Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Genetics & Development

Genetics of HbA1c: a case study in clinical translation

Aaron Leong[1,2,4] and Eleanor Wheeler[3,4]

Glycated hemoglobin (HbA1c) measures the amount of glucose in the blood in the previous 2–3 months and is used to test whether an individual has diabetes (HbA1c $\geq$ 6.5%), or how well they are managing their diabetes. Genome-wide association studies have successfully identified multiple genomic loci influencing HbA1c, through both glycemic (factors that affect the amount blood glucose levels) and erythrocytic (factors that affect the red blood cell) pathways. Inaccuracies in HbA1c, due to non-glycemic variants, could lead to suboptimal care or adverse health consequences. A recently published example is the erythrocytic variant (rs1050828) in *G6PD*, which leads to the artificial lowering of HbA1c and missed diagnosis of diabetes using current thresholds. In this review we will discuss recent insights into the genetic etiology of HbA1c, and how these can translate to the clinic.

Addresses
[1] Division of General Internal Medicine, Massachusetts General Hospital, Boston, MA 02114, USA
[2] Harvard Medical School, Boston, MA 02115, USA
[3] Department of Human Genetics, Wellcome Sanger Institute, Genome Campus, Hinxton, Cambridge CB10 1SA, UK

Corresponding author: Wheeler, Eleanor (ew2@sanger.ac.uk)
[4] Equal contribution.

Current Opinion in Genetics & Development 2018, **50**:79–85

This review comes from a themed issue on **Molecular and genetic basis of metabolic disease**

Edited by **Inês Barroso** and **Jose C Florez**

For a complete overview see the Issue and the Editorial

Available online 6th March 2018

https://doi.org/10.1016/j.gde.2018.02.008

Introduction

Type 2 diabetes mellitus (T2D) is a complex, heritable disease, characterized by defects in insulin secretion and/or insulin action leading to increased blood glucose levels. Diabetes prevalence is rising dramatically in both developed and developing countries, in 2014 there were 422 million adults with diabetes and this number is expected to reach 700 million by 2025 [1]. There are numerous complications associated with diabetes, including long-term microvascular and macrovascular complications (such as nephropathy, retinopathy, neuropathy, coronary artery disease, and stroke) and it is estimated that 1.6 million people die as a result of diabetes and its complications every year [2]. Despite the serious health complications, only half of prevalent T2D worldwide has been clinically diagnosed [3].

Glycated hemoglobin (HbA1c) is a convenient measure of long-term blood glucose concentrations. This test measures the proportion of glycated hemoglobin, an irreversible chemical modification of the hemoglobin molecule by blood glucose, which reflects average ambient glycemia over the previous 2–3 months, the life of a red blood cell [4]. Following a World Health Organisation (WHO) consultation which concluded that HbA1c could be used to diagnose diabetes [5], HbA1c is now an accepted diagnostic test for T2D and used for monitoring glycemic control in patients with diabetes [6]. Unlike direct measures of blood glucose, such as fasting glucose, which were historically used to diagnose diabetes, HbA1c is a convenient, and stable indicator of glycemic status, and a good predictor of T2D-related complications even in individuals without diabetes [7].

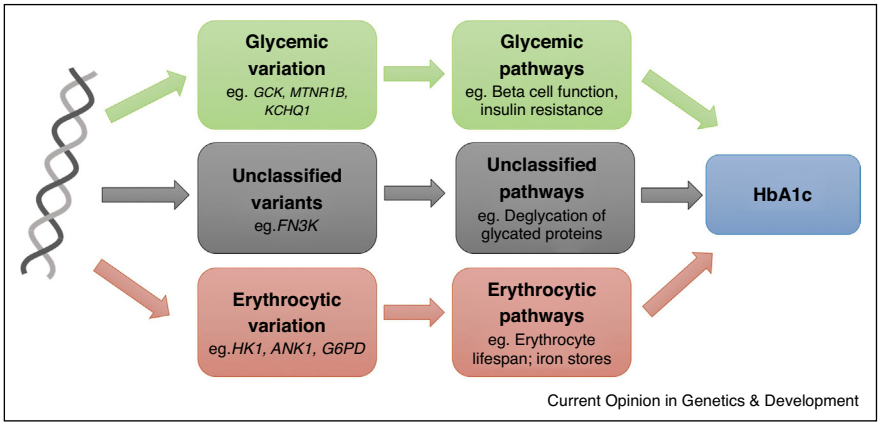
Heritability studies have shown that HbA1c is, in part, genetically determined in individuals with T2D, type 1 diabetes, and those without diabetes [8–10]. In this review, we present recent insights into the genetic etiology of HbA1c and important considerations for the use of HbA1c in clinical practice.

The genetic etiology of HbA1c

The advent of genome-wide association studies (GWAS), and combining multiple such studies through large international consortia such as the Meta-Analysis of Glucose and Insulin related traits Consortium (MAGIC), have successfully identified 61 loci influencing HbA1c [11–15,16••,17]. These studies suggest that some genetic variants influence HbA1c through glycemic pathways, whereas others influence HbA1c through nonglycemic pathways (Figure 1). Interestingly, although HbA1c and fasting glucose are both used to diagnose diabetes, the genetic correlation of these traits is only moderate (r_g = 0.412) [18,19], implying that the genetic determinants of HbA1c do not completely overlap those of glycemia.

Most recently, the MAGIC investigators performed the largest to date GWAS of HbA1c in up to 159 940 individuals without diabetes of European, African, East Asian, and South Asian ancestry [16••]. They identified 60 genetic variants, explaining 4%–14% of the trait variance, of which 19 were classified as influencing HbA1c through glycemic pathways, 22 through erythrocytic pathways, and 19 remained unclassified (Figure 2).

Figure 1



Overview of mechanisms through which genetic variants can influence HbA1c.

Nonglycemic pathways influencing HbA1c include factors affecting erythrocyte biology. Hereditary hemolytic anemia is associated with reduced erythrocyte lifespan and therefore likely to cause falsely lower HbA1c relative to average glycemia [20]. On the other hand, iron deficiency is known to raise HbA1c [21]. Further, thalassemias, other hemoglobin variants (e.g. HbS, HbC, HbD, HbE), and elevated fetal hemoglobin have been reported to interfere with some HbA1c laboratory assay methods [22,23]. As HbA1c is a diagnostic test for diabetes, genetic variation acting through nonglycemic pathways that falsely lower HbA1c result in missed T2D cases [23,24]. Conversely, falsely raised HbA1c can result in overdiagnoses.

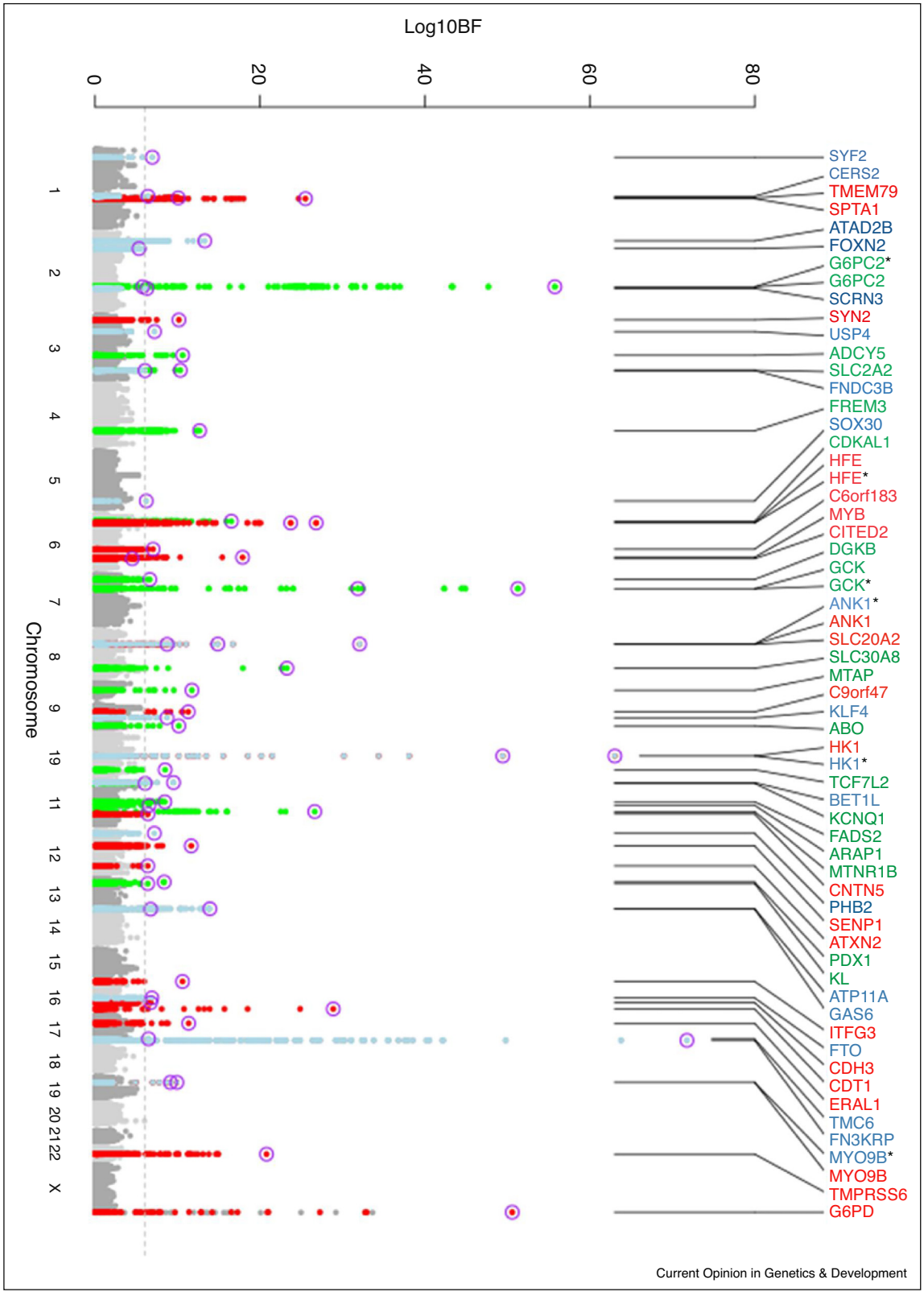
The role of unclassified genetic variants is unclear. It is possible that some of them may still act through glycemic or erythrocytic pathways, but studies to date may have been insufficiently powered to demonstrate any modest associations with glycemic or erythrocytic measures. Alternatively, they may be acting through mechanisms that are neither mediated through between-person variation in erythrocytic lifespan, erythrocytic biology or glycemia. For instance, the *FN3K* locus has been identified through GWAS to be associated to HbA1c but neither glycemic measures nor erythrocytic indices, and has therefore remained unclassified [16••]. The *FN3K* gene encodes fructosamine-3-kinase, an enzyme involved in deglycation of glycated proteins, a nonglycemic nonerythrocytic mechanism that likely influences HbA1c.

Implications of G6PD deficiency and sickle cell trait on the use of HbA1c in people from different genetic backgrounds

Wheeler *et al.* [16••] found that the genetic architecture of HbA1c in Europeans and Asians comprised multiple genetic variants with modest effect sizes. By contrast, the genetic architecture of HbA1c in African Americans was dominated by a single missense variant on the X-chromosome in the gene *G6PD*, rs1050828 (G202A, alleles C/T, p.Val68Met). *G6PD* codes for the erythrocyte enzyme glucose-6-phosphate dehydrogenase, and mutations in this gene can lead to G6PD deficiency, and hemolytic anemia [25,26] including favism [27]. The geographic distribution of G6PD deficiency is strongly correlated with the distribution of malaria, and *G6PD* allelic-variants have been found in malarial endemic regions around the world, specifically parts of the Middle East, Asia, South America, and most of Africa [28]. Thus, G6PD deficiency has implications on the diagnostic accuracy of HbA1c worldwide, including cosmopolitan areas where minority groups with recent non-European ancestry are more likely to carry the *G6PD* variant. The particular variant identified by Wheeler and colleagues was monomorphic in European and Asian populations, but had minor allele (T) frequency 10%–15% in African American individuals. The minor allele was associated with lower HbA1c; an absolute decrease of 0.81%-units (95% CI 0.66–0.96) for men carrying the T allele, and 0.68%-units (95% CI 0.38–0.97) in women with two copies (Figure 3). Importantly, to achieve certification for accurate laboratory reporting of HbA1c values, measured HbA1c ought to be within 6% of the standard reference laboratory mean values (i.e., 6.5 ± 0.4%-units) [29]. The effect of the *G6PD* variant on HbA1c exceeds these limits.

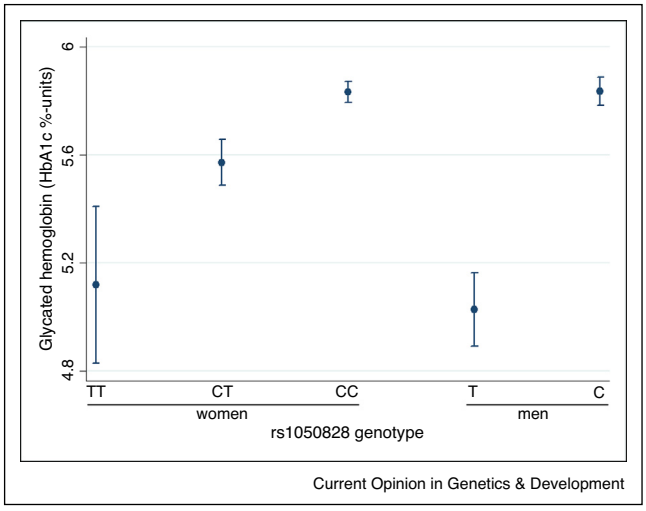
The findings of the study have direct clinical implications. If only a single measure of HbA1c were used to diagnose T2D, then 650 000 African Americans (430 000 men and 180 000 women) in the US (approximately 2% of out of an estimated 29.93 million) with T2D would remain undiagnosed due to the *G6PD* variant using the current threshold of 6.5%-units (Figure 4). Given individuals with G6PD deficiency are often asymptomatic and universal screening is not currently recommended worldwide [25,30], the study suggested investigating the benefits of screening for the *G6PD* genotype

Figure 2



Manhattan plot illustrating the 60 HbA1c-associated signals identified in Wheeler *et al*. [16••], colored by classification as erythrocytic (red), glycemic (green) or unclassified (blue). *Distinct secondary signal at the locus.

Figure 3



Mean glycated hemoglobin by genotype for chromosome X rs1050828.

when using HbA1c to diagnose diabetes in populations of African ancestry, or where G6PD deficiency is common. In those with G6PD deficiency, direct glucose measurements or adjusted diagnostic thresholds should be used to diagnose T2D, and it will be important to characterize the effect of other *G6PD* variants at this locus.

In an accompanying perspective [31•], Paterson noted that *G6PD* is subject to X-inactivation a process by which one of the copies of the X chromosome in females is inactivated. Depending on which X chromosome is inactive in red cell precursors, the artificial lowering of HbA1c may differ across heterozygous females (leading to a larger variance in HbA1c levels in heterozygous (CT) compared to homozygous (CC) females) despite having the same genotype at this variant [16••].

Similarly, another study examined whether sickle cell trait (SCT), defined as the presence of one abnormal allele for HbS at rs334, a variant on chromosome 11, was associated with differences in HbA1c among African Americans. Investigators found that, for a given fasting glucose level, HbA1c values were lower in those with SCT compared to those without (mean HbA1c difference, −0.29% (95%CI −0.35, −0.23). The prevalence of prediabetes and diabetes defined by HbA1c (5.7 to <6.5%-units, and ≥6.5%-units, respectively) in African Americans without a prior diagnosis of diabetes was found to be over 40% lower among those with SCT compared to those without. Conversely, they found no difference in the prevalence of prediabetes or diabetes when defined by fasting glucose or 2-hour glucose values. These findings suggest that HbA1c may systematically underestimate past glycemia in people with SCT, resulting in underdiagnosis of T2D in African Americans [32•].

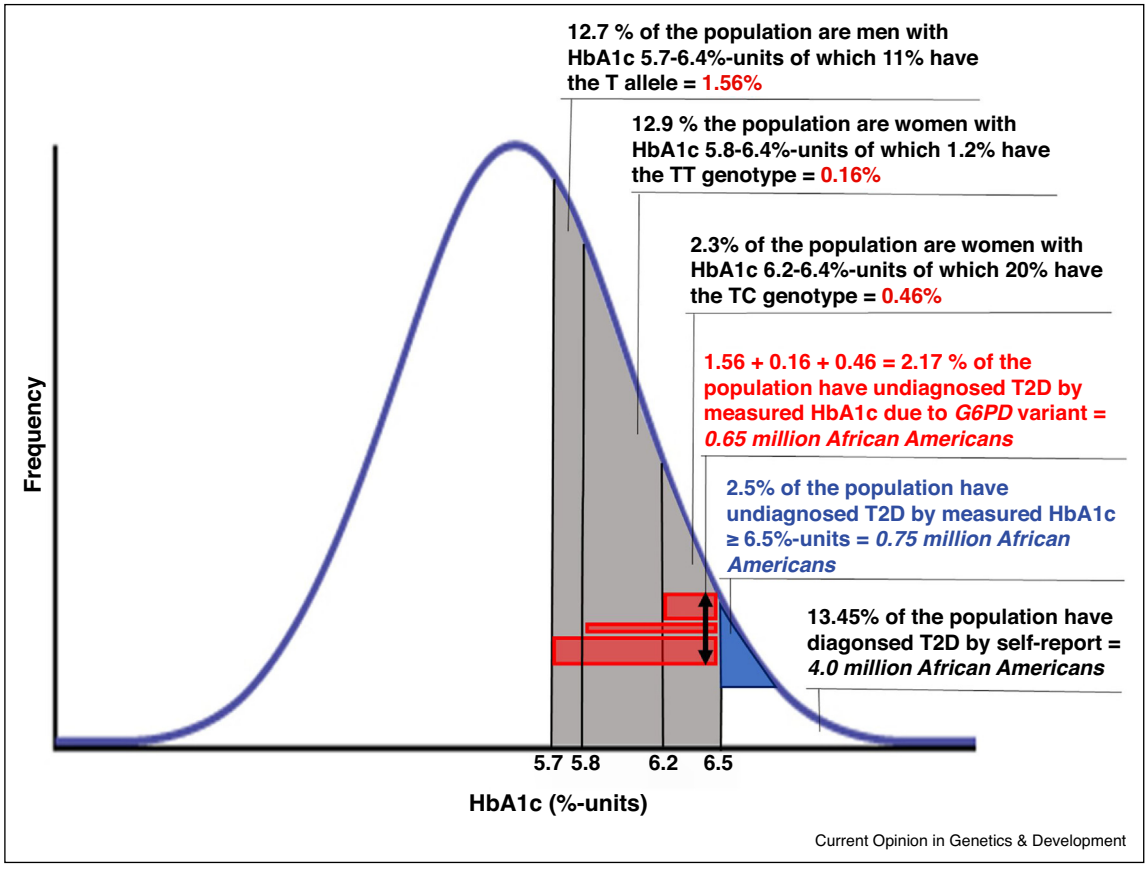
In the era of precision care, it has become increasingly important to progress beyond race-based medical practices to genotype-based medical practices [33]. Genetic investigations across different ancestral groups may allow us to eventually isolate the clinical implications of ancestry-specific HbA1c-related genetic variants from the social determinants of health that correlate with race/ethnic groupings. For instance, the mean HbA1c among Blacks is consistently slightly higher than among Whites [34,35]. Yet, HbA1c has similar predictive ability for longitudinal prediction of incident T2D in both races even after adjusting for fasting glucose and other clinical variables [36].

Nevertheless, these racial differences in mean HbA1c continue to spark debate over the extent to which these differences are genetically determined, and whether these differences can be explained by differences in glycemia, social factors, or the quality or access to healthcare. Further, it is unclear whether such differences are meaningful when using HbA1c in clinical practice [37]. Although the American Diabetes Association acknowledges in clinical practice guidelines that race/ethnicity can be taken into consideration when using HbA1c to diagnose diabetes [38], an individual's unique genotype, in addition to the socially defined concept of race/ethnicity, needs to be considered when using HbA1c in diabetes diagnosis and patient care. Applying a different diagnostic threshold to *all* individuals who self-identify as African American as an attempt to address diagnostic inaccuracy caused by genetics, such as the G6PD deficiency variant or sickle cell variant which only affect carriers, would be inappropriate, and could ironically create greater disparities in care. Similarly, completely avoiding the use of HbA1c in clinical practice is probably inappropriate as HbA1c is a convenient and valid test for the majority of people. Therefore, a detailed examination of genetic effects on HbA1c and the frequency by which these genetic variants differ across ancestrally diverse populations is a critical next step in elucidating the clinical implications of HbA1c genetics. Whether HbA1c genetics would be able to explain racial difference in mean HbA1c remains to be answered.

Implications of HbA1c genetics to HbA1c-glycemia discordance and precision medicine

Apart from its use as a diagnostic test, HbA1c is also used to assess overall glycemic control, disease progression, and response to therapy in people with diabetes [39]. Current management of T2D involves regular measurements of HbA1c and subsequent adjustment of treatment to lower the HbA1c towards a normal or near-normal level. As treatment decisions are based on HbA1c, patients with genetically raised HbA1c through nonglycemic mechanisms may overuse medical services, be over-treated, and have increased rates of side-effects; whereas patients with genetically lowered HbA1c

Figure 4



Estimated number of African Americans with type 2 diabetes (T2D) in the US whose diagnoses would be missed due to the glycose-6-phosphate dehydrogenase (*G6PD*) variant if screened with glycated hemoglobin (HbA1c). Using NHANES, a representative sample of US in 2013–2014, investigators estimated that 0.65 million African Americans with type 2 diabetes (T2D) in the US would remain undiagnosed if screened with glycated hemoglobin (HbA1c) ≥6.5%-units due to the glycose-6-phosphate dehydrogenase (*G6PD*) variant. The frequency of the HbA1c-lowering allele, T, was assumed to be 11% and well-distributed in the population. Total number of African Americans in the US in 2013 was 29.93 million; mean age was 41; 54% were women. Median HbA1c in this population was 5.5%-units and mean HbA1c was 5.7%-units (SD 0.6). Adjusted T2D diagnosis threshold for men with the T allele is 5.7%-units, 5.8%-units for women with the TT genotype, and 6.2% for women with the TC genotype.

through nonglycemic mechanisms may have delayed diagnoses, inadequate treatment, persistent hyperglycemia, and increased rates of T2D-related complications. It remains uncertain whether genetically induced discordance between HbA1c and glycemia leads to suboptimal care and adverse health consequences in ways that could be remedied by use of genetic knowledge.

Measures of the discordance between reported HbA1c levels and the expected HbA1c level estimated by glycemia include the hemoglobin glycation index and the glycation gap. The hemoglobin glycation index is calculated from the difference between the measured HbA1c level and the HbA1c level predicted from its regression on mean glucose levels. The glycation gap is calculated from its regression on fructosamine levels, a glycemic measure unrelated to erythrocytes. It has been shown that these measures of HbA1c-glycemia discordance are associated with important health outcomes, including mortality, microvascular disease, macrovascular disease and treatment-related hypoglycemia [40–42].

It is possible that genetic variants that influence HbA1c through nonglycemic pathways are determinants of these measures of HbA1c-glycemia discordance. Supporting this hypothesis is a previous study that showed that the glycation gap may be partly genetically determined and account for one third of the heritability of HbA1c [43]. Similar to how the sickle cell and G6PD deficiency variants artificially lower HbA1c through shortening of the erythrocytic lifespan, some common HbA1c-related genetic variants may contribute to individual differences in erythrocytic age. A previous study has shown that between-person variation in mean erythrocytic age explains nonglycemic variation in HbA1c and accounting for this variation likely reduces errors in its estimation of

average glycemia [44•]. Certain unclassified HbA1c-related variants that may not influence erythrocytic lifespan or glycemia may also contribute to this discordance. For instance, differences in fructosamine-3-kinase activity, which could be caused by variation in the *FN3K* gene, have been shown to be associated with the glycation gap in people with diabetes [45].

Determining which alleles at these HbA1c-related variants raise or lower the hemoglobin glycation index or glycation gap is necessary to accurately recalibrate HbA1c values for these genetic effects or derive genotype-specific diagnostic thresholds for HbA1c, and improve the diagnostic value and ability of HbA1c to evaluate glycemic control in patients with T2D regardless of their genetic background. Importantly, while the majority of these genetic variants are common in the populations studied, they have modest effects on HbA1c individually. Only people carrying multiple of these variants would have clinically significant discordance between HbA1c and overall glycemia.

Future directions and conclusions

Large-scale genetic discovery efforts on common human variation with the potential for direct clinical practice or public health translation are few and far between. Investigating how the application of HbA1c genetics can improve the clinical utility of this valuable biomarker that is central to T2D diagnosis, risk stratification, prediction, and patient care is an important next step. As illustrated by the discovery of the *G6PD* variant in African Americans, genetic analyses of ethnically diverse populations offer the possibility of uncovering novel genetic variants that impact the clinical utility of HbA1c in ethnic minorities. Similarly, studies focusing on low-frequency and rare variants, using newer genotyping arrays or denser imputation panels such as the 1000 Genomes Project [46], the Haplotype Reference Consortium [47], deep-coverage whole genome sequence data from The Trans-Omics for Precision Medicine (TOPMed) Program (https://www.nhlbiwgs.org/), or the African Genome Resources (AGR) panel (https://www.apcdr.org/), may identify additional variants with clinically meaningful effects on HbA1c. Findings have the potential to challenge the manner in which HbA1c is currently used as a diagnostic tool, inform the individualization of HbA1c-defined thresholds for screening, and HbA1c-defined goals to guide treatment decisions, while promoting health equity for people of different genetic backgrounds, and encouraging the practice of precision medicine [48], precision screening and precision public health.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Collaboration NCDRF: **Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants**. *Lancet* 2016, **387**:1513-1530.
2. Global Burden of Metabolic Risk Factors for Chronic Diseases C: **Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment**. *Lancet Diabetes Endocrinol* 2014, **2**:634-647.
3. Beagley J, Guariguata L, Weil C, Motala AA: **Global estimates of undiagnosed diabetes in adults**. *Diabetes Res Clin Pract* 2014, **103**:150-160.
4. Mortensen HB, Christophersen C: **Glucosylation of human haemoglobin a in red blood cells studied in vitro. Kinetics of the formation and dissociation of haemoglobin A1c**. *Clin Chim Acta* 1983, **134**:317-326.
5. *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a Who Consultation*. Geneva (2011).
6. American Diabetes A: **Diagnosis and classification of diabetes mellitus**. *Diabetes Care* 2012, **35(Suppl. 1)**:S64-S71.
7. Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL: **Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults**. *N Engl J Med* 2010, **362**:800-811.
8. Simonis-Bik AM, Eekhoff EM, Diamant M, Boomsma DI, Heine RJ, Dekker JM, Willemsen G, van Leeuwen M, de Geus EJ: **The heritability of HbA1c and fasting blood glucose in different measurement settings**. *Twin Res Hum Genet* 2008, **11**:597-602.
9. Mathias RA, Deepa M, Deepa R, Wilson AF, Mohan V: **Heritability of quantitative traits associated with type 2 diabetes mellitus in large multiplex families from south India**. *Metabolism* 2009, **58**:1439-1445.
10. 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, **50**:2858-2863.
11. Franklin CS, Aulchenko YS, Huffman JE, Vitart V, Hayward C, Polasek O, Knott S, Zgaga L, Zemunik T, Rudan I *et al.*: **The TCF7L2 diabetes risk variant is associated with HbA(1)(c) levels: a genome-wide association meta-analysis**. *Annals Hum Genetics* 2010, **74**:471-478.
12. Pare G, Chasman DI, Parker AN, Nathan DM, Miletich JP, Zee RY, Ridker PM: **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 Genetics* 2008, **4**:e1000312.
13. Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, Bouatia-Naji N, Langenberg C, Prokopenko I, Stolerman E *et al.*: **Common variants at 10 genomic loci influence hemoglobin A (1)(C) levels via glycemic and nonglycemic pathways**. *Diabetes* 2010, **59**:3229-3239.
14. Chen P, Takeuchi F, Lee JY, Li H, Wu JY, Liang J, Long J, Tabara Y, Goodarzi MO, Pereira MA *et al.*: **Multiple nonglycemic genomic loci are newly associated with blood level of glycated hemoglobin in east Asians**. *Diabetes* 2014, **63**:2551-2562.
15. Ryu J, Lee C: **Association of glycosylated hemoglobin with the gene encoding CDKAL1 in the Korean Association Resource (KARE) study**. *Hum Mutat* 2012, **33**:655-659.

16. •• Wheeler E, Leong A, Liu CT, Hivert MF, Strawbridge RJ, Podmore C, Li M, Yao J, Sim X, Hong J *et al.*: **Impact of common genetic determinants of hemoglobin A1C on type 2 diabetes risk and diagnosis in ancestrally diverse populations: a transethnic genome-wide meta-analysis**. *PLoS Med* 2017, **14**: e1002383.
This study conducted the largest to date trans-ethnic GWAS and identified 60 genetic variants associated with HbA1c and is the first to identify a genetic variant in *G6PD*, identified in individuals of African-American ancestry, which has a large effect on HbA1c levels with important clinical implications. The study also highlights that around a third of HbA1c-related variants are implicated in erythrocytic biology.

17. Chen P, Ong RT, Tay WT, Sim X, Ali M, Xu H, Suo C, Liu J, Chia KS, Vithana E *et al.*: **A study assessing the association of glycated hemoglobin A1C (HbA1c) associated variants with HbA1c, chronic kidney disease and diabetic retinopathy in populations of Asian ancestry**. *PLoS One* 2013, **8**:e79767.

18. Zheng J, Erzurumluoglu AM, Elsworth BL, Kemp JP, Howe L, Haycock PC, Hemani G, Tansey K, Laurin C, Early G *et al.*: **LD hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level gwas data for SNP heritability and genetic correlation analysis**. *Bioinformatics* 2017, **33**:272-279.

19. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Schizophrenia Working Group of the Psychiatric Genomics C, Patterson N, Daly MJ, Price AL, Neale BM: **LD score regression distinguishes confounding from polygenicity in genome-wide association studies**. *Nat Genet* 2015, **47**:291-295.

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

21. Coban E, Ozdogan M, Timuragaoglu A: **Effect of iron deficiency anemia on the levels of hemoglobin A1C in nondiabetic patients**. *Acta Haematol* 2004, **112**:126-128.

22. Little RR, Roberts WL: **A review of variant hemoglobins interfering with hemoglobin A1C measurement**. *J Diab Sci Technol* 2009, **3**:446-451.

23. Bry L, Chen PC, Sacks DB: **Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin**. *Clin Chem* 2001, **47**:153-163.

24. Venkataraman K, Kao SL, Thai AC, Salim A, Lee JJ, Heng D, Tai ES, Khoo EY: **Ethnicity modifies the relation between fasting plasma glucose and HbA1c in Indians, Malays and Chinese**. *Diab Med J Br Diab Assoc* 2012, **29**:911-917.

25. Leong A: **Is there a need for neonatal screening of glucose-6-phosphate dehydrogenase deficiency in Canada?** *McGill J Med MJM* 2007, **10**:31-34.

26. Motulsky AG, Stamatoyannopoulos G: **Clinical implications of glucose-6-phosphate dehydrogenase deficiency**. *Annals Internal Med* 1966, **65**:1329-1334.

27. Luzzatto L, Arese P: **Favism and glucose-6-phosphate dehydrogenase deficiency**. *N Engl J Med* 2018, **378**:60-71.

28. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, Hogg MM, Battle KE, Padilla CD, Baird JK, Hay SI: **G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map**. *PLoS Med* 2012, **9**:e1001339.

29. Little RR, Rohlfing CL, Sacks DB, National Glycohemoglobin Standardization Program Steering C: **Status of hemoglobin A1C measurement and goals for improvement: from chaos to order for improving diabetes care**. *Clin Chem* 2011, **57**:205-214.

30. Watchko JF, Kaplan M, Stark AR, Stevenson DK, Bhutani VK: **Should we screen newborns for glucose-6-phosphate dehydrogenase deficiency in the United States?** *J Perinatol* 2013, **33**:499-504.

31. • Paterson AD: **HbA1c for type 2 diabetes diagnosis in Africans and African Americans: personalized medicine now!** *PLoS Med* 2017, **14**:e1002384.
In this accompanying editorial to the Wheeler and colleagues paper, Andrew Paterson discussed the potential impact of X-inactivation on the effect of the G6PD variant on HbA1c in women.

32. • Lacy ME, Wellenius GA, Sumner AE, Correa A, Carnethon MR, Liem RI, Wilson JG, Sacks DB, Jacobs DR Jr, Carson AP *et al.*: **Association of sickle cell trait with hemoglobin A1C in African Americans**. *JAMA* 2017, **317**:507-515.
This study evaluated the association between sickle cell trait, defined as the presence of one abnormal allele for HbS at rs334, and HbA1c among African Americans and found that for a given fasting glucose, HbA1c values were lower in those with sickle cell trait compared to those without by an estimated 0.29% units.

33. Meier RJ: **A critique of race-based and genomic medicine**. *Coll Antropol* 2012, **36**:5-10.

34. Meigs JB, Grant RW, Piccolo R, Lopez L, Florez JC, Porneala B, Marceau L, McKinlay JB: **Association of African genetic ancestry with fasting glucose and HbA1c levels in non-diabetic individuals: the Boston Area Community Health (BACH) prediabetes study**. *Diabetologia* 2014, **57**:1850-1858.

35. Cavagnolli G, Pimentel AL, Freitas PA, Gross JL, Camargo JL: **Effect of ethnicity on HbA1c levels in individuals without diabetes: systematic review and meta-analysis**. *PLoS One* 2017, **12**:e0171315.

36. Leong A, Daya N, Porneala B, Devlin JJ, Shiffman D, McPhaul MJ, Selvin E, Meigs JB: **Prediction of type 2 diabetes by hemoglobin A1C in two community-based cohorts**. *Diabetes Care* 2017, **41**:60-68.

37. Selvin E: **Are there clinical implications of racial differences in HbA1c? A difference, to be a difference, must make a difference**. *Diabetes Care* 2016, **39**:1462-1467.

38. American Diabetes A: **2. Classification and diagnosis of diabetes**. *Diabetes Care* 2017, **40(Suppl. 1)**:S11-S24.

39. Kohnert KD, Heinke P, Vogt L, Salzsieder E: **Utility of different glycemic control metrics for optimizing management of diabetes**. *World J Diabetes* 2015, **6**:17-29.

40. Nayak AU, Nevill AM, Bassett P, Singh BM: **Association of glycation gap with mortality and vascular complications in diabetes**. *Diabetes Care* 2013, **36**:3247-3253.

41. Hempe JM, Liu S, Myers L, McCarter RJ, Buse JB, Fonseca V: **The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the accord trial**. *Diabetes Care* 2015, **38**:1067-1074.

42. Ahn CH, Min SH, Lee DH, Oh TJ, Kim KM, Moon JH, Choi SH, Park KS, Jang HC, Ha J *et al.*: **Hemoglobin glycation index is associated with cardiovascular diseases in people with impaired glucose metabolism**. *J Clin Endocrinol Metab* 2017, **102**:2905-2913.

43. Cohen RM, Snieder H, Lindsell CJ, Beyan H, Hawa MI, Blinko S, Edwards R, Spector TD, Leslie RD: **Evidence for independent heritability of the glycation gap (glycosylation gap) fraction of HbA1c in nondiabetic twins**. *Diabetes Care* 2006, **29**:1739-1743.

44. • Malka R, Nathan DM, Higgins JM: **Mechanistic modeling of hemoglobin glycation and red blood cell kinetics enables personalized diabetes monitoring**. *Sci Transl Med* 2016, **8**:359ra130.
Using a mechanistic mathematical model of hemoglobin glycation and red blood cell kinetics, combined with large sets of within-patient glucose measurements, this study found that between-patient variation in derived mean red blood cell age explained all glucose-independent variation in HbA1c.

45. Dunmore SJ, Al-Derawi AS, Nayak AU, Narshi A, Nevill AM, Hellwig A, Majebi A, Kirkham P, Brown JE, Singh BM: **Evidence that differences in fructosamine-3-kinase activity may be associated with the glycation gap in human diabetes**. *Diabetes* 2017, **67**:131-136.

46. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR: **A global reference for human genetic variation**. *Nature* 2015, **526**:68-74.

47. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A, Kang HM, Fuchsberger C, Danecek P, Sharp K *et al.*: **A reference panel of 64,976 haplotypes for genotype imputation**. *Nat Genet* 2016, **48**:1279-1283.

48. Florez JC: **Precision medicine in diabetes: is it time?** *Diabetes Care* 2016, **39**:1085-1088.