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SHORT COMMUNICATION

Association testing of *TCF7L2* polymorphisms with type 2 diabetes in multi-ethnic youth

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Abstract
Aim/hypothesis Common variants in the transcription factor 7-like 2 (*TCF7L2*) gene have been associated with type 2 diabetes in adults. However, it is not known whether *TCF7L2* variation increases the risk of early onset type 2 diabetes. Using a case–control design, we examined whether the reported variants [rs12255372 (T/G) and rs7903146 (T/C)] are associated with type 2 diabetes in SEARCH for Diabetes in Youth study participants.
Methods Variants were genotyped in 694 non-Hispanic white (NHW) youth (86 cases, mean age 15.5 years, mean BMI 34.8; and 608 controls, mean age 14.4 years, mean BMI 22.3) and 545 African-American (AA) youth (154 cases, mean age 15.9, mean BMI 37; and 391 controls, mean age 14.8, mean BMI 23.8). Logistic regression adjusted for age, sex, BMI and West African ancestry.
Results The association of the risk T allele with case/control status was different in AA and NHW youth (p=0.025). Among AA youth, each copy of the T allele (rs7903146) was associated with a 1.97-fold (1.37, 2.82) increased odds for type 2 diabetes (p<0.0001), after adjustment for age, sex, BMI and African ancestry. No significant association was detected in NHW youth (adjusted OR, 1.14; 0.73, 1.79).
Conclusion/interpretation TCF7L2 variation is associated with an increased risk of early-onset type 2 diabetes among AA youth, and the association appears to be stronger in AA

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than NHW youth. This suggests potential different contributions of genetic and environmental factors to early-onset type 2 diabetes by race.

Keywords *TCF7L2* · Type 2 diabetes · Youth

Abbreviations

AA	African-American
NHW	Non-Hispanic white
SNP	Single nucleotide polymorphism
TCF7L2	Transcription factor 7-like 2

Introduction

Recent studies have demonstrated that common variants of the transcription factor 7-like 2 (*TCF7L2*) gene are associated with adult-onset type 2 diabetes. Meta-analyses indicate that the association between *TCF7L2* and adult-onset type 2 diabetes is highly reproducible [1, 2] and suggest an additive effect [1].

Type 2 diabetes is now being reported in adolescents and even children. The SEARCH study identified youth with type 2 diabetes in all major racial/ethnic groups in the USA [3]. However, no studies have directly tested the association between *TCF7L2* and early-onset type 2 diabetes, where the size of effect may be larger. We hypothesised that there would be a significant association between *TCF7L2* risk-conferring variants (rs12255372 and rs7903146) and type 2 diabetes prevalence in 10- to 22-year-old youth of non-Hispanic white (NHW) and African-American (AA) origin, independent of age, sex, race/ethnicity and current levels of adiposity.

Methods

Identification of cases and controls Youth eligible for inclusion as cases were all youth age 10–22 years with a clinical diagnosis of type 2 diabetes, who participated in the SEARCH research visit, provided a DNA sample, and self-reported their race/ethnicity as NHW or AA. SEARCH is an on-going multicentre study that conducts population-based ascertainment of diabetes in youth [3]. Cases are identified in geographically defined populations in Ohio, Washington, South Carolina and Colorado and among youth enrolled in managed healthcare plans in Hawaii and California [3]. Control participants who were not diabetic were recruited from population-based studies in Ohio, Colorado and South Carolina [4] and group-matched to cases on race/ethnicity. Control participants reported no known family history of diabetes and were confirmed to be diabetes-free by fasting plasma glucose testing.

The study was approved by the local institutional review boards, and consent was given by all participants for DNA testing. Weight and height were measured and BMI was calculated. Among controls, fasting insulin and glucose levels were used to estimate insulin secretion and resistance using the homeostasis model assessment (HOMA-B and HOMA-IR) equations [5]. Among cases, fasting blood samples were obtained and analysed for glutamic acid decarboxylase-65 and insulinoma-associated-2 autoantibodies using a standardised assay protocol developed by the standardisation group sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases. Testing for the most common subtypes of maturity onset diabetes of the young (*HNF1A*, *GCK* and *HNF4A*α) was conducted in A. Hattersley's laboratory in Exeter, UK. Youth with diabetes were included if they were negative for both autoantibodies and negative for mutations in these genes, resulting in the exclusion of 35 participants with clinical type 2 diabetes and positive autoantibodies and 14 cases with MODY-related mutations.

TCF7L2 genotyping The single nucleotide polymorphism (SNP) variants rs7903146 and rs12255372 were genotyped using DNA extracted from peripheral blood leucocytes and quantified with Pico-Green analysis (Molecular Probes, Eugene, OR, USA). Genotyping was performed by allele-specific primer extension of singleplex amplified products, with detection by matrix-assisted laser desorption–ionisation time-of-flight mass spectroscopy on a Sequenom platform [6]. The allele frequencies for both SNPs in each of the two racial/ethnic groups were in Hardy–Weinberg equilibrium ($p>0.05$). In addition, 54 ancestry informative markers (AIMs) were genotyped to evaluate the proportion of African and European ancestry in each sample, using STRUCTURE (a computer software package available at http://www.stats.ox.ac.uk/~pritch/home.html) [7], trained on the HapMap CEU (European) and YRI (West African) populations. AIMs were chosen to maximally differentiate the proportion of European and African ancestry in the AA admixed population. No effort was made to include markers that distinguish between the Asian and European or Asian and African populations. Given the historical genealogy of AA, we do not believe that undetected Asian ancestry plays a significant role in the great majority of our participants.

Statistical analyses Logistic regression was used to calculate ORs and 95% CIs, for the association between the risk T allele and case/control status, stratified according to race/ethnicity, using an additive model. We adjusted for age, sex, BMI and proportion of African ancestry. Heterogeneity of results between NHW and AA youth was assessed by a χ^2 statistic. Among controls, analysis of variance was used to explore the association of the risk T

Table 1 Characteristics of study participants by race/ethnicity and presence (cases) and absence (controls) of type 2 diabetes

Variable	NHW		African-Americans		All	
	Cases	Controls	Cases	Controls	Cases	Controls
n	86	608	154	391	240	999
Age (years)	15.5 (3.0)	14.4 (2.3)	15.9 (2.8)	14.8 (2.2)	15.7 (2.8)	14.5 (2.3)
Male, *n* (%)	37 (43.0)	301 (49.5)	51 (33.0)	173 (44.0)	88 (37.0)	474 (47.0)
BMI (kg/m^2)	34.8 (8.2)	22.3 (5.1)	37.0 (11.1)	23.8 (6.7)	36.2 (10.2)	22.9 (5.8)
African ancestry	0.02 (0.11)	0.02 (0.08)	0.92 (0.15)	0.90 (0.16)		

Data are means (standard deviations), unless otherwise noted

allele with quantitative traits (BMI, glucose, insulin, HOMA-IR and HOMA-B levels).

Results

A total of 240 youth with type 2 diabetes and 999 control participants were included. Characteristics of the youth are shown in Table 1. The mean age was 15.7 years for those with diabetes and 14.5 years for control youth. As expected, youth with type 2 diabetes had higher BMI levels than the control youth. Mean estimated African ancestry was not statistically different among the case and control groups by self-reported race/ethnic group.

Table 2 shows that among AA youth, each copy of the T allele (rs7903146) was associated with a 1.97-fold (95% CI 1.37–2.82) increased adjusted odds for type 2 diabetes (p=0.0002). No significant association with this allele was detected in NHW youth (adjusted OR, 1.09; 95% CI 0.70–1.70; test for heterogeneity p=0.025). For rs12255372, the results were less conclusive and non-significant associations were noted for both racial/ethnic groups. Similar findings were noted for the association of *TCF7L2* genotypes and case/control status. Among AA youth, the adjusted OR for type 2 diabetes was 4.27 (95% CI 1.95–9.35) for homozygotes and 1.87 (95% CI 1.08–3.23) for heterozygotes at rs7903146, whereas no significant associations were observed for NHW youth (test for heterogeneity p=0.018). No significant associations were noted for the rs12255372 for either racial/ethnic group. Among controls, only glucose levels were higher for the T allele carriers at rs7903146 (p=0.02) and rs12255372 (p=0.004), although

Table 2 Genotype frequency, T allele frequency and association of the risk T allele with type 2 diabetes status in NHW and African-American youth

Variable	Genotype frequency		Minor allele frequency		Crude OR (95% CI)	Adjusted OR[a] (95% CI)	*p* value
	Cases	Controls	Cases	Controls			
rs7903146[b]							
NHW			0.33	0.29	1.22 (0.87–1.72)	1.09 (0.70–1.70)	0.70
CC	0.52	0.51					
CT	0.32	0.40					
TT	0.16	0.09					
African-American			0.44	0.27	2.11 (1.60–2.78)	1.97 (1.37–2.82)	0.0002
CC	0.39	0.53					
CT	0.40	0.40					
TT	0.21	0.07					
rs12255372[c]							
NHW			0.32	0.275	1.24 (0.88–1.75)	1.14 (0.73–1.79)	0.57
GG	0.56	0.53					
GT	0.32	0.39					
TT	0.12	0.8					
African-American			0.31	0.28	1.13 (0.84–1.50)	1.37 (0.94–2.00)	0.10
GG	0.49	0.51					
GT	0.41	0.42					
TT	0.1	0.07					

[a] Adjusted for age, sex, BMI, and African ancestry

[b] Test for heterogeneity, p=0.025

[c] Test for heterogeneity, p=0.5

there was also a non-significant tendency for HOMA-B levels to be lower among carriers (p=0.09). Results are presented in the Electronic supplementary material ESM Table 1.

Discussion

This is the first report on *TCF7L2* variation in a sample of well-characterised youth with type 2 diabetes and healthy controls in two racial/ethnic groups. We found a strong association among AA youth, with an effect size greater than previously seen in AA adults [2]. No significant association was observed in NHW youth.

These data are consistent with a previous study in Finland among 11 young adults with incident type 2 diabetes (OR 10.9) suggesting that an association with *TCF7L2* might have a more pronounced effect in participants with an earlier age at onset [8]. A study in Colorado also identified that the allele and genotype frequencies of the two SNPs are associated (OR up to 4.0) with antibody-negative, but not antibody-positive diabetes with onset before age 25 years [9]. The non-significant findings among NHW youth in our study may be the result of the relatively small sample of cases (n=86). Several meta-analyses of data from multiple studies in adults estimated an allelic risk around 40% [1, 2], which is not inconsistent with the effect seen in NHW youth in our study (adjusted OR 1.09, 95% CI 0.70–1.70).

However, the effect of *TCF7L2* on risk of adult onset type 2 diabetes was reported to be similar in NHW and AA populations [2]. Estimates of allele frequencies for the T risk allele among our controls are consistent in both racial/ethnic groups and with other published reports in adults [1], suggesting that our controls were reasonably representative of the NHW and AA in the USA. By virtue of their earlier age of onset of type 2 diabetes, youths may have higher frequencies of risk alleles than those seen in adult-onset cases. Our data suggest a greater contribution of *TCF7L2* variation to early-onset type 2 diabetes among youths of AA origin. Nevertheless, our 95% CI (1.37–2.82) spans the published OR (~1.4), so we cannot state with certainty that the effect is stronger in younger AA people. In contrast, different genes and environmental factors may be more important contributors to early-onset type 2 diabetes among NHW youth.

TCF7L2 variation leads to type 2 diabetes by an impairment in insulin secretion, rather than insulin resistance [10]. Our findings that the association of *TCF7L2* and type 2 diabetes in youth is independent of BMI, and the relationship between *TCF7L2* variation and quantitative traits among controls are consistent with previous reports. A recent study has shown that the rs7903146 polymorphism modifies an enhancer element [11]. The stronger effects seen for rs7903146 when compared with rs12255372 in AA youth supports the hypothesis that rs7903146 is a strong candidate for the functional variant.

Our study was limited by the small number of NHW youth with type 2 diabetes, because of the relative rarity of the condition. Additionally, our conclusions are limited to NHW and AA youth. Although the SEARCH study includes other racial/ethnic groups (Hispanics, Asian/Pacific Islanders and Native Americans), the samples sizes were small and no appropriate control groups were available. We focused on *TCF7L2* because it is the strongest type 2 diabetes association reported to date and the only one for which we had adequate statistical power. However, to date, our study is the largest case–control study of youth with type 2 diabetes that includes NHW and AA youth from a population-based study, with a well characterised phenotype.

In conclusion, *TCF7L2* variation is strongly associated with an increased risk of early-onset type 2 diabetes among AA youth in the USA. This association is independent of obesity, with the effect size being larger than previously seen in adults. The stronger association between *TCF7L2* variation and type 2 diabetes in AA youth than NHW youth suggests potential different contributions of genetic and/or environmental factors to early-onset type 2 diabetes in various racial/ethnic groups and requires further study.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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