The prevalence of Behçet's syndrome, familial Mediterranean fever, HLA-B51 and *MEFV* gene mutations among ethnic Armenians living in Istanbul, Turkey

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ABSTRACT

Objectives: *We investigated the prevalence of Behçet's syndrome (BS) among the ethnic Armenians in Istanbul using Familial Mediterranean Fever (FMF) as a comparator disease. We also studied HLA-B51 and MEFV mutations among a group of healthy Armenians and a non-Armenian population.*

Methods: *The prevalence study was conducted in 2 parts in the Armenian primary schools in Istanbul, using the enrolled students as index cases to study the core family. In Part I, a questionnaire seeking only whether either parent had previously been diagnosed as having BS or FMF by a physician was distributed to a total of 1873 index students registered at 10 schools. A total of 1380 parents filled in the questionnaire, yielding a response rate of 37% (1380 / 3746). In Part II, eight schools participated with a response rate of 83 % (1183/1428). Also, genomic DNA samples of 108 healthy (14 M/ 94 F) Armenians and 97 (45 M/ 52 F) non-Armenians, were studied for HLA-B51 and MEFV gene mutations.*

Results: *In Part I, none of the parents turned out to have been diagnosed as BS, whereas a total of 12 / 1380 (870/ 10^5) had been diagnosed as FMF. In the second part the estimated prevalence of BS was 90 /10^5 and that of FMF was 760/ 10^5. HLA-B51 carrier rate was found to be similar between the Armenian (27%, 29/108) and the non-Armenian participants (19%, 18/97), (p=0.158). Overall carrier rate of MEFV gene mutations was significantly higher in the Armenian group (36% vs. 20%, p=0.015).*

Conclusions: *The genetic load for FMF is considerably higher among the Armenians when compared to the load for BS among the same ethnic group. On the other hand, the rather low frequency of BS among the Armenians when compared to the frequency among the general population living in the same environment is further evidence for a genetic predisposition to BS. HLA- B51 does not seem to play a dominant role in the said predisposition. Finally, as we have used an unorthodox epidemiological methodology in data collection our results might need to be further verified by more conventional methods.*

Introduction

Behçet's syndrome (BS) is a multisystem vasculitis which is more prevalent in the Middle- Eastern and Mediterranean countries. Turkey has the highest prevalence rate reaching 420 per 10^5 (1). While the cause is not known, it is regarded as a complex disease with both genetic and environmental factors playing roles in disease causation. Strong predilection for certain ethnic populations, familial aggregation, high sibling recurrence rate and association with class I MHC complex (HLA-B51) are in favor of a genetic predisposition (2-6). On the other hand, the lack of cases of BS among Japanese immigrants in Hawaii and a lower prevalence among Turks living in Berlin compared to that seen in mainland Japan and Turkey in addition to the abundance of sporadic cases with unaffected families support environmental effects (7-8).

We had the longstanding impression that BS was not common among the ethnic Armenians, a closed Christian community with an estimated population of 30.000–50.000 living mostly in Istanbul, compared to the general population.

In this study, we formally surveyed the frequency of BS among the ethnic Armenians living in Istanbul. FMF is known to have a strong genetic back-

ground as evidenced by the *MEFV* association (9-10). It is well known historically to be common among the Armenians (11-13). Up to recent times, FMF had also been called the "Armenian disease" in Turkey. We used FMF, with its strong genetic component, as a comparator disease in our survey. We reasoned a) our hypothesis of less frequent BS among the Armenians and its genetic basis would be better tested by comparing its frequency to a more frequent condition with accepted and defined genetic association; and b) This control group would also help us to assess the validity of our survey method. Lastly our findings would also give us an estimate of the frequency of FMF among the ethnic Armenians in Turkey which, to our knowledge, had not been studied before. After we had the initial results from our survey (Part I, see below) we further investigated the frequency of HLA-B51 known to be increased among the BS patients (6) among a small sample of healthy Armenians and non-Armenians also living in Istanbul. In the same group we also studied the *MEFV* mutations, the well known associate of FMF (9-10).

Patients and methods

Prevalence of BS and FMF

The prevalence study was conducted in 2 parts in the Armenian primary and secondary schools in Istanbul. We utilised a convenience sample of all enrolled students (age range 7-15), as index cases to study the families. Out of 15 schools in total, we studied 10 schools which we had easy access through acquaintances in the school administration in the first part. We asked permission from all 15 schools in the second part, however only 8 agreed to collaborate, 3 of which had not participated in the first part.

Prevalence study – Part I

In the first part we used a questionnaire which, in addition to the demographic data, asked only the question whether either parent had previously been diagnosed as having BS or FMF by a physician. A total of 1873 index students registered at 10 schools were surveyed between April and December 2006. Each student was given a questionnaire by the school administration to be filled by both parents at home. Out of a total 3746, 1380 parents (657 fathers and 723 mothers) with a mean age of 41.5±5.9, SD years filled in the questionnaire, yielding a response rate of 37% (1380/3746).

As we collected data at this stage, we became worried that the survey participants interviewed were better informed about FMF as compared to BS. To assess this contention formally we assessed the knowledge of BS and FMF among the teachers and other school employees, all Armenian, with a multichoice short test (see Appendix). We saw that they indeed were better informed about FMF: 74% (90/121) gave the correct answers for FMF compared to only 33% (40/121) for BS ($p<0.001$). Thus we reasoned that the first part of our study with a low response rate of 37% could have been biased with BS perhaps being under recognised. We therefore planned a second part aiming to assess better reproducibility and seeking a higher questionnaire response rate.

Prevalence study – Part II

The same questionnaire was distributed to each of 1428 registered students of 8 schools to be filled in by both parents. This time, the questionnaire sought whether the parents, the index student and his or her siblings had previously been diagnosed as having BS or FMF by a physician. Also, we requested from the school administration that each student should be warned for bringing back the questionnaire as soon as answered by the parents. Between February and June 2009, a total of 1183/1428 (83%) families of each index student replied to our questionnaire. Six fathers and 1 mother were deceased. Information was available on a total of 4462 individuals (2171 M/ 2291 F; mean age: 27.6±16.3). There were 1177 fathers, 1182 mothers and 2103 children (994 M/1109 F).

In both parts of the study, those who were identified as having BS or FMF were invited for an interview to verify the diagnoses. For those who were not able to come, hospital records were investigated, related physicians were contacted or home visits were done. If all attempts were unsuccessful, telephone interviews were used to verify the diagnoses. The Cerrahpaşa Medical Faculty Ethics Committee approved both parts of the study and written consent was taken before each interview with the parents.

HLA-B51 and MEFV gene studies

A total of 115 (14 M/ 101F) volunteering staff from 7 Armenian primary schools and 100 (48 M/ 52 F) Turkish students attending Cerrahpaşa Medical Faculty (all non-Armenian) were included in our study between April and December 2007. Demographic characteristics and whether or not the individual had previously been diagnosed as BS or FMF by a doctor were also sought by the help of a questionnaire. Those who were previously diagnosed with BS or FMF were excluded. Saliva samples were collected for DNA isolation and kept in Oragene™ vials (DNA Genotek, Canada), in room temperature. Total genomic DNA was isolated according to the manufacturer's protocol. DNA isolation and genotyping studies were completed between May and July 2008. The hospital ethics committee approved the study protocol and written informed consent was obtained from all study participants.

a) HLA genotyping

HLA-B51 genotyping was done using Olerup SSP HLA and Micro SSP™ Seramates HLA Class I DNA typing kits (One Lambda) according to the manufacturer's instructions (Qiagen, Germany). The genotyping was done with Helmberg-Score® Virtual Sequencing software for the Olerup kit and with the table provided by the supplier for the One lambda Seramates kit.

b) MEFV genotyping

Mutations and polymorphisms were genotyped using established protocols based on PCR-RFLP analysis (14-15). The genotyping was done by analysing the agarose gel photos of the RE digested PCR products for the five most common mutations observed in *MEFV* gene namely, M694V, M694I, V720A,

M680I and E148Q. Each gel included two positive controls, one heterozygote carrier and one homozygote mutant and genotyping was done blindly by two researchers. Alleles and genotypes were counted and frequencies were obtained accordingly.

Statistical analysis

Comparisons of continuous and categorical variables were made using Student's *t*-tests and chi-square tests, respectively. In Part I we used the zero patient formula ($p<3/n$ where 'n' signifies number of the population free of disease) (16) to estimate the one-sided 95% confidence interval for the prevalence rate of BS. The remaining prevalence rates were expressed as two-sided 95% confidence intervals. The statistical analysis was performed using SPSS, version 15 (SPSS Inc., Chicago, IL, USA).

Results

The prevalence rates observed in both parts of the study are shown in Tables I–III.

Prevalence study - Part I
(Tables I and II)

a) BS

In the first part of the study, which included only parents, none of the parents turned out to have been diagnosed as BS. It can be estimated with 95% confidence that BS had a frequency of $<217/10^5$ (3/1380) (16) in this group.

b) FMF

In contrast to BS, a total of 12 parents (6 M/ 6F) reported that they had been diagnosed as having FMF. We confirmed these diagnoses by interviewing 5 (3 M/2 F) parents at the hospital and 2 (1 M/ 1 F) at home. We contacted the physicians who were following the other 5 parents (2 M/ 3 F). The diagnoses in all 12 were confirmed. One male had also concomitant diagnosis of ulcerative colitis. All were using colchicine regularly except for 1 female who had experienced no attacks for 5 years. This gave us an overall prevalence of $870/10^5$ (12/ 1380) (95% CI: 380-1360) for FMF. The prevalence was $913/10^5$ (6/657) among fathers and $830/10^5$ (6/723) among mothers.

Table I. Prevalence of Behçet's syndrome and Familial Mediterranean Fever in the first and second part of the study.

		Behçet's Syndrome			Familial Mediterranean Fever		
		n (M/F)	Prevalence per 10^5	95% CI per 10^5	n (M/F)	Prevalence per 10^5	95% CI per 10^5
Part 1.	Parents only n=1380 (657 M / 723 F)	0	0	< 217	12 (6/6)	870	380-1360
Part 2.	Parents and offspring n=4462 (2171 M / 2291 F)	4 (4/0)	90	0-180	34 (18/16)	760	510-1002

Table II. Age stratified prevalence for Familial Mediterranean Fever (FMF) in Part I of the study.

Age interval	Armenian population n (M/ F)	Patients with FMF n (M/ F)	Prevalence (per 10^5)	95% CI per 10^5
26–39	536 (152 / 384)	6 (2/4)	1120	230–2010
40–49	719 (407 / 312)	6 (4/2)	830	170–1490
50–62	125 (98 / 27)	0	0	0
Total	1380 (657 / 723)	12 (6/6)	870	380–1360

Table III. Age stratified prevalence for Behçet's syndrome (BS) and Familial Mediterranean Fever (FMF) in the second part of the study.

Age interval	Armenian population n. (M/ F)	BS n. (M/ F)	Prevalence for BS (per 10^5), 95 % CI per 10^5	FMF n. (M/ F)	Prevalence for FMF (per 10^5), 95 % CI per 10^5
0–9	820 (430 / 390)	0	0	5 (3/2)	610 (80–1140)
10–19	1090 (464 / 626)	0	0	7 (5/2)	640 (170–1110)
20–29	212 (96 / 116)	0	0	0	0
30–39	861 (258 / 603)	2 (2/0)	232 (-90–550)	10 (3/7)	1161 (440–1880)
40–49	1201 (695 / 506)	2 (2/0)	170 (-60–400)	12 (7/5)	1000 (440–1560)
50–59	260 (210 / 50)	0	0	0	0
60–69	18 (18 / 0)	0	0	0	0
Total	4462 (2171/ 2291)	4 (4/0)	90 (0–180)	34 (18/16)	760 (510–1010)

Prevalence study - Part II
(Tables I and III)

a) BS

In the second part of the study which included both parents and children, there were 4 fathers with BS among 4462 individuals ($90/10^5$). All 4 came from the schools which had not been included in the first part. All were diagnosed and followed at other medical institutions. When we contacted their physicians we found out that 1 had eye involvement and the remaining 3 had mucocutaneous disease, only. Interestingly, 1 father with BS was one of a dizygotic concordant twin pair. The other pair was not part of the study population. Both twins were concordant for BS which had started 12 and 7 years ago in the sibling and the father, respectively. They were also concordant for FMF which developed 7 and 2 years previously, respectively, with periodic abdominal pain and fever. No index student or their siblings had BS.

b) FMF

We identified 34 (18 M/16 F) patients with FMF among 4462 ($760/10^5$). There were 12 mothers, 10 fathers, 4 boys and 8 girls. Prevalence was $1015/10^5$ for mothers (12/1182), $850/10^5$ (10/1177) for fathers and $571/10^5$ (12/2103) for offspring. Familial recurrences were present in 2 families in the form of mother-son. Seven (5 M/2 F) patients had also been identified during the first part of our study and 5 (2 M/3 F) were already our registered patients at our outpatient unit. Six (3 M/3 F) were called to our outpatient clinic for diagnostic verification in-

cluding the aforementioned father who had a dizygotic twin sibling. We contacted private physicians in 3 (2 M/1 F) patients and obtained detailed information via telephone interview in the remaining 13 (6M/7 F). All were previously diagnosed with FMF and treated with colchicine.

Table II and III show age stratified prevalence rates of BS and FMF, respectively in either part of the survey. BS patients were all males, whereas males and females were similarly represented among the FMF patients. BS patients were in the 3rd and 4th decade, whereas FMF patients were between the 1st and 4th decade. Since BS starts rarely in the childhood, the adjusted prevalence for among those who are aged 10 years or over was calculated as 110/ 10^5 (4/3642) as previous studies have done (1, 17-21).

Genome DNA analysis

We excluded 5 Armenian females who had been previously diagnosed with FMF and one Turkish male who turned out to have BS. One female staff who was a Christian Arab and one other female whose sister was already included in the study were also excluded. Genome DNA samples of 2 non-Armenian students were inadequate; therefore we studied 108 (14 M/94 F) Armenians and 97 (45 M/52 F) Turkish genome DNA samples. Armenian participants were more likely to be female ($p<0.001$) and older compared to non-Armenians (mean age: 42±12 *vs*. 24±10, SD years, respectively) ($p<0.001$). Moreover there was significant difference between the two groups when we compared the place of birth of individuals. While Armenians were more likely to be born in Istanbul (Istanbul: 74%, Central Anatolia: 14% and Eastern Anatolia: 12%), non-Armenians were more likely to originate from various parts of Turkey (Istanbul: 18%, Marmara: 11%, Western Anatolia: 14%, Central Anatolia: 19%, Eastern Anatolia: 19%, Black Sea: 10%, Southern Anatolia: 9%).

a) HLA genotyping

HLA-B51 carrier rate was found to be similar between Armenians (27%, 29/108) and non-Armenian study participants (19%, 18/97), ($p=0.158$).

b) MEFV gene mutations

The genotype and allele frequencies of the *MEFV* mutations/polymorphisms are shown in Table IV. There are 3 compound heterozygotes in the Armenian group and 5 among the non-Armenians. The allele frequency of M694V, M694I and M680I were almost two times more among Armenians (6%, 2.8% and 2.8%, respectively) compared to that found among non-Armenians (3.6%, 1.5% and 0.5%, respectively), however the difference was not statistically significant. The allele frequency of V726A (2.8% vs 2.6%, for Armenians and non-Armenians, respectively) and E148Q (5.6% vs 6.7%) were also similar between two study groups. Total allelic mutation frequency was 20% for Armenian and 15% for non-Armenians, ($p=0.188$). However, overall carrier rate of any of the *MEFV* mutations was significantly higher among the Armenians compared to that among non-Armenians (36% vs 20%, respectively, $p=0.015$).

We also analysed *MEFV* mutations of 5 Armenian FMF patients: 4 carried mutations (M680I/ M680I, M680I/V726A, M694I/V726A and E148Q/N).

Table IV. Distribution of 5 *MEFV* mutations, allele frequency and carrier rate among Armenians and non-Armenians.

Mutation	Allele mutation frequency n., (%)		
	Armenian group (n=216 alleles)	Non-Armenian group (n=194 alleles)	*p*-value
M694V	13 (6.0)	7 (3.6)	0.258
M694I	6 (2.8)	3 (1.5)	0.396
M680I	6 (2.8)	1 (0.5)	0.077
V726A	6 (2.8)	5 (2.6)	0.900
E148Q	12 (5.6)	13 (6.7)	0.628
M694V/E148Q	1 (0.5)	–	–
M694V/M694I	–	1 (0.5)	–
M694V/V726A	1 (0.5)	–	–
M694I/V726A	–	2 (1)	–
M694I/E148Q	–	1 (0.5)	–
M694V/M694I/E148Q	1 (0.5)	–	–
E148Q/E148Q/ V726A	–	1 (0.5)	–
Total allelic mutation frequency	43 (20.0)	29 (15.0%)	0.188
Overall carrier rate (mutation per individual)	37/108 (36%)	19/97 (20%)	0.015

Discussion

Our study indicated that the frequency of BS among the Armenians we studied was indeed less than that of FMF. This differs from what had been observed among the non-Armenian population in Turkey.

Five field surveys previously investigated the prevalence of BS in Turkey (1, 17-20) as shown in Table V. All were cross-sectional; population based multidisciplinary surveys with similar methodologies. They were conducted in different locations in Turkey and screened a population aged 10 years and over with similar methodology: the target population was questioned for recurrent oral ulceration at the first stage, and then those candidates with oral ulcers were examined for other manifestations of BS. Estimated prevalence rates in decreasing order were: 420/10^5 in Istanbul (1), 370/10^5 in a town of Northern Anatolia (17), 120/10^5 in Ankara, Central Anatolia (18), 80/10^5 in a distant suburb of Istanbul in Thrace (19) and 20/10^5 in a border town in Thrace (20). Apart from these, in a preliminary study our group found a prevalence rate of 500/10^5 for BS among hospital workers again in Istanbul (21) (Table V). It is seen that BS reaches its highest prevalence in Istanbul the

Table V. Prevalence studies of Behçet's syndrome (BS) and Familial Mediterranean Fever (FMF) carried out in Turkey.

Ref. no.	Studies by city / region	Age range of the screened population	Patients with BS n. (M/ F)	Population screened (n.)	BS prevalence per 10^{5*} (95% CI)
21	Istanbul / Marmara	>18	10 (6/4)	1.996	500 (190–810)
1	Istanbul / Marmara	>10	101 (52/49)	23.986	420 (340–500)
17	Ordu / Black Sea	>10	19 (6/13)	5.131	370 (200–540)
18	Ankara / M. Anatolia	>12	16 (5/11)	13.894	120 (60–180)
19	Silivri / Thrace	>10	4 (-)	4.960	80 (0–160)
20	Edirne / Thrace	>10	1 (-)	4.861	20 (-20–60)
Ref. no.	Studies by city / region	Age range of the screened population	Patients with FMF n (M/ F)	Population screened (n)	FMF prevalence per 10^{5*} (95% CI)
24	Tokat / Black Sea	>18	9 (3/6)	1.095	820 (290–1350)
25	Sivas / M. Anatolia	0–70	10 (8/2)	3948	250 (90–410)
26	Ankara / Around Turkey	18–22 (among men)	50 (50/0)	45.745	110 (80–140)
27	Ankara / M. Anatolia	≤16	33 (-)	35.284	93 (60–120)
28	Denizli / West Anatolia	Between 7–17	2 (2/0)	7.389	27 (-10–70)

* studies are listed by decreasing order of frequency. M.: middle. (-): undefined in the manuscript.

largest city with $13x10^6$ population in Turkey and seems to be less common in Thrace, the European part of Turkey, populated almost homogenously by the Balkan immigrant Turks.

In the current study, we observed a lower frequency of BS among the 10 years or older ethnic Armenians living in Istanbul ($110/10^5$) compared to that found previously in the general population of Istanbul, ($420\text{-}500/10^5$) (1, 21). It is to be noted that the current and the previous studies are not strictly comparable because of different methodologies. Nevertheless, the relatively low prevalence found in this ethnic population is indeed further evidence for a genetic predisposition to BS. This assumes that ethnic Armenians share the same environment and the living conditions with the general population. Our impression, admittedly informal, is that this was so in the population we studied.

An ethnic predilection to BS has been shown also in other studies (8, 22-23). This is quite similar to what is observed in FMF (11-13, 24-25). For example, the frequency of BS was found to be 50 times higher in Turkish immigrants compared to the Germans living in Berlin (8) and about 14 times higher in North-Africans compared to the native Europeans living in a suburb of Paris in France (22). Furthermore, BS is reported to be significantly more frequent among Druzes or Arabs compared to ethnic Jews living in Israel (23). On the other hand, in the previous studies the environmental influences like hygiene cannot be easily excluded, this being particularly true for the French and the Israeli studies (22-23).

We suggest like others that genetic factors are important in the disease mechanisms in BS (3, 5-6, 22-23, 26-28). In addition, we think that genetic contribution seems to be more important in FMF than in BS. Assuming similar biological pathways as they relate to the genetic load are also carried in the same frequency are operative in disease expression both in BS and FMF and the environmental influences are the same then one would have expected a higher frequency of disease expression in the more closed population, the Armenians in our case. However this, theoretically would have been true for either disease. On the other hand in our survey this was only true for FMF. This final point also suggests that BS and FMF are not closely linked even though one patient identified in the second part had concomitant BS and FMF.

The prevalence of FMF among the Armenians, reached the highest ever observed in Turkey. It was estimated as $870/10^5$ among only the parents in the first part and as $760/10^5$ among both parents and the offspring in the second. It was calculated as $860/10^5$ (Table III) again approaching the earlier figure, when only those of the second part who were ≥20 years old were included (22/2552).

Although no prevalence study of FMF is available among the general population of Istanbul, 5 field studies were done in other Turkish cities (29-33) as shown in Table V. Like those in BS, all were cross-sectional, population based and used similar methodology. Target population was first screened for the clinical manifestations of FMF by the help of a questionnaire, then those answered positive to the questionnaire were interviewed for the second time for a definite diagnosis. While all other 4 reported considerably lower frequencies, the prevalence reported in the adult population of Tokat (a city in Northern Anatolia) ($800/10^5$) was interestingly close to that we found among the ethnic Armenians (29). It is to be underlined that Tokat is an area heavily populated by Armenians in the recent past. Other FMF surveys reported lower rates such as $250/10^5$ in both adults and children combined in Sivas, Central Anatolia (30), $110/10^5$ among young men who underwent routine physical assessment for military service in 53 centres around Turkey (31), $93/10^5$ in a juvenile population in Ankara, Middle Anatolia (32) and $27/10^5$ among only primary school student population in Denizli, Western Anatolia (33).

BS risk seems to be increased in populations where the frequency of HLA-B51 is high (6). As seen in Table V, there are significant differences in prevalence of BS in different areas of Turkey. Unfortunately the HLA-B51 frequency in the normal population is not known except for Istanbul (25–27%) (27-28) and Antalya – a city in the Mediterranean region - (24%) (34).

HLA-B51 antigen frequency we found in both healthy populations (Armenians: 27% and non-Armenians: 19%) were in line with previous observations (27-28, 34). Verity *et al*., referring to a workshop in a review (6) gave a lower carrier rate for Armenians than found in our study (10.4%). On the other

hand a study made in Armenia reported a range of 26.0–32.5% for HLA-B5 (51+52), a carrier rate closer to our result (35). Since a similar carrier rate of HLA-B51 was observed between the two populations, it is possible that genetic susceptibility factors other than HLA may also play role (26, 36-37) in disease expression in BS. There may be also genetic role of various single nucleotide polymorphisms as previously demonstrated (38-44).

Although allele frequencies of *MEFV*-gene mutations were not significantly different between the two groups, the overall carrier rate for having any one of the 5 mutations surveyed was significantly higher among the Armenians (36% *vs*. 20%, p=0.015). On the other hand, allele frequencies among the non-Armenian group in this study were in line with what had previously been reported (45-48) while, in fact, a lower carrier rate (20%) has been reported among 250 healthy controls in Armenia (13).

Our method of investigating the family through an index student in primary schools is, as far as we know, quite unique. The definition of affected cases was also different than the usual. We only accepted those cases who were previously diagnosed with FMF or BS by a physician. Hence, we may have missed milder cases that had not been diagnosed by a physician or true BS cases that could not be diagnosed by a physician. This can result in underestimation of the actual rate. On the other hand, "self-reported doctor-diagnosed arthritis" has been thought to provide a good estimate of arthritis prevalence, with acceptable sensitivity and specificity as had been validated (49-50) and used (51) in other settings. We propose this method as a simple, cheap and time saving way of doing a field survey. As parents know their immediate family better, we suggest that the population studied should be limited to the first degree relatives.

Since our method was mainly based on volunteered information, we were initially worried that the parents were more likely to give better information about FMF as compared to BS, especially for the results of the first part of the study where the response rate was quiet low (37%). Our worry lessened; however, when the survey response rate became much higher (83%) in the second part where, also, nearly the same prevalence rates for both diseases with overlapping 95% confidence intervals were observed. In this line it is to be noted that the all new cases of BS identified in the second part of the study came from the new schools, suggesting that the low response rate was not a very important factor in the observed absence of parents in the first part. Our inclusion of only "physician-diagnosed" cases, we reason, also reduces the likelihood of the bias being discussed. On the other hand, we do not know the reasons behind the low response rate of the first part. There was no apparent difference with regard to genetic background, socioeconomic status and environmental conditions -factors that could perhaps affect susceptibility to BS- between the populations studied in Part 1 and Part 2. It could have been due to general lack of interest to our survey, because as we pointed out earlier, the response rate increased only upon our warning.

Another consideration worth noting is that all 4 patients indentified in Part 2. were males, while the male/female ratio for BS ranges from 0.5 to 1.5 in the non-Armenian populations listed in Table V. It is well recognised that BS is more severe among the males (52) and it might be suggested that among the Armenians the phenotype is expressed only when it is severe enough, in the male gender. This however is post-hoc reasoning and should further be validated.

Our study had other limitations. We used historic controls instead of non-Armenian population to compare the disease prevalence. This may need to be prospectively verified. The studied population may not be representative of the Armenians living in Istanbul, since we may have missed those who were not able to have a child due to infertility or miscarriages and those who were educated in Turkish schools. However, our informal observations strongly suggest that the latter condition is very infrequent. Furthermore these limitations would reasonably be true, for either disease. In addition, it is also possible that those with BS might have not openly declared their disease due to a potential misconception that BS might be venereal.

Our sample sizes for comparing the frequencies of HLA-B51 and the *MEFV* mutations between the healthy Armenians and the non-Armenian Turks from the same geography were small and thus underpowered to detect small but significant differences between the 2 populations. However to search for the more precise frequencies of HLA-B51 and the *MEFV* mutations among the Armenian families surveyed was not the primary aim of our study. The molecular genetic studies were done after Part I, with which we had the initial indication that BS was indeed less frequent among the Armenians. We aimed to assess whether there was any immediate explanation of what we observed in phenotype grossly reflected itself in the genotype. We reason that what we observed among the healthy Armenians and non-Armenians did not in any way challenge the validity of our observations. A. The lower frequency of BS we observed among the Armenians was most probably not due to a lower carrier frequency of HLA-B51 in this population in that what we actually observed was a higher frequency. B. The higher frequency of being a carrier of any one of the *MEFV* alleles among the Armenians was, on the other hand, supportive of the higher frequency of FMF we saw in this group. It is well appreciated that the genetic contribution of the *MEFV* alleles to the FMF phenotype (9-10) is stronger than that of HLA-B51 to the BS phenotype and our observations in the current study are reflective of this contention. It is known that the HLA-B51 contributes only to a fraction (19%) of the genetic load to BS (53). In the light of the current data we suggest, as we also discuss above in the context of disease expression in a closed versus a more open population, a yet undescribed genetic marker(s) is/are associated with BS and this (these) may be less frequent among the Armenians, which is not true for HLA-B51.

Finally we did not formally assess whether genetic inbreeding in this

small and closed community which might have affected our observations. Having said that, the Jewish population in Israel – a similarly closed community with more strict religious rules of marriage – has a lower prevalence of FMF (100-200/10^5) (54). Also prevalence rates found in our study were quite similar when mothers and fathers were separately calculated in both parts (results not given) indicating that a potential consanguinity bias would not be very likely.

In conclusion the lower frequency of BS among the Armenians when compared to the frequency among the general population living in the same environment, is further evidence for a genetic predisposition to BS. On the other hand the genetic load for FMF seems to be considerably higher among the Armenians when compared to the load for BS among the same ethnic group.

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References

1. AZIZLERLI G, KÖSE AA, SARICA R *et al*.: Prevalence of Behçet's disease in Istanbul, Turkey. *Int J Dermatol* 2003; 42: 803-6.
2. NISHIURA K, KOTAKE S, ICHIISHI A, MATSUDA H: Familial occurrence of Behçet's disease. *Jpn J Ophthalmol* 1996; 40: 255-9.
3. GUL A, INANC M, OCAL L, ARAL O, KONICE M: Familial aggregation of Behçet's disease in Turkey. *Ann Rheum Dis* 2000; 59: 622-5.
4. FRESKO I, SOY M, HAMURYUDAN V *et al*.: Genetic anticipation in Behçet's syndrome. *Ann Rheum Dis* 1998; 57: 45-8.
5. MOLINARI N, KONE PAUT I, MANNA R, DEMAILLE J, DAURES JP, TOUITOU I: Identification of an autosomal recessive mode of inheritance in paediatric Behçet's families by segregation analysis. *Am J Med Genet A* 2003; 122: 115-8.
6. VERITY DH, MARR JE, OHNO S, WALLACE GR, STANFORD MR: Behçet's disease, the Silk Road and HLA-B51: historical and geographical perspectives. *Tissue Antigens* 1999; 54: 213-20.
7. HIROHATA T, KURATSUNE M, NOMURA A, JIMI S: Prevalence of Behçet's syndrome in Hawaii with particular reference to the comparison of the Japanese in Hawaii and Japan. *Hawaii Med J* 1975; 34: 244-6.
8. ZOUBOULIS CC, KÖTTER I, DJAWARI D *et al*.: Epidemiological features of Adamantiades-Behçet's disease in Germany and in Europe. *Yonsei Med J* 1997; 38: 411-22.
9. THE INTERNATIONAL FMF CONSORTIUM: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. *Cell* 1997; 90: 797-807.
10. THE FRENCH FMF CONSORTIUM: A candidate gene for familial Mediterranean fever. *Nat Genet* 1997; 17: 25-31.
11. ROGERS DB, SHOHAT M, PETERSEN GM *et al*.: Familial Mediterranean fever in Armenians: autosomal recessive inheritance with high gene frequency. *Am J Med Genet* 1989; 34: 168-72.
12. CAZENEUVE C, SARKISIAN T, PÊCHEUX C *et al*.: MEFV-Gene analysis in armenian patients with Familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-genetic and therapeutic implications. *Am J Hum Genet* 1999; 65: 88-97.
13. SARKISIAN T, AJRAPETYAN H, SHAHSUVARYAN G: Molecular study of FMF patients in Armenia. *Curr Drug Targets Inflamm Allergy* 2005; 4: 113-6.
14. TOUITOU I: The spectrum of Familial Mediterranean Fever (FMF) mutations. *Eur J Hum Genet* 2001; 9: 473-83.
15. BERNOT A, DA SILVA C, PETIT JL *et al*.: Non-founder mutations in the MEFV gene establish this gene as the cause of familial Mediterranean fever (FMF). *Hum Mol Genet* 1998; 7: 1317-25.
16. YAZICI H, BIYIKLI M, VAN DER LINDEN S, SCHOUTEN HJ: The 'zero patient' design to compare the prevalences of rare diseases. *Rheumatology* (Oxford) 2001; 40: 121-2.
17. YURDAKUL S, GUNAYDIN I, TUZUN Y *et al*.: The prevalence of Behçet's syndrome in a rural area in northern Turkey. *J Rheumatol* 1988; 15: 820-2.
18. IDIL A, GURLER A, BOYVAT A *et al*.: The prevalence of Behçet's disease above the age of 10 years. The results of a pilot study conducted at the Park Primary Health Care Center in Ankara, Turkey. *Ophthalmic Epidemiol* 2002; 9: 325-31.
19. DEMIRHINDI O, YAZICI H, BINYILDIZ P *et al*.: The prevalence of Behçet's disease in Fener village (Silivri, Istanbul) and its surroundings [in Turkish]. *Cerrahapasa Tip Fak Derg* 1981; 12: 509-14.
20. CAKIR N, DERVIS E, BENIAN O *et al*.: Prevalence of Behçet's disease in rural western Turkey: a preliminary report. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S53-5.
21. COSGUN S, CELIK S, MELIKOGLU M *et al*.: Prevalence of Behçet's syndrome and rheumatoid arthritis among hospital workers. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S85.
22. MAHR A, BELARBI L, WECHSLER B *et al*.: Population-based prevalence study of Behçet's disease: differences by ethnic origin and low variation by age at immigration. *Arthritis Rheum* 2008; 58: 3951-9.
23. KRAUSE I, YANKEVICH A, FRASER A *et al*.: Prevalence and clinical aspects of Behçet's disease in the north of Israel. *Clin Rheumatol* 2007; 26: 555-60.
24. SAMUELS J, AKSENTIJEVICH I, TOROSYAN Y *et al*.: Familial Mediterranean fever at the millennium. Clinical spectrum, ancient mutations, and a survey of 100 American referrals to the National Institutes of Health. *Medicine* (Baltimore).1998; 77: 268-97.
25. SALEHZADEH F, JAHANGIRI S, EMAMI D, EMAMI D, SHIARI R: Familial Mediterranean fever in Iranian children. First report from Iran. *Clin Exp Rheumatol* 2010; 28: 287.
26. KARASNEH J, GÜL A, OLLIER WE, SILMAN AJ, WORTHINGTON J: Whole-genome screening for susceptibility genes in multicase families with Behçet's disease. *Arthritis Rheum* 2005; 52: 1836-42.
27. GÜL A, UYAR FA, INANÇ M *et al*.: A weak association of HLA-B*2702 with Behçet's disease. *Genes Immun* 2002; 3: 368-72.
28. YAZICI H, AKOKAN G, YALGIN B, MUFTUOGLU A: A high prevalence of HLA-B5 in Behçet's disease. *Clin Exp Immunol* 1977; 30: 259-61.
29. KISACIK B, YILDIRIM B, TASLIYURT T *et al*.: Increased frequency of familial Mediterranean fever in northern Turkey: a population-based study. *Rheumatol Int* 2009; 29: 1307-9.
30. ONEN F, SUMER H, TURKAY S, AKYUREK O, TUNCA M, OZDOGAN H: Increased frequency of familial Mediterranean fever in Central Anatolia, Turkey. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S31-3.
31. DINC A, PAY S, TURAN M, SIMSEK I: Prevalence of familial Mediterranean fever young Turkish men. *Clin Exp Rheumatol* 2000; 18: 292.
32. OZEN S, KARAASLAN Y, OZDEMIR O *et al*.: Prevalence of juvenile chronic arthritis and familial Mediterranean fever in Turkey: a field study. *J Rheumatol* 1998; 25: 2445-9.
33. COBANKARA V, FIDAN G, TÜRK T, ZENCIR M, COLAKOGLU M, OZEN S: The prevalence of familial Mediterranean fever in the Turkish province of Denizli: a field study with a zero patient design. *Clin Exp Rheumatol* 2004; 22 (Suppl. 34): S27-30.
34. ALPSOY E, YILMAZ E, COŞKUN M, SAVAŞ A, YEĞIN O: HLA antigens and linkage disequilibrium patterns in Turkish Behçet's patients. *J Dermatol* 1998; 25: 158-62.
35. NERSISYAN VM, MARTIROSYAN IG, MUSAELYAN NO, MELIKSETYAN NO: Distribution of HLA Antigens in the Armenian Population of Nagornyi Karabakh. *Russian Journal of Genetics* 2002; 38: 843-6.
36. LI K, ZHAO M, HOU S, DU L, KIJLSTRA A, YANG P: Association between polymorphisms of FCRL3, a non-HLA gene, and Behçet's disease in a Chinese population with ophthalmic manifestations. *Mol Vis* 2008; 14: 2136-42.
37. MIZUKI N, INOKO H, SUGIMURA K *et al*.: RFLP analysis in the TNF-beta gene and the susceptibility to alloreactive NK cells in Behçet's disease. *Invest Ophthalmol Vis Sci* 1992; 33: 3084-90.
38. BEN DHIFALLAH I, LACHHEB J, HOUMAN H, HAMZAOUI K: Toll-like-receptor gene polymorphisms in a Tunisian population with Behçet's disease. *Clin Exp Rheumatol* 2009; 27 (Suppl. 53): S58-62.
39. GUNESACAR R, ERKEN E, BOZKURT B *et al*.:

Analysis of CD28 and CTLA-4 gene polymorphisms in Turkish patients with Behçet's disease. *Int J Immunogenet* 2007; 34: 45-9.

40. WALLACE GR, KONDEATIS E, VAUGHAN RW *et al*.: IL-10 genotype analysis in patients with Behçet's disease. *Hum Immunol* 2007; 68: 122-7.
41. BARANATHAN V, STANFORD MR, VAUGHAN RW *et al*.: The association of the PTPN22 620W polymorphism with Behçet's disease. *Ann Rheum Dis* 2007; 66: 1531-3.
42. KARASNEH JA, HAJEER AH, SILMAN A, WORTHINGTON J, OLLIER WE, GÜL A: Polymorphisms in the endothelial nitric oxide synthase gene are associated with Behçet's disease. *Rheumatology* (Oxford). 2005; 44: 614-7.
43. JANG WC, NAM YH, AHN YC *et al*.: Interleukin-17F gene polymorphisms in Korean patients with Behçet's disease. *Rheumatol Int* 2008; 29: 173-8.
44. OZEN S: Mutations/polymorphisms in a monogenetic autoinflammatory disease may be susceptibility markers for certain rheumatic diseases: lessons from the bedside for the benchside. *Clin Exp Rheumatol* 2009; 27 (Suppl. 53): S29-31.
45. KOCA SS, ETEM EO, ISIK B *et al*.: Prevalence and significance of MEFV gene mutations in a cohort of patients with rheumatoid arthritis. *Joint Bone Spine* 2010; 77: 32-5.
46. YIGIT S, BAGCI H, OZKAYA O, OZDAMAR K, CENGIZ K, AKPOLAT T: MEFV mutations in patients with familial Mediterranean fever in the Black Sea region of Turkey: Samsun experience [corrected]. *J Rheumatol* 2008; 35: 106-13.
47. YILMAZ E, OZEN S, BALCI B *et al*.: Mutation frequency of Familial Mediterranean Fever and evidence for a high carrier rate in the Turkish population. *Eur J Hum Genet* 2001; 9: 553-5.
48. LACHMANN HJ, SENGÜL B, YAVUZŞEN TU *et al*.: Clinical and subclinical inflammation in patients with familial Mediterranean fever and in heterozygous carriers of MEFV mutations. *Rheumatology* (Oxford) 2006; 45: 746-50.
49. SACKS JJ, HARROLD LR, HELMICK CG, GURWITZ JH, EMANI S, YOOD RA: Validation of a surveillance case definition for arthritis. *J Rheumatol* 2005: 32: 340-7.
50. BOMBARD JM, POWELL KE, MARTIN LM, HELMICK CG, WILSON WH: Validity and reliability of self-reported arthritis: Georgia senior centers, 2000-2001. *Am J Prev Med* 2005; 28: 251-8.
51. HELMICK CG, FELSON DT, LAWRENCE RC *et al*.; NATIONAL ARTHRITIS DATA WORKGROUP: Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part I. *Arthritis Rheum* 2008; 58: 15-25.
52. KURAL-SEYAHI E, FRESKO I, SEYAHI N *et al*.: The long-term mortality and morbidity of Behçet syndrome: a 2-decade outcome survey of 387 patients followed at a dedicated center. *Medicine* (Baltimore) 2003; 82: 60-76.
53. GUL A, HAJEER AH, WORTHINGTON J, BARRETT JH, OLLIER WE, SILMAN AJ: Evidence for linkage of the HLA-B locus in Behçet's disease, obtained using the transmission disequilibrium test. *Arthritis Rheum* 2001; 44: 239-40.
54. BEN-CHETRIT E, TOUITOU I: Familial Mediterranean Fever in the world. *Arthritis Rheum* 2009; 61: 1447-53.

Appendix 1

We kindly ask you to answer the questions below prepared to measure your knowledge about Familial Mediterranean Fever and Behçet's syndrome. We thank you for your contribution.

1) Which one is common to see in Familial Mediterranean Fever?

a) Periodic abdominal pain with fever
b) Gallbladder infection
c) Hair loss
d) Cataract
e) I do not know

2) Which one describes best Familial Mediterranean Fever?

a) The disease begins in middle age or in the elderly with morning stiffness. Pain and swelling in the hand joints are common and may lead to hand deformity if untreated.
b) The disease starts usually in the childhood or between the first and second decade with episodic abdominal pain and fever. The attacks develop with unknown periodicity and usually last 1-3 days. The patient is normal between the attacks. Patients may also experience chest and or joint pain during the attacks.
c) The disease starts usually in women of childbearing age. Fatigue, fever, joint pain and weight loss are the most common complaints. An erythematous rash can be seen on the cheeks. The disease may involve multiple organ systems including kidneys and nervous system.
d) The disease starts in the 2nd or 3rd decade. It is characterized by recurrent oral and genital ulcers. Acne like lesions and red nodular lesions are also frequently seen on the extremities. Sight threatening eye disease may be seen in half of the patients.
e) I don't know.

3) Which one is infrequently seen in Behçet's syndrome?

a) Recurrent oral ulcers
b) Genital ulcers
c) Eye disease and or blindness
d) Extremity vein thromboses
e) Periodic abdominal pain with fever
f) I don't know.

4) Which one describes best Behçet's syndrome?

a) The disease starts in the 2nd or 3rd decade. It is characterized by recurrent oral and genital ulcers. Acne like lesions and red nodular lesions are also frequently seen on the extremities. Sight threatening eye disease may be seen in half of the patients.
b) The disease begins in middle age or in the elderly with morning stiffness. Pain and swelling in the hand joints are common and may lead to hand deformity if untreated.
c) The disease starts usually in the childhood or between the first and second decade with episodic abdominal pain and fever. The attacks develop with unknown periodicity and usually last 1-3 days. The patient is normal between the attacks. Patients may also experience chest and or joint pain during the attacks.
d) The disease is most common in middle aged men. It is also called as 'gout'. It can cause sudden burning pain, redness and swelling in a joint, usually a big toe.
e) I don't know.