Association of mitochondrial polymorphism m.709G>A with Behçet's disease

The involvement of nuclear genes in Behçet's disease (BD) risk has been investigated, but the role of the mitochondrial DNA (mtDNA) has been completely neglected. Mitochondria are the main intracellular source of reactive oxygen species produced during normal aerobic metabolism via the electron transport chain and since mitochondrial dysfunction may underlie a multitude of clinical features in multifactorial and multisystemic diseases such as BD, we assessed whether mtDNA single nucleotide polymorphisms (SNPs) and haplogroups confer susceptibility to BD.

A total of 615 patients and 434 controls from Iran were enrolled in this study. BD diagnosis was made according to the revised International Criteria for Behçet's Disease[1] (ICBD cases). A total of 494 patients also fulfilled the International Study Group[2] criteria for diagnosis of BD (ISG cases). We genotyped 19 mtDNA SNPs which are sufficient for classifying our Iranian cohort into their most prevalent haplogroups: West Eurasian R0, H, V, J, T, U, K, N1, N1e'I, I, X and W haplogroups; Eastern Eurasian macrohaplogroup M, D, N (except for haplogroups N1, N1e'I, I, W and X) and R (lineages except R0, JT and UK) haplogroups; and African L haplogroup (figure 1).[3–6] Using a panel of 89 autosomal ancestry informative markers, no evidence of population admixture was found in our cohort

	SNP ID	m.709G>A	m.1719G>A	m.4216T>C	m.4580G>A	m.5178C>A	m.7028T>C	m.8251G>A	m.8701A>G	m.9055G>A	m.10034T>C	m.10398A>G	m.10400C>T	m.10873T>C	m.11719G>A	m.12308A>G	m.12705C>T	m.13368G>A	m.14470T>C	m.15607A>G	
	SNP type	ncod	ncod	p.Y304H	syn	p.L237M	syn	syn	p.T59A	p.A177T	ncod	p.T114A	syn	syn	syn	ncod	syn	syn	syn	syn	Haplogroup frequency (%)
Haplogroup	H						C								G		C				16.4
	R0				G		T								G		C				13.6
	V				A		T								G						0.0
	J	G	G	C								G			A		C	G			14.0
	T	A		C			T					A			A		C	A		G	9.1
	U						T		A	G				T		G	C			A	20.6
	K								A	A						G	C			A	6.5
	R*					C			A					T	A	A	C			A	2.1
	N*		G					G	A		T					A	T		T		1.2
	N1		A					G	A		T	A					T				0.9
	N1e'I		A						A		T						T				2.1
	I		A					A	A		C	G					T				3.0
	W	A	G					A	A			A				A	T				2.6
	X								A								T		C		1.6
	L								G		T	G	C	C			T				0.7
	M*					C			G		T		T	C			T				4.0
	D					A							T	C							1.6
SNP frequency (%)	Controls	14.5	7.0	23.4	0.0	1.6	83.2	7.5	6.3	7.0	3.0	29.0	5.6	6.3	70.1	27.1	17.5	9.3	1.9	9.3	
	ICBD cases	19.6	7.2	24.5	0.3	1.5	80.9	7.9	5.1	7.9	2.6	28.0	4.6	5.2	69.7	25.5	16.5	11.3	2.1	11.0	
	ISG cases	21.4	6.9	25.5	0.4	1.2	82.3	8.4	4.9	7.3	2.2	27.3	4.3	4.9	70.5	24.6	17.1	12.0	2.4	11.6	
ICBD cases vs controls	p (unadjusted)	**0.038**	0.980	0.714	0.641	0.963	0.299	0.914	0.474	0.696	0.832	0.782	0.548	0.550	0.952	0.631	0.778	0.365	0.945	0.458	
	OR (95% CI)	1.46 (1.03 to 2.02)																			
	p (adjusted)	0.053	0.997	0.665	0.972	0.859	0.296	0.667	0.436	0.635	0.843	0.836	0.514	0.517	0.959	0.562	0.862	0.394	0.856	0.503	
	OR (95% CI)	1.40 (1.00 to 1.97)																			
ISG cases vs controls	p (unadjusted)	**0.007**	0.924	0.459	0.537	0.807	0.690	0.702	0.442	0.905	0.595	0.683	0.439	0.446	0.784	0.449	0.972	0.213	0.704	0.292	
	OR (95% CI)	1.61 (1.14 to 2.27)																			
	p (adjusted)	**0.013**	0.890	0.505	0.971	0.759	0.585	0.536	0.395	0.839	0.579	0.671	0.413	0.437	0.867	0.397	0.981	0.238	0.585	0.330	
	OR (95% CI)	1.56 (1.10 to 2.21)																			

R*: all lineages inside haplogroup R except for haplogroups JT and UK; N*: all lineages inside haplogroup N except for haplogroups N1, I, W and X; M*: macro-haplogroup M except for haplogroup D.

Figure 1 Characterisation and association of the investigated mitochondrial markers and haplogroups. Each haplogroup was determined by the combination of alleles in bold, and the alleles not in bold aided in the phylogenetic assignment. The polymorphisms are named after their base pair position and alleles. The type of the variant is indicated as 'ncod' for non-coding single nucleotide polymorphisms (SNPs), 'syn' for synonymous SNPs, and the amino acid substitution is also shown for non-synonymous SNPs. The haplogroup frequencies in controls and the SNP frequencies of the derived allele (second allele in the SNP ID) are indicated. The results of mitochondrial SNP association testing with Behçet's disease risk using the ICBD cases or ISG cases are shown. Unadjusted and gender-adjusted p values are presented and significant p values (<0.05) are highlighted in bold. ORs and 95% CIs are shown only for allele A of m.709G>A. ICBD, International Criteria for Behçet's Disease; ISG, International Study Group Behçet's Disease.

(data not shown). Extensive genotyping quality control checks were implemented.

The associations with BD risk were assessed using Pearson's χ^2 tests and logistic regression analyses with gender as a covariate. Each haplogroup was compared with all other haplogroups pooled together. Results were considered significant below the conventional level of p=0.05. Since some of the markers are in linkage disequilibrium and the haplogroup comparisons are not independent, we did not perform corrections for multiple testing and uncorrected p values are reported.

The general characteristics (table 1) and the observed haplogroup frequencies (figure 1) in our cohort are in agreement with those previously reported in the Iranian population.[7 8] In the ICBD dataset, m.709G>A (A allele in 14.5% of controls, 19.6% of ICBD cases) was significantly associated with BD prior (p=0.038) and marginally after adjustment for gender (p=0.053). In ISG cases, m.709G>A was also the only marker associated with BD prior (p=0.007, A allele in 21.4% of ISG cases) and after adjustment for gender (p=0.013). This marker does not define any haplogroup by itself. None of the haplogroups tested showed an association with BD risk.

Our findings link the mtDNA m.709G>A non-coding variant in the 12S rRNA (MT-RNR1) locus with risk for BD. Consistent with the multisystemic nature and the gene–environmental interaction of BD, ribosomal RNAs are among the very few genes present in all cells. 12S rRNA is a 959-nucleotide molecule participating in the assembly of amino acids into functional proteins. Mutations in MT-RNR1 are known to cause maternally inherited non-syndromic and antibiotic-induced deafness. Further investigation is required to determine if m.709G>A contributes to BD susceptibility by impairing the ability of mitochondria to produce proteins and enhancing oxidative stress or through another mechanism. Increased oxidative stress and impaired antioxidant defence system observed in blood of patients with BD[9 10] may significantly contribute to the disease pathophysiology.

Interestingly, we found a stronger association of mtDNA SNP m.709G>A with BD in the subset of samples fulfilling the more specific ISG criteria than in the entire dataset of BD cases, supporting the notion that the ISG subset may be a genetically more homogeneous group of cases.

Table 1 General characteristics of the study sample

Characteristic	Controls	ICBD cases	ISG cases
N	434	615	494
Gender, n/N (% males)	407/434 (93.8)	498/615 (81.0)	409/494 (82.8)
Mean age at examination, years±SD	41.4±11.6	38.7±10.4	39.2±10.7
Mean age at diagnosis, years±SD	–	31.5±8.5	31.9±8.9
Oral aphtosis, n/N (%)	92/434 (21.2)	607/615 (98.7)	494/494 (100)
Genital aphtosis, n/N (%)	0/434 (0.0)	372/615 (60.5)	343/494 (69.4)
Skin lesions, n/N (%)		364/615 (59.2)	357/494 (72.3)
Pseudofolliculitis		290/615 (47.2)	286/494 (57.9)
Erythema nodosum		133/615 (21.6)	129/494 (26.1)
Skin aphtosis		18/615 (2.9)	17/494 (3.4)
Ophthalmological manifestations, n/N (%)		399/615 (64.9)	312/494 (63.2)
Anterior uveitis		290/615 (47.2)	221/494 (44.7)
Posterior uveitis		341/615 (55.4)	264/494 (53.4)
Retinal vasculitis		235/615 (38.2)	192/494 (38.9)
Joint manifestations, n/N (%)		205/615 (33.3)	175/494 (35.4)
Arthralgia		84/615 (13.7)	66/494 (13.4)
Arthritis		133/615 (21.6)	120/494 (24.3)
Ankylosing spondylitis		12/615 (2.0)	8/494 (1.6)
Neurological manifestations, n/N (%)		38/615 (6.2)	33/494 (6.7)
Vascular involvement, n/N (%)		38/615 (6.2)	33/494 (6.7)
Gastrointestinal manifestations, n/N (%)		17/615 (2.8)	16/494 (3.2)
Epididymitis, n/N (%)		21/498 (4.2)	18/409 (4.4)
Cardiac involvement, n/N (%)		4/615 (0.7)	4/494 (0.8)
Pleuropulmonary involvement, n/N (%)		5/615 (0.8)	5/494 (1.0)
Pathergy phenomenon, n/N (%)		281/603 (46.6)	275/483 (56.9)
Family history of Behçet's disease, n/N (%)		52/586 (8.9)	40/468 (8.5)

ICBD, International Criteria for Behçet's Disease; ISG, International Study Group Behçet's Disease.

This study links for the first time the mtDNA with BD susceptibility and suggests that, in addition to multiple nuclear genes and environmental contributions, BD risk might also be governed by mitochondrial genomic background. This preliminary association warrants further validation and investigation in BD and in other rheumatic diseases.

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Acknowledgements We thank Dr Majid Zeidi, Iranian Blood Transfusion Organization, for his valuable support. We also thank to Doctor Sirous Zeinali, Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran and Doctor Kayvan Saeedfar for their valuable help. We are also deeply grateful to all study participants and to the genotyping unit at the Instituto Gulbenkian de Ciência.

Funding This work was supported by the grant from the Research Committee of the Tehran University of Medical Sciences under the registration number 132/714, the PTDC/SAU-GMG/098937/2008 grant and a doctoral fellowship (JMX) from the Portuguese Fundação para a Ciência e a Tecnologia, and a fellowship from the Portuguese Instituto do Emprego e Formação Profissional (JMX).

Competing interests None.

Patient consent Obtained.

Ethics approval This study received ethics approval from the ethics committees at the Rheumatology Center, Tehran University for Medical Sciences, Iran, and from the Portuguese Institute of Rheumatology, Lisbon, Portugal.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 23 January 2011
Published Online First 22 February 2011

Ann Rheum Dis 2011;**70**:1514–1516.
doi:10.1136/ard.2010.143537

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Ann Rheum Dis 2011 70: 1514-1516 originally published online February 22, 2011
doi: 10.1136/ard.2010.143537

Notes