Journal of Thrombosis and Haemostasis, **9**: 33–38 DOI: 10.1111/j.1538-7836.2010.04103.x

ORIGINAL ARTICLE

Plasma glutathione peroxidase in pediatric stroke families

U. NOWAK-GÖTTL,* B. FIEDLER,† A. HUGE,‡ T. NIEDERSTADT,§ S. THEDIECK,* T. SEEHAFER‡ and M. STOLL‡

**Department of Pediatric Hematology/Oncology; †Department of Pediatric Neurology, University Hospital of Münster; ‡Leibniz-Institute for Arteriosclerosis Research at the University Muenster, Genetic Epidemiology of Vascular Disorders; and §Institute of Clinical Radiology/Neuroradiology, University Hospital of Münster, Germany*

To cite this article: Nowak-Göttl U, Fiedler B, Huge A, Niederstadt T, Thedieck S, Seehafer T, Stoll M. Plasma glutathione peroxidase in pediatric stroke families. *J Thromb Haemost* 2011; **9**: 33–8.

Summary. *Background/objectives:* Promoter polymorphisms in the plasma glutathione peroxidase gene (*GPX3*), which encodes a major antioxidant enzyme implicated in post-translational modification of fibrinogen, have been implicated as risk factors for arterial ischemic stroke (AIS) and cerebral sinovenous thrombosis (CSVT) in young adults. However, the contribution of these polymorphisms could not be confirmed by other studies. *Patients/methods:* The aim of the present study was to investigate the association of three haplotype-tagging single-nucleotide polymorphisms (htSNPs) in *GPX3* in a large family-based study sample comprising 268 nuclear families with different pediatric AIS subtypes, i.e. arteriopathy stroke (AS) and thromboembolic stroke (TS). In addition, an independent study sample comprising 154 nuclear families of pediatric CSVT was investigated. Single-point and haplotype association was assessed with the transmission disequilibrium test implemented in HAPLOVIEW. *Results:* Single-point analysis revealed that the G allele of htSNP rs8177412 was significantly overtransmitted to affected AS children (T/U = 25 : 11, $\chi^2 = 5.54$, $P = 0.019$), but not to affected TS children (T/U = 49 : 40, $\chi^2 = 0.91$, $P = 0.34$). The corresponding GG haplotype (H2: frequency 0.18) was also significantly overtransmitted to AS children (T/U = 23 : 11, $\chi^2 = 4.28$, $P = 0.03$), but not to TS children or in children with CSVT. These results remained significant following 10 000 bootstrap permutations. Our findings indicate that genetic variants of *GPX3* are risk factors for AS, but not for thromboembolic AIS or CSVT, in children. *Conclusions:* Our results further highlight the need to analyze the contribution of genetic variants to pediatric AS, TS or CSVT separately, as these subcategories probably result from different combinations of risk-conferring and protective genetic variations.

Correspondence: Ulrike Nowak-Göttl, Department of Pediatric Hematology/Oncology, University Children's Hospital, University of Münster, Albert-Schweitzer-Street 33, 48149 Münster, Germany.
Tel.: +49 251 8347936; fax: +49 251 8347941.
E-mail: leagottl@uni-muenster.de

Received 29 June 2010, accepted 29 September 2010

Keywords: cerebral sinovenous thrombosis, *GPX3*, pediatric stroke.

Introduction

Stroke in children is a heterogeneous disorder associated with significant morbidity and mortality. The incidence of stroke in neonates and children, i.e. arterial ischemic stroke (AIS) or cerebral sinovenous thrombosis (CSVT), is estimated to be between 2.6 and 6.4 per 100 000 per year [1–4]. The most frequent reported risk factors for pediatric AIS or CSVT include underlying medical conditions such as cardiac disorders, metabolic diseases, cerebrovascular pathologies, and infectious diseases [2]. In addition, within the last decade, many genetic and acquired prothrombotic abnormalities have been evaluated in children with cerebrovascular disease, and have been found to be more common in children with AIS/CSVT than in healthy controls [3,5,6].

Glutathione peroxidase (GPX) is one of the primary antioxidant enzymes that scavenge hydrogen peroxide and organic hydroperoxides with glutathione as the hydrogen donor during normal metabolism or following oxidative insults. This selenium-dependent enzyme family was first described in 1957, and is predominantly found in renal proximal tubular cells and parietal epithelial cells of Bowman's capsule [7]. Because the GPX family decomposes hydrogen peroxide and lipid hydroperoxides, GPX prevents peroxide-induced DNA damage, lipid peroxidation, and protein degradation [8–10]. Plasma GPX (GPX3) is unique among the members of the GPX family, because it is the only extracellular isoform, and the gene has been mapped to the q32 region of chromosome 5 [11]. Kryukov *et al.* [9] showed that low plasma GPX3 activity correlates with reduced selenoprotein expression. In addition, lower GPX3 activity levels have been found in pediatric and young adult AIS families [12,13]. In 2004, a study identified a *GPX3* promoter polymorphism responsible for downregulation of gene transcription, resulting in decreased plasma GPX3 activity levels. One of the described promoter haplotypes, originating from the three most 5′ single-nucleotide polymorphisms (SNPs), –942A → C, –927T → C, and –861A → T, as well as –568T → C, –518T → C,

–302A → T, –287T → C, and –65T → C (H2: CCTCCTTC), was identified as an independent risk factor for AIS in the young cohorts investigated [14]. The same working group of Voetsch *et al.* [15] found a similar strong risk association with the onset of CSVT. As neither finding, i.e. the risk association of the H2 haplotype with AIS or CSVT, could be confirmed in two replication studies including three further populations [16,17], the present family-based cohort study investigating pediatric stroke subgroups was performed.

Patients and methods

The present study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki, and was approved by the medical ethics committee of the University of Münster, Germany.

In the German study center (Münster), plasma and DNA samples of 268 families of children with AIS (arteriopathy stroke [AS], $n = 64$; thromboembolic stroke [TS], $n = 204$) and 154 families of children with CSVT, i.e. samples of the propositus, non-affected brothers or sisters, and biological parents, were collected between January 2002 and September 2009 (Fig. 1). As described previously, patients and family members were routinely revisited as per study protocol in the pediatric–hematologic stroke department at least yearly for clinical and laboratory follow-up [5].

As recently described, AIS/CSVT types were confirmed by standard imaging methods, i.e. magnetic resonance imaging and computerized tomography or magnetic resonance venography/angiography [5,18]: all patients were investigated with vascular imaging. Stroke subtypes of the children enrolled were reclassified according to explicit predefined criteria based on the TOAST criteria modified for children, by substituting arteriopathy for large vessel atherosclerosis, by an independent blinded team of neuroradiologists [18–20]. As recently described in detail, the arteriopathy subgroup included children with intracranial large vessel stenosis (55%), spontaneous dissection associated with infections within the 3 months prior to onset of symptomatic AIS (30%), and vasculitis (10%); in 5% of children with arteriopathy, a combination of large vessel stenosis and vasculitis was diagnosed [18,20]. Childhood AIS not classified as arteriopathy, i.e. AIS of cardiac origin or AIS resulting from paradoxical embolism, including neonates with a patent foramen ovale ($n = 136$), was considered to be thromboembolic AIS [20]. Children with moyamoya arteriopathy, Ehlers–Danlos syndrome, Marfan syndrome or α_1-antitrypsin deficiency were not enrolled [18]. With written parental consent, term neonates, children with confirmed diagnosis of AS/TS/CSVT not older than 18 years at onset and available biological parents were enrolled. Premature births (≤ 36 gestational weeks) and patients older than 18 years at stroke onset were excluded. In addition, children with missing parents, families with non-paternity, children lost to follow-up and patients without parental consent were excluded. Inclusion and exclusion criteria are shown in Fig. 1.

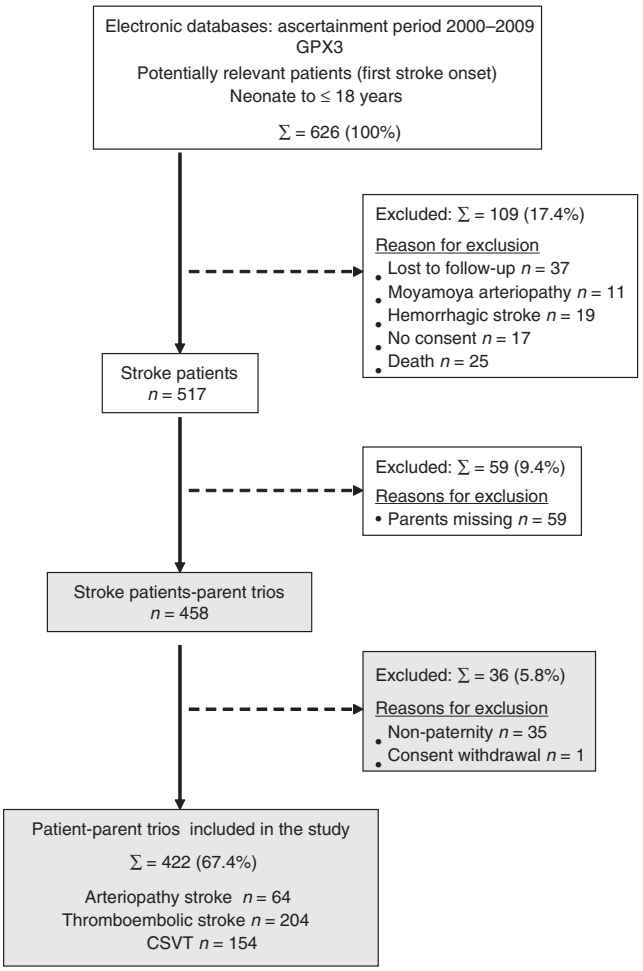

Fig. 1. Inclusion and exclusion criteria for patients enrolled in the study are shown (flow chart). CSVT, cerebral sinovenous thrombosis; GPX3, plasma glutathione peroxidase.

Blood sample collection and genetic analysis

Blood sample collection from patients and family members was performed 6–12 months after onset of stroke (index patient) in the morning after a 12-h fasting period (for infants, 4–6 h), and DNA extraction was performed with a spin column procedure (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

We identified three haplotype-tagging SNPs (htSNPs) capturing 97.5% of the genetic variation in *GPX3*, using the genotype information from Centre d'Etude du Polymorphisme Humain (CEPH) families (Utah residents with northern and western European ancestry: CEU) available from HAPMAP (http://www.hapmap.org) and the SNPtagger-tool as implemented in HAPLOVIEW [21–23]. These three htSNPs located in *GPX3* are rs8177412, rs870407, and rs870406. The htSNP rs8177412 is identical to one of the eight polymorphisms (rs1946234, rs1946235, rs1946236, rs8177404, rs8177406, rs8177409, rs6888961, and rs8177412) recently reported by Voetsch *et al.* and by Grond-Ginsbach *et al.* [14–17]. All of these 10 SNPs are in tight linkage disequilibrium (LD)

(D' between 0.97 and 1.0). Genotyping was performed with the TaqMan allelic discrimination method on a 384-well H7900 (Applied Biosystems, Foster City, CA, USA), using 2 ng of genomic DNA. For quality control, each plate contained eight positive CEPH controls and eight no-template controls, to ensure accuracy of genotyping. The genotyping efficiency was > 99.5%.

Statistical analysis

Hardy–Weinberg equilibrium for each htSNP was tested with chi-squared analysis across all samples. Family-based association was determined with the transmission disequilibrium test in the trios comprising unaffected parents and the affected child [24]; haplotypes were inferred with the Expectation Maximization Algorithm as implemented in HAPLOVIEW (v. 4.2; release 27, phase III) [24]. Significance of association was assessed with a Pearson chi-squared test, and empirical P-values were determined with 10 000 bootstrap permutations for single SNPs and haplotypes, accepting a failure detection rate of 10% as implemented in HAPLOVIEW [23,24].

Further analyses were performed on the AIS index cohorts. Carrier rates of the G allele (rs8177412) were calculated across AIS subtypes, including neonatal patients ($n = 136$) and non-neonatal patients ($n = 68$) with TS. These index subgroups were compared by chi-squared analysis. In a second step, multivariate analysis (logistic regression) adjusted for fibrinogen concentration (increase per g L^{-1}), established thrombophilic risk factors (factor V G1691A, FII G20210A, elevated lipoprotein[a], protein C, protein S and antithrombin deficiencies: presence vs. absence) and age (months) at AIS onset was performed to compare the presence of the G allele (rs8177412) in children with AS with that in children with thromboembolic AIS. Odds ratios (ORs) and 95% confidence intervals (CIs) are given.

Results

Characteristics of patient–parent trios are depicted in Table 1. Gender distribution, presence of thrombophilic genetic traits, smoking habits and use of oral contraceptives or hormone replacement therapy with respect to stroke subtypes AS/TS vs. CSVT are shown. At the time of investigation, 5.8% of parents with a median age of 37 years had also suffered from cardiovascular events, i.e. stroke ($n = 4$), deep vein thrombosis ($n = 37$), or myocardial infarction ($n = 8$).

Distribution of htSNPs in the cohorts investigated

The distribution of the three htSNPs is shown in Table 2. Higher, but not statistically significant, differences were found for the htSNP distribution between AS, TS and trios with CSVT with respect to observed/predicted heterozygosity, non-missing genotypes, and minor allele frequencies.

Single-point association between GPX3 htSNPs and AIS subtypes/CSVT

Single-point analysis revealed that the G allele of htSNP rs8177412 was significantly overtransmitted to affected AS children (transmitted versus untransmitted allele (T/U) = 25 : 11, $\chi^2 = 5.44$, $P = 0.019$), but not to affected TS patients (T/U = 49:40, $\chi^2 = 0.91$, $P = 0.34$).

Subgroup analysis in AIS index cohorts

The G allele carrier rates were 43.8% in patients with AS (28 of 64) and 23% in children with TS (47 of 204), with no significant difference between neonatal and non-neonatal TS patients (neonates: 34 of 136 [25%]; non-neonates, 13 of 68 [19%]; $P = 0.44$). In multivariate analysis adjusted for fibrinogen, established thrombophilic risk factors, and age at first stroke onset, children with AS and the G allele (rs8177412) showed an increased OR as compared with those with TS and the G allele (OR 2.97, CI 1.42–6.19). Furthermore, multivariate analysis revealed that the risk of suffering from AIS of vascular origin was elevated with increasing age (OR 1.26, CI 1.17–1.37). In contrast, neither the gradual increase in fibrinogen (OR 1.003,

Table 1 Characteristics of patient–parent trios

Characteristics	AS/TS children ($n = 268$)	CSVT children ($n = 154$)	Parents ($n = 844$)
Disease/health status			
Stroke/VT/MI	268/–/–	–/154/–	4/37/8
Age at blood collection (years), median (min.–max.)	4 (0.1–18)	6 (0.1–18)	37 (17–64)
Gender (male), no. (%)	148 (55)	82 (53)	422 (50)
BMI (kg m^{-2})	16.4 (11.2–30.6)	17.5 (7.2–30.7)	24.6 (17.7–46.9)
Risk factors			
FVG1691A, no. (%)	32 (11.9)	27 (17.5)	59 (7.0)
FII G20210A, no. (%)	16 (6.0)	12 (7.8)	28 (3.3)
Antithrombin/protein C/protein S deficiency/APS, No.	0/2/0/8	4/2/4/5	3/3/4/6
Lp(a) > 30 mg dL^{-1}, no. (%)	83 (30.9)	53 (34.4)	136 (16.1)
Smoking under age 12 years, no. (%)	4 (1.5)	8 (5.2)	129 (15.3)
Use of oral contraceptives/HRT (> tanner 2), no. (%)	3 (1.1)	14 (9.0)	52 (6.2)

APS, antiphospholipid syndrome; AS, arteriopathy stroke; BMI, body mass index (mass/height^2); CSVT, cerebral sinovenous thrombosis; F, factor; HRT, hormone replacement therapy; Lp(a), lipoprotein(a); MI, myocardial infarction; TS, thromboembolic stroke; VT, venous thrombosis.

Table 2 Distribution of haplotype-tagging single-nucleotide polymorphisms (htSNPs) in pediatric trios with stroke (arteriopathy stroke [AS], 64; thromboembolic stroke [TS], 204) and cerebral sinovenous thrombosis (CSVT) (n = 154)

htSNP ID no. Position	rs8177412 150380280	rs870407 150380780	rs870406 150380794	χ^2 *P*-value
Observed heterozygosity				
AS	0.35	0.30	0.30	0.97
TS	0.24	0.26	0.23	
CSVT	0.25	0.26	0.24	
Predicted heterozygosity				
AS	0.31	0.31	0.28	0.99
TS	0.24	0.26	0.23	
CSVT	0.23	0.25	0.23	
Non-missing genotype				
AS	97.2	98.8	100	0.99
TS	98.9	98.8	99.9	
CSVT	99.3	100	99.9	
Minor allele frequency				
AS	0.19	0.19	0.17	99.9
TS	0.14	0.15	0.13	
CSVT	0.14	0.15	0.13	

CI 0.99–1.008) nor the presence of established thrombophilic risk factors (OR 0.49, CI 0.17–1.36) was associated with AIS of arteriopathic origin.

In an independent study sample comprising 154 nuclear families with pediatric CSVT, none of the *GPX3* htSNPs was associated with thrombosis. In addition, the A allele of htSNP rs870406 was overtransmitted to affected children with AS (T/U = 23 : 11, χ^2 = 4.23, P = 0.039).

Association between GPX3 haplotypes and stroke subtypes/CSVT

Rs8177412, rs870407 and rs870406 are in tight LD (D' between 0.91 and 1.0), and the combination of rs8177412 and rs970407 defined four haplotypes, with HT1 and HT2 capturing at least 96% of the genetic variation for this single LD block of the entire *GPX3* gene (not extending into a neighboring gene), whereas HT3 and HT4 are rare (each < 2%) (Table 3). It is of note that rs870406 was redundant, and was thus left out of the haplotype analysis. Whereas the corresponding GG haplotype (frequency 0.18) was significantly overtransmitted in AS (T/U = 23 : 11, χ^2 = 4.28, P = 0.03), no such association was found in children with TS or CSVT. The AA haplotype (frequency 0.79) was significantly undertransmitted (T/U = 12 : 26, χ^2 = 5.1, P = 0.024) in children with AS. All results remained significant following 10 000 bootstrap permutations.

Discussion

In association studies, the definition of haplotype blocks of SNPs as markers has been proposed to efficiently describe human genetic variation [25]. In addition, it has been proposed that one can distinguish between block haplotypes with a minimal set of htSNPs, which would then efficiently describe the variation in the human genome through genotyping of only a subset of SNP loci [26]. In the present candidate gene association study, comprising a cohort of white German pediatric AIS subtypes/CSVT patients and their biological parents (nuclear families), we have used the latter approach. In the present cohort, we found that the *GPX3* H2 haplotype is a genetic marker for arteriopathy AIS in children, but not for TS or CSVT, and also that the H1-AA haplotype may be protective in children with AS.

Platelet activation and aggregation play an important role in the pathogenesis of AIS. In 1996, it was shown by Freedman *et al.* [12] that plasma from two brothers with stroke could inactivate the antiplatelet effects of nitric oxide (NO), and that this effect was caused by decreased levels of plasma GPX3. NO, which was originally identified as a vasodilator produced of the endothelium. Although NO is also present in platelets, limited effects upon platelet activation, adhesion and aggregation were found. On the basis of the finding that the platelet-derived NO pool has the ability to limit recruitment of platelets to the platelet-rich thrombus, NO insufficiency has been proposed to promote arterial thrombosis [27]. Thus, distinct from their influence on post-translational modification of fibrinogen, polymorphisms in *GPX3* associated with plasma GPX3 deficiency also reduce the vascular bioavailability of NO, and, in turn, result in a prothrombotic state. Interestingly, however, in our cohort of patients, the fibrinogen concentration did not independently influence the increased risk of the

Table 3 Association between *GPX3* haplotypes and pediatric stroke subtypes/cerebral sinovenous thrombosis (CSVT)

Stroke subgroups (no. of trios)	Block	Haplotype	Frequency	T/U	χ^2	*P*-value
AS (n = 64)	HT1	AA	0.790	12 : 26	5.10	0.0239
	HT2	GG	0.176	23 : 11	4.28	0.0385
	HT3	GA	0.017	4 : 0	3.86	0.0493
	HT4	AG	0.017	0 : 2.1	2.09	0.1477
TS (n = 204)	HT1	AA	0.83	53 : 53	0	0.9948
	HT2	GG	0.127	45 : 47	0.043	0.8363
	HT3	GA	0.020	10 : 7.1	0.504	0.4778
	HT4	AG	0.001	0 : 1	1.017	0.3133
CSVT (n = 154)	HT1	AA	0.862	33 : 43	1.31	0.2517
	HT2	GG	0.127	40 : 29	1.76	0.1854

AS, arteriopathy stroke; TS, thromboembolic stroke.

htSNP rs8177412 G allele in children with AS. The fact that this postulated underlying pathophysiology of the htSNP rs8177412 G risk allele and the *GPX3* H2 haplotype produce a prothrombotic state via activation of platelet adhesion and aggregation in the AS group, but not in the TS group, underlines the current knowledge that the molecular mechanisms in thromboses of arterial origin (platelet-rich thrombi) and venous origin (fibrin-rich thrombi including a smaller amount of aggregated platelets: [28]) are quite different.

In line with the concept of underlying genetics and heritability in familial AIS and deep vein thrombosis [29,30], the finding of Freedman *et al*. [13] was confirmed in a larger Israeli family study, in which at least a second family member had suffered a stroke episode. Three probands and their affected parents of four extensively studied families showed a 50% decrease in plasma GPX3 activity. The association between decreased GPX3 plasma levels and decreased GPX3 transcription/expression was determined separately in an in situ anephric mouse model in 1994 [31], and was additionally confirmed in patients with renal dysfunction [32,33].

Since then, contradictory data have been reported for *GPX3* haplotypes associated with AIS and CSVT. On one hand, Voetsch *et al*. [14] reported that the H2 promoter haplotype was more frequently associated in: (i) 82 young adults and children with stroke; and (ii) in 23 young adult patients with CSVT. The highest risk in this cohort was found in women with hormonal risk factors, i.e. the use of oral contraceptives and pregnancy [15]. On the other hand, two replication studies enrolling 318 patients and 374 controls with different adult AIS subtypes from Germany and Italy, and a further investigation in a cohort of 79 adults with non-septic CSVT, could not confirm the aforementioned risk association with the H2 haplotype [16,17]. Our findings obtained here in children with AIS are in line with results obtained from young adults and children, suggesting that the H2 promoter haplotype exhibits a significant risk association with AS onset [14]. The negative association with TS, however, confirmed the results from the adult Heidelberg–Brescia stroke cohort [16]. Finally, we confirmed the data reported in the adult German non-septic CSVT population, as the H2 haplotype did not contribute to the risk of CSVT in white German children [17]. The reported absence of an association between GPX3 and stroke or CSVT, however, may be explained by phenotypic heterogeneity and differences in age demographics.

There are some limitations in the present family-based candidate gene study. First, the study included only white children with AIS or CSVT and their families. Thus, it is unknown whether our findings can be extrapolated to other ethnic groups. Apart from phenotypic heterogeneity and differences in age demographics, the former may also serve as an explanation for the different results reported previously on this topic. Second, because of the small number of patients with AS, we were unable to divide the patients into more subgroups as suggested by the TOAST classification. Thus, to further evaluate the role of GPX3 in children and young adults with AS, multicenter international cooperative projects are mandatory to analyze the direct association between *GPX3* risk SNPs/haplotypes and the modified TOAST subtypes. Third, in the patients and their family members investigated, we did not evaluate plasma GPX3 activity levels. Therefore, the aforementioned genotype–phenotype interaction between the risk haplotype investigated and plasma levels could not be reported.

In conclusion, while keeping in mind the limitations of the study, the data presented here highlight the complex nature of pediatric AIS/CSVT subtypes and the importance of evaluating gene–gene interactions in association studies of complex, polygenic diseases. To better understand the broad spectrum of AIS subtypes in children, possible contributory genetic variants should be investigated separately, as these subcategories are probably caused by different combinations of risk-conferring and protective genetic variants. Further research is necessary to include genetic risk profiles into a set of clinical targets for possible therapeutic interventions, also including prevention and treatment trials of AIS in children.

Addendum

All investigators had full access to the data and took part in the design, execution, data analysis, and writing of the report. M. Stoll and U. Nowak-Göttl were responsible for the statistical analysis.

Acknowledgements

We wish to acknowledge G. Kenet for helpful discussions and help in editing this manuscript.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

References

1 Earley CJ, Kittner SJ, Feeser BR, Gardner J, Epstein A, Wozniak MA, Wityk R, Stern BJ, Price TR, Macko RF, Johnson C, Sloan MA, Buchholz D. Stroke in children and sickle-cell disease: Baltimore-Washington-Cooperative Young Stroke Study. *Neurology* 1998; **51**: 169–76.

2 Kirkham FJ, Prengler M, Hewes DK, Ganesan V. Risk factors for arterial ischemic stroke in children. *J Child Neurol* 2000; **15**: 299–307.

3 Haywood S, Liesner R, Pindora S, Ganesan V. Thrombophilia and first arterial ischaemic stroke: a systematic review. *Arch Dis Child* 2005; **90**: 402–5.

4 DeVeber G, Andrew M, Adams C, Bjornson B, Booth F, Buckley DJ, Camfield CS, David M, Humphreys P, Langevin P, MacDonald EA, Gillett J, Meaney B, Shevell M, Sinclair DB, Yager J, Canadian Pediatric Ischemic Stroke Study Group. Cerebral sinovenous thrombosis in children. *N Engl J Med* 2001; **345**: 417–23.

5 Kenet G, Kirkham F, Niederstadt T, Heinecke A, Saunders D, Stoll M, Brenner B, Bidlingmaier C, Heller C, Knöfler R, Schobess R, Zieger B, Sébire G, Nowak-Göttl U, European Thromboses Study Group. Risk factors for recurrent venous thromboembolism in the European collaborative paediatric database on cerebral venous thrombosis: a multicentre cohort study. *Lancet Neurol* 2007; **6**: 595–603.

6 Kenet G, Lütkhoff LK, Albisetti M, Bernard TJ, Bonduel M, Brandao LR, Chabrier S, Chan A, Fiedler B, Fullerton HJ, Ganesan V, Goldenberg NA, Golomb M, Grabowski EF, Heller C, Holzhauer S, Iorio A, Journeycake J, Kirkham FJ, Kurnik K, *et al*. Impact of thrombophilia on arterial ischemic stroke or cerebral venous sinus thromboses in children: a systematic review & meta-analysis of observational studies. *Circulation* 2010; **121**: 1838–47.

7 Mills GC. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J Biol Chem* 1957; **229**: 189–97.

8 Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med* 1990; **8**: 583–95.

9 Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. *Science* 2003; **300**: 1439–43.

10 Bierl C, Voetsch B, Jin RC, Handy DE, Loscalzo J. Determinants of human plasma glutathione peroxidase (GPx-3) expression. *J Biol Chem* 2004; **279**: 26839–45.

11 Yoshimura S, Suemizu H, Taniguchi Y, Arimori K, Kawabe N, Moriuchi T. The human plasma glutathione peroxidase-encoding gene: organization, sequence and localization to chromosome 5q32. *Gene* 1994; **145**: 293–7.

12 Freedman JE, Loscalzo J, Benoit SE, Valeri CR, Barnard MR, Michelson AD. Decreased platelet inhibition by nitric oxide in two brothers with a history of arterial thrombosis. *J Clin Invest* 1996; **97**: 979–87.

13 Kenet G, Freedman J, Shenkman B, Regina E, Brok-Simoni F, Holzman F, Vavva F, Brand N, Michelson A, Trolliet M, Loscalzo J, Inbal A. Plasma glutathione peroxidase deficiency and platelet insensitivity to nitric oxide in children. *Arterioscler Thromb Vasc Biol* 1999; **19**: 2017–23.

14 Voetsch B, Jin RC, Bierl C, Benke KS, Kenet G, Simioni P, Ottaviano F, Damasceno BP, Annichino-Bizacchi JM, Handy DE, Loscalzo J. Promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene. A novel risk factor for arterial ischemic stroke among young adults and children. *Stroke* 2007; **38**: 41–9.

15 Voetsch B, Jin RC, Bierl C, Deus-Silva L, Camargo ECS, Annichino-Bizacchi JM, Handy DE, Loscalzo J. Role of promoter polymorphisms in the plasma glutathione peroxidase (GPx-3) gene as a risk factor for cerebral venous thrombosis. *Stroke* 2008; **39**: 303–7.

16 Grond-Ginsbach C, Lichy C, Padovanti A, Pezzini A. GPx-3 gene promoter variation and the risk of arterial ischemic stroke. *Stroke* 2007; **38**: e23.

17 Grond-Ginsbach C, Arnold ML, Lichy C, Grau A, Renner K. No association of the plasma glutathione peroxidase (GPx-3) gene with cerebral venous thrombosis in the German population. *Stroke* 2009; **40**: e34.

18 Bernard TJ, Goldenberg NA, Tripputi M, Manco-Johnson MJ, Niederstadt T, Nowak-Göttl U. Anticoagulation in childhood-onset arterial ischemic stroke with nonmoyamoya arteriopathy. *Stroke* 2009; **40**: 2869–71.

19 Williams LS, Garg BP, Cohen M, Fleck JD, Biller J. Subtypes of ischemic stroke in children and young adults. *Neurology* 1997; **49**: 1541–5.

20 Kirkham F, Sebire G, Steinlin M, Sträter R. Arterial ischaemic stroke in children. Review of the literature and strategies for future stroke studies. *Thromb Haemost* 2004; **92**: 697–706.

21 Zhang W, Collins A, Morton NE. Does haplotype diversity predict power for association mapping of disease susceptibility? *Hum Genet* 2004; **115**: 157–64.

22 Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**: 506–16.

23 deBakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005; **37**: 1217–23.

24 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–5.

25 Goldstein DB. Islands of linkage disequilibrium. *Nat Genet* 2001; **29**: 109–11.

26 Johnson GCL, Esposito L, Barrett BJ, Amith AN, Heward J, DiGenova DG, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, *et al*. Haplotype tagging for the identification of common disease genes. *Nat Genet* 2001; **29**: 233–7.

27 Loscalzo J. Nitric oxide insufficiency, platelet activation, and arterial thrombosis. *Circ Res* 2001; **88**: 756–62.

28 Bockenstedt P. Laboratory methods in hemostasis. In: Loscalzo J, Schafer AI, eds. *Thrombosis and Hemorrhage*, 2nd edn. Baltimore: Williams & Wilkins, 1998: 405–18.

29 Souto JC, Almasy L, Borrell M, Garí M, Martínez E, Mateo J, Stone WH, Blangero J, Fontcuberta J. Genetic determinants of hemostasis phenotypes in Spanish families. *Circulation* 2000; **101**: 1546–51.

30 Nowak-Göttl U, Langer C, Bergs S, Thedieck S, Sträter R, Stoll M. Genetics of hemostasis. Differential effects of heritability and household components influencing lipid concentrations and clotting factor levels in 282 pediatric stroke families. *Environ Health Perspect* 2008; **116**: 839–43.

31 Avissar N, Ornt DB, Yagil A, Horowitz S, Watkins RH, Kerl EA, Takahashi K, Palmer IS, Cohen HJ. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. *Am J Physiol* 1994; **266**: C367–75.

32 Yoshimura S, Suemizu H, Nomoto H, Sakai H, Katasuoka Y, Kawamura N, Moriuchi T. Plasma glutathione peroxidase deficiency caused by renal dysfunction. *Nephron* 1996; **73**: 207–11.

33 El-Far MA, Bakr MA, Farahat SE, Abd El-Fattah EA. Glutathione peroxidase activity in patients with renal disorders. *Clin Exp Nephrol* 2005; **9**: 127–31.