

Migraine and antiphospholipid antibodies: no association found in migraine-discordant monozygotic twins

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Migraine headache (with and without aura) is common in the general population and is known to be influenced by genetic factors with heritability estimates between 34–57%. Antiphospholipid syndrome (APS) is a hypercoagulable state characterized by clinical features including venous and arterial thromboses, pregnancy loss and migraine, and by association with antiphospholipid antibodies (aPL). Numerous small studies have investigated whether aPL are associated with migraine in the general population—with contradictory results. In this study, the question was addressed by studying the prevalence of aPL in members of monozygotic (MZ) twin pairs differing in their migraine status. Such twins provide a unique natural experiment, matched as they are for age, sex and genetic factors, and allow the role of environmental factors, such as aPL, to be determined. Despite 95% power to detect a difference of 0.59 IgG units per litre in anticardiolipin antibody IgG titres, no difference in prevalence of aPL could be detected in migraine-discordant MZ twins. □ *Migraine, antiphospholipid, anticardiolipin, twin, monozygotic*

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Introduction

Migraine is a common form of headache with an estimated prevalence of 13–34% across European populations (1). A severe, throbbing headache, it is characteristically unilateral and associated with other symptoms including nausea and intolerance of light and sound. An attack of migraine may or may not be preceded by aura. The pathophysiology is still to be fully elucidated, but aberration in the trigeminovascular system seems to be important (2). Genetic factors have been shown to play a significant role in the variation of expression of migraine, with reported heritability estimates from twin studies of 34–57% (3). Migraine is associated with stroke and forms, with hypertension,

Raynaud's phenomenon and coronary artery disease, the vasospastic phenotype. We have shown these traits to be attributable to a single underlying genetic factor (4). Although several susceptibility genes have been identified for the rare Mendelian trait familial hemiplegic migraine, the genetic loci responsible for migraine in the general population are not the same and remain elusive (reviewed in (5)).

The antiphospholipid syndrome (APS) is a thrombophilic disorder characterized by arterial and/or venous thrombosis and/or pregnancy morbidity, associated with the presence of a specific group of autoantibodies called antiphospholipid antibodies (aPL). Migraine is one of the most prominent complaints in patients with APS (6, 7),

but its association with aPL is controversial (8). It is not clear whether, in the absence of APS, aPL play a role in migraine in the general population. This is important to answer for a number of reasons. First, it would shed light on the pathogenic mechanisms behind at least a proportion of migraine, and may lead to its subdivision into those having, and not having, aPL. Second, a further avenue of therapeutics could be explored, such as aspirin and anticoagulation. Finally, it would raise the epidemiological question of whether migraineurs having aPL should be included in the definition of APS in future.

The role aPL play in migraine has been studied before, and there is evidence both for and against aPL involvement in children (9–12) and adults (13–16). Many of the studies reported to date were small and poorly controlled. As approximately half of variation in migraine expression is due to genetic factors, we sought to control for this and other factors known to influence migraine (age, sex) by examining identical or monozygotic (MZ) twins discordant for the presence of migraine. Such adult discordant MZ twins had been identified as part of on-going studies into migraine and vasospasm. Their stored sera were examined for the presence of a number of aPL, including anticardiolipin antibodies (aCL), anti- β_2 glycoprotein I (anti- β_2 GPI), antibodies to oxLDL- β_2 GPI complex and oxLDL- β_2 GPI antigen level. The discordant MZ design is a powerful method for addressing a question such as this because it controls strongly for confounders such as genes, age and sex and partly for environment and upbringing. By such a method, questions can be answered using many fewer subjects than would be required in a random population sample.

Patients and methods

Twins were identified from the TwinsUK register (17) and Ethics Committee approval for this study had been obtained. The register comprises MZ and dizygotic (DZ) twin volunteers who have been recruited since 1992 using twin registers and successive national media campaigns. For historical reasons, most of the enrolled twins are female. This well-studied population of MZ and DZ twins is sent regular questionnaires for self-completion concerning wide-ranging health issues. Questions relating to migraine were contained within large questionnaires sent to the twins in 1998 and 2000 as part of previous studies (4, 18). Questions to determine the presence of migraine were taken from the University College of San Diego Migraine

Questionnaire (19), which were themselves based on International Headache Society migraine criteria. Twins were considered to have a diagnosis of migraine if they had been: significantly bothered by recurrent headaches (five or more episodes), not due to definite cause (e.g. tumour). Subjects had to be pain free between attacks and, if left untreated, headaches lasted 4–72 h. Characteristics of the headache included at least two of: 'pulsating', 'unilateral', 'severe enough to decrease or stop activity' and 'made worse by physical activity'. In addition, headache was accompanied by at least one of the following: 'nausea and vomiting', 'sensitivity to light' and 'sensitivity to noise'. The questionnaires also contained standard questions relating to zygosity assignment, which is accurate in 95% (20). In addition, 54% of the respondents had their zygosity assigned with certainty by multiplex DNA fingerprinting using variable tandem repeats on venous blood samples taken on attendance at the Twin Unit.

Venous blood was collected from twin volunteers by venepuncture from the antecubital vein into plain tubes. Blood was allowed to clot at room temperature and then centrifuged, aliquoted and stored frozen at -80°C until use. The following aPL were examined: IgG and IgM aCL, anti- β_2 GPI, antibodies to oxLDL- β_2 GPI complex and oxLDL- β_2 GPI antigen level. All were quantified by enzyme-linked immunosorbent assay (ELISA), in duplicate, by laboratory personnel blinded to migraine status.

Anticardiolipin antibodies

The aCL ELISA was performed according to the standardized technique. Briefly, microtitre plates (Immulon I; Dynatech, Chantilly, VA, USA) were coated with 50 $\mu\text{g}/\text{ml}$ bovine cardiolipin in ethanol at 4°C overnight. Wells were blocked with 10% fetal calf serum in phosphate-buffered saline (10% FCS-PBS) for 1 h at room temperature, and 50 μl of serum diluted in 10% FCS-PBS at 1:50 was added in duplicate. Plates were then incubated for 2 h at room temperature, followed by alkaline phosphatase-conjugated goat antihuman IgG or IgM at the appropriate dilution and substrate.

Anti- β_2 glycoprotein I antibodies

Anti- β_2 GPI was detected by ELISA using irradiated plates. Briefly, irradiated microtitre plates (Nunc Maxisorp, Roskilde, Denmark) were coated with 4 $\mu\text{g}/\text{ml}$ of purified human β_2 GPI (Yamasa, Chiba,

Japan) in PBS at 4°C overnight. Wells were blocked with 3% gelatin in PBS for 1 h at 37°C. After three washes with PBS-Tween, 50 µl of serum diluted 1:50 in PBS containing 1% bovine serum albumin was added in duplicate. Plates were incubated for 1 h at room temperature, followed by alkaline phosphatase-conjugated goat antihuman IgG or IgM and substrate. Anti-β2GPI value for each sample was derived from a standard curve according to the dilutions of the positive control.

Antibodies to oxidized-LDL-β₂ glycoprotein I complex

A commercially available enzyme-linked immunoassay for the determination of IgG and IgM antibodies to complexes formed by the interaction of oxidized low-density lipoprotein (oxLDL) with β2GPI in human serum (Anti-AtherOx™ Test Kit; Corgenix, Denver, CO, USA) was used, according to the manufacturer's instructions. Briefly, 100 µl of calibrators, controls or samples, all diluted 1:100 in sample diluent, was added in duplicate and incubated for 1 h at room temperature. After four washes with PBS-Tween 20, 100 µl of IgG or IgM anti-human horseradish peroxidase (HRP)-conjugated antibody solution (provided by the manufacturer) was added and incubated for another hour at room temperature. After four more washes, 100 µl of substrate was added and incubated for 30 min in the dark before stopping the reaction with 100 µl of 0.36 sulphuric acid. Optical densities were read at 450/620 nm within 5 min. The value for each sample was obtained from the calibrator curve and a normal range of 0–20 IgG or IgM units was used as established based on manufacturer's calculations from 100 normal subjects. Lyophilized human sera with known values of IgG and/or IgM anti-oxLDL-β2GPI were included as positive and normal assay controls.

Oxidised LDL-β2GPI antigen complex levels

Quantitative determination of oxLDL-β2GPI antigen complex levels in plasma was performed by a commercially available sandwich ELISA kit serum (AtherOx™ Test Kit; Corgenix), according to the manufacturer's instructions. Briefly, several dilutions of a reference solution and samples diluted 1:100 were added to the pre-coated plates and incubated for 1 h at room temperature. After four washes, 100 µl of HRP-conjugated antibody solution was added and incubated for a further hour. Substrate was added and plates incubated in the dark for

30 min followed by 100 µl of 0.36 sulphuric acid. Optical densities were read at 450/620 nm within 5 min. Values for each sample were derived from a standard curve produced with serial dilutions of the calibrator provided. Normal values were set by the manufacturer at 0–7 units. Lyophilized human plasma containing known oxLDL-β2GPI antigen complex levels was included as positive and negative assay controls.

Data obtained from the twins were analysed according to antibody positivity as well as by antibody titre. Data were log transformed to create normally distributed variables where necessary, and examined using the χ^2 and paired *t*-tests.

Results

One hundred and seven migraine discordant MZ twin pairs (214 individuals) were included in the study. Twins having and not having migraine were perfectly matched for age (mean 53.8; s.d. 12.1, range 27–80 years) and sex (105 female, two male pairs).

By migraine status, the number of subjects positive for each type of aPL was determined from each antibody's normal range (Table 1). There was no significant difference in the numbers of subjects positive for an antibody by migraine status. In addition, the actual titre of antibody was studied and these are recorded in Table 2. There was no detectable difference in titre between the two groups, even without correction for multiple comparisons. Interestingly, the few subjects who were found aPL+ were not usually members of the same twin pair, except in the case of the antigen complex oxLDL-β2GPI Ag, where six positive subjects were members of three twin pairs. Two subjects, not from the same twin pair, were positive for both anti-β2GPI and oxLDL-β2GPI, in one case with IgG and the other IgM, the latter also being found to be positive for IgM anti-oxLDL-β2GPI.

Discussion

Genetic factors account for considerable variation in the expression of migraine. This study was designed to control for these and answer the question of whether aPL play a role in migraine in the general population. The discordant MZ design is a powerful method, because it controls completely for important confounders such as genes, age and sex, and partly for environment and upbringing. Also, this study used volunteer subjects, so is more likely to be applicable to the general population

Table 1 Antiphospholipid antibody positivity by migraine status

Antibody test	Migraine positive	Migraine negative
No. positive IgG aCL	1/107	3/107
No. positive IgM aCL	0/107	3/107
No. positive IgG β 2GPI	1/107	1/107
No. positive IgM β 2GPI	0/107	1/107
No. positive IgG anti-oxLDL- β 2GPI	4/107	5/107
No. positive IgM anti-oxLDL- β 2GPI	2/107	3/107
No. positive oxLDL- β 2GPI antigen level	4/107	3/107

Number of twins positive for each antibody is shown out of total of 107 for each migraine state. No significant difference was detected for any antibody tested.

aCL, anticardiolipin antibodies; β 2GPI, β ₂ glycoprotein I; oxLDL, oxidized low-density lipoprotein.

Table 2 aPL titres on MZ twins, by migraine status

	Normal value	Migraine positive twins	Migraine negative twins
IgG aCL	< 2 GPL	0.13 (0.6)	0.23 (0.94)
IgM aCL	< 3.2 MPL	0.05 (0.13)	0.21 (1.01)
IgG or M anti- β 2GPI		0.01 (0.1)	0.02 (0.14)
IgG oxLDL- β 2GPI	< 20 AU	9.33 (5.48)	9.08 (5.2)
IgM oxLDL- β 2GPI	< 20 AU	5.38 (7.34)	4.95 (4.31)
oxLDL- β 2GPI antigen level	0–7 units	2.68 (2.39)	2.72 (2.88)

aPL titre, mean (and standard deviation) is shown.

aPL, antiphospholipid antibodies; MZ, monozygotic; aCL, anticardiolipin antibodies; β 2GPI, β ₂ glycoprotein I; oxLDL, oxidized low-density lipoprotein; GPL, IgG units per litre; MPL, IgM units per litre; AU, antibody units.

than studies using hospital-based subjects with migraine. The question of how twins differ from singletons needs to be considered. There is evidence that results from twin studies are generalizable, and we have shown our adult twins to be similar to a singleton adult population for a range of health and lifestyle characteristics (21).

The main limitation of the study was the way in which the diagnosis of migraine was made, using questionnaires which raise the issues of ascertainment bias, validity of self-reporting and case definition. Attempts were made to reduce these sources of error, where possible. Questions used to form the diagnosis were based on validated methods. Prevalence of migraine in the twin sample from which these discordant twins were selected was consistent with population estimates (4), and the prevalence of aPL was similar to that reported for normal healthy blood donors (22). In a previous study of soft tissue rheumatic diagnoses we compared questionnaire responses with family practitioner records and found no evidence of recall bias (23). Data on the presence or absence of aura were incomplete and so were not included in the analysis. It is possible, therefore, that we failed to detect a disease

subgroup being influenced by aPL, but with so few subjects identified as aPL+ this seems unlikely. It remains debated whether aura signifies a different pathogenic mechanism, but there is evidence that the two forms of migraine defined by aura constitute opposite ends of a phenotype spectrum (24). As in any cross-sectional study, it is possible that subjects are misclassified both by migraine status and aPL status—we did not serially test serum as suggested by the clinical recommendations. As there is no validated questionnaire method of diagnosing APS, we did not examine this, but twins were asked to record any medical illness and none volunteered a diagnosis of APS or 'Hughes syndrome'. The sample group in this study was predominantly female, and although there is a higher prevalence of migraine in women than in men in the general population, these results do not pertain to men. Finally, one form of aPL, the lupus anticoagulant, has not been studied, as this may be examined only in citrated plasma, which was not available at the time of the study.

One of the commonest reasons why studies have negative findings is type 2 error. This is usually because small sample sizes lead to the study having

insufficient power to detect a difference between two groups even if one exists. This study had 95% power to detect a difference of 0.59 IgG units per litre in aCL IgG titres between migraine and the non-migraine cotwins. We could find no evidence of any of the aPL tested being up-regulated in the MZ twins with migraine. This suggests that screening patients attending a migraine clinic for the presence of aPL may not be useful. In summary, aPL do not appear to play a role in migraine in well-matched volunteer subjects, and testing for aPL in the general migraine population is not recommended.

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