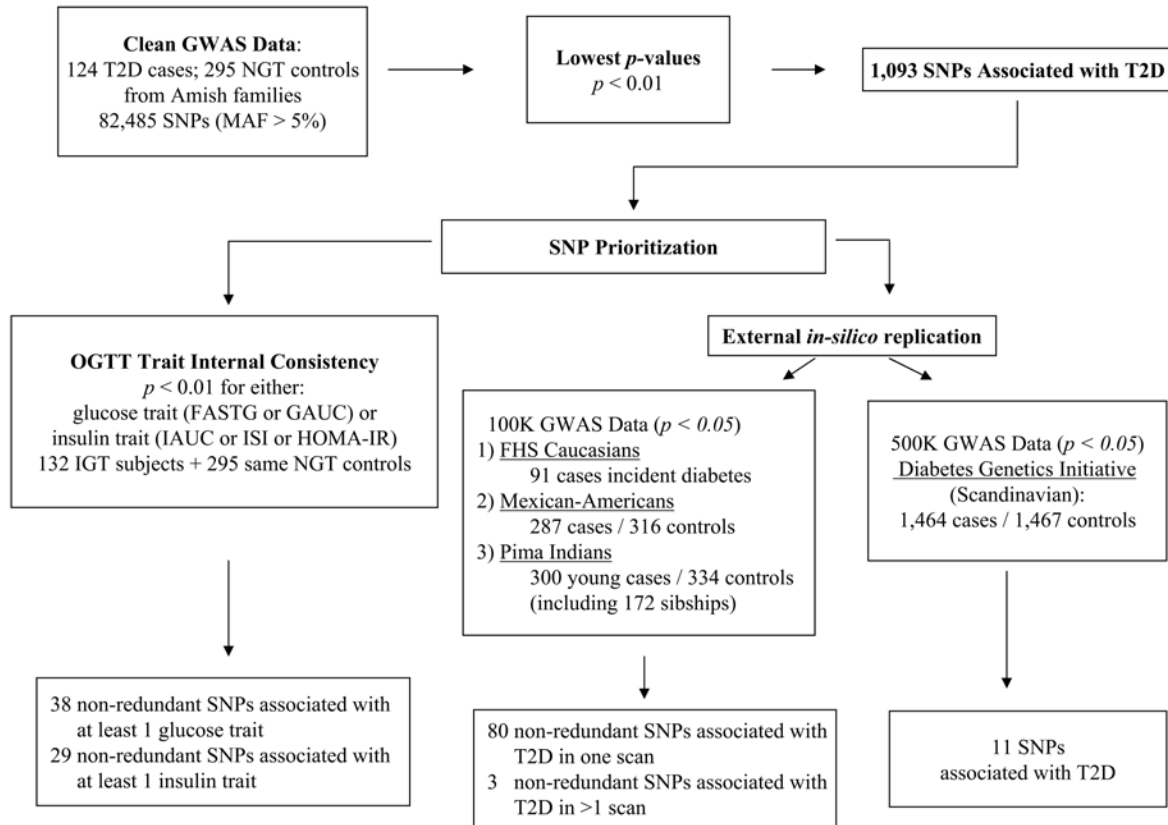


Figure 2

