Atherosclerosis 282 (2019) 148–153

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Glucose-6-phosphate dehydrogenase deficiency and risk of cardiovascular disease: A propensity score-matched study

Giovanni Mario Pes[a,*], Guido Parodi[a], Maria Pina Dore[a,b]

[a] Dipartimento di Scienze Mediche, Chirurgiche e Sperimentali, Clinica Medica, University of Sassari, Italy
[b] Baylor College of Medicine, One Baylor Plaza, Houston, TX, USA

HIGHLIGHTS

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency increases the cardiovascular risk up to 70%.
- The risk conferred by G6PD deficiency is moderate compared with the impact of primary cardiovascular risk factors.
- G6PD deficient patients would be eligible for additional preventive measures against cardiovascular disease.

ARTICLE INFO

Keywords:
Glucose-6-phosphate dehydrogenase deficiency
Cardiovascular disease
Risk factors
Propensity score matching

ABSTRACT

Background and aims: Cardiovascular disease (CVD) is associated with high morbidity and mortality. Studies in animal models and humans suggested that glucose–6–phosphate dehydrogenase (G6PD) deficiency, a genetically inherited condition causing haemolytic anemia, may be a risk factor for CVD. This hypothesis was tested in a large cohort from Northern Sardinia, where the population prevalence of G6PD deficiency is the highest in the Mediterranean area.

Methods: A retrospective observational case–control study was performed using clinical records of 9604 patients undergoing digestive endoscopy between 2002 and 2017, with a known G6PD status and a complete clinical history including CVD and leading CVD risk factors. To circumvent covariates imbalance between cases and controls, a 1:2 propensity score–matched analysis was performed.

Results: Major predictors of CVD, as expected, were age (OR 1.07; 95%CI 1.06–1.08), male sex (1.63; 95%CI 1.29–2.06), high blood pressure (OR 1.46; 95%CI 1.16–1.84), smoking (OR 3.03; 95%CI 2.42–3.79), diabetes (OR 1.65; 95%CI 1.23–2.21) and hypercholesterolemia (OR 2.20; 95%CI 1.71–2.84). The propensity score matching procedure resulted in 1123 G6PD deficient patients and 2246 patients with normal enzyme activity. When G6PD status was regressed on the CVD, including propensity score as a continuous covariate, an OR of 1.71 (95%CI 1.17–2.49; $p = 0.006$) was obtained.

Conclusions: G6PD deficiency is significantly associated with increased risk of CVD, although the underlying mechanisms are still poorly understood. The loss of important protective pathways against oxidative stress, especially in the early stages of atherogenesis, might play a crucial role.

1. Introduction

Cardiovascular disease (CVD), which comprises a broad range of disorders including coronary artery disease, cerebrovascular disease, and peripheral artery disease, remains the leading cause of mortality worldwide, with an estimated 422.7 million cases and 17.9 million deaths every year, nearly 31% of all global deaths [1,2]. The incidence of CVD is variable across different countries and racial groups, ranging from 94 per 100,000 inhabitants per year in Japan to 1752 deaths per 100,000 inhabitants per year in Russia [3]. This wide variability is the result of lifelong exposure to several risk factors including “modifiable” factors such as smoking, high blood pressure, obesity, dyslipidemia and type 2 diabetes, and “not modifiable” factors including age, sex and a positive family history of CVD [4]. Among non–modifiable factors, heredity plays a considerable role, as proven by the greater CVD mortality in offspring of parents with history of atherosclerosis [5]. By using genome–wide association studies, several DNA gene variants have been identified able to raise the risk of CVD [6,7]. However, few gene

* Corresponding author. Clinica Medica, Viale San Pietro 8, I-07100, Sassari, Italy.
E-mail address: gmpes@uniss.it (G.M. Pes).

https://doi.org/10.1016/j.atherosclerosis.2019.01.027
Received 30 October 2018; Received in revised form 9 January 2019; Accepted 15 January 2019
Available online 28 January 2019
0021-9150/

variants may reduce, rather than increase, the risk of developing CVD, including loss–of–function mutations in *PCSK9*, *ApoAI*, interleukin–10, adiponectin and many other genes [8].

Several *in vitro* and *in vivo* studies in the past decades suggested that hereditary deficiency of glucose–6–phosphate dehydrogenase (G6PD), an enzyme catalysing the first reaction in the pentose phosphate pathway (PPP) to produce NADPH, may be protective against CVD. These studies have been conducted mostly in the Sardinian population where the frequency of G6PD deficiency is one of the highest in the Mediterranean area, ranging between 12% and 24% [9–10]. Subjects with this condition may experience episodes of hemolysis after infections, assumption of a variety of drugs, or exposure to plants like *Vicia faba* [11]. In virtually all cases the underlying mutation is the *Mediterranean* mutation (a C→T transition at nucleotide 563 of the coding gene) [9–10]. An epidemiological study in 1998, in a cohort of 1756 Sardinian men with G6PD deficiency, reported a relatively lower standardized mortality ratio for CVD (0.28 versus the reference value of 1.0 for ischemic heart disease, and 0.22 versus 1.0 for cerebrovascular disease) [12]. A further Sardinian study found a 40% reduction of CVD risk associated to G6PD deficiency after adjustment for family history, hypertension, diabetes, and smoking [13]. Whereas this apparent protective effect of G6PD deficiency on cardiovascular risk was observed in earlier studies [12–14], more recent surveys seem instead to support an association with increased risk [15–17]. These divergent results may be partially attributed to the weak statistical power of early investigations and lack of randomization, which may have distorted the true relationship between G6PD deficiency and CVD. Therefore, a definitive conclusion on the impact of G6PD deficiency on the cardiovascular system cannot yet been drawn.

In this study we tested for potential association of G6PD deficiency with CVD risk in a large patient cohort from Northern Sardinia, by using propensity score (PS) matching and adjusting for conventional CVD risk factors.

2. Patients and methods

2.1. Study population

This was a single–center retrospective case–control study. Data were retrieved from a large digitalized database of patients undergoing digestive endoscopy from January 2002 through January 2017. Patients were referred to the endoscopy service (Department of Medical, Surgical and Experimental Medicine, University of Sassari, Italy) by general practitioners and/or specialists with different indications (as reported in full previously [18–21] mostly for dyspepsia, reflux symptoms, coeliac and liver disease and cancer follow–up).

2.2. Patients eligibility

All patients with a known G6PD status and full availability of all demographic and clinical data including age, sex, socioeconomic status (SES), smoking habits, body mass index (BMI), occurrence of CVD, blood pressure, presence of hypercholesterolemia and diabetes were considered eligible for the study. The clinical history was collected by a trained physician, and only diagnoses ascertained by the specialist were recorded in the database. Diagnoses of CVD, high blood pressure, hypercholesterolemia and type 1 and 2 diabetes were made by the specialists according to national and international guidelines progressively developed and usually followed in clinical practice. Self–reported CV diagnoses were not considered as study endpoints. The specific outcomes of interest were classified as: (i) nonfatal acute myocardial infarction, (ii) history of effort–induced angina or coronary revascularization, (iii) stenosis ≥70% of lower limb artery, (iv) ischemic stroke). Patients younger than 18 years, or who were not born to Sardinian parents, were excluded from the study. For patients with multiple digestive endoscopy procedures in the study interval, only the data from the last endoscopy were included.

2.3. G6PD-deficiency assessment

In all study participants the enzyme activity had been measured quantitatively using a biochemical assay based on G6PD/6GPD ratio in erythrocytes according to WHO recommendations [22]. Subjects were defined as deficient when the ratio was lower than 0.10 (G6PD, Nurex Diagnostici s.r.l. Sassari, ITALY).

2.4. Ethical approval

An Institutional Review Board approval was obtained from *Comitato di Bioetica, Azienda Ospedaliero-Universitaria di Sassari* (Prot N° 3004/CE, 2016).

2.5. Statistical analysis and propensity score matching

The results were expressed as mean and standard deviation (SD) for continuous variables, and as frequencies for categorical variables. Comparative analysis between normal and G6PD deficient subjects was performed by the χ^2 test for categorical variables and by the two–-sample Student's *t*-test for continuous variables.

Body mass index was calculated as weight/height^2 (kg/m^2) and treated as a continuous variable. For smoking habits, patients were stratified into never smokers and current or former smokers. Socioeconomic status was inferred from current or past occupation and recoded into two categories as previously reported [23]. Briefly, high–SES including graduated professionals, technicians and administrators (non–graduated); and low–SES including clerks and salesmen, semiskilled and unskilled workers, and uneducated shepherds and peasants. Since previous observations reported a lower frequency of G6PD deficiency in patients with malignancies compared with patients with normal G6PD enzyme activity [20,21], all patients diagnosed with cancer were excluded from the analysis.

In order to avoid the potential bias of covariates not evenly distributed between G6PD deficient and wild–type patients, a PS matching was performed using the open–source R software (http://www.r-project.org/). This procedure is frequently used in quasi-experimental studies, to generate an unbiased control group enabling the exploration of causal relationships using observational data [24]. In our study the PS was based on demographic variables (age, sex, and socioeconomic status), as well as on factors that are known to affect CVD risk (smoking, BMI, high blood pressure, hypercholesterolemia, diabetes mellitus). Using the *MatchIn* package installed in R, 1123 patients with G6PD deficiency were matched with 2246 patients with similar PS using G6PD as the grouping variable. A 1:2 greedy nearest neighbors matching method was used, within PS calipers of ± 0.2 SD [25]. Further, a post–matching conditional logistic regression analysis was conducted to check whether the distribution of potential confounding covariates was equal in both cases and controls. Finally, logistic regression analysis was performed with CVD classified in a binary manner (presence/absence) as the main dependent variable, and G6PD status as the main predictor variable; as PS was used to obtain information on confounding variables, it was included as a covariate in the regression model [26]. The regression coefficients β and their standard error (SE) of each covariate were calculated, as well as the ORs and their 95% CIs. The logistic regression analysis was performed using SPSS statistical software (version 16.0, Chicago, IL, USA). *P* values lower than 0.05 were considered statistically significant.

3. Results

A total of 9604 clinical records of patients (3558 men and 6046 women; mean age 51.31 ± 17.42 years) who underwent digestive endoscopy between 2002 and 2017 were collected (Table 1). All

Table 1
Demographic and clinical features of the study population according to glucose–6–phosphate dehydrogenase (G6PD) status.

Features	Cases (G6PD deficiency)	Controls (G6PD normal)	Total
No. patients	1123 (11.7%)	8481 (88.3%)	9604
Sex (M/F)	279/844	3279/5202	3558/6046
Age (mean ± SD, years)	51.16 ± 16.98	51.33 ± 17.48	51.31 ± 17.42
Civil status			
Single	295 (26.3%)	2502 (29.5%)	2797 (29.1%)
Married	702 (62.5%)	5046 (59.5%)	5748 (59.9%)
Widow	93 (8.3%)	651 (7.7%)	744 (7.7%)
Divorced	33 (2.9%)	282 (3.3%)	315 (3.3%)
Socioeconomic status			
Low	688 (61.3%)	4711 (55.5%)	5399 (56.2%)
High	435 (38.7%)	3770 (44.5%)	4205 (43.8%)
High blood pressure			
No	860 (76.6%)	6462 (76.2%)	7322 (76.2%)
Yes	263 (23.4%)	2019 (23.8%)	2282 (23.8%)
Smoking habits			
No	850 (75.7%)	6395 (75.4%)	7245 (75.4%)
Yes	273 (24.3%)	2086 (24.6%)	2359 (24.6%)
Body mass index (kg/m^2)			
< 25	556 (49.5%)	3962 (46.7%)	4518 (47.0%)
25–29	481 (42.8%)	3864 (45.6%)	4345 (45.2%)
≥30	86 (7.7%)	655 (7.7%)	741 (7.7%)
Diabetes mellitus			
No	1045 (93.1%)	7907 (93.2%)	8952 (93.2%)
Yes	78 (6.9%)	574 (6.8%)	652 (6.8%)
Hypercholesterolemia			
No	1018 (90.7%)	7669 (90.4%)	8687 (90.5%)
Yes	105 (9.3%)	812 (9.6%)	917 (9.5%)
Cardiovascular disease			
None	1071 (95.4%)	8157 (96.2%)	9228 (96.1%)
Nonfatal myocardial infarction	32 (2.9%)	229 (2.7%)	261 (2.7%)
Angina	8 (0.7%)	26 (0.3%)	34 (0.4%)
Stroke	6 (0.5%)	42 (0.5%)	48 (0.5%)
Peripheral artery disease	6 (0.5%)	27 (0.3%)	33 (0.3%)
Total CV disease	52 (4.6%)	324 (3.8%)	376 (3.9%)

patients were white from Northern Sardinia, Italy. In the studied cohort, the proportion of patients with G6PD deficiency (cases) was 11.7% (1123/9604) quite similar to the frequency previously reported in the general population of Northern Sardinia [9,10]. Patients with G6PD deficiency were more likely to report CVD than subjects with normal enzyme activity (4.6% *vs.* 3.8%) although the difference was not statistically significant (Table 1). Despite the small number of patients with CVD (376) a sub analysis was conducted according to the specific CV endpoint. No statistically significant differences were found in the frequency distribution of CV endpoints between cases and controls, although the frequency of angina and/or revascularization procedures was more than twice higher among G6PD deficient patients compared with controls (0.7% *vs.* 0.3).

The 1:2 PS matching procedure yielded 1123 G6PD deficient patients matched to 2246 controls with normal enzyme activity. G6PD deficiency showed a trend, statistically significant, for an increased frequency of CVD (4.6% *vs.* 2.8%; $p = 0.006$) (Table 2). The ORs and their 95%CI for G6PD deficiency and the conventional CVD risk factor, before and after PS matching, are shown in Table 3. As expected, major predictors of CVD were age (OR 1.07; 95%CI 1.06–1.08), male sex (1.63; 95%CI 1.29–2.06), high blood pressure (OR 1.46; 95%CI 1.16–1.84), smoking (OR 3.03; 95%CI 2.42–3.79), diabetes (OR 1.65; 95%CI 1.23–2.21) and hypercholesterolemia (OR 2.20; 95%CI 1.71–2.84). When G6PD deficiency was regressed on the probability of CVD, also including PS as a continuous variable, an OR of 1.71 (95%CI 1.17–2.49) was obtained.

4. Discussion

In this observational case–control study we detected a 71% greater

Table 2
Covariate distribution by glucose–6–phosphate dehydrogenase (G6PD) status in the overall population, PS–Matched population with 1:2 ratio.

	Unadjusted		Propensity score matched 1:2	
	G6PD deficient	G6PD normal	G6PD deficient	G6PD normal
No. patients	1123	8481	1123	2246
Mean age, y (SD)	51.2 (16.9)	51.3 (17.5)	51.3 (16.9)	51.1 (17.4)
Male, %	24.8	38.7	24.8	24.8
High socioeconomic status, %	38.7	44.5	38.7	38.7
Current or former smokers, %	24.3	24.6	24.3	24.4
Body mass index (kg/m^2)	25.1 (3.7)	25.4 (3.6)	25.1 (3.7)	25.1 (3.6)
High blood pressure, %	23.4	23.8	23.4	23.4
Hypercholesterolemia, %	9.3	9.6	9.3	9.3
Diabetes mellitus, %	6.9	6.8	6.9	6.9
Outcome cardiovascular disease, %	4.6	3.8	4.6	2.8**

**$p < 0.01$.

Table 3
Pre- and post-PS-matching logistic regression in patients with known G6PD status. The presence/absence of cardiovascular disease was the outcome variable.

	Pre–matching[a]		Propensity Score Matched[b] 1:2	
	OR[d] (95% CI[e])	*p*-value	OR[d] (95% CI[e])	*p*-value
Age (years)	1.07 (1.06–1.08)	< 0.0001	5.43 (3.35–8.80)	< 0.0001
Male sex	1.63 (1.29–2.06)	< 0.0001	1.62 (1.05–2.51)	0.031
High socioeconomic status	0.86 (0.68–1.08)	0.196	0.74 (0.47–1.15)	0.175
Current or former smokers	3.03 (2.42–3.79)	< 0.0001	2.83 (1.91–4.21)	< 0.0001
Body mass index (kg/m^2)	1.25 (0.90–1.73)	0.178	1.02 (0.54–1.90)	0.959
High blood pressure	1.46 (1.16–1.84)	0.001	1.84 (1.20–2.82)	0.005
Hypercholesterolemia	2.20 (1.71–2.84)	< 0.0001	1.97 (1.24–3.13)	0.004
Diabetes mellitus	1.65 (1.23–2.21)	0.001	1.33 (0.62–2.85)	0.460
G6PD deficiency	1.46 (1.06–2.01)	0.021	1.78 (1.20–2.64)	0.004

[a] Logistic regression with unmatched sample (n = 9604).
[b] Logistic regression with 1:2 matched sample (n = 3369).
[d] Odd Ratio.
[e] Confidence Interval.

risk of developing CVD in patients with G6PD-deficiency compared with controls, after adjusting for conventional risk factors. Our findings are apparently in contrast with the results of previous investigations in Sardinia which supported a significant protective effect of G6PD deficiency against CVD [12–14]. This discrepancy may be partly accounted for by different design and methods. For example, in the longitudinal study of Cocco et al. [12] the outcome was CVD mortality, and, the test used at that time to assess G6PD status, was the qualitative Beutler's fluorescent spot test [27] which may be less accurate than the quantitative test [28]. In the study of Meloni et al. the number of subjects aged 80 and older was relatively low, and a possible age unbalance occurred between cases and controls [13]. More importantly, those studies were not randomized, making difficult the balance among covariates between cases and controls. Our study was exempt from such limitations, since the PS matching provided a control group very similar to what may be achieved in a randomised controlled trial.

Our findings are consistent with results from a recent large–scale study performed in U.S. military medical centres which found 40% greater odds of developing CVD in G6PD-deficient individuals compared with individuals with normal enzyme levels [17]. Since in our study the risk was superior (71%) we speculate that this may be due to a genetic defect which entails lower levels of residual enzyme activity. In fact, in the Sardinian population more than 95% of G6PD deficient case are due to the so-called *Mediterranean* mutation (a C→T transition at nucleotide 563 of the coding gene) which causes severe (Class II) enzyme deficiency, whereas in the U.S. population of African descent the

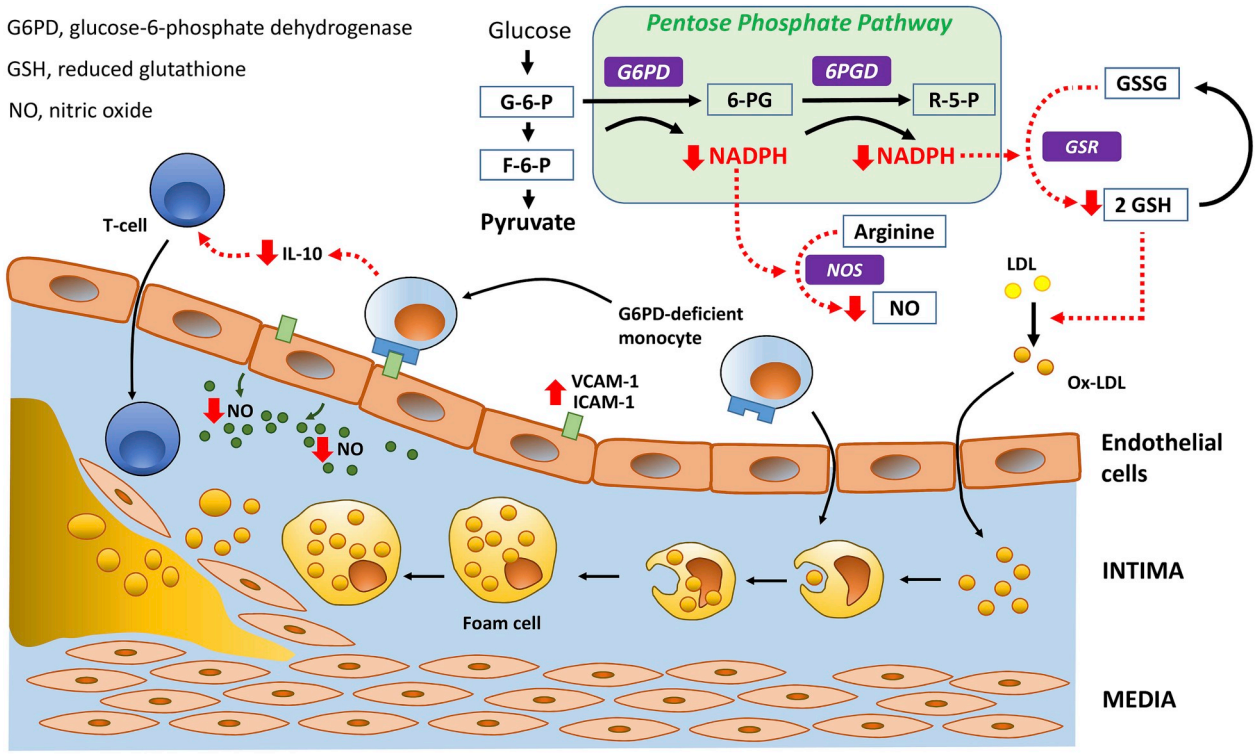

Fig. 1. Putative mechanisms involved in the atherogenic effect of G6PD deficiency.
The cartoon shows that intracellular NADPH depletion due to G6PD deficiency leads to a reduction of NO and GSH synthesis, and to a greater LDL oxidizability. Monocytes, through adhesion molecules such as ICAM-1 and VCAM-1, overexpressed by G6PD-deficient endothelial cells, enter the intima of the vessel differentiating into macrophages; this, in turn, results in IL-10 reduction. The shift toward pro–inflammatory state attracts T cells into the plaque area. G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; NOS, nitric oxide synthase; GSH, reduced glutathione; GSSG, glutathione disulfide; GSR, glutathione reductase; IL-10, interleukin 10; VCAM-1 and ICAM-1, adhesion molecules.

most frequent mutation is G6PD A– (mutations at nucleotides 202A/376G), whose phenotype is less severe (Class III) [9–11].

To explain our finding, a working hypothesis based on *in vitro* studies is that defective G6PD may activate *per se* several molecular mechanisms involved in the early stages of atherogenesis (Fig. 1). It was suggested that G6PD deficient cells cannot generate enough NADPH in the PPP to detoxify the excess of hydrogen peroxide and other oxygen reactive species [11], therefore, according to this hypothesis, impaired defence against oxidative stress induced by G6PD deficiency is the main responsible for the increased risk [29]. Depletion of cellular GSH increases the cytotoxicity of oxidized low-density lipoprotein to human monocytes and macrophages, a major factor in the development and progression of atheroma [30]. On the other hand, NADPH is also required for nitric oxide (NO) formation by NO synthase. This signalling molecule is a powerful vasodilator and a major regulator of vascular tone [31]: its synthesis depends upon the availability of NADPH produced by the G6PD activity. Thus, a depletion of cellular stores of NADPH induced by a defective G6PD gene may alter NO generation leading to endothelial disfunction via production of cytokines and other pro-inflammatory mediators [32] (Fig. 1). In addition, *in vitro* experiments in monocytes, where G6PD had been inactivated through siRNA-mediated RNA interference, showed increased levels of cell adhesion molecules (ICAM-1, VCAM-1, L–selectin, ITGB1 and 2), which play a major role in early stages of atheroma plaque formation [32]. Moreover, in contrast to the widely held belief, a blockade in NADPH production stimulates the activity of NADPH oxidase and in turn upregulation of reactive oxygen species and MCP-1 production, and decrease in GSH and NO levels [33]. This notion is further corroborated by experiment in rodents, where G6PD overexpression was conducive to life span extension through increased NADPH production and consequent shielding from the adverse effects of free radicals [34]. Moreover, an alteration of the cytokine network was documented in G6PD deficient cells of the immune system. *Ex vivo* monocytes from subjects with the *Mediterranean* form of G6PD deficiency have been shown to produce less interleukin–10 (IL-10) than non-deficient cells in response to phagocytic challenges [35], and a decreased IL-10 production was observed also in monocytes with G6PD A− upon lipopolysaccharide challenge [36]. Since IL–10 is a key mediator of inflammation and innate immunity [37], it may turn out that G6PD deficient individuals might have a cytokine balance skewed toward pro–inflammatory upregulation (Fig. 1). This conjecture is in line with recent studies in animal models and in humans [38,39]. Double knockout $ApoE^{-/-} \times IL\text{–}10^{-/-}$ mice show accelerated atheroma formation, a procoagulant state, enhanced thrombotic response and secretion of matrix metalloproteinases, which are potentially conducive to plaque surface erosion and rupture [38]. Also, in humans IL–10 is emerging as a protective and plaque-stabilizing mediator in atherosclerotic disorders [39]. Thus, decreased IL–10 production driven by G6PD deficiency may have pathogenetic implications in CVD and may contribute to accelerate its progression.

An interesting possibility that deserves further testing, is that G6PD deficiency may have a different impact, in some respects antithetical, in the early phases of atherogenesis and later, when ischaemic events have already arisen. More specifically, despite the greater susceptibility to develop atherosclerotic lesions and, hence, ischemic outcomes, G6PD deficient subjects may eventually experience a lower CVD mortality. This may be conjectured from findings obtained in animal models of protein aggregation cardiomyopathy crossed with G6PD deficient mice ($G6PD^{mut}$) which carry a mutation at the 5' untranslated sequence of the G6PD gene [16]. In these experiments G6PD enzyme seems to provide a potential causative mechanism for the initiation of myofibrils aggregation, thus it is tempting to infer that G6PD deficiency might prevent cardiac remodelling and ventricular dysfunction in coronary syndromes.

Our study may have a number of potential methodological weaknesses. An obvious limitation lies on its observational design, that we tried to counterbalance using PS matched analysis. Even doing so, the possibility of uncontrolled confounding remains. For example, CVD family history, a well-known risk factor for CVD, especially among younger patients, was not included in the analysis due to missing data. However only 16 patients out of 376 (4.2%) younger than 40 years experienced CVD. In addition, up to 10% of patients might have been beta–thalassemia carriers, whose G6PD activity above the threshold caused by reticulocytosis [40] could have falsely classified them as G6PD normal. However, due to the large size of our cohort it is unlikely that beta–thalassemia carriers were differently distributed among cases and controls. Furthermore, it can be argued against the use of a database of patients undergoing endoscopy. However, there is no *a priori* reason to think this may have introduced a selection bias. Proof of that is the similar frequency of G6PD-deficiency found in our study and that reported in the literature for the same population [12]. Finally, in our study, patients with cancer, where G6PD deficiency is rarer, have been excluded from analysis, contrary to most researches conducted previously, and the analysis covered all birth cohorts in the last century. Since our study population was not at high risk for CVD, and due to the multifactorial nature of atherosclerosis, we believe that the enrolment of patients with different cardiovascular risk profile might have modified the strength of association between G6PD deficiency and CVD.

Although the effect of G6PD deficiency is moderate compared with primary CVD risk factors, a better knowledge of this inherited, and hence unmodifiable, condition influencing CVD risk would result in more effective recommendations to prevent the premature onset of the disease in at–risk patients [41]. Longitudinal studies are needed to evaluate whether systematic determination of G6PD status in patients with CVD risk may eventually result in better prevention of adverse outcomes.

4.1. Conclusion

In the present study, a propensity score–matched analysis showed that a genetic defect in the G6PD gene is associated with an increased risk of CVD independently from other risk factors. This finding is consistent with *in vitro* and *in vivo* studies suggesting that G6PD enzyme activity is important for antioxidant defence not only in erythrocytes but also in nucleated cells.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

References

[1] G.A. Roth, C. Johnson, A. Abajobir, F. Abd-Allah, S.F. Abera, G. Abyu, et al., Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015, J. Am. Coll. Cardiol. 70 (2017) 1–25, https://doi.org/10.1016/j.jacc.2017.04.052.

[2] D. Mozaffarian, Global scourge of cardiovascular disease. Time for health care systems reform and precision population health, JACC (J. Am. Coll. Cardiol.) 70 (2017) 26–28, https://doi.org/10.1016/j.jacc.2017.05.007.

[3] A.N. Nowbar, J.P. Howard, J.A. Finegold, P. Asaria, D.P. Francis, 2014 global geographic analysis of mortality from ischaemic heart disease by country, age and income: statistics from World Health Organisation and United Nations, Int. J. Cardiol. 174 (2014) 293–298, https://doi.org/10.1016/j.ijcard.2014.04.096.

[4] S.A. Patel, M. Winkel, M.K. Ali, K.M. Narayan, N.K. Mehta, Cardiovascular mortality associated with 5 leading risk factors: national and state preventable fractions estimated from survey data, Ann. Intern. Med. 163 (2015) 245–253, https://doi.org/10.7326/M14-1753.

[5] D.M. Lloyd-Jones, B.H. Nam, R.B. D'Agostino Sr, D. Levy, J.M. Murabito, T.J. Wang, P.W. Wilson, C.J. O'Donnell, Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: a prospective study of parents and offspring, J. Am. Med. Assoc. 291 (2004) 2204–2211, https://doi.org/10.1001/jama.291.18.2204.

[6] S. Kathiresan, D. Srivastava, Genetics of human cardiovascular disease, Cell 148 (2012) 1242–1257, https://doi.org/10.1016/j.cell.2012.03.001.

[7] H. Schunkert, M. von Scheidt, T. Kessler, B. Stiller, L. Zeng, B. Vilne, Genetics of coronary artery disease in the light of genome-wide association studies, Clin. Res.

Cardiol. 107 (2018) 2–9, https://doi.org/10.1007/s00392-018-1324-1.
[8] K. Dai, S. Wiernek, J.P. Evans, M.S. Runge, Genetics of coronary artery disease and myocardial infarction, World J. Cardiol. 8 (2016) 1–23, https://doi.org/10.4330/wjc.v8.i1.1.
[9] G. De Vita, M. Alcalay, M. Sampietro, M.D. Cappelini, G. Fiorelli, D. Toniolo, Two point mutations are responsible for G6PD polymorphism in Sardinia, Am. J. Hum. Genet. 44 (1989) PMID: 2912069.
[10] G. Fiorelli, T. Meloni, V. Palomba, C. Manoussakis, S. Villa, M.D. Cappellini, Gene frequency of glucose–6–phosphate dehydrogenase (G6PD) polymorphic variants in Sardinia, Gene Geogr 4 (1990) 139–142 PMID: 2129615.
[11] L. Luzzatto, R. Notaro, Malaria. Protecting against bad air, Science 293 (2001) 442–443.
[12] P. Cocco, P. Todde, S. Fornera, M.B. Manca, P. Manca, A.R. Sias, Mortality in a cohort of men expressing the glucose-6-phosphate dehydrogenase deficiency, Blood 91 (1998) 706–709 PMID: 9427729.
[13] L. Meloni, M.R. Manca, I. Loddo, G. Cioglia, P. Cocco, A. Schwartz, S. Muntoni, S. Muntoni, Glucose-6-phosphate dehydrogenase deficiency protects against coronary heart disease, J. Inherit. Metab. Dis. 31 (2008) 412–417, https://doi.org/10.1007/s10545-008-0704-5.
[14] S. Muntoni, B. Batetta, S. Dessi, S. Muntoni, P. Pani, Serum lipoprotein profile in the Mediterranean variant of glucose-6-phosphate dehydrogenase deficiency, Eur. J. Epidemiol. 8 (Suppl 1) (1992) 48–53.
[15] P.A. Hecker, V. Lionetti, R.F. Ribeiro Jr., S. Rastogi, B.H. Brown, K.A. O'Connell, J.W. Cox, K.C. Shekar, D.M. Gamble, H.N. Sabbah, J.A. Leopold, S.A. Gupte, F.A. Recchia, W.C. Stanley, Glucose 6-phosphate dehydrogenase deficiency increases redox stress and moderately accelerates the development of heart failure, Circ. Heart. Fail. 6 (2013) 118–126, https://doi.org/10.1161/CIRCHEARTFAILURE.112.969576.
[16] P.A. Hecker, J.A. Leopold, S.A. Gupte, F.A. Recchia, W.C. Stanley, Impact of glucose-6-phosphate dehydrogenase deficiency on the pathophysiology of cardiovascular disease, Am. J. Physiol. Heart Circ. Physiol. 304 (2013) H491–H500, https://doi.org/10.1152/ajpheart.00721.2012.
[17] J.E. Thomas, S. Kang, C.J. Wyatt, F.S. Kim, A.D. Mangelsdorff, F.K. Weigel, Glucose-6-Phosphate dehydrogenase deficiency is associated with cardiovascular disease in U.S. Military centers, Tex. Heart Inst. J. 45 (2018) 144–150, https://doi.org/10.14503/THIJ-16-6052.
[18] G.M. Pes, A. Errigo, A. Bitti, M.P. Dore, Effect of age, period and birth-cohort on the frequency of glucose-6-phosphate dehydrogenase deficiency in Sardinian adults, Ann. Med. 50 (2018) 68–73, https://doi.org/10.1080/07853890.2017.1390247.
[19] M.P. Dore, G. Marras, C. Rocchi, S. Soro, G.M. Pes, G6PD deficiency does not enhance susceptibility for acquiring Helicobacter pylori infection in Sardinian patients, PLoS One 11 (2016) e0160032, https://doi.org/10.1371/journal.pone.0160032 PMID: 27467818.
[20] M.P. Dore, A. Davoli, N. Longo, G. Marras, G.M. Pes, Glucose-6-phosphate dehydrogenase deficiency and risk of colorectal cancer in Northern Sardinia: a retrospective observational study, Medicine (Baltim.) 95 (2016) e5254, https://doi.org/10.1097/MD.0000000000005254 PMID: 27858887.
[21] M.P. Dore, G. Vidili, G. Marras, S. Assy, G.M. Pes, Inverse association between glucose–6–phosphate dehydrogenase deficiency and hepatocellular carcinoma, Asian Pac. J. Cancer Prev. APJCP 19 (2018) 1069–1073, https://doi.org/10.22034/APJCP.2018.19.4.1069.
[22] A. Mosca, R. Paleari, E. Rosti, M. Luzzana, S. Barella, C. Sollaino, R. Galanello, Simultaneous automated determination of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in whole blood, Eur. J. Clin. Chem. Clin. Biochem. 34 (1996) 431–438 PMID: 8790979.
[23] G.M. Pes, A. Ganau, E. Tognotti, A. Errigo, C. Rocchi, M.P. Dore, The association of adult height with the risk of cardiovascular disease and cancer in the population of Sardinia, PLoS One 13 (2018) e0190888, https://doi.org/10.1371/journal.pone.0190888.
[24] P.R. Rosenbaum, D.B. Rubin, The central role of the propensity score in observational studies for causal effects, Biometrika 70 (1983) 41–55.
[25] J.J. Randolph, K. Falbe, A.K. Manuel, J.L. Balloun, L. Joseph, A step-by-step guide to propensity score matching in R. Practical assessment, Res. Eval. 19 (2014) 1–6.
[26] S. Guo, M.W. Fraser, Propensity Score Analysis: Statistical Methods and Applications, SAGE Publications Inc, 2014.
[27] E. Beutler, M. Mitchell, Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency, Blood 32 (1968) 816–8.
[28] L. Thielemans, G. Gornsawun, B. Hanboonkunupakarn, M.K. Paw, P. Porn, P.K. Moo, B. Van Overmeire, S. Proux, F. Nosten, R. McGready, V.I. Carrara, G. Bancone G, Diagnostic performances of the fluorescent spot test for G6PD deficiency in newborns along the Thailand-Myanmar border: a cohort study, Wellcome Open Res 3 (1) (2018) 3, https://doi.org/10.12688/wellcomeopenres.13373.1.
[29] M. Mari, A. Morales, A. Colell, C. Garcia-Ruiz, J.C. Fernandez-Checa, Mitochondrial glutathione, a key survival antioxidant, Antioxidants Redox Signal. 11 (2009) 2685–2700, https://doi.org/10.1089/ARS.2009.2695.
[30] N. Gotoh, A. Graham, E. Nikl, V.M. Darley-Usmar, Inhibition of glutathione synthesis increases the toxicity of oxidized low-density lipoprotein to human monocytes and macrophages, Biochem. J. 296 (Pt 1) (1993) 151–154.
[31] R.O. Cannon 3rd, Role of nitric oxide in cardiovascular disease: focus on the endothelium, Clin. Chem. 44 (1998) 1809–1819.
[32] J.A. Leopold, A. Cap, A.W. Scribner, R.C. Stanton, J. Loscalzo, Glucose-6-phosphate dehydrogenase deficiency promotes endothelial oxidant stress and decreases endothelial nitric oxide bioavailability, FASEB J. 15 (2001) 1771–1773.
[33] R. Parsanathan, S.K. Jain, L-Cysteine in vitro can restore cellular glutathione and inhibits the expression of cell adhesion molecules in G6PD-deficient monocytes, Amino Acids 50 (2018) 909–921, https://doi.org/10.1007/s00726-018-2559-x.
[34] S. Nóbrega-Pereira, P.J. Fernandez-Marcos, T. Brioche, M.C. Gomez-Cabrera, A. Salvador-Pascual, J.M. Flores, J. Viña, M. Serrano, G6PD protects from oxidative damage and improves healthspan in mice, Nat. Commun. 7 (2016) 10894, https://doi.org/10.1038/ncomms10894.
[35] B. Mordmuller, F. Turrini, H. Long, P.G. Kremsner, P. Arese, Neutrophils and monocytes from subjects with the Mediterranean G6PD variant: effect of Plasmodium falciparum hemozoin on G6PD activity, oxidative burst and cytokine production, Eur. Cytokine Netw. 9 (1998) 239–245.
[36] A.M. Liese, M.Q. Siddiqi, J.H. Siegel, E.A. Deitch, Z. Spolarics, Attenuated monocyte IL-10 production in glucose-6-phosphate dehydrogenase-deficient trauma patients, Shock 18 (2002) 18–23.
[37] A. Ledeboer, J.J. Brevé, S. Poole, F.J. Tilders, A.M. Van Dam, Interleukin-10, interleukin-4, and transforming growth factor-beta differentially regulate lipopolysaccharide-induced production of pro-inflammatory cytokines and nitric oxide in co-cultures of rat astroglial and microglial cells, Glia 30 (2000) 134–142.
[38] G. Caligiuri, M. Rudling, V. Ollivier, M.P. Jacob, J.B. Michel, G.K. Hansson, A. Nicoletti, Interleukin-10 deficiency increases atherosclerosis, thrombosis, and low-density lipoproteins in apolipoprotein E knockout mice, Mol. Med. 9 (2003) 10–17.
[39] B. Halvorsen, T. Waehre, H. Scholz, J.K. Damås, A. Yndestad, P. Aukrust, Role of interleukin-10 in atherogenesis and plaque stabilization, Future Cardiol. 2 (2006) 75–83.
[40] A. Minucci, B. Giardina, C. Zuppi, E. Capoluongo, Glucose-6-phosphate dehydrogenase laboratory assay: how, when, and why? IUBMB Life 61 (2009) 27–34, https://doi.org/10.1002/iub.137.
[41] M. Houston, The role of noninvasive cardiovascular testing, applied clinical nutrition and nutritional supplements in the prevention and treatment of coronary heart disease, Ther. Adv. Cardiovasc. Dis. 12 (2018) 85–108, https://doi.org/10.1177/1753944717743920.