Corneal endothelial cells and central corneal thickness in patients with neurofibromatosis type 1

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Purpose: The aim of this study was to evaluate the morphological properties of corneal endothelial cells and central corneal thickness (CCT) in patients with neurofibromatosis type 1 (NF1) and to compare them with age-matched healthy controls. **Methods:** Nineteen NF1 patients and 38 healthy individuals were recruited. All participants underwent complete ophthalmological examination as well as noncontact specular microscopy to measure endothelial cell density (ECD), average cell area (AVG), coefficient of variation of cell area (CV), the percentage of hexagonal cells, and CCT. Eyes with previous ocular trauma, inflammation or surgery, and preexisting corneal and ocular surface diseases were excluded. **Results:** NF1 patients had higher ECD compared to healthy controls of the same age (2764.2 ± 270.4 versus 2570.4 ± 449.2 cells/mm^2, respectively), although at a borderline level (P = 0.051). Patients with NF1 presented significantly lower CV and AVG when compared to controls (32.9 ± 4.6 versus 37.8 ± 9.5%, P = 0.011 and 364.9 ± 34.4 versus 406.0 ± 107.4 μm^2, P = 0.038, respectively). The NF1 group had significantly higher hexagonality in comparison with controls (55.7 ± 6.5 versus 50.5 ± 9.9%, P = 0.025). CCT was similar between the two groups (P = 0.955). **Conclusion:** Our results show that corneal endothelium has more favorable morphological characteristics in NF1 patients compared to healthy individuals of the same age.

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Neurofibromatosis type 1 (NF1), also known as von-Recklinghausen disease, or peripheral neurofibromatosis, is one of the most common autosomal dominant diseases encountered in clinical practice.[1] With a penetrance of essentially 100% and spontaneous mutations causing 50% of cases,[2] NF1 affects approximately one in 2500 births.[3] It is caused by mutations in the tumor suppressor gene NF1, which is located on chromosome 17q11.2, encoding the neurofibromin protein that is mainly expressed in neurons, nonmyelinated Schwann cells, astrocytes, leukocytes and oligodendrocytes.[4,5] This is a 220 kDa guanosine triphosphate (GTP) ase-activating cytoplasmic protein that regulates multiple growth control pathways, including the Rat Sarcome (RAS) and its downstream elements, such as the mitogen-activated protein kinase (MAPK) signaling pathway.[6] Nonfunctional neurofibromin results in an excess of the RAS-GTP active form, promoting excessive cell growth and leading to dysregulation and tumorigenesis.[7]

Patients with NF1 have defects in neural-crest derived tissues, leading to a wide spectrum of clinical presentations, including developmental, pigment or neoplastic aberrations of the skin, nervous system, bones, endocrine organs, blood vessels and the eyes.[8] Lisch nodules, the most common feature of the anterior segment of the eye,[9] as well as optic pathway gliomas and plexiform neurofibromas are included in the diagnostic criteria of NF1.[10] A characteristic hypertrophy of corneal intrastromal nerves also known as "lignes grises" is also associated with NF1.[11] Recently, abnormal corneal fiber length was found in patients with NF1 suggesting that small-fiber neuropathy may be common in this population.[12]

Corneal endothelium, the cell layer with the lowest mitotic activity,[13] is embryologically derived from the neural crest.[14] Given the importance of its function, damage of this layer is potentially serious leading in cell loss and irreversible damage to the endothelial cytoskeleton with direct harmful alterations of visual function.[15] The aim of our study was to evaluate the morphological characteristics of the corneal endothelial cells and thickness alterations in 19 patients with NF1 and to compare them with those of 38 healthy age-matched controls.

Methods

Nineteen consecutive patients with NF1 and 38 healthy individuals were recruited. To eliminate the significant confounding effect of age in the evaluation of corneal endothelium morphology parameters, we matched 19

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NF1 patients with 38 healthy controls for this crucial factor (2 controls per case). This case-control study was conducted between March 2018 and January 2020 at the Ophthalmologic Clinic of our University Hospital in accordance with the Declaration of Helsinki and legal regulations. The study protocol was approved by the institutional review board of our hospital and the local ethics committee. All patients and healthy participants signed a written informed consent form after explanation of the study protocol.

Diagnosis of NF1 was based on having at least two or more of the seven clinical criteria defined by the National Institutes of Health which include: six or more café au lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in postpubertal individuals, frecklings over the axillary or inguinal regions, two or more Lisch nodules (iris hamartomas), two or more neurofibromas or one plexiform neurofibroma, optic pathway glioma, characteristic skeletal dysplasia (sphenoid wing dysplasia, long-bone dysplasia) and first-degree relative with NF1.[10]

All patients underwent complete ophthalmological examination, including best-corrected visual acuity measurement with Snellen chart, slit-lamp biomicroscopy and intraocular pressure examination as well as dilated fundoscopy. Demographic characteristics (age, gender) of all participants were recorded. General medical history and comorbidities that could interfere with our studied parameters (hypertension, diabetes mellitus, gout, chronic kidney disease, cancer, rheumatoid arthritis, systemic lupus erythematosus, and sleep disorders) and ophthalmological history such as previous ocular trauma or surgery, preexisting corneal and ocular surface diseases, mature cataract, intraocular inflammation, contact lens use, glaucoma, pseudoexfoliation, retinal disease, regular use of any eye drops or known tear-interfering systemic drugs (such as hormone-replacement, anti-histamines, and antidepressants drugs) were accepted as exclusion criteria. Finally, patients diagnosed with hypermetropia greater than +4 dioptres (D), myopia greater than –5D and astigmatism exceeding ±1D were also excluded from the study, not only because of the dubious effect of excessive refractive errors, and especially high myopia, on corneal endothelium morphology, but also in order to optimally match NF1 patients with healthy controls.[16,17]

Furthermore, all participants underwent noncontact corneal specular microscopy using TOMEY EM-3000 by the same examiner. The patient was asked to fixate for a few seconds on the red light inside the device for a clear image of the corneal endothelium to be taken automatically, measuring at the same time the central corneal thickness (CCT). All the measurements were taken from the central clear area of the cornea.

All measurements were performed at least three times in a row using the "center" method, the most common manual analysis method in which the user marks the center of each cell in a contiguous group and the Tomey Cell Count software then counts the number of cells by determining cell area from a polygon digitization by locating cell border intersections.[18] At least 100 contiguous cells in the evaluated corneal field were included in each measurement and the mean values were calculated. Endothelial cell density (ECD) (cells/mm^2), average cell area (AVG) (μm^2), coefficient of variation of cell area (CV) as an index of the extent of variation in cell area (polymegathism), the percentage of hexagonal cells as an index of variation in the cell shape (pleomorphism) and CCT of each eye were analyzed. The intra-observer reproducibility of all corneal endothelium morphology parameters was excellent as indicated by the intraclass correlation coefficient (>0.9).

Statistical analysis was performed using the SPSS statistical package (IBM Corp., version 21.0, Armonk, NY, USA). Although both eyes were examined, one eye was randomly chosen per patient to be analyzed, so as to avoid bias due to the intercorrelation of values between the eyes of the same patient. Variables were tested for normality by the Kolmogorov–Smirnov test and histograms. Normally distributed variables are presented as mean values ± standard deviation and categorical variables as frequencies. Comparisons among the two groups were performed using independent samples *t* test for continuous variables and Chi-square test for categorical variables. The level of statistical significance was set at $P < 0.05$ for all comparisons. Having our results, we calculated the Cohen's d (effect size) of our groups at above 0.6 for the CV and the %Hex variables and performed a post hoc power calculation with respect to α-error probability of 0.05 using the G* power software. The power of our study for $\delta = 2.5$ and $\alpha = 0.05$ was estimated at 0.67.

Results

The demographics and baseline characteristics of 19 patients with NF1 and their 38 matched controls are shown in Table 1. The mean age of NF1 patients and controls was 41.8 ± 18.2 years and 42.3 ± 17.7 years, respectively. There was no statistically significant difference between NF1 patients and healthy controls regarding age ($P = 0.925$), gender ($P = 1.000$) and refractive status expressed as spherical equivalent ($P = 0.875$). Lisch nodules were found in 34 eyes (89.5%) of the NF1 group.

Table 2 shows the results of the corneal specular microscopy and the comparison between controls and NF1 participants. When compared to healthy controls, NF1 patients presented increased ECD (2764.2 ± 270.4 versus 2570.4 ± 449.2 cells/mm^2 for NF1 and control group, respectively) although at a borderline level ($P = 0.051$). The mean AVG was significantly lower in NF1 patients compared to the controls (364.9 ± 34.4 versus 406.0 ± 107.4 μm^2, $P = 0.038$, respectively). Furthermore, a statistically significant decrease of CV in NF1 patients was observed when compared to healthy individuals (32.9 ± 4.6 versus 37.8 ± 9.5%, $P = 0.011$, respectively). As far as hexagonality is concerned, NF1 patients presented significantly higher values

Table 1: Characteristics of 19 patients with Neurofibromatosis type 1 (NF1) and 38 healthy controls

	NF1 (19 patients)	Controls (38 patients)	*P*
Age (mean±SD, years)	41.8±18.2	42.3±17.7	0.925
Gender (*n*, %)			1.000
Male	6 (31.6)	13 (34.2)	
Female	13 (68.4)	25 (65.8)	
Lisch nodule (*n*,%)			-
Positive	34 (89.5)	NA	
Negative	4 (10.5)	NA	

Data are presented as mean±SD for continuous and as percentage (%) for categorical variables. NA: Not applicable

Table 2: Comparison of corneal endothelial and thickness parameters between Neurofibromatosis type 1 (NF1) patients and healthy controls

	NF1 (19 patients)	Controls (38 patients)	*P*
Endothelial cell density (mean±SD, cells/mm^2)	2764.2±270.4	2570.4±449.2	0.051
Average size (mean±SD, μm^2)	364.9±34.4	406.0±107.4	0.038
Cell size coefficient of variation (mean±SD, %)	32.9±4.6	37.8±9.5	0.011
Hexagonality (mean±SD, %)	55.7±6.5	50.5±9.9	0.025
Central Corneal Thickness (mean±SD, μm)	535.9±43.6	535.2±42.1	0.955

Data are presented as mean±SD for continuous and as percentage (%) for categorical variables

in comparison with healthy individuals (55.7 ± 6.5 versus 50.5 ± 9.9%, P = 0.025, respectively). In addition, the mean CCT of patients with NF1 was similar with the control group (535.9 ± 43.6 and 535.2 ± 42.1, respectively, P = 0.955) [Fig. 1].

Discussion

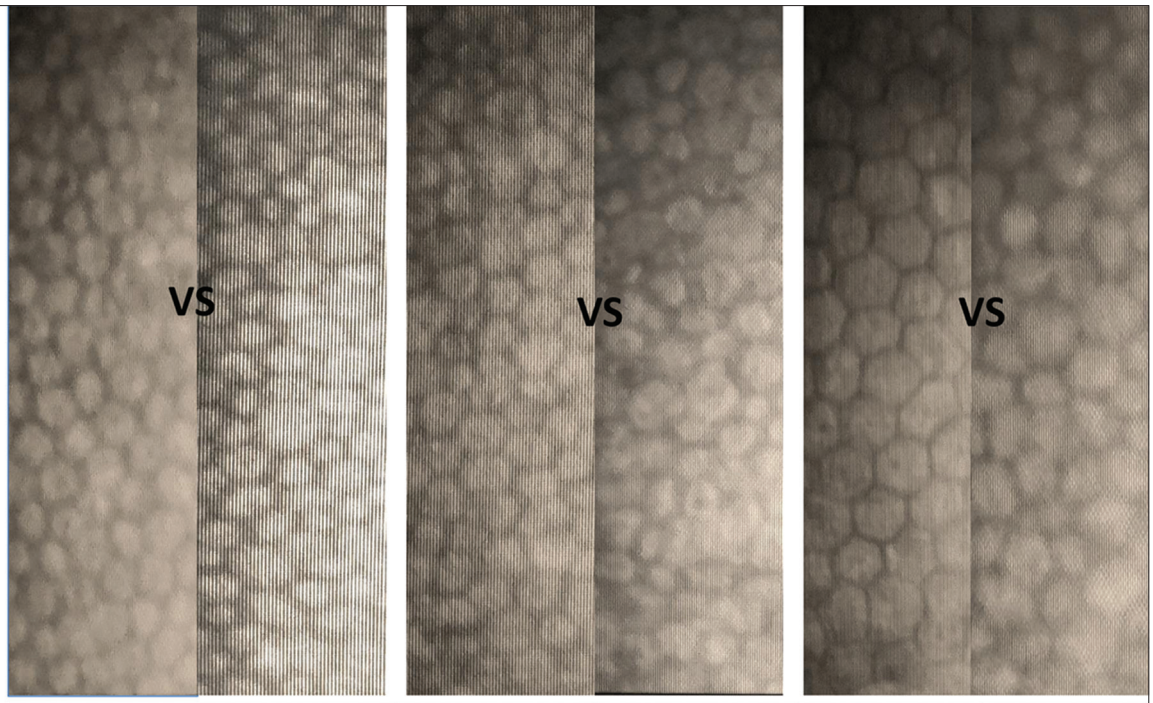
To our knowledge, this is the first study that evaluates overall the morphological properties of corneal endothelial cells in patients with NF1. The assessment of corneal endothelium by specular microscopy is a crucial process as this tissue is directly involved in maintaining the constant thickness and metabolic homeostasis of the cornea.[19] It is the metabolically most active layer of the cornea, but at the same time, the most sensitive to potential damage. We found that NF1 patients had statistically significant lower CV and AVG but higher values of hexagonality compared to healthy controls of the same age, while ECD was increased in NF1 patients although at a borderline level. CCT did not differ significantly between NF1 group and controls.

Our findings are in line with the results of the recent study by Moramarco *et al*.[20] that showed increase of corneal endothelial cell density in 28 patients with NF1 compared to 14 healthy participants. However, in our study NF1 patients presented increased ECD (2764.2 ± 270.4 versus 2570.4 ± 449.2 cells/mm^2 for NF1 and control group, respectively) although at a borderline level (P = 0.051). The small sample size of both studies, imposed by the low prevalence of the disease, the difference in patients: controls ratio used, as well as the different characteristics of studied populations, mainly the distribution of age, could explain this discrepancy. Edward *et al*.[21] observed endothelial cells proliferation in some NF1 patients with ectropion uvea and glaucoma. The functional inactivation of the NF1 gene leads to activation of the MAPK pathway[6,22] which results in cellular proliferation of many neural-crest derived cells and development of neurofibromas and pigmentary abnormalities in NF1. Consequently, it has been hypothesized that activation of RAS pathway may also induce corneal endothelial cells proliferation[21,23] explaining our results.

Corneal endothelium, the cellular monolayer on the posterior surface of the cornea that is responsible for corneal clarity, is unique, as constitutes the only tissue with living cells that can be examined at high magnification repeatedly and noninvasively. The human corneal endothelial cells do not have a significant capacity for *in vivo* regeneration, thus making them unable to replace dead or damaged cells.[24] Therefore, their response to minor damage is stretching and centripetal migration into the injured area, in order to maintain an intact barrier function and active transport mechanism.[17] This procedure is a reflection of the normal endothelial cell movement that characterizes the normal wound repair mechanism, therefore there is always some degree of variation in cell size (polymegathism) in the corneal endothelium. Both rates of polymegathism and pleomorphism (variation in cell shape) are reflecting the endothelial cell functional reserve and are highly sensitive indicators of incipient endothelial instability. CV increase and HEX decrease are signs of an overactive wound repair mechanism, related to a subsequent reduction of ECD and are considered to be its precursors. When the limits of endothelial functional reserve are approximated, compromised corneal endothelial function can disturb the balance of stromal hydration, leading to corneal edema, changes in corneal transparency and reduced corneal sensitivity.[14,25,26] Polymegathism and pleomorphism also increase with age.[27,28]

An interesting finding of our study, which is investigated for the first time in NF1 patients, was that CV and AVG were statistically significant lower in this group when compared to healthy controls (P = 0.011 and P = 0.038, respectively). In addition, significantly higher hexagonality was found in NF1 patients when compared to healthy controls (P = 0.025). A cornea with these characteristics is implied to have a greater endothelial monolayer functional reserve and could therefore be privileged to withstand the inevitable stress caused by insults as prolonged or complicated ocular surgery and severe trauma or even glaucoma and uveitis.[29,30] Further research is needed to confirm this indirect hypothesis and define its potential clinical application. *In vitro* study of the corneal endothelium of NF1 patients post mortem would be a difficult and longstanding project, given the rarity of the studied disease, but also a very ambitious idea concerning the therapeutic management of patients in need for transplant because of endothelial dystrophy or endothelial damage post-surgery or trauma. Finally, as far as CCT is concerned, there was no statistically significant difference between NF1 patients and controls in our study (P = 0.935), which is in accordance with the findings of the study conducted by Duru *et al*.[31] in 17 NF1 patients and 17 age- and gender-matched healthy individuals (P = 0.875).

Strength of our study was that the significant confounding effect of age in the evaluation of corneal endothelium morphology parameters was eliminated by the matching for age with healthy controls, as well as the fact that all measurements were performed by one examiner. The relatively small sample size of the study presents a potential limitation, which was however compensated by the 1:2 ratio between participating patients and healthy controls. However, future prospective studies with larger sample sizes could be conducted to confirm our findings and clarify the underlying pathophysiological



	NF1	Control	NF1	Control	NF1	Control
Age(years)	27	28	38	39	60	59
ECD (cells/mm^2)	3078	3030	2938	2804	2333	2273
CV (%)	32	35	32	37	31	34
AVG (µm^2)	305	333	352	364	380	395
HEX (%)	65	46	61	47	58	55
CCT (µm)	557	549	514	508	517	520

Figure 1: Representative noncontact corneal specular microscopy images of corneal endothelial cells and corresponding morphological measurements of 3 NF1 patients and their matched-controls

mechanisms leading to corneal morphological alterations in this population.

Conclusion

The results of our study show that corneal endothelium has more favorable morphological characteristics in patients with NF1, as compared to healthy individuals of the same age.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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