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Evaluation of four novel genetic variants affecting hemoglobin A1c levels in a population-based type 2 diabetes cohort (the HUNT2 study)

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Abstract

Background: Chronic hyperglycemia confers increased risk for long-term diabetes-associated complications and repeated hemoglobin A1c (HbA1c) measures are a widely used marker for glycemic control in diabetes treatment and follow-up. A recent genome-wide association study revealed four genetic loci, which were associated with HbA1c levels in adults with type 1 diabetes. We aimed to evaluate the effect of these loci on glycemic control in type 2 diabetes.

Methods: We genotyped 1,486 subjects with type 2 diabetes from a Norwegian population-based cohort (HUNT2) for single-nucleotide polymorphisms (SNPs) located near the *BNC2*, *SORCS1*, *GSC* and *WDR72* loci. Through regression models, we examined their effects on HbA1c and non-fasting glucose levels individually and in a combined genetic score model.

Results: No significant associations with HbA1c or glucose levels were found for the *SORCS1*, *BNC2*, *GSC* or *WDR72* variants (all *P*-values > 0.05). Although the observed effects were non-significant and of much smaller magnitude than previously reported in type 1 diabetes, the *SORCS1* risk variant showed a direction consistent with increased HbA1c and glucose levels, with an observed effect of 0.11% ($P = 0.13$) and 0.13 mmol/l ($P = 0.43$) increase per risk allele for HbA1c and glucose, respectively. In contrast, the *WDR72* risk variant showed a borderline association with reduced HbA1c levels ($\beta = -0.21$, $P = 0.06$), and direction consistent with decreased glucose levels ($\beta = -0.29$, $P = 0.29$). The allele count model gave no evidence for a relationship between increasing number of risk alleles and increasing HbA1c levels ($\beta = 0.04$, $P = 0.38$).

Conclusions: The four recently reported SNPs affecting glycemic control in type 1 diabetes had no apparent effect on HbA1c in type 2 diabetes individually or by using a combined genetic score model. However, for the *SORCS1* SNP, our findings do not rule out a possible relationship with HbA1c levels. Hence, further studies in other populations are needed to elucidate whether these novel sequence variants, especially rs1358030 near the *SORCS1* locus, affect glycemic control in type 2 diabetes.

Background

Good glycemic control may slow or prevent long-term diabetes-associated complications, preserve β-cell function, and improve long-term outcomes in both type 1 and type 2 diabetes [1,2]. Chronic hyperglycemia is also a risk factor for cardiovascular disease and all-cause mortality in persons without diabetes [3,4]. Individuals with diabetes often have difficulties attaining the recommended HbA1c goals, and inter- and intra-individual variability in HbA1c is commonly observed, even for patients using the same treatment regimen. Medical conditions that influence erythrocyte turnover, as well as genetic hereditary anemia and iron storage disorders, affect the HbA1c level. Moreover, several twin and family studies have demonstrated a heritable component in both HbA1c and fasting blood glucose levels, but these measures are not genetically correlated to each

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other [5-7]. Although emerging data now suggest that also common genetic variants may affect HbA1c and fasting glucose in both diabetic and non-diabetic individuals via both glycemic and non-glycemic pathways [5,8-19], little is known about the genetic background of HbA1c in type 2 diabetes.

Recently, Paterson and colleagues conducted a genome-wide association study (GWAS) on longitudinal repeated measures of HbA1c in 1,441 patients with type 1 diabetes collected from the Diabetes Control and Complications Trial (DCCT). They reported evidence of one major locus for glycemic control near *SORCS1*, as measured by both HbA1c and glucose, and three other loci (near *BNC2*, *GSC* and *WDR72*) achieving association close to genome-wide significance [20]. The clinical and biological significance of these findings remains to be demonstrated. They may, however, point to new pathways relevant for glycemic physiology [21]. We aimed to evaluate the individual and cumulative effect of the four novel loci on glycemic control in unselected individuals with type 2 diabetes collected from a Norwegian population-based study (HUNT2).

Methods

HUNT2 subjects and ethics

The study population has recently been described [22-24]. In short, the participants were ≥20 years of age (range 21-97) and comprised the total diabetes population drawn from an extensive population-based study (the HUNT2 Study). Diagnosis of diabetes was self-reported or identified by standard tests if random glucose was >8.0 mmol/l. Genomic DNA was available for 1,850 (94%) diabetic participants. Eight subjects with genetically verified maturity-onset diabetes of the young [24] and 205 subjects evaluated as having type 1 diabetes were excluded. More detailed inclusion and exclusion criteria for the diabetic participants have been described previously [22]. Of the 1,637 type 2 diabetic participants enrolled in the study, 73 subjects had missing data on HbA1c and another 44 subjects had missing BMI data. For those subjects with data, the range was 4.1-16.7% and 16.9-49.5 kg/m^2 for HbA1c and BMI, respectively. In addition, 34 individuals were excluded due to low genotyping quality or missing DNA. The study group finally consisted of 1,486 individuals with type 2 diabetes. The study was approved by the Regional Committee for Research Ethics and the Norwegian Data Inspectorate, and was performed according to the latest version of the Helsinki Declaration. All participants gave written informed consent.

SNP selection, genotyping and quality control

We included only the four SNPs from Paterson et al. [20] which had shown the strongest association with glycemic control in type 1 diabetes. These are the non-coding SNPs rs10810632, rs1358030, rs11624318 and rs566369 located in or close to the *BNC2*, *SORCS1*, *GSC* and *WDR72* genes, respectively. The genotyping was carried out by the multiplex MassARRAY® *iPLEX*™ System (SEQUENOM Inc., San Diego, CA, USA) at the technology platform CIGENE, Ås, Norway. The final genotyping success rate was >95% for each SNP, with an average of 98.5%. For the internal controls, the genotyping concordance rate was 100% (n = 80 concordant calls). All SNPs examined were consistent with Hardy-Weinberg equilibrium ($P > 0.05$).

Statistical analysis

We assessed the effect of each risk variant on single cross-sectional HbA1c levels and on non-fasting glucose levels using linear regression models assuming additive effects of allele dosage. Subsequently, we studied the combined SNP effect by using an allele counting method to assign a genetic risk score to each subject according to the total number of risk alleles that they carried. The allele counting method assumed equal and additive effects for each of the different variants. All analyses were conducted using age, sex and BMI as covariates, and none of the phenotypes analyzed were logarithmically transformed since a transformation did not influence the distributions and results noticeably. Detailed information regarding medical treatment was not available. Since our results represents a basic replication of previously reported findings, *P*-values presented in this study are two-sided, but was not corrected for the number of test performed. All analyses were carried out using the PLINK software [25] and Stata SE v10.0 for Windows (Stata Corp LP, Brownsville, TX, USA). We had >80% power to detect a total QTL variance of ≥0.5% at the 0.05-level, assuming additive effects, allele frequency of 0.1 or more [26].

Results

Table 1 shows the clinical characteristics for the 1,486 individuals analyzed in the present study. Age, sex and

Table 1 Clinical characteristics of the 1,486 type 2 diabetic participants included in the study

Individuals (*n*)	1,486
Sex (male/female)	706/780
Age (years at examination)	68.1 ± 11.9
BMI (kg/m^2)	29.2 ± 4.8
HbA1c (%)	8.1 ± 1.8
Non-fasting serum glucose (mmol/l)	9.6 ± 4.2
Serum triglyceride (mmol/l)	2.5 ± 1.6
Serum cholesterol (mmol/l)	6.2 ± 1.3
Serum HDL cholesterol (mmol/l)	1.2 ± 0.4

Values are presented as means ± SD.

BMI were included in the regression models as covariates. The risk alleles were defined according to Paterson et al. [20] and we assumed an additive model for all four SNPs throughout this study, based on the results reported in the DCCT study [20]. The results did not change notably in view of dominant or recessive genetic models (not shown). We observed allele frequencies similar to the frequencies reported in individuals with type 1 diabetes [20]. The mean HbA1c by genotype for each of the SNPs are presented in Table 2.

In the individual SNP analysis, none of the risk alleles reached statistical significance with either increased HbA1c measures or increased non-fasting serum glucose levels (all *P*-values > 0.05, Table 3). Although the observed effects were non-significant and of much smaller magnitude than previously reported in type 1 diabetes, the *SORCS1* risk variant showed a direction consistent with increased HbA1c and glucose levels, with an observed effect of 0.11% ($P = 0.13$) and 0.13 mmol/l ($P = 0.43$) increase per risk allele for HbA1c and glucose, respectively (Table 3). In contrast, the *WDR72* risk variant showed a borderline association with reduced HbA1c levels ($\beta = -0.21$, $P = 0.06$, Table 3), and direction consistent with decreased glucose levels ($\beta = -0.29$, $P = 0.29$, Table 3).

Even though the four examined loci were not significantly associated with increased HbA1c values at an individual level, three of the four risk variants showed concordance in allelic direction in which individuals carrying the risk allele had higher HbA1c. When we included all four variants in a combined genetic score model we observed, however, no evidence for a relationship between increasing number of risk alleles and increasing HbA1c levels ($P = 0.38$). Each additional risk allele demonstrated an increase in HbA1c of approximately 0.04% (Table 3, Figure 1).

Discussion

To our knowledge, this study is the first attempt to evaluate the effect of the SNPs found by Paterson [20] with regard to glycemic control in type 2 diabetes. None of the SNPs were found associated with glycemic control in type 2 diabetes, either individually or combined by applying an allele count score. Hence, we were not able to confirm the strong associations recently reported in the DCCT genome-wide association study for HbA1c in the context of treated type 1 diabetes [20].

Using the same definition of the risk alleles as the DCCT study, the *WDR72* SNP showed a borderline association with reduced and not increased HbA1c levels in our study. Thus, our results do not support a role of the *WDR72* SNP on glycemic control as found in the DCCT study. The different pathophysiology between type 1 and type 2 diabetes could be one of the explanations why our results do not lend support to the finding that the four SNPs reported in the DCCT genome-wide association study [20] are genetic susceptibility factors for glycemic control in type 2 diabetes. In addition to a strong association with HbA1c, the *BNC2* and *SORCS1* risk alleles have revealed associations with mean glucose levels in type 1 diabetes [20], suggesting that these genetic variations affect HbA1c through their effects on glucose. We obtained no support for any associations between the *BNC2* and *SORCS1* SNPs and non-fasting serum glucose. The *SORCS1* risk allele indicated, however, an effect consistent in direction with its effect on HbA1c.

There are some prior data supporting a role of the *SORCS1* gene in glycemic traits. *SORCS1* encodes a sortilin-related vacuolar protein sorting 10 domain-containing receptor, which binds to platelet-derived growth factor. A quantitative trait locus for fasting insulin in the syntenic region in mice has been described [27], with further independent evidence obtained in rats for post-intra-peritoneal glucose tolerance [28]. Two studies have also demonstrated modest evidence for association between SNPs in *SORCS1* and fasting insulin, insulin sensitivity and insulin resistance in humans [29,30]. However, no association has been found with type 2 diabetes. Considering our results in light of the previous reported results and features for *SORCS1*, we can not refute a possible link between *SORCS1* and glycemic control in type 2 diabetes.

The *BNC2*, *WDR72* and *GSC* genes encode a zinc finger protein, a putative β propeller expected to be involved in protein-protein interactions and a transcription factor of

Table 2 Genotype-specific means for single cross-sectional HbA1c levels in 1,486 subjects with type 2 diabetes

Nearest gene	SNP	Common homozygote			Heterozygote			Rare homozygote			Minor allele*	Major allele*	MAF	MISS #
		N	Mean	SD	N	Mean	SD	N	Mean	SD				
BNC2	rs10810632	1215	8.05	0.52	257	8.12	0.11	13	7.95	0.65	**C**	T	0.09	1
SORCS1	rs1358030	648	7.94	0.07	622	8.16	0.07	139	8.02	0.16	**C**	T	0.32	77
GSC	rs11624318	908	8.07	0.06	500	8.02	0.08	76	8.11	0.21	A	**C**	0.22	2
WDR72	rs566369	1218	8.02	0.05	251	8.18	0.12	10	8.89	0.74	A	**G**	0.09	7

Risk alleles are defined according to Paterson et al. [20], and underlined and highlighted in bold.

MAF = minor allele frequency.

MISS# = number of individuals with missing genotype data.

*Alleles are indexed from the forward strand of the human reference sequence NCBI Build 36.

Table 3 Effects observed for the individual risk alleles and for the combined genetic scores on HbA1c and non-fasting serum glucose levels in 1,486 individuals with type 2 diabetes

Individual SNP effects			HbA1c				Non-fasting serum glucose			
Gene region	**SNP**	**RAF**	**Effect size**	**Std Error**	**P-value**	**Sample size**	**Effect size**	**Std Error**	**P-value**	**Sample size**
BNC2	rs10810632	0.09 (C)	0.07	0.11	0.57	1485	-0.00	0.26	0.99	1484
SORCS1	rs1358030	0.32 (C)	0.11	0.07	0.13	1409	0.13	0.17	0.43	1408
GSC	rs11624318	0.78 (C)	0.03	0.08	0.75	1484	-0.14	0.18	0.45	1483
WDR72	rs566369	0.91 (G)	-0.21	0.12	0.06	1479	-0.29	0.27	0.29	1478
Combined SNP effect based upon an allele count score			0.04	0.04	0.38	1403	-0.05	0.1	0.66	1402

All effect sizes represent the change in HbA1c or non-fasting serum glucose per risk allele. Age, sex and BMI were included as covariates in the regression models. *P* values are two-sided and are unadjusted for multiple testing.

RAF: risk allele frequency.

the paired homeobox family of proteins, respectively. Their exact function is unknown. Except for the results reported by Paterson and colleagues [20] none of these gene regions have previously been shown to be associated with glycemic traits in humans or animals. We found no evidence of association for any of these loci with glycemia in our type 2 diabetes cohort. The possibility nevertheless exists that the analysed SNP or genetic variants in strong linkage disequilibrium with these SNPs, are involved in glycemia, but that they have weak effects and/or are population specific. Our results therefore emphasize the need for further replication studies if one is to be successful in defining the true genetic risk factors involved in glycemic-related traits.

There are limitations of our study. We had access to only one HbA1c and non-fasting blood glucose value for each case, in contrast to the repeated measurements used by the DCCT investigators during the course of a carefully controlled clinical trial. Furthermore, the use of HbA1c as a quantitative trait modulated by genetic factors must be taken with caution in the context of pharmacological treatment, since treatment as an environmental variable may overwhelm the genetic signal. Whereas the DCCT investigators attempted to control for this, we had no access to information on medical treatment in the current study. Thus, our data may be confounded by environmental factors and cannot be considered a straight-forward replication study.

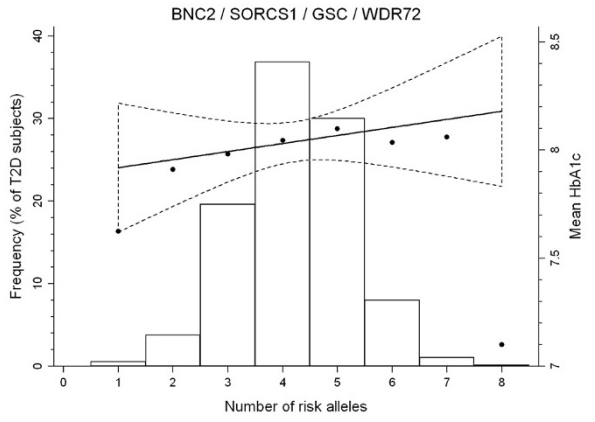

Figure 1 Mean HbA1c (black circles) and frequency (bars) of type 2 diabetes individuals plotted against the number of risk alleles carried, and the relationship between *BNC2* (rs10810632), *SORCS1* (rs1358030), *GSC* (rs11624318) and *WDR72* (rs566369) combined genotypes and mean HbA1c. Only individuals genotyped for all variants are included (n = 1,403). The black line is the fitted HbA1c linear regression line with the area between the dashed curves representing the 95% confidence interval.

Our study has, however, also several important strengths. The HUNT cohort is a well-characterized, stable (net emigration around 0.3% per year) and ethnically uniform (less than 3% of the people are of non-Caucasian origin) population from a clearly defined region of Norway [31]. Our study participants were part of an all-population-inclusive survey with high attendance. Hence, possible selection biases that can arise when studying referral patients or patients selected for inclusion in clinical intervention studies were avoided. The HUNT samples have previously been validated by genotyping of known type 2 diabetes risk variants [23,32] indicating that the HUNT population contains a representative diabetes cohort. Furthermore, we observed allele frequencies similar to the frequencies reported by Paterson et al [20], arguing against problems with population stratification. Finally, our study was conducted in one data set avoiding loss of power and, although the design was different than that of the initial report [20], we tested identical SNPs.

Conclusions

The four recently reported loci affecting glycemic control in type 1 diabetes patients had no apparent effect on HbA1C levels in type 2 diabetes, neither individually nor by using a combined genetic score model. For the *SORCS1* SNP however, we cannot refute a possible relationship with HbA1c. Hence, further studies in other

populations are needed to elucidate whether these novel sequence variants, especially rs1358030 near the *SORCS1* locus, affect glycemic control in type 2 diabetes.

Abbreviations

DCCT: Diabetes Control and Complications Trial; GWA: genome-wide association; HbA1c: glycosylated hemoglobin; HUNT: Helseundersøkelsen i Nord-Trøndelag; SNP: single-nucleotide polymorphism.

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Authors' contributions

JKH contributed to the study design, performed the statistical analyses, researched and interpreted the data, and wrote the manuscript. SJ contributed to the study design, directed the genotyping analyses, researched and interpreted the data, involved in drafting the manuscript. HR assisted in the study design, researched data, contributed to discussion and reviewed and edited the manuscript. CGPP researched data, contributed to discussion, and reviewed and edited the manuscript. KM contributed to discussion, and reviewed and edited the manuscript. KH contributed to discussion, and reviewed and edited the manuscript. AM assisted with study design, interpreted the data, contributed to discussion and helped to draft the manuscript. PRN conceived of the study, participated in the study design and coordination, interpreted the data, contributed to discussion and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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