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Chapter 5

Determinants of HbA_{1c} in non-diabetic Dutch adults: genes, environment and their interactions. The LifeLines cohort study

Submitted for publication.

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ABSTRACT

Background: HbA_{1c} is associated with cardiovascular risk in persons without diabetes, and its use has been recommended for diagnosing diabetes. Therefore, it is important to get better insight in determinants of HbA_{1c}.

Objective: To investigate the effects of environmental factors, genetic loci and gene-environment interactions on HbA_{1c} in non-diabetic adults.

Design: Population based cohort study.

Setting: Three Northern provinces of The Netherlands
Participants: 2,921 non-diabetic adults participating in the population-based LifeLines Cohort Study.

Measurements: BMI, waist circumference, HbA_{1c}, fasting plasma glucose (FPG) and erythrocyte indices were measured. Data on current smoking and alcohol consumption were collected by questionnaires. Genome-wide genotyping was performed, 12 previously identified SNPs were selected for replication and categorized in "glycemic" and "non-glycemic" SNPs according to the presumed way they act on HbA_{1c}. Genetic risk scores (GRSs) were calculated by adding up the weighted effect of HbA_{1c}-increasing alleles.

Results: Age, gender, BMI, FPG, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), current smoking and alcohol consumption were independent predictors of HbA_{1c}, together explaining 26.2% of the variance in HbA_{1c}, with FPG only contributing 10.9%. We replicated three of the previously identified SNPs, namely rs1402837, rs4737009 and rs1046896 and the GRSs were also independently associated with HbA_{1c}. We found a smaller effect of the "non-glycemic GRS" in females compared to males and an attenuation of the effect of the GRS of all 12 SNPs with increasing BMI.

Limitations: The lack of other measures of glycemia besides FPG.

Conclusions: Our results suggest that a substantial part of HbA_{1c} is determined by non-glycemic factors, raising serious questions about the use of HbA_{1c} as a diagnostic test for diabetes.

INTRODUCTION

HbA_{1c} has been firmly established as an index of long term glucose control and as a predictor of complications in patients with diabetes mellitus[1]. In addition, several studies have shown an association between HbA_{1c} and cardiovascular risk in people without diabetes[2],[3]. An international expert committee recommended using HbA_{1c} as indicator for the diagnosis of diabetes[4], but this is still fiercely debated[5-7]. Therefore, it is important to get better insight in factors determining HbA_{1c} in non-diabetic adults and the mechanisms through which they act.

Despite its standing as the most validated and widely used measure for average glycemic control over time, discordance between HbA_{1c} and other measures of glycemic control are commonly observed. These mismatches between blood glucose data and HbA_{1c} levels have led to a controversy regarding the role of the remaining sources of variation in HbA_{1c}[8]. Among 223 adults without diabetes, differences in glucose intolerance explained only one third of the variance in glycated hemoglobin levels[9]. In addition, only moderate correlations of glucose with HbA_{1c} were found in a population of 2,122 randomly selected non-diabetic adults aged 40-65 years[10]. This suggests that HbA_{1c} and glucose partly reflect different processes, particularly in the non-diabetic range of glucose tolerance.

Variation in HbA_{1c} is thought to be subject to environmental and genetic determinants[11]. Environmental factors may explain about 40-60% of the HbA_{1c} level in non-diabetic persons[12,13]. Two environmental clinical variables that are most evidently identified as influencing HbA_{1c} levels are red cell survival and blood glucose concentrations. However, there are several other (glycemia independent) variables that can influence HbA_{1c}, including race, smoking and age[14-17].

The heritability of HbA_{1c} is estimated to be 40-60%[12,13]. Two genome-wide association studies (GWASs) identified fifteen independent single nucleotide polymorphisms (SNPs) that were significantly associated with HbA_{1c} in non-diabetic adults[18,19]. The physiological mechanisms through which these genetic loci regulate HbA_{1c} levels remain unclear. Some SNPs are considered to modulate glycemic physiology[20], while others are supposed to regulate non-glycemic factors like red blood cell function[21].

In the current study we investigated a wide range of determinants of HbA_{1c} in non-diabetic Dutch adults participating in the population-based LifeLines Cohort Study[22]. We selected genetic loci that have been established in published GWASs[18,19] and studied the individual and combined effects of these SNPs plus environmental factors and gene-environment interactions on HbA_{1c}.

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METHODS

LifeLines participants

The study population was derived from the LifeLines Cohort Study, a three-generation population-based study[22]. In short, LifeLines is an observational follow-up study in a large representative sample of the population of the three northern provinces of The Netherlands covering three generations. All participants received a number of questionnaires and a medical examination at baseline, and are being followed longitudinally with extensive standardized measurements. The study was approved by the Ethics Committee of the University Medical Center Groningen.
For the current study, 3,367 unrelated participants of western European descent who completed the baseline medical examination, who filled out the questionnaires and from whom genome-wide data of good quality were available were selected. We excluded participants with diabetes mellitus (n=156), either self-reported or diagnosed by a fasting plasma glucose (FPG) ≥ 7.0 mmol/l. In addition, we excluded participants with significant anemia (n=86), defined by a hemoglobin level < 8.2 mmol/l for males and <7.0 mmol/l for females, and those without an HbA_{1c} value (n=13), erythrocyte indices (n=10) or FPG (n=181). The final study population consisted of 2,921 non-diabetic adults.

Anthropometry

Weight was measured to 0.1 kg and height to 0.1 cm by trained research staff using calibrated measuring equipment, with participants wearing light clothing. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). Overweight and obesity are defined as BMI > 25 kg/m^2 and BMI > 30 kg/m^2, respectively. Waist-circumference, to the nearest 0.1 cm, was measured twice midway between the lowest rib and the top of the iliac crest at the end of gentle expiration. The mean of the two measurements was used in the analysis.

Laboratory analyses

For HbA_{1c} analysis, a fasting whole blood sample (EDTA) was taken and analysed using a turbidimetric inhibition immunoassay on a Cobas Integra 800 CTS analyzer (Roche Diagnostics Nederland BV, Almere, the Netherlands). This method has been standardized against the reference method of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). Results (mmol HbA_{1c} /mol Hb) were converted to units (% HbA_{1c}) traceable to the Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Program (DCCT/NGSP) using the Roche master-equation. Between-batch imprecision (coefficient of variation) was 2.1% for a mean HbA_{1c} of 5.5% and 1.9% for a mean HbA_{1c} of 10.6%.

FPG was measured from fasting fresh venous plasma using the Roche glucose-assay (hexokinase/glucose-6-phosphate dehydrogenase enzymatic reactions) on the Modular P (Roche Diagnostics, Burgdorf, Switzerland).
Hemoglobin (Hb), hematocrit (Ht), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were assessed using a Sysmex XE-2100 automated hematology analyser (Sysmex Corporation, Kobe, Japan). Gender-specific z-scores were calculated for Hb, Ht, RBC, MCV, MCH and MCHC, to take differences between sexes into account. These gender-specific z-scores were used in all modelling.

Current smoking and alcohol consumption
Data on current smoking and alcohol consumption were obtained from the questionnaires. Current smoking was defined as any smoking in the previous month and alcohol consumption was defined as any alcohol consumption in the previous month, both answered with yes or no. Data on alcohol consumption were missing for 89 participants.

"Environmental" factors
Although some cannot be considered true environmental factors, in contrast to the measured genotypes (SNPs), age, gender, BMI, waist circumference, current smoking, alcohol consumption, FPG, Hb, Ht, RBC, MCV, MCH and MCHC are all considered environmental determinants of HbA_{1c} for the purpose of this paper.

Genotyping, quality control and imputation
For the present study genome-wide genotyping was performed in all participants using the Illumina HumanCytoSNP12 v2 beadchip assay (Illumina, Inc.; San Diego, USA). Genotypes were called with the Illumina GenomeStudio software package (Illumina, Inc.; San Diego, USA). SNPs were excluded if the genotype call rate was <95%, the minor allele frequency was <1% or the Hardy-Weinberg p-value was <10-4. Samples were excluded if the sample call rate was <95%, they were non-Caucasian (evaluated with EigenStrat[23]), or if they were highly related to another individual[24] and had a lower call rate. The resulting data set contained 257,581 SNPs of 3,367 individuals. Imputation of untyped SNPs was done with IMPUTE using the HapMap CEU Phase II reference set (release 22, build 36)[25].

Selected SNPs

For this study we selected genome-wide significant SNPs from two large GWASs[18,19]. Soranzo et al. identified 11 independent SNPs and Paré et al. found four independent SNPs for HbA_{1c}[18,19]. Of these 15 SNPs, two SNPs had an imputation quality score < 0.5 (rs1387153 and rs16926246) in our sample, and rs730497 was in perfect LD with rs1799884. These three SNPs were excluded from the analyses and the remaining 12 SNPs were used for replication in the current study (Appendix Table I).

We categorized the 12 SNPs according to the presumed way they act on HbA_{1c}. The loci of SNPs rs552976, rs1402837, rs1799884 and rs13266634 have previously been associated with fasting glucose or reduced glucose-induced insulin secretion[26-29]. Consequently, we expect that these "glycemic" loci act on HbA_{1c} through a glycemic pathway. In contrast, the other SNPs are thought to influence HbA_{1c} via "non-glycemic" erythrocyte and iron biology pathways[18,21].

A genetic risk score (GRS) was calculated for each individual by adding up the HbA_{1c}-increasing allele dosages of all 12 SNPs weighted by their respective effect sizes as observed in the studies of Soranzo et al. and Paré et al.[18,19]. In addition, we calculated a GRS for the four "glycemic" SNPs and eight "non-glycemic" SNPs separately.

Statistical methods

We used multiple linear regression to investigate the relation of all potential determinants with HbA_{1c}. First a model with only age and gender was analyzed. In the next steps, each determinant was added to the age and gender model separately. Next a forward-stepwise approach with all environmental factors was used to derive a full predictive model of HbA_{1c} including only significant terms. To this end, age and gender were forced in the model and the other environmental factors were added subsequently by stepwise modelling. Since BMI and waist circumference were highly correlated ($r=0.80$, $p<0.001$) we initially only used BMI in the modelling. The GRSs were each separately added to this so-called full predictive model to determine their effect on HbA_{1c} as well as the proportion of variance explained.

Gene-environment interactions were calculated by multiplying the GRS of all 12 SNPs by the environmental determinants. First, all gene-environment interactions were tested separately for their association with HbA_{1c} in the age and gender model. Thereafter, significantly associated gene-environment interactions were added to the full predictive model to test their independent association with HbA_{1c}.

A level of significance of $p<0.05$ was applied for all analyses, which were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The characteristics of the study population are displayed in Table I.

Table I. Characteristics of the study population

	Values
Age (yrs.)	55.5 ± 9.9
Gender: Male (%)	1,176 (40.3)
BMI (kg/m^2)	26.5 ± 4.1
Normal (%)	1,112 (38.1)
Overweight (%)	1,322 (45.3)
Obese (%)	487 (16.7)
Waist circumference (cm)	92.9 ± 11.7
Current smoking: n (%)	566 (19.4)
Alcohol consumption: n (%)	2,344 (80.2)
HbA_{1c} (%)	5.6 ± 0.3
HbA_{1c} (mmol/mol)	38 ± 3
Fasting plasma glucose (mmol/l)	5.1 ± 0.5
Hemoglobin (mmol/l)	8.7 ± 0.7
Hematocrit (l/l)	0.42 ± 0.03
RBC (x 10-12/l)	4.7 ± 0.4
MCV (fl)	90.4 ± 4.2
MCH (fmol)	1.9 ± 0.1
MCHC (mmol/l)	20.6 ± 0.6

Values are means ± SD or number (%)
Abbreviations: BMI, body mass index; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count

Environmental factors and HbA_{1c}

We found a positive association of age, BMI, waist circumference, FPG, hematocrit, RBC and current smoking with HbA_{1c} and a negative association with hemoglobin level, MCV, MCH, MCHC and alcohol consumption (Table II). Gender was not associated with HbA_{1c} in the baseline age and gender model.

In a forward-stepwise multiple linear regression model, age, gender, BMI, FPG, MCH, MCHC, current smoking and alcohol consumption, were independent predictors of HbA_{1c}. This full predictive model with eight environmental factors explained 26.2% of the variance in HbA_{1c} (Table III).

Note that FPG alone explained only 10.9%. We also used forward-stepwise modelling to derive a full predictive model with waist circumference instead of BMI; the results remained largely the same.

Genotypic factors and HbA_{1c}

In the current study we replicated three of the twelve SNPs previously identified from GWASs for HbA_{1c}, namely rs1402837, rs4737009 and rs1046896 (Table II). SNP rs7072268 was also significantly associated with HbA_{1c}, but surprisingly, we found a negative effect of the T-allele, in contrast to other studies[19,21]. Hence this finding cannot be considered a replication. The loci nearest to the three replicated SNPs are G6PC2, ANK1 and FN3K, respectively. Thus we replicated one "glycemic" locus and two "non-glycemic" loci. The three GRSs were all positively and significantly associated with HbA_{1c}, indicating a cumulative effect of the different loci (Table II). Adding the GRS of all 12 SNPs to the full model with independent environmental risk factors increased the percentage of explained variance in HbA_{1c} with 0.2% (Table III). In the full predictive model with the eight independent environmental risk factors, the effect of the "non-glycemic GRS" on HbA_{1c} attenuated markedly while the effect of the "glycemic GRS" remained largely the same.

Gene – environment interactions

Two interactions between the GRS of all 12 SNPs and environmental factors remained significant after adding them to the full multivariate model (Table IV). The effect of the GRS of all 12 SNPs on HbA_{1c} was significantly lower in females compared to males. In addition, the effect of the GRS of all 12 SNPs attenuated with an increasing BMI. Both interactions together added 0.3% to the explained variance in HbA_{1c}.

To get further insight in the interactions between the GRSs and gender, we stratified the population according to gender and calculate the effect of the three GRSs on HbA_{1c} in males and females separately (Table Va). In males we found a positive and highly significant

Table II. Associations of HbA_{1c} with environmental and genetic risk factors

	HbA_{1c} (mmol/mol)			
	Beta	CI	p-value	R^2 (%)
Baseline model				10.8
Age (yrs.)	0.11	0.10 – 0.12	<0.001	
Gender	-0.13	-0.37 – 0.11	0.276	
Environmental factors:	Beta*	CI	p-value	Δ R2 (%)
BMI (kg/m²)	0.13	0.10 – 0.16	<0.001	2.5
Waist circumference (cm)	0.05	0.04 – 0.06	<0.001	2.4
FPG (mmol/l)	2.29	2.07 – 2.51	<0.001	11.2
Hemoglobin †	-0.13	-0.25 – -0.02	0.027	0.1
Hematocrit †	0.17	0.05 – 0.28	0.006	0.2
RBC †	0.23	0.12 – 0.35	<0.001	0.5
MCV †	-0.14	-0.25 – -0.02	0.025	0.1
MCH †	-0.45	-0.57 – -0.34	<0.001	1.8
MCHC †	-0.62	-0.73 – -0.50	<0.001	3.2
Current smoking	0.70	0.40 – 1.00	<0.001	0.6
Alcohol consumption	-0.94	-1.26 – -0.63	<0.001	1.1‡
Genotypic factors: SNP	Beta*	CI	p-value	Δ R^2 (%)
rs552976	0.10	-0.07 – 0.27	0.235	0.0
rs1402837	0.28	0.08 – 0.48	0.007 §	0.2
rs1799884	0.19	-0.04 – 0.41	0.099	0.1
rs13266634	-0.02	-0.20 – 0.16	0.862	0.0
rs2779116	0.03	-0.16 – 0.22	0.745	0.0
rs1800562	0.14	-0.21 – 0.50	0.436	0.0
rs6474359	0.35	-0.05 – 0.75	0.088	0.1
rs4737009	-0.26	-0.46 – -0.05	0.014 §	0.2
rs7072268	-0.34	-0.54 – -0.14	0.001	0.3
rs7998202	0.17	-0.11 – 0.44	0.236	0.0
rs1046896	0.20	0.00 – 0.40	0.046 §	0.1
rs855791	-0.11	-0.28 – 0.06	0.206	0.0
GRS All 12 SNPs	0.32	0.15 – 0.48	<0.001	0.4
“Glycemic GRS”	0.28	0.05 – 0.51	0.018	0.2
“Non-glycemic GRS”	0.32	0.09 – 0.54	0.005	0.2

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; GRS, genetic risk score; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count; SNP, single nucleotide polymorphisms

* Linear regression model with the risk factors separately added to the baseline model with age and gender.

† Erythocyte indices are expressed as z-scores

‡ For alcohol consumption the age and gender model consisted of n=2,832 participants with a R^2 (%) of 9.6.

§ Replicated SNPs

R^2: Explained variance in HbA_{1c}. Δ R^2: Added explained variance compared to the explained variance of the age and gender model.

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Table III. Multivariate associations of HbA_{1c} with environmental and genetic risk factors

	HbA_{1c} (mmol/mol)			
	Beta*	CI	p-value	R^2 (%)
Model I				9.6
Age (yrs)	0.11	0.10 – 0.12	<0.001	
Gender	-0.14	-0.38 – 0.10	0.263	
Model II				20.5
Age (yrs)	0.08	0.07 – 0.09	<0.001	
Gender	0.35	0.12 – 0.58	0.003	
FPG (mmol/l)	2.25	2.03 – 2.48	<0.001	
Model III				26.2
Age (yrs)	0.07	0.06 – 0.09	<0.001	
Gender	0.25	0.03 – 0.48	0.029	
BMI (kg/m^2)	0.05	0.02 – 0.08	0.001	
FPG (mmol/l)	2.13	1.91 – 2.36	<0.001	
MCH	-0.21	-0.33 – -0.09	0.001	
MCHC	-0.56	-0.68 – -0.44	<0.001	
Current smoking	0.81	0.53 – 1.09	<0.001	
Alcohol consumption	-0.73	-1.02 – -0.44	<0.001	
	Beta*	CI	p-value	ΔR^2 (%)
Model IV				
GRS all 12 SNPs	0.20	0.05 – 0.35	0.010	0.2
Model V				
"Glycemic GRS"	0.23	0.02 – 0.44	0.032	0.2
Model VI				
"Non-glycemic GRS"	0.15	-0.06 – 0.35	0.151	0.1

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; GRS, genetic risk score; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SNP, single nucleotide polymorphisms
R^2: Explained variance in HbA_{1c} (%).
ΔR^2: Added explained variance of the GRSs to the model (%).
* Multiple linear regression model.
Model IV: Model III + GRS all 12 SNPs
Model V: Model III + "Glycemic GRS"
Model VI: Model III + "Non-glycemic GRS"

Table IV. Full multivariate model for HbA_{1c} including gene-environment interactions

	HbA_{1c} (mmol/mol)			
	Beta*	CI	p-value	R^2
Model				26.7
Age (yrs)	0.07	0.06 – 0.09	<0.001	
Gender	1.52	0.52 – 2.52	0.003	
BMI (kg/m²)	0.20	0.08 – 0.32	0.001	
FPG (mmol/l)	2.11	1.88 – 2.34	<0.001	
MCH	-0.21	-0.33 – -0.09	0.001	
MCHC	-0.55	-0.67 – -0.43	<0.001	
Current smoking	0.83	0.55 – 1.11	<0.001	
Alcohol consumption	-0.73	-1.02 – -0.44	<0.001	
GRS all 12 SNPs	2.13	0.98 – 3.27	<0.001	
Interaction GRS all 12 SNPs * Gender	-0.40	-0.71 – -0.09	0.011	
Interaction GRS all 12 SNPs * BMI	-0.05	-0.09 – -0.01	0.012	

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; GRS, genetic risk score; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SNP, single nucleotide polymorphisms
R^2: Explained variance in HbA_{1c} (%).
* Multiple linear regression model.

Table Va. Effect of genetic risk scores on HbA_{1c} in males and females

	Male (n=1,134) HbA_{1c} (mmol/mol)			Female (n=1,698) HbA_{1c} (mmol/mol)		
	Beta*	CI	p-value	Beta*	CI	p-value
GRS all 12 SNPs	0.42	0.18 – 0.67	0.001	0.05	-0.14 – 0.24	0.634
GRS Glycemic SNPs	0.29	-0.06 – 0.63	0.102	0.19	-0.08 – 0.45	0.169
GRS Non-glycemic SNPs	0.49	0.16 – 0.82	0.004	-0.09	-0.35 – 0.17	0.494

Abbreviations: GRS, genetic risk score; SNP, single nucleotide polymorphisms
* Linear regression model adjusted for the full predictive model with age, BMI, FPG, MCH, MCHC, current smoking and alcohol consumption

Table Vb. Effect of genetic risk scores on HbA_{1c} in normal, overweight and obese persons

	Normal (n=1,091) HbA_{1c} (mmol/mol)			Overweight (n=1,278) HbA_{1c} (mmol/mol)			Obese (n=463) HbA_{1c} (mmol/mol)		
	Beta*	CI	p-value	Beta*	CI	p-value	Beta*	CI	p-value
GRS all 12 SNPs	0.30	0.07 – 0.53	0.012	0.28	0.05 – 0.51	0.017	-0.25	-0.66 – 0.15	0.218
GRS Glycemic SNPs	0.43	0.11 – 0.75	0.009	0.21	-0.11 – 0.54	0.197	-0.19	-0.74 – 0.37	0.512
GRS Non-glycemic SNPs	0.13	-0.18 – 0.45	0.410	0.33	0.01 – 0.64	0.041	-0.27	-0.79 – 0.26	0.326

Abbreviations: GRS, genetic risk score; SNP, single nucleotide polymorphisms
* Linear regression model adjusted for the full predictive model with age, gender, BMI, FPG, MCH, MCHC, current smoking and alcohol consumption

association between the “non-glycemic GRS” and HbA_{1c}, while in females there was no significant association. Thus the observed difference in the effect of the GRS of all SNPs on HbA_{1c} between sexes is largely explained by a difference in the effect of the “non-glycemic GRS” on HbA_{1c}.
In addition, we stratified the population in normal, overweight and obese individuals and we calculated the effect of the three GRSs on HbA_{1c} in these three groups separately (Table Vb). We found a positive association between all three GRSs and HbA_{1c} in normal and overweight individuals, but a negative association between all three GRSs and HbA_{1c} in obese individuals, although not significant.

DISCUSSION

The aim of this study was to investigate environmental and genetic determinants of HbA_{1c} in non-diabetic adults to get further insight in the relative contribution of the different factors (glycemic versus non-glyemic and environmental versus genotypic) to HbA_{1c} levels in non-diabetic adults. We found age, gender, BMI, FPG, MCH, MCHC, current smoking and alcohol consumption to be independently associated with HbA_{1c}. A full predictive model with these eight environmental factors explained 26.2% of the variance in HbA_{1c} and FPG contributed only less than half to this explained variance namely 10.9%. In addition, association between three out of 12 previously identified SNPs and HbA_{1c} could be replicated in our study population. The GRSs were also independently associated with HbA_{1c} and explained 0.2% of the variance in HbA_{1c}. We found a lower effect of the “non-glycemic GRS” in females compared to males and an attenuation of the effect of the GRS of all 12 SNPs with increasing BMI.
Since erythrocytes are freely permeable to glucose, the rate of formation of HbA_{1c} is directly proportional to the ambient glucose concentration. However, it is common to find discordance between HbA_{1c} and other measures of glycemic control in diabetes patients[30,31]. These mismatches still remain unexplained and have led to a controversy as to the role of the remaining sources of variation in HbA_{1c}. From a recent study investigating the relationship among HbA_{1c}, fasting plasma glucose and 2-hour postload plasma glucose it was concluded that the correlation between glucose and HbA_{1c} was only moderate in the general population[10]. Twenty years ago, Yudkin et al. already suggested that the degree of glucose intolerance may explain only one third of the variance in HbA_{1c} in a non-diabetic population[9]. These conclusions are in line with our findings; our full predictive model with eight environmental factors explained 26.2% of the variance in HbA_{1c}, but FPG contributes only less than half to this explained variance namely 10.9%.
Glycation of HbA begins during erythropoiesis and continues throughout the lifespan of hemoglobin

in the circulation. Consequently, erythrocyte life span determines the duration of exposure of hemoglobin to glucose and thereby HbA_{1c} levels. Increased erythrocyte turnover, as observed in hemolytic anemia, results in lower HbA_{1c} levels[32]. In contrast, patients with iron deficiency anemia showed higher HbA_{1c} levels, probably because of a relatively high proportion of old erythrocytes[33,34]. In line with our findings, Koga et al. demonstrated that in pre-menopausal women MCH was negatively associated with HbA_{1c} and they explained this by pre-menopausal menstrual blood loss that causes iron-deficiency and thereby relatively low levels of MCV and MCH[35]. As our study population consisted of men and women (pre- as well as post-menopausal), pre-menopausal blood loss could not be the only explanation of the observed relation between MCH and HbA_{1c}. Possibly, also a relative lack of iron caused by other factors than blood loss leads to a relatively longer life-span of erythrocytes in persons with lower MCH. Cohen et al. concluded that erythrocyte survival varies sufficiently among hematologically normal persons to cause differences in HbA_{1c}[36]. If MCH is related to this variance in erythrocyte survival, this could explain the observed differences in HbA_{1c}. Certainly more studies are needed to investigate the mechanisms through which erythrocyte indices influence HbA_{1c} levels. However, the relatively high contribution of MCHC and MCH to the explained variance in HbA_{1c}, underlines that other factors besides glycemia determine HbA_{1c} levels. In line with other studies, we found a negative association between alcohol consumption and HbA_{1c}[37,38]. Facchini et al. found that light-to-moderate alcohol consumption in healthy men and women was associated with enhanced insulin-mediated glucose uptake and lower plasma glucose and insulin concentrations in response to oral glucose[39]. The resulting lower levels of glycemia could explain the lower levels of HbA_{1c} related to alcohol consumption. Our finding of higher HbA_{1c} levels in current smokers is also in line with results of earlier studies[16,40,41]. Even after adjusting for potential confounders like abdominal obesity, there is a positive association between current smoking and HbA_{1c}. Glycotoxins found in cigarette smoke may induce the higher rate of glycation of HbA[42] or the relative higher tissue hypoxia[43] may explain increased HbA_{1c} levels in smokers[44].

In the current study we could replicate three of the previously genome-wide significant SNPs for HbA_{1c}, but the contribution of these SNPs to the explained variance in HbA_{1c} is rather low. In contrast to other studies, we found a negative association of the T-allele of SNP rs7072268 with HbA_{1c}, which may have been a false positive finding[19,21]. Fluctuation of effects of individual SNPs is no longer a problem when the effects of all SNPs are added to calculate a GRS. This GRS gives the opportunity to get insight into the extent of the effect of all SNPs together on HbA_{1c}. The GRS of all 12 SNPs is independently associated with HbA_{1c}, but contributes only 0.2% to the explained variance in HbA_{1c}.

In the full predictive model with independent environmental risk factors the effect of the "non-glycemic GRS" attenuated markedly. This marked attenuation could be explained by mediation

of MCHC and/or MCH. If (part of) these SNPs acts on HbA_{1c} through its effect on MCHC and/or MCH, the effect of the GRS will attenuate when MCHC and/or MCH is also in the model. The effect of the "glycemic GRS" on the other hand largely remained the same.

The "non-glycemic GRS" was positively associated with HbA_{1c} in males but not in females, demonstrating a difference in the effect of these non-glycemic SNPs on HbA_{1c} between sexes. In addition, the effect of the GRS of all 12 SNPs attenuated with increasing BMI. Possibly, the effect of the environmental factor being obese overrides the potential genetic effect of the SNPs.

An important strength of our study is that we were able to investigate the association between a wide range of "environmental" risk factors and HbA_{1c} in a large population-based study population. Consequently, we could adjust for potential confounding and investigated the independent associations between the risk factors and HbA_{1c}. An important limitation of our study is the lack of other measures of glycemia besides FPG. However, repeated daily measurements of glucose levels are not feasible in a cohort study with non-diabetic participants. And more importantly, in spite of this lack of other measures of glycemia the contribution of non-glycemic factors to the explained variance in HbA_{1c} remains indisputable.

In conclusion, age, gender, BMI, FPG, MCH, MCHC, current smoking and alcohol consumption are "environmental" factors independently associated with HbA_{1c}. In addition, the association between three previously identified SNPs and HbA_{1c} could be replicated in our study population. The added effect of the previously identified non-glycemic SNPs is different in men and women and the added effect of all 12 SNPs attenuated with increasing BMI. These results suggest that HbA_{1c} is determined by other factors besides preceding glucose levels which raises serious questions about the use of HbA_{1c} for diagnosing diabetes.

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References

1 Wolffenbuttel BH, Giordano D, Founds HW, Bucala R. Long-term assessment of glucose control by haemoglobin-AGE measurement. Lancet 1996 Feb 24;347(9000):513-515.

2 Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med 2004 09/21;141(6):413-420.

3 Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010 Mar 4;362(9):800-811.

4 International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009 Jul;32(7):1327-1334.

5 Herman WH, Cohen RM. Hemoglobin A1c: teaching a new dog old tricks. Ann Intern Med 2010 Jun 15;152(12):815-817.

6 Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. Is haemoglobin A1c a step forward for diagnosing diabetes? BMJ 2009 Nov 10;339:b4432.

7 Fonseca V, Inzucchi SE, Ferrannini E. Redefining the diagnosis of diabetes using glycated hemoglobin. Diabetes Care 2009 Jul;32(7):1344-1345.

8 McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA_{1c} predicts risk of retinopathy and nephropathy in type 1 diabetes. Diabetes Care 2004 Jun;27(6):1259-1264.

9 Yudkin JS, Forrest RD, Jackson CA, Ryle AJ, Davie S, Gould BJ. Unexplained variability of glycated haemoglobin in non-diabetic subjects not related to glycaemia. Diabetologia 1990 Apr;33(4):208-215.

10 van 't Riet E, Alssema M, Rijkelijkhuizen JM, Kostense PJ, Nijpels G, Dekker JM. Relationship between A1C and glucose levels in the general Dutch population: the new Hoorn study. Diabetes Care 2010 Jan;33(1):61-66.

11 Florez JC. A genome-wide association study of treated A1C: a genetic needle in an environmental haystack? Diabetes 2010 Feb;59(2):332-334.

12 Meigs JB, Panhuysen CI, Myers RH, Wilson PW, Cupples LA. A genome-wide scan for loci linked to plasma levels of glucose and HbA(1c) in a community-based sample of Caucasian pedigrees: The Framingham Offspring Study. Diabetes 2002 Mar;51(3):833-840.

13 Snieder H, Sawtell PA, Ross L, Walker J, Spector TD, Leslie RD. HbA(1c) levels are genetically determined even in type 1 diabetes: evidence from healthy and diabetic twins. Diabetes 2001 Dec;50(12):2858-2863.

14 Herman WH, Dungan KM, Wolffenbuttel BH, Buse JB, Fahrbach JL, Jiang H, et al. Racial and ethnic differences in mean plasma glucose, hemoglobin A1c, and 1,5-anhydroglucitol in over 2000 patients with type 2 diabetes. J Clin Endocrinol Metab 2009 May;94(5):1689-1694.

15 Ziemer DC, Kolm P, Weintraub WS, Vaccarino V, Rhee MK, Twombly JG, et al. Glucose-independent, black-white differences in hemoglobin A1c levels: a cross-sectional analysis of 2 studies. Ann Intern Med 2010 Jun 15;152(12):770-777.

16 Higgins T, Cembrowski G, Tran D, Lim E, Chan J. Influence of variables on hemoglobin A1c values and nonheterogeneity of hemoglobin A1c reference ranges. J Diabetes Sci Technol 2009 Jul 1;3(4):644-648.

17 Pani LN, Korenda L, Meigs JB, Driver C, Chamany S, Fox CS, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care 2008 Oct;31(10):1991-1996.

18 Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, et al. Common variants at ten genomic loci influence hemoglobin A1C levels via glycemic and non-glycemic pathways. Diabetes 2010 Sep 21.

19 Pare G, Chasman DI, Parker AN, Nathan DM, Miletich JP, Zee RY, et al. Novel association of HK1 with glycated hemoglobin in a non-diabetic population: a genome-wide evaluation of 14,618 participants in the Women's Genome Health Study. PLoS Genet 2008 Dec;4(12):e1000312.

20 Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2010 Feb;42(2):105-116.

21 Bonnefond A, Vaxillaire M, Labrune Y, Lecoeur C, Chevre JC, Bouatia-Naji N, et al. Genetic variant in HK1 is associated with a proanemic state and A1C but not other glycemic control-related traits. Diabetes 2009 Nov;58(11):2687-2697.

22 Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, et al. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol 2008;23(1):67-74.

23 Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet 2006 Dec;2(12):e190.

24 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007 Sep;81(3):559-575.

25 Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet 2007 Jul;39(7):906-913.

26 Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, et al. Variations in the G6PC2/ABCB11 genomic region are associated with fasting glucose levels. J Clin Invest 2008 Jul;118(7):2620-2628.

27 Bouatia-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, Cavalcanti-Proenca C, et al. A polymorphism within the G6PC2 gene is associated with fasting plasma glucose levels. Science 2008 May 23;320(5879):1085-1088.

28 Weedon MN, Clark VJ, Qian Y, Ben-Shlomo Y, Timpson N, Ebrahim S, et al. A common haplotype of the glucokinase gene alters fasting glucose and birth weight: association in six studies and population-genetics analyses. Am J Hum Genet 2006 Dec;79(6):991-1001.

29 Staiger H, Machicao F, Stefan N, Tschritter O, Thamer C, Kantartzis K, et al. Polymorphisms within novel risk loci for type 2 diabetes determine beta-cell function. PLoS One 2007 Sep 5;2(9):e832.

30 Hempe JM, Gomez R, McCarter RJ,Jr, Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. J Diabetes Complications 2002 Sep-Oct;16(5):313-320.

31 Cohen RM, Snieder H, Lindsell CJ, Beyan H, Hawa MI, Blinko S, et al. Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA_{1c} in nondiabetic twins. Diabetes Care 2006 Aug;29(8):1739-1743.

32 Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. Glycosylated hemoglobins (GHb): an index of red cell survival. Blood 1982 Jun;59(6):1348-1350.

33 El-Agouza I, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes: possible consequences for clinical diagnosis. Clin Lab Haematol 2002 Oct;24(5):285-289.

34 Tarim O, Kucukerdogan A, Gunay U, Eralp O, Ercan I. Effects of iron deficiency anemia on hemoglobin A1c in type 1 diabetes mellitus. Pediatr Int 1999 08;41(4):357-362.

35 Koga M, Morita S, Saito H, Mukai M, Kasayama S. Association of erythrocyte indices with glycated haemoglobin in pre-menopausal women. Diabet Med 2007 Aug;24(8):843-847.

36 Cohen RM, Franco RS, Khera PK, Smith EP, Lindsell CJ, Ciraolo PJ, et al. Red cell life span heterogeneity in hematologically normal people is sufficient to alter HbA_{1c}. Blood 2008 Nov 15;112(10):4284-4291.

37 Harding AH, Sargeant LA, Khaw KT, Welch A, Oakes S, Luben RN, et al. Cross-sectional association between total level and type of alcohol consumption and glycosylated haemoglobin level: the EPIC-Norfolk Study. Eur J Clin Nutr 2002 Sep;56(9):882-890.

38 Boeing H, Weisgerber UM, Jeckel A, Rose HJ, Kroke A. Association between glycated hemoglobin and diet and other lifestyle factors in a nondiabetic population: cross-sectional evaluation of data from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition Study. Am J Clin Nutr 2000 May;71(5):1115-1122.

39 Facchini F, Chen YD, Reaven GM. Light-to-moderate alcohol intake is associated with enhanced insulin sensitivity. Diabetes Care 1994 Feb;17(2):115-119.

40 Gulliford MC, Ukoumunne OC. Determinants of glycated haemoglobin in the general population: associations with diet, alcohol and cigarette smoking. Eur J Clin Nutr 2001 Jul;55(7):615-623.

41 Nilsson PM, Gudbjornsdottir S, Eliasson B, Cederholm J, Steering Committee of the Swedish National Diabetes Register. Smoking is associated with increased HbA_{1c} values and microalbuminuria in patients with diabetes--data from the National Diabetes Register in Sweden. Diabetes Metab 2004 Jun;30(3):261-268.

42 Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, et al. Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci U S A 1997 Dec 9;94(25):13915-13920.

43 Sagone AL,Jr, Lawrence T, Balcerzak SP. Effect of smoking on tissue oxygen supply. Blood 1973 Jun;41(6):845-851.

44 Smith RJ, Koenig RJ, Binnerts A, Soeldner JS, Aoki TT. Regulation of hemoglobin AIc formation in human erythrocytes in vitro. Effects of physiologic factors other than glucose. J Clin Invest 1982 May;69(5):1164-1168.

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Appendix Table I. The twelve selected SNPs used for replication in the current study

SNP	Chr	Position*	Effect allele/ Other allele	Nearest locus
"Glycemic"				
rs552976	2	169499684	G / A	G6PC2/ABCB11
rs1402837	2	169465600	T / C	G6PC2
rs1799884	7	44195593	T / C	GCK
rs13266634	8	118253964	T / C	SLC30A8
"Non-glycemic"				
rs2779116	1	156852039	T / C	SPTA1
rs1800562	6	26201120	G / A	HFE
rs6474359	8	41668351	T / C	ANK1
rs4737009	8	41749562	G / A	ANK1
rs7072268	10	70769919	T / C	HK1
rs7998202	13	112379869	G / A	ATP11A/TUBGCP3
rs1046896	17	78278822	T / C	FN3K
rs855791	22	35792882	G / A	TMPRSS6

Abbreviations: Chr, chromosome; Pos, position; SNP, single nucleotide polymorphisms
* NCBI Genome build 36, release 22