A twin study in Behçet's syndrome

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ABSTRACT

Objectives. *Case reports on monozygotic (MZ) twins with Behçet's syndrome (BS) have been few and we are not aware of formal twin studies. We sought the frequency of MZ and dizygotic (DZ) twin births in BS and compared it to a healthy population sample from the same geography. We also looked for the concordance rate among the MZ and DZ twins.*

Methods. *1705 (1039 M/ 666 F) patients attending a dedicated BS outpatient clinic and 7761 (3848 M/ 3913 F) medical school students were asked about having a MZ or DZ twin sibling. MZ and DZ twins thus identified among both patients and controls were individually seen at the clinic. In addition, HLA, DNA microsatellite markers and blood groups were typed to further confirm twin- ship. All twins were contacted 8 years later for new emergence of disease.*

Results. *There were 14 (0.82%) patients with BS and 120 (1.55 %) controls who had a twin sibling (p=0.022). Of these, 8 (0.47%) patients with BS and 92 (1.19%) controls had a DZ twin sibling (p=0.009). MZ twin frequency was similar between BS patients (6/ 1705; 0.35 %) and control population (28/ 7761; 0.36 %). The pairwise concordance rate for BS was 2/6 (95% CI: -0.21- 0.88) for MZ and 1/8 (95% CI: -0.17- 0.42) for DZ twins (p=0.538). Genetic effects accounted for 41% of the phenotypic variance for BS among twins. After 8 years of follow-up, 4 of 6 MZ and 6 of 7 DZ twin pairs were still discordant.*

Conclusions. *The frequency of MZ twin births in BS is not different than that in the general population while the DZ twins were seen less frequently among the BS patients. The concordances for BS were higher in MZ compared with DZ twins, suggesting genetic predisposition. On the other hand, the persistence of discordance after 8 years of follow up among the remaining MZ twins demands further research to understand non- genetic factors in causation of BS.*

Introduction

Behçet's syndrome (BS) is a multisystem vasculitis of unknown etiology (1). It may deteriorate the quality life of patients, cause several serious morbidities, and lead to a fatal outcome (2-4). It is likely to result from complex interaction of genetic and environmental factors. High prevalence in a unique geographical area and in certain ethnic populations, familial aggregation, high sibling recurrence rate and association with a class I MHC complex (HLA-B51) suggest genetic predisposition for BS (5-11).

Twin studies help us to better understand the genetic and environmental components of disease causation (12-13). Comparison of concordance rates between symptomatic monozygotic (MZ) and dizygotic (DZ) pairs of twins provide information whether the familial pattern could be due to hereditary or environmental factors or both.

Twin concordance studies of BS have not been available. There were only 3 case reports describing 4 pairs of MZ twins with BS, of which 2 were concordant (14- 16). One concordant pair reported by our group were HLA-B51 positive brothers who lived under the same roof for 23 years until disease onset (14). This MZ twin pair is also included in the current report (See Results). The other concordant pair, reported by Kobayashi *et al*., were HLA-B51 negative MZ twin brothers who were raised together until 17 years of age (16). One brother developed BS at age 17 and the other at 18. Both had gastrointestinal involvement. Gul *et al*., on the other hand reported 2 discordant MZ twin pairs (15). They were both HLA-B51 negative and both remained discordant through at least 5 years of follow-up.

Apart from the consideration of concordance between MZ and DZ twins for disease presentation, we were also concerned about the mere paucity of reports about MZ twins in BS and whether this represented a true biological phenomenon (in utero deaths) or simple underreporting.

In this formal twin study in BS, we aimed to determine genetic and environmental contributions to BS. We also compared the frequency of MZ and DZ twining of BS patients to a sample from the general population.

Patients and methods

A total of 1705 consecutive (1039 M/ 666 F; mean age: 37.2±10.8, years) patients attending a dedicated multidisciplinary outpatient clinic for BS at Cerrahpasa Medical Faculty of Istanbul University were asked whether they had a twin sibling. Information on having a twin or not was obtained by interviews in the outpatient clinic in 723 and by telephone in 74 patients between March 2001 and July 2002. In the remaining 908, a retrospective review of patient charts was done to seek this information which had been taken routinely at the first visit. As healthy controls, 7761 (3848 M/ 3913 F; mean age: 18.5±1.4 years) first year students at University of Istanbul were asked about having a twin sibling during their initial school registration.

The patients and controls were questioned as first "Do you have a sibling who was born on the same day with you?" If they answered affirmatively to this question, monozigosity was assessed by asking "Are you as similar as two halves of an apple?" MZ twins thus identified among both patients and healthy controls were individually seen at the clinic and their resemblance was verified. Those who answered negative to the aforementioned question were considered as DZ twins. Signs and symptoms of BS were evaluated in both MZ and DZ twin siblings of BS probands. However, skin pathergy, urate tests and blood tests to verify MZ twin-ship were done only in probands with BS and their MZ co-twin. All MZ and DZ twin siblings were questioned about how many years they have lived in the same environment with their co-twin after their birth.

Blood group and subgroup analyses were done using using standard tube agglutination methods. DNA and HLA genotyping were done to further confirm twin-ship and determine HLA B-51 carrier status in MZ BS twin pairs only.

All discordant MZ or DZ twins were contacted 8 years later for the assessment of new emergence of disease.

This study was conducted in accordance with the declaration of Helsinki and oral consent was taken from MZ twins before blood analysis.

DNA isolation and genotyping

Ten ml of venous blood was collected in EDTA containing tubes and genomic DNA is isolated using standard salting out techniques for extracting DNA (17). Polymerase chain reaction was carried out using primer sets of microsatellite markers (D1S519, D10S570, D10S1227 and D10S1430) that are obtained from Research Genetics Inc (Huntsville, AL, USA). Microsatellite markers with high PIC (Polymorphism Information Content) values were used for their high heterozygosity and chosen randomly from a panel of microsatellite markers for whole genome scans. The products were run on 8% polyacrylamide gels and visualised via silver nitrate staining.

Zygosity analysis

Zygosity testing was done to calculate the chance of monozygosity. Here the allele frequencies were obtained from the genome databases based on different populations.

HLA analysis

Patient peripheral blood was drawn by venipuncture into heparinised tubes. Peripheral blood lymphocytes of the patients were isolated by ficoll-hypaque density-gradient centrifugation. Following isolation, lymphocytes were counted in RPMI+ 10% FCS and cell number was adjusted to 3x 10^6/mm^3. Samples with >95% viability were included into the assay. One μl of lymphocyte cell suspensions were placed into each well of the prepared Terasaki plates including multiple anti-HLA-B5, cross-reactive anti-B35, HLA negative and HLA positive control sera. Plates were incubated at 22°C for 30 minutes and then 5 μl of rabbit complement was added to each well to facilitate the antigen (HLA-B5) and antibody (anti-HLA-B5) interaction. Following incubation with the complement for 1 hour, 3 μl of eosine dye to visualize the lyses of the cells by dye uptake and 5μl formaldehyde to inhibit the effect of complement was added. Finally, 5μl PBS was also added to each well. Plates were cover-slipped avoiding air bubbles and incubated for overnight at 4°C. Interpretation of the plates were based on a scoring system considering each well positive if more than half of the lymphocytes in the well were dead as a result of antigen-antibody reaction.

Statistical analysis

Twin frequencies and concordance rates were expressed as percentage and as 95% confidence intervals. Chi-square tests or Fisher exact tests were used to assess the twin prevalence rates and pairwise concordance rates for MZ and DZ twin pairs. Comparisons of continuous variables were made by using Student's *t*-tests. Heritability is calculated by doubling the difference between MZ and DZ concordance rate (h^2=2 (cMZ- cDZ)) (18-19). All statistical analyses were carried out using a statistical software (SPSS, version 13.0 for Windows; SPSS, Chicago, IL).

Results

There were 14 (0.82%) patients with BS and 120 (1.55%) healthy controls who had a twin sibling (p=0.022). Of these, 8 (0.47%) patients with BS and 92 (1.19%) healthy controls had a DZ twin sibling (p=0.009). The MZ twin frequency however was almost identical among both BS patients (6/ 1705; 0.35%) and controls (28/7761; 0.36%) (p=1.0). The blood subgroups were Dce/Dce Kell negative and each MZ twin pair shared the same genotype for the polymorphic microsatellite markers (D10S519, D10S570, D10S1227 and D10S1430). Of the 6 MZ twin pairs, 2 were concordant for BS while 4 were discordant when they were first identified. As shown in Table I, the syndrome

Table I. Clinical features of MZ twins concordant for BS.

Monozygotic twin pair no.		Sex	Age at onset	Duration of living together	HLA B51	Pathergy test	Urate skin test	OA	GU	Skin lesions	Ocular disease
1	Proband	F	29	16	+	–	+	+	+	+	–
	Sibling	F	30	16	+	–	+	+	+	+	–
2*	Proband	M	23	23	+	+	+	+	+	+	+
	Sibling	M	25	23	+	+	+	+	+	+	+

*Previously reported (Ref. no. 10), OA: oral aphtae, GU: genital ulcer,

Table II. Clinical features of MZ twins discordant for BS.

Monozygotic twin pair no.		Sex	Age at onset (years)	Duration of living together (years)	HLA B51	Pathergy test	Urate skin test	OA	GU	Skin lesions	Ocular disease
3	Proband	M	29	26	+	+	+	+	+	+	–
	Sibling	M		26	+	–	–	–	–	–	–
4	Proband	F	45	17	–	+	+	+	–	+	–
	Sibling	F		17	–	–	–	–	–	–	–
5	Proband	M	33	31	–	–	–	+	–	+	–
	Sibling	M		31	–	–	–	–	–	–	–
6	Proband	M	33	33	+	–	–	+	+	–	+
	Sibling	M		33	+	–	–	–	–	–	–

OA: oral aphtae, GU: genital ulcer.

had developed within 12 and 18 months, in the first and second pairs, respectively and the organ systems involved were quite similar. All were HLA B-51 positive. Pair no. 2 is the already reported pair (14). Clinical characteristics of 4 discordant MZ pairs are shown in Table II. Half of them (2/4) were HLA B-51 carriers. None of the discordant MZ siblings of the remaining 4 probands developed the syndrome after 8 years of follow- up.

Among 8 (4 M / 4 F) DZ twins, one DZ twin sibling of a proband had died at the age of 15. The proband himself developed BS at the age of 32. Of the remaining 7 DZ twin pairs, none were concordant at the time of the study, however, 2 probands had a non-twin sibling with BS. During 8 years of follow-up only 1 DZ sibling of a proband developed BS. The proband of this concordant pair was a 35 years old male who developed oral and genital ulcerations and thrombosis in the vena cava superior vein 13 years ago. He also suffered from papulopustular lesions and mild eye disease. He was treated with azathioprine and acetylsalicylic acid. His DZ twin brother started to suffer from recurrent oral ulcers, acne-like lesions and arthritis 5 years ago when he was 30 years old. He responded well to colchicine and non-steroidal anti-inflammatory drugs. He had been raised together with his twin brother and shared the same roof of their home until the age of 31. Similarly, the remaining DZ twin probands were brought up together with their DZ twin siblings and shared the same environment for a considerable period of time (15, 18, 18, 22, 25, 26 and 35 years).

It was seen that both MZ and DZ twin pairs had lived under the same roof for a considerable period of time which ranged between 17 and 33 years for MZ twins and between 15 and 35 years for DZ twins.

The pairwise concordance rate was 2/6 (95% CI: -21% - 88%) for MZ and 1/8 (95% CI: -17% - 42%) for DZ twins (p=0.538). Using the formula described above, the heritability was estimated as 41%. We also made the same calculations excluding the dizygotic twin pair in whom one co-twin had died at the age of 16. Then, the pairwise concordance rate for DZ twins was found as 1/7 (95% CI: (- 12% - 40%) which was still smaller than the MZ concordance rate. In this case estimated heritability would be 38%.

Discussion

In this study we demonstrated that the concordance rate for BS was higher in MZ twins (2 of 6 pairs) compared with DZ twins (1 of 8 pairs). We estimated the heritability as 41 %. We observed that 4 of 6 MZ and 6 of 7 DZ twin pairs remained discordant after 8 years of follow-up. Finally, we found that the frequency of MZ twins with BS (0.35%) was identical to that found in a representative sample of general population (0.36%).

With obvious due caution to the small number of twin pairs that could be studied, the concordance rate in MZ twins with BS in this study is lower than that reported for some complex inherited diseases such as autism, bipolar disorders, schizophrenia, ankylosing spondylitis and diabetes Type 1 and Type 2 (ranging between 50–90%), (12, 13, 20-24) whereas higher than that report-

ed for systemic lupus erythematosus, multiple sclerosis, Grave's disease, rheumatoid arthritis or scleroderma (12, 13, 25-28). The concordance rate in MZ twins we describe remains the same (3/9) when we add the previously reported 3 MZ twin pairs (14-16).

The concordance rate in MZ twins (2/6) was higher than in DZ twins (1/8) in this study, indicating genetic effects. The MZ twin concordance rate reached even 2/4, when only those MZ twin pairs who carried HLA-B51 were taken into account, again supporting the role of heredity. Similarly a higher concordance rate in excess of MZ twins has been reported in autism, bipolar disorders, multiple sclerosis and rheumatoid arthritis (20, 21, 25, 27).

All these findings together with the considerably high sibling recurrence rate in BS (4.2%) (8) give some support for heritability in this disorder. Close association with HLA B51, a predilection for a certain geographical area and presence of genetic anticipation were other evidences (1, 5, 9-10). The high prevalence of BS among Turkish or North African immigrants compared to native continental people living in Berlin or Paris, respectively, again suggests a hereditary rather than an environmental basis for BS (11, 29).

On the other hand, the fact that concordant MZ twin pairs had lived under the same roof for a considerable period of time before developing the disease makes difficult to eliminate the role of a shared environment. However, it is to be noted that, both discordant MZ twin pairs and DZ twin pairs similarly had lived under the same roof for long time in our study, this may somewhat neutralize environmental influences. Still, a discordant disease course for 8 years in 4 of 6 MZ twin siblings gives some support for the proposition that environmental factors are also operative in BS, like in a true to form complex disease.

Interestingly, of the 6 monozygotic pairs 4 twin pairs were HLA-B51 positive. Among these 4, 2 were discordant for BS suggesting that being carrier of HLA-B51 is not all important for disease generation. This is in line with the previous observation of a weak contribution of HLA-B locus to the overall genetic susceptibility to BS (30). Considering the age of our probands, we also think that discordance time was probably adequate for BS to develop, since the syndrome usually starts during the 2nd or 3rd decade (1).

Although 7 twin siblings of the 8 DZ twin probands had no symptoms or signs regarding BS, 2 of the probands had an elder non-twin brother with BS. This shows familial aggregation which emphasizes once more the role of genetic influences.

It is reported that twinning rates vary considerably across time and geographical localization (31). For example the highest rate is reported for South Africa (20 per 1000 maternities) and lowest for Japan and Taiwan (2–6 per 1000 maternities). Turkey seems to be in the intermediate prevalence group considering overall twinning rates reported in our study (0.82–1.55%). We found only one study giving data about twinning rate in Turkey (32). Duyar and Güntay-Ayaz surveyed 110.419 maternities from 8 maternity hospitals in Ankara between 1987 and 1988 and reviewed previous twin research studies done in several regions in Turkey between 1959 and 1985 (32). They found an overall twinning rate of 0.85% (937/110.419), a MZ twinning rate of 0.31% and a DZ twinning rate of 0.54%. They further reported that while MZ twin birth frequency was clustered about 0.30% that of DZ twin births varied widely between 0.54% and 1.45%. These findings are in line with what we have found in this study.

While MZ twinning rate generally occurs at a constant rate, in any case being about 3–5 per 1000, the frequency of DZ twin births shows considerable variability ranging from 5 per 1000 to 20 per 1000 (31). Heritability, race, maternal age, parity, nutrition, oral contraceptives and ovulation inducing agents are factors that have been shown to influence DZ twinning frequency (31). We found significant difference in DZ twinning between BS patients and general population in this study. This could be due to: a) significant age difference between BS patients and control population ($p<0.001$) which indicates a temporal variation and b) possible difference in socio-economical status and geography between patients and controls.

In our study, monozygous couples were identified first by asking the candidates if they were as similar as two halves of an apple. Thus identified twins were later seen by a physician (S.M.). DNA analysis to confirm zygosity was done however only in BS patients and their co-twins. We relied only on visual similarity among the healthy controls. On the other hand this should not be of major concern since the accuracy of the visual approach has been reported to be high (97.6%) (12).

This study has other limitations. As we mentioned earlier the number of twin pairs was small therefore caution is needed in interpretation of results. There was no disease control group is our study. Finally, studying MZ twins reared apart could have been more helpful in establishing the significance of genetic and environmental factors regarding susceptibility to complex disorders (33).

Conclusions

With due caution to small numbers that could be studied, higher concordance rate of MZ twins compared to DZ twins suggests significant genetic influences on the development of BS. However, the role of environmental factors should also not be neglected since a discordant disease course has been observed in 4/6 of MZ twin pairs. Finally, the frequency of MZ twin births in BS is not different to that observed in the general population in Turkey and around the world.

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