Association of Mitochondrial Haplogroups H and R With Keratoconus in Saudi Arabian Patients

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Purpose. Keratoconic corneas exhibit more mitochondrial DNA (mtDNA) damage than do normal corneas and thus mtDNA may represent a potential candidate for genetic susceptibility studies in keratoconus. To test this hypothesis we determined mitochondrial haplogroups in Saudi patients with keratoconus and healthy controls of same ethnicity.

Methods. Mitochondrial haplogrouping was performed by polymerase chain reaction–based automated Sanger sequencing in 114 patients with keratoconus and 552 healthy controls.

Results. Mitochondrial haplogroups H and R were significantly overrepresented in patients with keratoconus (28.9% vs. 8.5%, $P < 0.0001$ and 17.5% vs. 3.1%, $P < 0.0001$, respectively) as compared to healthy controls.

Conclusions. Our data suggest that individuals with mitochondrial haplogroups H and R are at increased risk to develop keratoconus. In addition, the results provide further evidence for a plausible role of mtDNA in keratoconus etiology.

Keywords: keratoconus, cornea, mitochondria, haplogroup, Arabs, genetics

Keratoconus (OMIM 148300) is a corneal ectatic disease characterized by the noninflammatory thinning and anterior corneal protrusion of corneal stroma, resulting in bilateral and asymmetrical corneal distortion, altered refractive power, and reduced vision.[1,2] The estimated incidence of keratoconus varies between 1/500 to 1/2000 in the general population worldwide.[3] It is a multifactorial disease and the pathogenesis may involve genetic,[2,4] environmental, and behavioral factors.[5,6] Most cases of keratoconus are sporadic, but a proportion (5%–10%) are familial.[7,8] In the latter cases, both autosomal recessive and dominant patterns of inheritance have been reported.[9–11] Although the exact etiology of keratoconus is not known, the involvement of oxidative stress in this disease has been reported.[12,13] However, the underlying mechanisms of oxidative damage in keratoconus corneas are still unclear.

Keratoconus corneas exhibit more mitochondrial DNA (mtDNA) damage than do normal corneas.[14] Earlier studies have reported the role of mitochondria as mediators of oxidative damage in aging and human diseases,[15] and recently we have shown the possible role of mtDNA mutations in keratoconus pathogenesis.[16]

In human genetics, a human mtDNA haplogroup is a haplogroup defined by differences in human mtDNA. Haplogroups are used to represent the major branch points on the mitochondrial phylogenetic tree. During evolution, several mutations have accumulated in mtDNA, including ethnic-specific single-nucleotide polymorphisms (SNPs) that have allowed human populations to be categorized into various mtDNA haplogroups. Understanding the evolutionary path of the female lineage has helped population geneticists trace the matrilineal inheritance of modern humans back to human origins in Africa and the subsequent spread across the globe.

The letter names of the haplogroups run from A to Z. As haplogroups are named in the order of their discovery, they do not reflect the actual genetic relationships.

In certain populations, these haplogroups confer resistance against type 2 diabetes,[17] influence energy-dependent processes such as sperm motility and the risk of developing late-onset neurodegenerative diseases,[15] and contribute to the development of various types of cancer,[18] Parkinson disease,[19] and multiple sclerosis.[20] We have previously reported the association of various mitochondrial haplogroups in the different types of glaucoma.[21] In this study we investigated the possible role of mitochondrial haplogroups in keratoconus among patients from Saudi Arabia.

Materials and Methods

Patients and Controls

The study adhered to the tenets of the Declaration of Helsinki, and all participants signed an informed consent. The study was approved by the College of Medicine (King Saud University, Riyadh, Saudi Arabia) Ethical Committee (proposal No. 09–659). All study subjects were self-identified of Saudi Arabian ethnicity. Family names were all present in the database of Arab families of Saudi Arabian origin. Patients were selected from the anterior segment clinic at King Abdulaziz University Hospital after examination. Patients were diagnosed with keratoconus if the Schimpff-flow–based elevation map showed posterior corneal elevation within the central 5 mm $\geq +20$ μm, the

Table 1. Haplogroup Distribution in Glaucoma Patients and Controls

Mitochondrial Haplogroup	Controls, $n = 552$, No. (%)	Cases, $n = 114$, No. (%)	Odds Ratio	95% CI	*P* Value*
H	47 (8.5)	33 (28.9)	4.37	2.64–7.24	**<0.0001**
I	5 (0.9)	0	0.43	0.024–7.91	0.57
J	116 (21)	23 (20.1)	0.95	0.57–1.56	0.84
K	22 (4)	1 (0.9)	0.21	0.03–1.60	0.13
L0	6 (1.1)	0	0.36	0.02–6.56	0.49
L1	3 (0.5)	1 (0.9)	1.61	0.17–15.71	0.67
L2	20 (3.6)	5 (4.4)	1.22	0.45–3.32	0.69
L3	22 (4)	9 (7.9)	2.06	0.92–4.61	0.08
L4	1 (0.2)	3 (2.6)	14.89	1.53–144.48	0.02
L5	4 (0.7)	0	0.53	0.03–9.95	0.67
M	17 (3.1)	0	0.13	0.008–2.24	0.16
M1	19 (3.5)	0	0.12	0.007–1.99	0.14
N	41 (7.4)	10 (8.8)	1.19	0.58–2.46	0.62
preHV1	99 (17.9)	0	0.02	0.001–0.32	0.006
R	17 (3.1)	20 (17.5)	6.69	3.38–13.25	**<0.0001**
T	34 (6.2)	0	0.065	0.004–1.08	0.056
U	58 (10.5)	9 (7.9)	0.73	0.35–1.52	0.40
W	6 (1.1)	0	0.36	0.02–6.56	0.50
X	15 (2.7)	0	0.15	0.009–2.55	0.19

* Since we have 19 mitochondrial haplogroups, the Bonferroni correction should be $0.05/19 = 0.0026$. Therefore, a P value < 0.0026 was considered significant. When comparing the haplogroup distribution among each of the three different glaucoma groups with the controls, the threshold was further reduced to 0.00087**.** The only significant P values are in bold.

inferior–superior dioptric asymmetry value > 1.2 diopters (D), and the steepest keratometry > 47 D. Patients were considered as sporadic cases after examining the immediate family members and identifying the patient as an isolated case of keratoconus. Age range for keratoconus patients was 34 ± 6 years. The exclusion criteria were based on the presence of post–laser-assisted in situ keratomileusis (LASIK) ectasia and refusal to participate. All keratoconus cases secondary to causes such as trauma, surgery, Ehlers-Danlos syndrome, osteogenesis imperfecta, and pellucid marginal degeneration were excluded from the study. All keratoconus patients were from different parts of Saudi Arabia; as judging by family names, they cover all five provinces of Saudi Arabia.

Controls ($n = 552$) were recruited from the general ophthalmology clinic and had no ocular disease(s) or previous ophthalmic surgeries. Their slit lamp examination showed clear cornea and their Schimpff-flow–based elevation map was within normal limits. Controls were from the five major provinces of Saudi Arabia.

Sequencing Analyses of Haplogroup Diagnostic Positions

Mitochondrial haplogrouping was performed as described previously.[21] To detect coding-region diagnostic haplogroup polymorphisms, a fragment spanning the diagnostic position was amplified by using any of the 32 overlapping pairs of primers that cover the whole mtDNA genome, with the PCR conditions previously published.[22] However, a polymorphism at nucleotide position 12,308 was amplified by using a reverse mismatch primer as described by Torroni et al.[23] The amplified fragments were analyzed by Sanger sequencing. For Eurasian haplogroups (H, HV, preHV1, J, T, R, U, K, I, N, X, and M) diagnostic positions were recompiled from Richards et al.[24]; for African haplogroups L0, L1, and L3, from Chen et al.[25]; and for L2, L4, and L5, from Kivisild et al.[26] Finally, diagnostic positions for preHV1 were taken from Abu-Amero et al.[27] and for M1, from Gonzalez et al.[28]

Data Analysis

The frequency of each haplogroup among cases and controls was compared with the χ^2 test (Fisher's exact test where appropriate), and the risk of having the disease if you have a certain haplogroup as compared to not having that specific haplogroup was estimated by computing the odds ratio and its 95% confidence interval. A P value less than 0.05 was considered significant. Bonferroni correction was used to adjust the significance level of a statistical test to protect against type I errors when multiple comparisons were being made. Since we have 19 mitochondrial haplogroups, the Bonferroni correction should be $0.05/19 = 0.0026$. Therefore, a P value less than 0.0026 was considered significant.

Results

Our study cohort consisted of 114 unrelated keratoconus patients (72 males and 45 females) of Saudi ethnicity. The family name data indicated that all patients were from different parts of Saudi Arabia, confirming their Saudi origin. Of the 114 keratoconus cases, 17 were found to be familial and 107 were sporadic cases. The control group included 552 unrelated healthy individuals free of keratoconus and any other ophthalmic diseases as established by extensive eye examination. Table 1 shows the mitochondrial haplogroup distribution among the keratoconus patients and controls. There was no statistically significant difference between patients and controls for all mitochondrial haplogroups tested except for mitochondrial haplogroups H ($P < 0.0001$) and R ($P < 0.0001$). Table 2 summarizes almost all literature available regarding various mitochondrial haplogroups and their associated disease risk, including the findings from this study.

Discussion

Keratoconus is a complex condition of multifactorial etiology. Both genetic and environmental factors are associated with keratoconus. Evidence of genetic etiology includes the

Table 2. Different Mitochondrial Haplogroups and Their Disease Risk

Mitochondrial Haplogroup	Population	Disease Risk	Reference
H	Spanish	Ischemic cardiomyopathy	34
H	Iranian (Persian)	Alzheimer's disease	35
H	Australian	Age-related macular degeneration	36
H	Polish	Breast cancer	41
I	Polish	Breast cancer	41
J	Spanish	Ischemic cardiomyopathy	34
J	Caucasian	Parkinson disease	42
J	Various populations	Leber hereditary optic neuropathy	33,40
K	Caucasian	Parkinson disease	42
K	Iranian (Persian)	Multiple sclerosis	43
K	European	Breast cancer	44
L, L2	Saudi Arabian	Primary open angle glaucoma	32
		Pseudoexfoliation glaucoma	31
M	Chinese	Breast cancer	45
N	Indian	Breast and esophageal cancer	46
N9a	Japanese	Type 2 diabetes	17
HV cluster	Polish	Alzheimer's disease	47
R	Han Chinese	Severe sepsis	37
T	Saudi Arabian	Pseudoexfoliation glaucoma	31
T	Austrian	Coronary artery disease	48
		Diabetic retinopathy	
U	North American white	Prostate cancer	49
		Renal cancer	
U	Iranian (Persian)	Alzheimer's disease	35
U	European	Alzheimer's disease	50
U	European-American	Breast cancer	44
U	Australian	Retinal pigment abnormalities	36
A	Iranian (Persian)	Multiple sclerosis	43
H	Saudi Arabian	Keratoconus	This study
R	Saudi Arabian	Keratoconus	This study

conditions of familial inheritance, discordance between dizygotic twins, and its association with other known genetic disorders. Environmental factors include contact lens wear, chronic eye rubbing, and atopy of the eye. Despite being a condition with multifactorial etiology, there are several chromosomal loci and genes reported to be associated with keratoconus,[7] some of which were eventually excluded, while others showed no confirmed association with the disease. This is not the case for the visual system homebox 1 (*VSX1*) gene: mutations associated with keratoconus cases have been found in different studies, although other studies have not found *VSX1* mutations in cohorts of keratoconus patients from various populations.[29] This indicates that keratoconus is a complex condition of multifactorial etiology and that mutations in the *VSX1* gene do not account for all the cases of keratoconus. We previously investigated whether Saudi patients with keratoconus have mutations in the *VSX1* gene; however, the study failed to show any association.[29] Additionally, we could not detect any crucial abnormalities in a group of Saudi patients with isolated keratoconus.[30]

Table 3. Possible Time and Place of Origin for Various Mitochondrial Haplogroups

Haplogroup	Possible Time of Origin	Possible Place of Origin
H	>35,000 years ago	Western Asia
I	30,000 years ago	Caucasus or North-East Europe
J	45,000 years ago	Near East or Caucasus
K	16,000 years ago	Near East
L0	160,000 years ago	East Africa
L1	>120,000 years ago	Africa
L2	87,000–107,000 years ago	East and North Africa
L3	84,000–104,000 years ago	East Africa and Arabia
L4	75,000 years ago	East Africa and Horn of Africa
L5	62,000–70,000 years ago	Tanzania, Kenya, Sudan, Nubia (Egypt), Saudi Arabia
M	60,000 years ago	Africa and Eurasia
M1	40,000 years ago	Eurasia
N	75,000 years ago	India or South Asia
preHV1	50,000 years ago	Near East
R	70,000 years ago	India or South Asia
T	17,000 years ago	Mesopotamia
U	60,000 years ago	North-East Africa or South-West Asia
W	25,000 years ago	North-East Europe or North-West Asia
X	>30,000 years ago	North-East Europe

Although the underlying mechanisms of oxidative damage in keratoconus are not yet completely elucidated, there is evidence of critical involvement of oxidative stress in the etiology of this disease.[12,13] Studies have shown that there is increased mtDNA damage in keratoconus corneas compared to normal corneas.[14] We have recently reported the presence of potentially pathogenic mtDNA mutations in a group of keratoconus patients from Saudi Arabia,[16] thus enforcing the notion that oxidative stress may play a role in keratoconus pathogenesis.

In addition to mtDNA mutations, mtDNA haplogroups have been shown to be associated with various diseases (see Table 2) including ophthalmic diseases such as glaucoma[21,31,32] and Leber hereditary optic neuropathy (LHON).[33] Here we investigated the possible association of mitochondrial haplogroups with keratoconus. We found that mitochondrial haplogroups H and R were significantly associated with keratoconus in our population. Mitochondrial haplogroup H is believed to have originated more than 35,000 years ago and possibly from Western Asia (Table 3). Mitochondrial haplogroup H has been reported as a disease risk for ischemic cardiomyopathy,[34] Alzheimer's disease,[35] and age-related macular degeneration,[36] whereas mitochondrial haplogroup R has been reported to be a disease risk for severe sepsis in Han Chinese[37]; the latter haplogroup is believed to have originated more than 70,000 years ago in India and South Asia.

Table 2 shows updated information of disease risk with various mitochondrial haplogroups and clearly indicates that there is considerable research in this area. However, the underlying mechanism(s) by which mitochondrial haplogroups contribute to disease development is not yet known and more research is needed to unveil the exact cause. One plausible

hypothesis is that the polymorphism(s) in the mtDNA sequence defining the different mtDNA haplotype could be causative factors in disease development. A good example supporting this hypothesis comes from patients with LHON who harbor one of the three primary LHON mutations and are also found to belong to mitochondrial haplogroup J. This indicates that mitochondrial haplogroup J is a risk factor for LHON and that mtDNA sequence polymorphisms defining mitochondrial haplogroup J (4216C, 13708A, 15452A) act as background genetic factors modulating LHON development in the presence of 11778 or 3416 primary LHON mutations.[33,38,39] Our previous study indicates that all LHON patients with 11778 and 3416 primary LHON mutations belonged to mitochondrial haplogroup J.[33] Another possibility is that this phenomenon can be caused by a founder effect. This seems very likely for the T14484C primary LHON mutation, where approximately 75% of cases belong to haplogroup J.[40] However, to unveil the genetic and physiologic causes of these associations, further research is needed. We further believe that the incorporation of the mtDNA SNPs, which define the most important haplogroups worldwide, into the genome-wide association studies would help to detect those nuclear–mitochondrial gene interactions that predispose to or protect from illnesses against polygenic diseases such as keratoconus.

Although haplogroup testing is not a routine procedure, we think that in the future, it should be added to the required tests to support the diagnosis of keratoconus for individuals whose clinical and topographic picture is not conclusive. Furthermore, finding group H or R in a Saudi individual during haplogrouping might warrant further ophthalmic testing, including clinical examination, refraction, and corneal topography, to detect any changes of keratoconus if the individual is not already known to have keratoconus. This is especially emphasized for adolescent age groups where adolescents might have early subclinical keratoconus and might benefit from corneal collagen cross-linking to arrest the disease before it progresses. In addition, haplogroup testing in family members of a keratoconus patient might help with other ophthalmic testing to identify persons at risk, especially children, and to observe them closely as they grow into adolescence when keratoconus usually manifests. Therefore, we advise haplogroup testing for all the children of a keratonus patient and watching more closely for those having groups H and R.

Another thing to consider in an individual with an H or R haplogroup who seeks corneal refractive surgery (e.g., LASIK) and has a suspicious corneal topography of keratoconus changes, is that it is safer to avoid the corneal refractive surgery to prevent the possible aggravation of the keratoconic changes, ending in the devastating post-LASIK frank ecstasia. Those individuals might be better advised to undergo non-corneal refractive surgery, such as phakic intraocular lenses or refractive lens exchange, if they insist on surgery. In conclusion, we found that H and R mitochondrial haplogroups confer susceptibility to keratoconus. We report a fairly small group of patients from a restricted ethnic population, and this type of evaluation needs to be repeated in other populations and in larger cohorts in order to establish a relationship. If confirmed, these findings will be extremely helpful for early intervention and better management of keratoconus patients.

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