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Short communication

Glucose-6-phosphate dehydrogenase deficiency and metabolic profiling in adolescence from the Chinese birth cohort: "Children of 1997"

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ABSTRACT

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects 6.0% of the global population. G6PD deficiency has been associated with lower risk of cardiovascular disease and higher risk of diabetes, which could be etiologically informative, but these relations are uncertain. To clarify, we assessed the associations of G6PD deficiency with serum metabolite profiles in late adolescence.
Methods: In a nested case-control study of 50 G6PD-deficient late adolescents (~17.5 years) and 150 sex-matched non-G6PD-deficient controls from a Chinese birth cohort: "Children of 1997", we compared 80 serum metabolites analyzed by nuclear magnetic resonance spectrometry using adjusted linear regression with Bonferroni correction for testing 12 traits ($p < 0.0042$).
Results: G6PD-deficiency was inversely associated with serum levels of total cholesterol (−0.27 mmol, 95% confidence interval (CI) −0.46, −0.09, $p = 0.004$), free cholesterol (−0.08 mmol, 95% CI −0.13, −0.03, $p = 0.003$) and creatinine (−0.004 mmol, 95% CI −0.007, −0.001, $p = 0.003$), adjusted for sex and parental education. G6PD deficiency was not associated with fatty acids, amino acids, glucose or related metabolites, ketone bodies or glycoprotein.
Conclusions: G6PD deficiency is associated with lower serum levels of cholesterol and creatinine, but not other serum metabolites. Whether such differences are transient or become more evident in adulthood warrant further investigations.

1. Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is relatively common in settings where malaria has been endemic because it confers some protection against malaria. Observationally, G6PD deficiency also appears to be associated with a lower risk of cardiovascular disease [1–3] and a higher risk of diabetes [4,5], for a number of possible reasons. G6PD deficiency primarily affects mature erythrocytes due to instability of G6PD activity resulting in hemolysis under oxidative stress [6]. Excess oxidative stress could affect the cardiovascular and metabolic system. G6PD, as a cytoplasmic enzyme producing most nicotinamide adenine dinucleotide phosphate (NADPH) in cytoplasm, is expressed in adipocytes, cardiac myocytes, smooth muscle, and testis, meaning NADPH-dependent pathways inhibited by G6PD deficiency in other tissues could be relevant [7]. G6PD deficiency could inhibit the NADPH-dependent 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase similar to how statins lower cholesterol synthesis, and impair glucose metabolism [3,8]. G6PD deficiency could also act via the NADPH-dependent cytochrome P450 enzymes that reduce steroidogenesis and hence indirectly lower serum cholesterol, and possibly glucose uptake [9,10]. To clarify, we compared metabolite profile, particularly of lipids, of young Hong Kong Chinese at ~17.5 years with and without G6PD deficiency.

2. Methods

2.1. Data source

A nested case-control study of 50 G6PD-deficient and 150 sex-matched non-G6PD-deficient controls at ~17.5 years were randomly selected from a population-representative Chinese birth cohort: Hong Kong's "Children of 1997" ($n = 8327$) that covered 88.0% of all births in Hong Kong in April and May 1997, described in detail elsewhere [11]. Families were recruited at the first postnatal visit to any of the 49 Maternal and Child Health Centers (MCHCs) in Hong Kong, which parents of all newborns are strongly encouraged to attend. A biobank clinical follow-up was conducted from 2013 to 2016 including biological specimen collection. Participants fasted overnight for ≥8 h before attending a clinic the following morning. Blood samples were collected using venipuncture, centrifuged and aliquoted into serum samples which were stored at −80 °C in the Centre for Genomic

Abbreviations: BMI, body mass index; CI, confidence interval; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; G6PD, glucose-6-phosphate dehydrogenase; MCHC, Maternal and Child Health Center; NADPH, nicotinamide adenine dinucleotide phosphate; NMR, nuclear magnetic resonance.

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Sciences of the University of Hong Kong. The serum samples, shipped and stored at −80 °C, were analyzed using automated high-throughput nuclear magnetic resonance (NMR) spectroscopy by the Brainshake metabolomics laboratory in Finland. NMR spectroscopy has the advantage of quantifying many circulating metabolites in a single test using a small sample and gives comparable estimates of standard clinical biomarkers as conventional biochemical assays.

2.2. G6PD status

In Hong Kong, a free-of-charge universal (99% coverage) neonatal screening program assesses erythrocyte G6PD activity from umbilical cord blood at birth using a spectrophotometric assay conducted by the Genetic Screening Unit of the Department of Health [12]. The assay has high sensitivity (100%) and specificity (99%) for G6PD activity in hemizygous boys and homozygous girls, but not heterozygous girls. Parents of newborns with G6PD activity below 25% of the mean are informed and followed-up by the Clinical Genetic Service of the Department of Health. When newborns attended the MCHCs, G6PD status, categorized as "G6PD-deficient" or "non-G6PD-deficient", was recorded.

2.3. Metabolites

Of 228 metabolites quantified, we considered the concentration of 80 metabolites as 12 traits ranging from standard clinical biomarkers, such as serum levels of cholesterol and triglycerides, to emerging potential biomarkers, including apolipoproteins and fatty acids, HMG-CoA reductase and amino acids, ketones and glycolysis-related metabolites for diabetes.

2.4. Statistical analysis

We assessed the adjusted associations of G6PD deficiency with serum metabolites in absolute concentration units using linear regression with Bonferroni correction to account for multiple testing of 12 traits ($p < 0.0042$). Confounders included were sex and highest parental education [13], because G6PD status varies by sex and could affect serum metabolite profiles, while socio-economic position may affect serum metabolite profiles; race/ethnicity was not considered because all participants are Chinese. For comparability, metabolites were scaled to standard deviation (SD) units, from which mean differences in SD and 95% confidence intervals (CIs) were presented. To minimize false-positives due to the small sample size, we excluded metabolites with >15% missing values (i.e. extremely large very low-density lipoprotein (VLDL) particles and very large VLDL particles) [14]. As a sensitivity analysis, we re-ran the analysis using log-transformed metabolites to check the normality assumption. Statistical analyses were performed using Stata version 13 (Stata Corp, College station, Texas, USA) and R version 3.3.1 (R Development Core Team, Vienna, Austria).

3. Results

Given G6PD deficiency is X-linked recessive, more boys ($n = 180$) were included than girls ($n = 20$). Table 1 shows that the cases and controls were similar in birth order, parental birthplace, and highest parental education.

Fig. 1 shows that G6PD deficiency was negatively associated with serum levels of total cholesterol, free cholesterol and creatinine, adjusted for sex and parental education. G6PD deficiency was not associated with triglycerides, phospholipids, lipoproteins, fatty acids, amino acids, glucose or related metabolites, ketone bodies, albumin or glycoprotein. G6PD deficiency was not clearly associated with LDL-cholesterol ($p = 0.009$). Sensitivity analysis using log-transformed metabolites showed similar associations; esterified cholesterol was also associated with G6PD deficiency (data not shown).

Table 1
Baseline characteristics by glucose-6-phosphate dehydrogenase deficiency (G6PD) status for 200 adolescents from Hong Kong's "Children of 1997" birth cohort, Hong Kong, China, 1997–2016.

Characteristics	G6PD status				p-Value
	Deficient ($n = 50$)		Non-deficient ($n = 150$)		
	No.	%	No.	%	
Child's sex					<0.001
Female	5	10.0	15	10.0	
Male	45	90.0	135	90.0	
Birth order					0.60
1st	25	51.0	64	43.5	
2nd	19	38.8	69	46.9	
3rd or above	5	10.2	14	9.5	
Mode of delivery					0.30
Natural labour	23	46.9	77	52.7	
Assisted natural labour	7	14.3	29	19.9	
Caesarean birth	19	38.8	40	27.4	
Secondhand smoke exposure					0.86
None	16	32.7	42	29.6	
Non-parental household smoking	16	32.7	55	38.7	
Paternal smoking	14	28.6	39	27.5	
Maternal smoking	3	6.1	6	4.2	
Breastfeeding					0.12
Never breastfed	28	56.0	79	53.0	
Partially breastfed or exclusively breastfed for <3 months	17	34.0	65	43.6	
Exclusively breastfed for 3+ months	5	10.0	5	3.4	
Type of hospital at birth					0.19
Public	32	64.0	109	73.6	
Private or overseas	18	36.0	39	26.4	
Mother's birthplace					0.30
Mainland China or elsewhere	24	48.0	59	39.6	
Hong Kong	26	52.0	90	60.4	
Father's birthplace					0.19
Mainland China or elsewhere	21	42.9	48	32.4	
Hong Kong	28	57.1	100	67.6	
Highest parental education at recruitment					0.38
Grade 9 or below	16	32.0	49	32.7	
Grade 10–11	25	50.0	61	40.7	
Grade 12 or above	9	18.0	40	26.7	
Household income per head at recruitment[a]					0.47
1st quintile	12	26.7	25	17.7	
2nd quintile	6	13.3	30	21.3	
3rd quintile	11	24.4	29	20.6	
4th quintile	6	13.3	28	19.9	
5th quintile	10	22.2	29	20.6	
Type of housing at recruitment					0.79
Public estate	21	42.9	64	43.5	
Subsidized home ownership flat	10	20.4	24	16.3	
Private flat	18	36.7	59	40.1	

[a] Mean (standard deviation) for household income per head at recruitment in quintiles (in Hong Kong dollar; pegged at a rate of 7.8 dollar = 1 U.S. dollar) were 1st quintile: $1812 (357), 2nd quintile: $2901 (321), 3rd quintile: $4257 (545), 4th quintile: $6964 (960) and 5th quintile: $13,400 (5433).

4. Discussion

In this ethnically homogeneous Chinese nested case-control study with minimal confounding by race/ethnicity or population stratification, G6PD-deficiency was associated with lower serum levels of total cholesterol, free cholesterol and creatinine, but no differences in other lipids, amino acids, glucose and related metabolites, ketone bodies, albumin or glycoprotein. Our findings suggest G6PD deficiency may result in lower serum cholesterol and hence possibly lower cardiovascular disease risk [1–3], whereas glucose metabolism or amino acids are unlikely relevant to the potentially higher diabetes risk.

This is the first study revealing lower serum cholesterol associated with G6PD deficiency in late adolescence which cannot be explained by treatment, ill health or adult lifestyle. To date, four studies of the association of G6PD deficiency with lipids in adulthood have been conducted, with mixed results [3,8,15,16]. However, these mostly case-control studies are open to selection bias given the controls were selected from clinics or workplaces rather than from the same underlying population. Their findings are inconsistent with our inverse associations of G6PD deficiency with serum levels of total cholesterol and nominally with LDL-cholesterol ($p = 0.009$), but are consistent with our null associations with HDL-cholesterol, triglycerides and apolipoproteins. In vitro cholesterol synthesis but not LDL receptor expression is lower in G6PD-deficient human cells. Moreover, inconsistent with a previous study [17], we found G6PD deficiency associated with lower creatinine, which has been associated with lower cardiovascular risk, although whether as a downstream marker or a causal factor is unclear.

Taken together, higher susceptibility to oxidative stress from unstable G6PD activity cannot explain our inverse association of G6PD deficiency with serum cholesterol and creatinine. Oxidative stress has

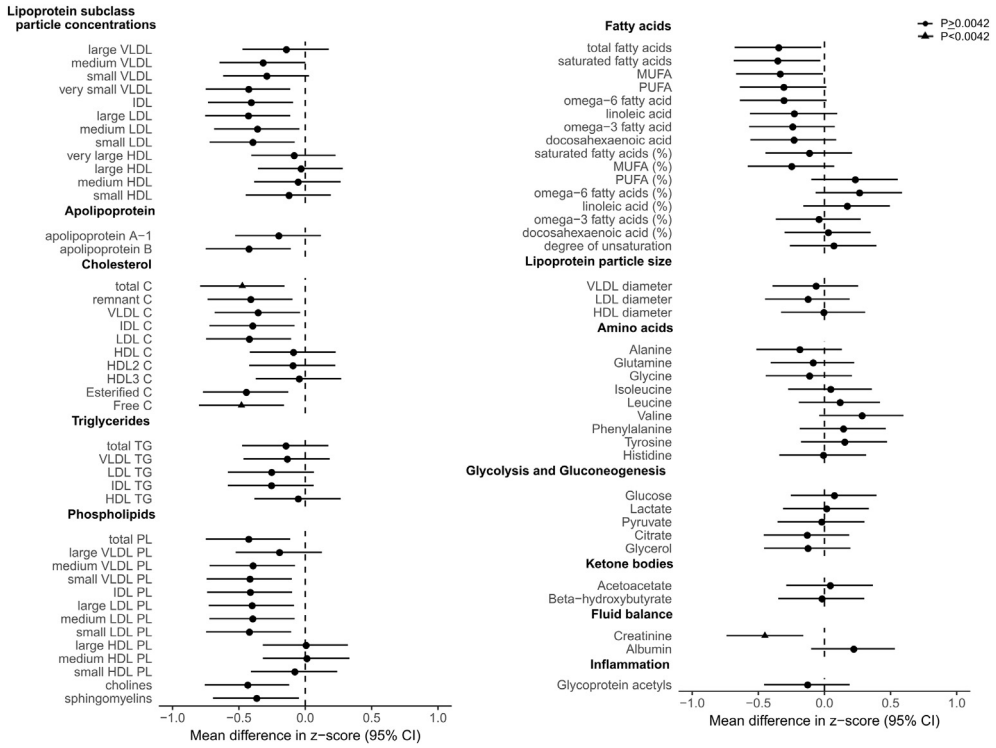

Fig. 1. Adjusted[a] associations of glucose-6-phosphate dehydrogenase deficiency (G6PD) status with serum metabolites at ~17.5 years, from which mean difference in terms of standard deviation and 95% confidence intervals (CIs) of 80 metabolites among 50 G6PD-deficient adolescents compared with 150 non-G6PD-deficient adolescents with Bonferroni-corrected *p*-value accounting for 12 metabolic traits ($p < 0.0042$) are presented, from Hong Kong's "Children of 1997", Hong Kong, China, 1997–2016. Abbreviation: CI, confidence interval; G6PD, glucose-6-phosphate dehydrogenase deficiency; z-score, standard deviation score. [a]Adjusted for sex and highest parental education.

been hypothesized to be detrimental, but randomized controlled trials show antioxidant vitamins do not prevent cardiovascular disease [18]. Intriguingly several factors related to red blood cells traits have recently emerged as novel cardiovascular risk factors, including blood group associated with lipids, and both reticulocytes and clonal hematopoiesis of indeterminate potential associated with heart disease [19]. Whether a unifying explanation encompassing G6PD-related red blood cells traits exists is unknown. Conversely, G6PD deficiency could act as a naturally occurring statin that inhibits the NADPH-dependent HMG-CoA reductase and hence directly reduces cholesterol synthesis [3,8]. Alternatively, G6PD could act via inhibiting NADPH-dependent cytochrome P450 enzymes and thereby reducing steroidogenesis with indirectly lowering serum cholesterol as a by-product [9]. Our previous study in this cohort found G6PD deficiency associated with lower childhood body mass index (BMI) gain and later onset of pubic hair development, but not with birthweight or infant BMI gain [13]. Lower serum cholesterol or creatinine associated with G6PD deficiency could possibly be via childhood weight changes. Moreover, considering G6PD deficiency is more common in boys, reduced production of sex steroids, particularly androgens, may also contribute to lower cardiovascular risk, and healthier lipid profile [10]. Whether G6PD deficiency provides an example of the evolutionary biology trade-off between survival against reproduction remains to be established. The potential role of sex steroids and/or childhood weight changes as the mediating pathways warrant further study.

Our null associations of G6PD deficiency with glucose and related metabolites and amino acids suggest pathways other than glucose or amino acid metabolism may be more relevant to the possible link between G6PD deficiency and diabetes. Such negative findings are unlikely related to higher susceptibility to excess oxidative stress arising from unstable G6PD activity. We cannot rule out the possibility that G6PD deficiency might impair glucose metabolism perhaps via HMG-CoA reductase inhibition [20]. Alternative pathways such as impaired insulin secretion and sensitivity, or reduced muscle mass which acts as a sink for glucose disposal could be further scrutinized to disentangle the observed higher diabetes risk.

Limitations exist. First, we do not have erythrocyte G6PD activity, thus cannot assess any graded association with health outcomes. We may also have missed girls with heterozygous G6PD deficiency, but we included few girls in this study. Second, detailed metabolite profiling was only available for a small subset, so we cannot replicate findings in a validation cohort, or detect very small differences that might be important at a population level even if not clinically meaningful. Thirdly, we only considered fasting serum glucose as insulin and insulin resistance are not currently available. Future studies with more comprehensive glycemic traits are needed to clarify the role of G6PD deficiency. Finally, we are limited by the age of the cohort, so we could not consider cardiovascular disease and diabetes as outcomes, and do not have subclinical measures of atherosclerosis.

Authors' contributions

MK Kwok performed the literature review, conducted data analysis, interpreted findings and drafted the manuscript. GM Leung and SL Au Yeung interpreted findings and critically reviewed the manuscript. CM Schooling conceptualized ideas, designed and directed analytic strategy, interpreted findings, revised drafts of the manuscript critically and supervised the study from conception to completion. All authors have read and approved the submission of the manuscript and take full responsibility for the manuscript.

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Conflict of interests

The authors declare that they have no competing interests.

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