ORIGINAL ARTICLE

Thrombophilia and first arterial ischaemic stroke: a systematic review

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Aims: To undertake a systematic review of the literature reporting the prevalence of thrombophilia in children with a first arterial ischaemic stroke (AIS).

Methods: Systematic review of case-control studies reporting data for prevalence of protein C, S, and antithrombin (AT) deficiencies, activated protein C resistance (APCr), total plasma homocysteine >95th centile, the thrombophilic mutations factor V1691 GA, prothrombin 20210GA, and MTHFR C677T in children with first, radiologically confirmed, AIS.

Results: Of 1437 potentially relevant citations, 18 met inclusion criteria. A total of 3235 patients and 9019 controls had been studied. Results of meta-analyses were expressed as pooled odds ratios (OR) relating the prevalence of the thrombophilic condition in children with AIS to that in controls. The pooled OR (and 95% CI) were: protein C deficiency, 6.49 (2.96 to 14.27); protein S deficiency, 1.14 (0.34 to 3.80); AT deficiency, 1.02 (0.28 to 3.67); APCr, 1.34 (0.16 to 11.52); FV1691 GA, 1.22 (0.80 to 1.87); PT20210GA, 1.10 (0.51 to 2.34); MTHFR C677T, 1.70 (1.23 to 2.34); and total plasma homocysteine >95th centile, 1.36 (0.53 to 3.51). There was no statistical heterogeneity within these data.

Conclusions: All factors examined were more common in children with first AIS than in controls, and significantly so for protein C deficiency and the MTHFR C677T mutation. The implications of thrombophilia for prognosis and recurrence need to be established before clinical recommendations can be made regarding investigation and treatment of children with AIS.

A rterial ischaemic stroke (AIS) affects 2.7/100 000 children annually,[1] with recurrence in at least 10% of cases. Multiple risk factors commonly coexist in individual patients.[2] Studies investigating whether thrombophilia is associated with first AIS in childhood have produced conflicting results and have been confounded by small patient numbers, lack of controls, and technical variability.[3–5] A systematic review could provide a more conclusive answer to this question,[6] which is clinically important, as anticoagulation could be considered for both acute and prophylactic treatment.

METHODS

We aimed to identify all case-control studies investigating the prevalence of any of the following thrombophilic conditions in children with a first AIS: protein C, protein S, antithrombin (AT) deficiencies, activated protein C resistance (APCr), the thrombophilic mutations factor V Leiden (FVL), thermolabile methylene tetrahydrofolate reductase (MTHFR C677T), prothrombin G20210A (PT20210), and raised total plasma homocysteine (>95th centile). The following inclusion and exclusion criteria were used: (1) the diagnosis of AIS had to be confirmed on brain imaging; (2) cases of recurrent AIS were excluded due to the potential for introducing bias; (3) only patients aged more than 28 days old and less than 18 years at the time of AIS were included; (4) laboratory tests had to have been undertaken in the same way for cases and contemporaneous age matched controls; and (5) studies where the majority of data were from patients with AIS secondary to sickle cell disease and moyamoya syndrome were excluded due to the specific pathophysiological factors encountered in these patients.

Searches were undertaken in electronic bibliographic databases (Medline (1966 to March 2002), Premedline,

Cochrane Database of Systematic Reviews in the Cochrane Library, Registered Titles (protocols under development), Embase (Excerpta Medica) (1980 to March 2002), Healthstar, Scisearch (Science Citation Index), NRR (National Research Register), BLDSC (British Library Document Supply Centre), EDINA (includes Periodical Contents Index, Scottish Academic Libraries, SERials (SALSER)), reference lists from primary and review articles, and research registers (Cochrane Stroke Group). Additional data were sought from the grey literature (thesis,[7] unpublished/prepublished data, conference proceedings, SIGLE (system for information on grey literature) and the internet (using listed search strategies; see *ADC* website, fig A)). Authors of original studies and known experts in the field were contacted and the Cochrane Stroke Group website was searched for published reviews and published protocols. The searches included all languages.

Studies were assessed for inclusion by two reviewers (SH and VG). Authors were contacted if further information was required. The third reviewer (RL), a paediatric haematologist, arbitrated if there was disagreement between the first two reviewers, and also reviewed the laboratory methods in detail. Specifically, as the normal range for protein C and S and antithrombin may very between laboratories, she confirmed that the studies including these factors were comparable. If a paper contained data that may have been duplicated in several studies, the authors were contacted and the most methodologically satisfactory study was included. The relevant data from all included studies was extracted and tabulated.

The summary statistic used was the odds ratio (OR) with 95% confidence intervals (CI). The data were combined using

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Abbreviations: AIS, arterial ischaemic stroke; APCr, activated protein C resistance; AT, antithrombin; FVL, factor V Leiden

a fixed effects meta-analysis model using the Cochrane Group RevMan program. The fixed effect model was used as little heterogeneity was expected in the results. The χ^2 test with $k-1$ degrees of freedom was applied to identify heterogeneity. Where meta-analysis was not possible with RevMan (that is, where a zero value was recorded in the AIS groups), the data were reanalysed using the statistical package, Intercooled Stata 7.0.

RESULTS

Of the 214 papers identified, 211 full text articles were obtained and reviewed, including 17 foreign language papers (see *ADC* website, fig B).

Qualitative assessment of studies

Authors of 46 papers were contacted for further information. Authors of 16 papers provided all the information requested and authors of six of the papers provided some information. Several authors were contacted to distinguish adult and child data,[8–10] and to confirm which tests were performed,[11] their results,[8] and control selection methodology. All the laboratory assays were technically valid. To exclude acquired protein deficiencies due to consumption, measurement of protein C, S, and AT has to be carried out beyond the acute period. Most studies did this[5 9 10 12–14] (and personal correspondence[15–16]), but the time interval was not clear in some.[5 17] Some studies specified homozygosity or heterozygosity for the thrombophilic mutations, but the numbers of each were too small to enable separate analysis. In the case of the MTHFR C677T mutation, the majority were homozygous or not specified.

All but one study was performed in a tertiary centre.[13] The three main groups of patients were from Turkey,[16 18] Germany,[11 15] and London.[4] Overall, eight studies listed exclusion criteria. The most common factor leading to exclusion from this review was lack of matched controls. Some studies (particularly those reporting on homocysteine levels) used published reference values. Most were excluded from the meta-analysis as these controls were not formally matched for age and were often derived from a different time and population.[19–21] The only studies included applied the same exclusion criteria for the controls as the study group.[22 23] Some authors described controls briefly, but said little about

matching[8–10 13 14 16 24] or included controls only for the children undergoing genetic analysis.[4 23] Five papers described the matching of the study groups with controls of the same ethnic group (Caucasian[11 15 20 23] and Indian[9 10]). Sträter *et al* described the children in their study group as white and as having been matched with children from the same geographic area.[24] Prengler *et al* described the ethnic mix of their group but were unable to match with controls.[25] The other papers made reference to nationality or geography but not to the ethnicity of the patients or controls. There was a variety of information given regarding family history, other aetiology of AIS, and combination of thrombophilia factors, but none of this data was could be explored in the quantitative analysis due to lack of detail.

Quantitative analysis

Eighteen studies were included.[4 5 8–18 20 22–25] These included data from 3235 patients and 9019 controls. The pooled OR and 95% CI are summarised in table 1 (see *ADC* website). In all the conditions, there were positive odds ratios, consistent with an increased frequency of the thrombophilic factors examined in children with first AIS. However, with the exception of protein C deficiency (see fig A) and the MTHFR C677T mutation, the confidence intervals crossed 1, reflecting the small sample size for most of the conditions examined. Thus, analysis of the pooled data established an increased prevalence of protein C deficiency and the MTHFR C677T mutation in children with first AIS, but there were insufficient data to conclusively establish increased prevalence of the other factors examined.

Qualitative assessment of data

There was no significant heterogeneity in any of the meta-analyses (see final column in table 1, *ADC* website).

DISCUSSION

This systematic review of the literature has found that all the thrombophilic factors examined are more common in children with first AIS than in healthy children. This conclusion is most robust for patients with protein C deficiency and the MTHFR C677T mutation. Although all other thrombophilic factors appeared to be more common in
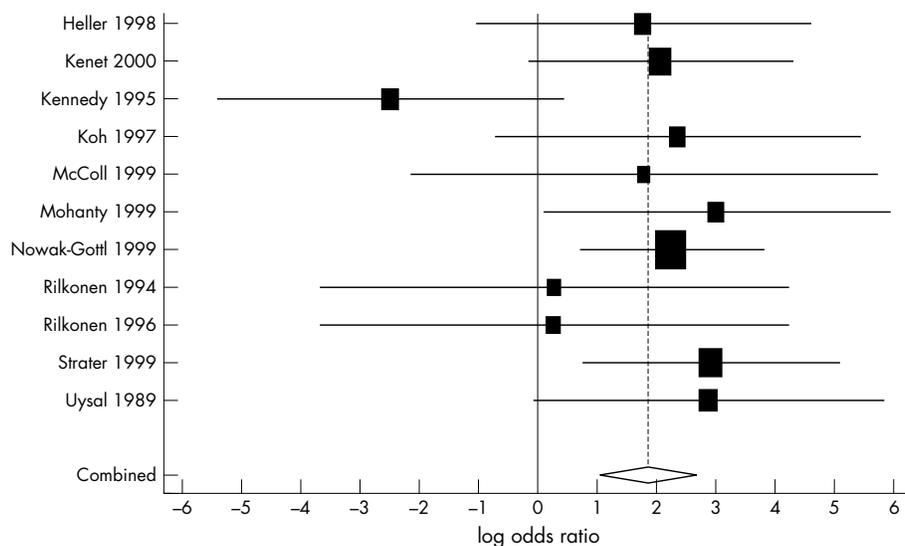
Figure 1 Forest plot showing the meta-analysis for studies comparing prevalence of protein C in children with first AIS to controls. The log OR is depicted on the horizontal axis; a positive OR indicates that protein C deficiency was more common in the patients and a negative OR indicates that it was more common in controls. Each horizontal line represents an individual study (listed on the left). The size of each filled box is proportional to the weight given to the individual study and the limits of the horizontal line represent the 95% CIs. The open diamond indicates the results of the meta-analysis, with the widest point indicating the pooled odds ratio and the horizontal limits indicating the 95% confidence intervals.

What is already known on this topic

- Studies investigating whether thrombophilia is associated with first arterial ischaemic stroke (AIS) in children have produced conflicting results and have been confounded by small patient numbers, lack of controls, and technical variability

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What this study adds

- All the thrombophilia factors examined (protein C, protein S, antithrombin deficiency APCr, plasma homocysteine >95th centile, and the thrombophilia mutations FV1691 GA, PT20210GA, MTHFR C677T) are more common in children with first AIS than in healthy children, and significantly so for protein C deficiency and MTHFR C677T
- Thrombophilia screening is justified in children presenting with first AIS

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the AIS patients, insufficient patients were included for these findings to be conclusive. The increased frequency of these thrombophilic factors does not, however, establish that there is a direct causative relation between them and first AIS in childhood. As there is increasing evidence that both childhood AIS and thrombosis are multi-factorial disorders,[2 23–26] it is likely that AIS is the result of the interaction between thrombophilia and other genetic and environmental risk factors for both thrombosis and AIS. However, the presence of thrombophilia could influence the severity of the ischaemic lesion, for example, by leading to more extensive thrombosis in the acute phase, or may increase the incidence of recurrent AIS.

The reliability of systematic reviews is dependent on inclusion of high quality, methodologically similar studies. Although the inclusion and exclusion criteria for this study aimed to ensure this, these data are still subject to several sources of bias. For example, as most of the papers included originated from North American or European centres, and ethnicity has a strong influence on prevalence of genetic thrombophilia, it is questionable whether these results are applicable to other populations. The lack of detail regarding the ethnicity of both cases and controls in the majority of papers meant that undertaking separate analyses in different ethnic groups was impossible. Thrombophilia screening was not comprehensive in all the included patients and there may have been specific factors selecting children who underwent more thorough evaluation (for example, they may have had a more complicated clinical course). As the majority of the patients were recruited retrospectively, patients who died or who were lost to follow up could not have been included. Although all cases were radiologically confirmed as having cerebral infarction, vascular imaging was rarely undertaken to clearly distinguish cases due to cerebral venous ischaemia. Despite these considerations, there was no significant statistical heterogeneity identified in the results.

A large amount of published data could not be included in the review, usually because of lack of controls. Matching for ethnicity, an important consideration when considering the prevalence of thrombophilia, was reported very infrequently. In addition, it was not always possible to distinguish between homozygous and heterozygous gene mutations from the data provided. In the case of the MTHFR C677T mutation, the majority of cases were homozygous for the mutation, but the results represent pooled data. All of these factors are relevant when considering the robustness of the conclusions from individual studies and are also relevant to interpretation of the results of this systematic review. In order to establish whether there is indeed a significant relation between the prothrombotic factors other than protein C deficiency and the MTHFR C677T mutation and childhood AIS, a large study, with adequate consideration of the factors discussed, above is required. This will only be feasible with international, multicentre collaboration.

Thrombophilia is more commonly associated with venous rather than arterial thrombosis. Uncertainty about whether there is an association between thrombophilia and first childhood AIS has meant that it has been difficult to know whether or not to undertake thrombophilia evaluation in these patients. Thrombophilia screening is expensive and involves drawing a large volume of blood. Serial testing is often necessary to confirm abnormalities. Although this systematic review has established that the prevalence of thrombophilia is increased in children with AIS, the implications of thrombophilia for clinical outcome and AIS recurrence remain unclear. The first of these questions has not been systematically investigated. However, a recent study by Sträter and colleagues[27] identified protein C deficiency as one of several risk factors associated with recurrence of childhood AIS. The relatively uniform ethnic composition of the patients included in the Sträter et al study means that it is questionable whether these findings can be generalised to other populations. This was confirmed by analysis of pooled data from three countries (Canada, Germany, United Kingdom), which found that overall, thrombophilia was associated with a relatively low risk of AIS recurrence.[28]

The clinical implications of this study are that it is reasonable to undertake thrombophilia screening in a children presenting with first AIS. Diagnosis of a thrombophilic tendency may be important in relation to the patients' overall management, in particular, in relation to prevention of venous thromboembolism. However, at present there is no evidence to suggest that specific management strategies should be employed either in the acute stage or in the longer term.

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ARCHIVIST ....

PEG 3350 for constipation

There have been no large randomised controlled trials of treatments for childhood constipation. Lactulose may cause bloating and abdominal pain because of its fermentation by colonic bacteria and it has been suggested that low-dose polyethylene glycols (PEG) could be a less troublesome alternative. Systemic absorption of PEG is said to be very low. Now Dutch workers (W Voskuijl and colleagues. *Gut* 2004;**53**:1590–4**)** have compared lactulose with PEG 3350 (Transipeg; polyethylene glycol with electrolytes).

They randomly assigned 100 patients aged 6 months to 15 years to PEG 3350 or lactulose (under 6 years, one sachet (2.95 g PEG 3350 or 6 g lactulose) per day; over 6 years two sachets per day; dose adjusted according to response) for 8 weeks. The children had constipation defined as at least two of four symptoms or findings: less than three bowel movements per week, encopresis (more than once a week), large amounts of stool every 7–30 days, or palpable faecal abdominal or rectal mass. Enemas were given to clear the rectum before starting the trial medication.

Ninety-one children completed the study. After 8 weeks of treatment success (at least three bowel movements a week and no more than one episode of encopresis every 2 weeks) was achieved in 26/46 in the PEG group and 13/45 in the lactulose group. The mean increase in frequency of bowel movement and decrease in frequency of encopresis were similar in the two groups. Patients taking lactulose were significantly more likely to complain of abdominal pain or of pain or straining at defaecation, and patients taking PEG 3350 were significantly more likely to complain of the taste.

The authors of this paper consider that PEG 3350 should be the first choice treatment for childhood constipation.