Published in final edited form as:
Hum Genomics. 2011 January ; 5(2): 79–89.

Ancestry informative markers on chromosomes 2, 8 and 15 are associated with insulin-related traits in a racially diverse sample of children

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Abstract

Type-2 diabetes represents an increasing health burden. Its prevalence is rising among younger age groups and differs among racial/ethnic groups. Little is known about its genetic basis, including whether there is a genetic basis for racial/ethnic disparities. We examine a multiethnic sample of 253 healthy children to evaluate associations between insulin-related phenotypes and 142 ancestry informative markers (AIMs), while adjusting for sex, age, Tanner stage, genetic admixture, total body fat, height and socio-economic status. We also evaluate the effect of measurement errors in estimation of the individual ancestry proportions on the regression results. We find that European genetic admixture is positively associated with insulin sensitivity (S_I), and negatively associated with acute insulin response to glucose, fasting insulin, and homeostasis model assessment of insulin resistance. Our analysis reveals associations between individual AIMs on Chromosomes 2, 8, and 15 and these phenotypes. Most notably, marker rs3287 at chromosome 2p21 was found to be associated with S_I ($p=5.8 \times 10^{-5}$). This marker may be in admixture linkage disequilibrium with nearby loci (THADA and BCL11A) that have previously been reported to be associated with diabetes and diabetes-related phenotypes in several genome-wide association and linkage studies. Our results provide further evidence that variation in the 2p21 region containing THADA and BCL11A is associated with type-2 diabetes. Importantly, we have implicated this region in the early development of diabetes-related phenotypes, and in the genetic etiology of population differences in these phenotypes.

Keywords

insulin sensitivity; genetic admixture; type-2 diabetes; genetic association; ancestry informative marker

INTRODUCTION

Type-2 diabetes prevalence in the pediatric population is increasing, while age at onset is decreasing (1;2). Type-2 diabetes also disproportionately affects racial/ethnic minorities in

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the US (3). Twin and familial studies have shown a substantial genetic component to the disease as well as its related phenotypes (4-10). Although much is known about environmental contributions to type-2 diabetes, only a very small proportion of the variation due to genetic factors is currently explainable by identified genetic polymorphisms (11-13). Similarly, little is known about the specific genetic factors that may contribute to population differences in diabetes prevalence. Because origins of type-2 diabetes are likely rooted in childhood, a better understanding of genetic determinants among pediatric populations can lead to better insight into the etiology of type-2 diabetes and eventually improved prediction and prevention.

Endo-phenotypes can be useful in closely dissecting the genetic basis of eventual disease status (14). For type-2 diabetes, several such measurable phenotypes exist, typically examining measures of glucose and insulin homeostasis. These measures serve as indicators of insulin response and action that may presage type-2 diabetes (15;16). Further, previous studies have suggested a genetic basis for racial/ethnic differences in insulin dynamics (17;18). Examining the genetic basis for these detailed phenotypes therefore allows for a much better understanding of the link between the genetic and metabolic pathways that underlie the development of type-2 diabetes.

Since the loci recently identified by genome-wide association studies (GWAS) (19-21) were identified predominately among individuals of European descent, there is considerable uncertainty regarding whether these associations translate to other populations. Mexican and African Americans suffer from higher rates of type-2 diabetes than European Americans (22;23), and similar differences exist for endo-phenotypes among children, regardless of disease status (24-28). Admixed populations such as Hispanic, African, and European Americans can be examined to determine if there is a genetic basis for these population differences, and to identify specific genetic regions associated with both ancestry and insulin-related outcomes (29). Previous studies have shown that admixture is a strong predictor of diabetes and insulin-related traits, but also that this relationship may be mediated through environmental factors such as income or educational level that are correlated with genetic admixture (30-33).

Genetic mapping methods such as admixture mapping capitalize on population differences in a trait and the extended blocks of linkage disequilibrium (LD) created after the admixture process (34). They are therefore most applicable to recently admixed populations such as Hispanic and African Americans. To our knowledge, only one previous study has used this type of method to localize genetic variants associated with type-2 diabetes (35). Given the large differences in type-2 diabetes and the plausible genetic origin of these differences, there is a great need for additional studies that attempt to pinpoint population-specific genetic risk factors. In this study we also capitalize on population differences in a phenotype and extended LD blocks resulting from admixture in an attempt to identify specific genetic regions that may be involved in the etiology of population differences in insulin-related traits.

Specifically, we examine the association between genetic admixture and four insulin-related outcomes: insulin sensitivity (S_I), acute insulin response to glucose (AIRg), fasting insulin (FI), and homeostasis model assessment of insulin resistance HOMA-IR). We then examine the association between 142 ancestry informative markers (AIMs) and each of these traits to identify genetic regions that potentially account for ethnic/racial differences. We performed this study among a multi-ethnic sample of children, adjusting for known influential covariates, including genetic admixture as a control for genetic background, and socio-economic status. We place our findings in the context of other studies that have found associations in similar regions.

METHODS

Study Participants

A total of 253 children between the ages of 7 and 12 (52% male) were recruited as part of a cross-sectional cohort study examining population differences in metabolic phenotypes among children with no major illnesses or medical diagnoses. The children were classified by parental report as African American (AA) (n=87), European American (EA) (n=108), Hispanic American (HA) (n=52), and Bi-racial (n=6). All children were pubertal stage ≤3 as assessed by a pediatrician according to the criteria of Marshall and Tanner (36). Written informed assent and consent were obtained from children and parents respectively, as approved by the University of Alabama at Birmingham Institutional Review Board. All measurements were taken between 2004 and 2008 at the University of Alabama at Birmingham General Clinical Research Center (GCRC) and Department of Nutrition Sciences.

Anthropometric Measurements

In the first of two sessions completed by participants, pubertal status, anthropometric measurements, and body composition were assessed. Height was measured without shoes to the nearest centimeter using a stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, WA). Body composition was assessed by dual-energy x-ray absorptiometry (DXA) using a GE Lunar Prodigy densitometer (GE LUNAR Radiation Corp., Madison, WI) as previously described (37). Participants were measured while lying flat on their backs with arms at their sides, wearing light clothing. Analysis of DXA scans was performed using pediatric software (Encore 2002 version 6.10.029).

Insulin-related measurements

At the second visit (which took place within thirty days of the first visit), participants were admitted to the GCRC for an overnight visit. Following an overnight fast, blood samples were obtained to establish the basal levels of glucose and insulin, and a frequently sampled intravenous glucose tolerance test (FSIGTT) was performed as described elsewhere (38-40). S_I (the increase in fractional glucose disappearance per unit of insulin increase) and AIRg (the area above baseline insulin concentration during 10 minutes following exposure to glucose) were estimated from the FSIGTT using minimal modeling (41). HOMA-IR, a surrogate measure of insulin resistance, derived as: fasting glucose (mg/dl) * fasting insulin (uU/ml))/405 (42), was also calculated.

Socio-economic status (SES)

SES was measured by using the Hollingshead 4-factor index of social class, which combines information on the education and occupational prestige of parents (43). Scores range from 8 to 66, with higher scores representing higher status.

Genetic Analysis

DNA from blood was obtained from all study participants and was typed at 142 AIMs by Prevention Genetics (Marshfield, WI). Each marker (single nucleotide polymorphism) was genotyped using a fluorescent allele specific PCR (AS-PCR) based assay (44). Reaction components were assembled on an array tape platform (www.douglasscientific.com) using nanoliter volumes (500-1000nl). PCRs are carried out in a water bath thermocycler using standard 3-stage parameter (denature, primer annealing, primer extension). The specific parameters of each PCR vary depending on the nature of the primers and the SNP being genotyped. The array tape is scanned post PCR and the ratio of fluorescent signals is used to

determine the genotype (homozygous for one allele, or heterozygous). A subset of these AIMs is described elsewhere (45).

These markers were chosen because they exhibit large frequency differences between ancestral West African, Amerindian and West European populations. Individual West African, Amerindian, and European genetic admixture estimates were obtained by maximum likelihood estimation (46), using the genotypes at each AIM and an estimate of the allele frequencies of these AIMs in the three ancestral parental populations (see Supplementary Table).

Statistical Analyses

Differences between racial/ethnic groups in mean values for phenotypes were examined using ANOVA. Multiple linear regression analyses were used to test the association between European admixture and total fat and the four insulin-related phenotypes, and to examine the association between each of 142 SNPs and four insulin-related phenotypes. For S_i, FI, and HOMA-IR the model was defined by age, Tanner stage, sex, SES, European admixture, Amerindian admixture, total fat, and height. By controlling for two of three admixture estimates, we prevent the introduction of colinearity in the statistical models, since the three admixture estimates add up to 1. For AIRg, the model was additionally adjusted for S_I. To conform to the assumptions of regression, all models were evaluated for residual normality, and logarithmic transformation was performed when appropriate. Outliers were removed based on whether residuals were greater than three standard deviations away from the mean.

Genotyped SNPs were tested for association with the four insulin-related phenotypes using linear regression under additive, dominant, recessive and 2-degrees-of-freedom genotypic models. Considering each phenotype and each genetic model separately, we applied a Bonferroni multiple correction to the marker association tests; a p-value cutoff of 3.6×10^{-4} keeps the nominal type I error rate at 0.05. To determine the extent to which measurement error in admixture estimates could skew the results, we applied the method as described by Divers et al. (47). Basically, we obtained an estimate of the measurement error covariance and applied the simulation extrapolation (SimEx) algorithm (48) to retest for association between each marker and phenotype, for each mode of inheritance model. Analyses were carried out with PLINK (49), SAS 9.1 software (SAS Institute, Cary, NC) and R (50).

RESULTS

Descriptive Characteristics

Table 1 shows the descriptive characteristics of the sample. Differences in total fat, AIRg, FI, and HOMA-IR were statistically significant between racial/ethnic groups (all at $p<0.01$). HA had higher total fat, FI and HOMA-IR than both EA and AA. AA had higher AIRg, and lower S_I than other groups. EA had the highest S_I values ($p<0.0001$).

Association between genetic admixture and insulin-related phenotypes

The associations between European genetic admixture and total fat and all insulin-related phenotypes were statistically significant ($p<0.05$; Table 2). Individuals with higher European admixture had less total fat, higher S_I, and lower AIRg, FI, and HOMA-IR. In these models, there were significant associations of tanner stage and total fat with Si, FI and HOMA-IR ($p<0.01$), and total fat was also associated with AIRg ($p=0.04$). Upon analyzing these associations within racial/ethnic groups, we found significant associations of European genetic admixture only among HA for S_I, FI, and AIRg ($p<0.05$). These results suggest that specific genetic variants may exist contributing to population differences for these phenotypes.

Association between single markers and insulin-related phenotypes

Results of single SNP analyses are presented in Table 3. Marker rs3287 located at 2p21 is significantly associated with S_I under the recessive ($p=5.8 \times 10^{-5}$) and genotypic ($p=1.9 \times 10^{-4}$) models. Allele G at this SNP is associated with decreased S_I, and is at a higher frequency in the West African parental population (0.75) compared to both the European (0.18) and Amerindian (0.18) parental populations. In our sample, the frequency of the G allele is 0.58 among AA, 0.25 among EA, and 0.21 among HA. We found no associations that withstand Bonferroni correction ($p<3.6 \times 10^{-4}$) within each racial/ethnic group.

Marker rs1373302 located at 8q13 is significantly associated with AIRg and DI under the dominant model ($p=9.7 \times 10^{-5}$, $p=8.9 \times 10^{-5}$, respectively). Allele T at this marker is associated with increased AIRg, and is at a higher frequency among the West African (0.65) and European (0.73) parental populations than among the Amerindian parental population (0.08). We found no significant associations within racial/ethnic groups for this marker after adjusting for Bonferroni correction. Marker rs2671110 located at 7q32.3 is significantly associated with AIRg among AA under the recessive model ($p=1.4 \times 10^{-4}$). Allele A at this marker is associated with increased AIRg and is at a higher frequency among the West African parental population (0.94) than among the Amerindian (0.0) and European (0.13) parental populations.

Marker rs12439722 is significantly associated with FI and HOMA-IR under the recessive and genotypic models ($p=2.8 \times 10^{-4}$, 1.4×10^{-4}, respectively). Allele A at this marker is associated with increased FI, and is at a higher frequency in the West African (1.0) and European (0.97) parental populations than in the Amerindian (0.17) parental population.

The application of the measurement error correction methods did not yield results that were significantly different than those we observed with the naïve analyses. For example, we observed a p-value of 7.68×10^{-5} for the association between rs3287 and S_I under the recessive model after accounting for the measurement error vs. 5.9×10^{-5} without the adjustment. This result confirms that individual ancestry proportions are very well measured. Therefore, with 142 AIMs, we can be confident our results are not driven by measurement errors in the estimation of individual ancestry.

DISCUSSION

We sought to examine the potential genetic basis for population differences in insulin-related phenotypes in a racially/ethnically diverse sample of children. We found that European genetic admixture is associated with insulin related phenotypes. Next, we determined whether any of the individual 142 AIMs scattered throughout the genome were associated with any of the insulin-related phenotypes. We find a strong association between S_I and an AIM at chromosome 2p21 (rs3287), explaining 4.14% of the variance of the trait. Although this effect size may appear large compared to other genetic association studies, our use of refined phenotypes, the inclusion of many covariates, and the use of admixed individuals likely increased our ability to detect an effect size of this magnitude. We also found weaker, but statistically significant associations between AIRg and an AIM at chromosome 8q13 (rs1373302) located in the TRPA1 gene, and between FI and HOMA-IR and an AIM at chromosome 15q22 (rs12439722) in the HERC1 gene. It should be noted that although we have used a multiple correction for the 142 markers tested, we have not corrected for each of the genetic models tested. If we were to use a Bonferroni correction for all markers and models tested, the p-value threshold would be 8.8×10^{-5}. In this case, only the association between rs3287 and S_I ($p=5.8 \times 10^{-5}$) would be considered statistically significant. However, the four genetic models are likely to be correlated, thus making such a correction overly conservative.

Our finding that insulin-related phenotypes are associated with European admixture is in agreement with previous findings (51). European admixture is positively associated with favorable insulin-related phenotypes (higher S_I, and lower AIRg, FI, and HOMA-IR). When we examine the association of European admixture within racial/ethnic groups, they are only statistically significant among HA. However, it is difficult to interpret these results because of the different sample sizes and different admixture proportion distributions by racial/ethnic group. Among the other covariates examined, we find that total body fat and Tanner stage are the strongest risk factors associated with these insulin-related traits. This result is consistent with those of other studies that show that adiposity is a major risk factor for these traits (52;53). Insulin-related traits have also previously been found to be associated with pubertal stage (54;55).

The 2p21 chromosomal region has been previously identified as being associated with type-2 diabetes and related traits via both linkage scans and GWAS. Marker rs3287 is located at 2p21, between two loci, *THADA* (thyroid adenoma associated) and *BCL11A* (B-cell CLL/lymphoma 11A). These loci have been previously identified in two recent GWAS meta-analyses of type-2 diabetes (56;57). This region was also identified in linkage scans for insulin- and diabetes- related traits (58;59). It is plausible that through their effects on cell apoptosis (60) and/or nutrient transport (61), these loci may be associated with the progression of type-2 diabetes and/or that different pathways may be involved across populations. Given that the rs3287 risk allele is higher in the West African parental population compared to the European and Amerindian parental populations, and that AA tend to have lower S_I, this or another nearby variant that is in admixture linkage disequilibrium may explain part of the observed differences in type-2 diabetes susceptibility between African and European Americans. The markers that we have found to be associated with FI and HOMA-IR are in a region on chromosome 15 that has previously been found in a linkage scan to be associated with insulin-related traits (62).

Unlike other association studies, we have identified these associations relatively early in the lifespan. It could be that the children with unfavorable insulin-related phenotypes are already on the path towards developing type-2 diabetes. In the long-term, these markers could inform prediction and treatment strategies for early-onset type-2 diabetes and explain population differences. The fact that none of the AIMs showed any significant association with any of the insulin related phenotypes when performing analyses within racial/ethnic groups may be related to the reduced power due to a smaller sample size. Furthermore, our ability to find significant associations by race is strongly influenced by the frequency of the variants, and the fact that AIM alleles tend to have a low frequency in one group and a high frequency in another group. By using a multiethnic approach we have the advantage of having more intermediate allele frequencies represented, thus increasing the power to detect associations.

This study has several strengths. First, the use of several endo-phenotypes that are likely to be proximal to the development of type-2 diabetes may more effectively pinpoint the genetics factors that eventually lead to disease phenotypes. Second, the inclusion of individuals from different racial/ethnic backgrounds, and the use of markers that differ in frequency between populations can lead to a better understanding of the genetic basis for population differences in insulin-related phenotypes and the prevalence of diabetes. Third, the inclusion of environmental and phenotypic measurements enhances the ability to pinpoint the genetic regions that directly influence the disease causing phenotype.

The study also has some limitations. There was a relatively small number of genetic markers used, reducing our ability to provide a high level of resolution with regards to the precise location of potential risk variants. The main weakness of the study lies in the small sample

size, which raises concerns about the statistical power of the study. Of all the reported associations, the one between marker rs3287 and S_I will require the greatest level of statistical power in order to reject the null hypothesis. A power calculation for this association model reveals that at a p-value of 6×10^{-5}, our data provides 67% power to estimate the R-squared effect of 0.45 that we obtained for the full model, with a semi-partial R-squared for the marker of 0.04. Although this level of power might not seem sufficient, the concerns of this association being a type-1 error are dissipated by the fact that it represents a form of replication of several previously reported findings at chromosome 2p21. Evidently, this level of detection with a small sample size was aided by the use of precise phenotyping, the consideration of physiological parameters and the inclusion of admixture estimates, as previously discussed.

In conclusion, we have shown that regions on chromosome 2, 8 and 15 are associated with insulin-related traits in this sample. These results suggest that these regions may harbor causal variants that may also explain population differences in the insulin-related phenotypes and ultimately type-2 diabetes prevalence, since the markers tested exhibit large frequency differences between groups. Future studies must combine detailed phenotypic, environmental and genetic measures on similarly diverse, but larger sample sizes. The inclusion of different populations is of paramount importance if we are to understand the genetic basis for population differences and fairly implement effective prevention, intervention, and treatment strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the participants of the AMERICO study and their families, and Nicholas Pajewski for help with statistical analyses.

Funded by: NIH R01-DK067426 and NIH T32HL007457 from the National Heart, Lung, and Blood Institute, Clinical Nutrition Research Center Grant P30-DK56336, UAB Diabetes Research and Training Center Grant P60DK079626, and by General Clinical Research Center Grant M01RR00032.

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Table 1

Populationcharacteristics for total sample and for each racial/ethnic group (mean ± s.d.)

	Total Sample	EA	AA	HA	Other
Female/Male (n)	122/131	51/57	40/47	28/24	3/3
Total Fat	8.76 ± 5.49	8.35 ±5.11	8.13 ±6.02	10.77 ± 5.13	7.22 ± 4.52
AIRg	903.9 ± 708.4	619.1 ± 425.4	1235.4 ± 827.5	940.8 ±748.6	768.0 ± 328.8
S_I	5.65 ± 3.05	6.69 ± 2.55	4.59 ± 2.61	5.32 ± 3.88	6.07 ± 3.24
FI	12.51 ± 5.89	10.91 ± 4.14	13.27 ± 5.74	14.58 ±7.92	10.5 ± 5.13
HOMA-IR	3.02 ± 1.51	2.64 ± 1.06	3.12 ± 1.41	3.64 ± 2.11	2.47 ± 1.25

Units: Total fat (kg), AIRg (μIU/ml × 10 min), S_I ($\times 10^{-4}$/min/(μIU/ml)), FI (μIU/ml)

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Table 2

Association between European admixture and phenotypes (Standardized parameter estimate and p-value shown, respectively)

	Entire Sample	Hispanic Americans	African Americans	European Americans
Total Fat	0.13 (p=0.037*)	-0.12 (p=0.40)	0.17 (p=0.090)	0.02 (p=0.80)
Si	0.34 (p<0.0001*)	0.25 (p=0.031*)	-0.01 (p=0.93)	0.13 (p=0.18)
AIRg	-0.28 (p<0.0001*)	-0.23 (p=0.038*)	-0.13 (p=0.19)	-0.09 (p=0.37)
FI	-0.20 (p=0.0013*)	-0.27 (p=0.045*)	-0.07 (p=0.45)	-0.02 (p=0.79)
HOMA-IR	-0.16 (p=0.0095*)	-0.26 (p=0.064)	-0.11 (p=0.25)	-0.005 (p=0.96)

Covariates: age, sex, Tanner stage, SES, total fat, height (for AIR, we also controlled for SI)

*denotes p<0.05.

Table 3

Summary of strongest associations by phenotype (p-value cutoff with Bonferroni correction: 3.6×10^{-4})

Gene	Chr.; loc	SNP	Pheno	Model	Group	Uncorrected p-value
SPTBN1	2; 54.7M	rs3287	S_I	Recessive	All	5.8×10^{-5}
SPTBN1	2; 54.7M	rs3287	S_I	Genotypic	All	1.9×10^{-4}
HERC1	15;61.7M	rs12439722	S_I	Genotypic	All	5.4×10^{-4}
TRPA1	8; 73.1M	rs1373302	AIRg	Dominant	All	9.7×10^{-5}
PLXNA4	7;131.5M	rs2671110	AIRg	Recessive	AA	1.4×10^{-4}
HERC1	15;61.7M	rs12439722	FI	Recessive	All	2.8×10^{-4}
HERC1	15;61.7M	rs12439722	FI	Genotypic	All	1.4×10^{-4}
HERC1	15;61.7M	rs12439722	HOMA-IR	Recessive	All	8.0×10^{-4}
HERC1	15;61.7M	rs12439722	HOMA-IR	Genotypic	All	4.8×10^{-4}

Covariates: age, sex, Tanner stage, SES, European admixture, Amerindian admixture, height, total fat (for AIR - control for SI as well).

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