Nasal Nitric Oxide in Patients With Inherited Retinal Dystrophies

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Background: Ciliopathies refer to a wide variety of diseases in which mutations in the genes encoding proteins involved in ciliogenesis or protein transport to the primary cilia play pathogenetic roles, and in such diseases, retinal involvement may be present. Nitric oxide (NO) plays an important role in airway physiology, including regulation of ciliary motility and host defense. In primary ciliary dyskinesia, a syndromic ciliopathy, nasal NO (nNO) levels were reported to be extremely low compared with controls, possibly reflecting molecular defects leading to structural and functional ciliary abnormalities. We investigated whether decreased nitric levels were also present in patients with retinal inherited dystrophies.

Methods: Nasal NO was measured in a group of patients with syndromic and nonsyndromic inherited retinal dystrophies.

Results: Patients with inherited retinal dystrophies, both syndromic and nonsyndromic, had mean nNO levels that were lower than healthy controls. Seven patients had particularly low levels of nNO: 3 patients with retinitis pigmentosa and 4 individual patients with Mainzer-Saldino syndrome, Bardet-Biedl syndrome, Usher syndrome, and cone-rod disease.

Conclusions: These findings provide evidence that there is an underlying abnormal ciliary function involving the nasal epithelium in some patients with inherited retinal dystrophies.

Key Words: inherited retinal dystrophies, nitric oxide, exhaled breath analysis, nose, ciliopathies

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Nitric oxide (NO) plays an important role in airway physiology, including the regulation of ciliary motility and host defense.[1,2] Cilia are evolutionarily conserved structures that play key roles in many cells of different tissues. Mutations in genes encoding proteins involved in ciliogenesis or protein transport to the primary cilia can lead to a wide variety of diseases commonly referred to as ciliopathies.[3] In primary ciliary dyskinesia (PCD), a ciliopathy that combines respiratory symptoms, male infertility, and in nearly 50% of cases, situs inversus, nasal NO (nNO) levels have been reported to be extremely low compared with controls; in fact, the measurement of nNO is commonly used as a reliable screening test in PCD diagnostic centers.[4,5] The mechanisms of low NO production in PCD is presently unknown[6]; however, impaired NO production may be related to the underlying molecular defects leading to structural and functional ciliary abnormalities. Supporting this hypothesis is the observation that even healthy parents (obligate heterozygotes for mutated genes associated with PCD) of subjects with PCD have lower than normal nNO levels.[7] Whether NO is involved in ciliary diseases other than PCD is not known.

Inherited retinal dystrophies are a clinically and genetically heterogeneous group of diseases, some of which are ciliopathies.[8] The abnormal function of proteins expressed in photoreceptor cells, which are ciliated sensory cells specialized for light detection, is known to cause photoreceptor cell death and retinal degeneration.[9]

In this study, our goal was to investigate whether decreased NO levels were present in patients with inherited retinal dystrophies. With this aim, nNO levels were measured in a group of patients with syndromic and nonsyndromic inherited retinal dystrophies.

MATERIALS AND METHODS

Patients and Controls

Thirty-three patients (19 males; mean age, 43.1 years; age range, 17–77 years) with syndromic (n = 10) or nonsyndromic (n = 23) inherited retinal dystrophies were recruited among members of the local retinitis pigmentosa (RP) patients association that were evaluated at the genetic counseling department within the RP outpatient clinic of our hospital. Of the nonsyndromic patients, 19 had RP, one had cone-rod dystrophy (CRD), and 3 had Leber congenital amaurosis (LCA). Among patients with syndromic inherited retinal dystrophies, Usher syndrome was diagnosed in 7, Bardet-Biedl syndrome in 2, and Mainzer-Saldino syndrome in 1 patient. None of the patients had a diagnosis of cystic fibrosis, and all of the patients were nonsmokers. All of the patients were asked about previous diagnoses of rhinitis and/or chronic rhinosinusitis.

Thirty nonatopic, nonsmoking individuals (12 males and 18 females; mean age, 34 years; age range, 21–57 years) made up the control group for nNO measurement.

This study was approved by the institutional ethics committee, and written patient consent was obtained to use their clinical data for this study.

Nasal Nitric Oxide

Measurements were obtained 2 hours after breakfast using a chemiluminescence NO analyzer (NIOX; Aerocrine AB, Solna, Sweden) calibrated with a certified NO calibration gas mixture according to the European Respiratory Society/American Thoracic Society recommendations.[10] The patients were relaxed and were in a sitting position and then were asked to insert a NIOX nasal olive into 1 nostril. They then inhaled to total lung capacity for more than 2 to 3 seconds through open mouths, after which they closed their mouths and held their breath while NO was continuously measured at an aspiration flow rate of 5 mL/s. We took into consideration the NO levels that were recorded at the plateau, which occurs after 20 to 30 seconds in most patients. The nasal olive was then placed in the other nostril, and the test was repeated. Measurements were made in triplicate for both nostrils, and the highest mean value, from which the ambient NO level was subtracted, was considered.

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TABLE 1. Clinical and Demographic Characteristics of Patients and Healthy Controls

Patient ID	Age, y	Sex	Atopy	Syndromic Inherited Retinal Dystrophies	Disease	nNO (ppb)
1	23	Female	No	Yes	Usher	863
2	57	Male	No	Yes	Usher	539
3	46	Female	Yes	Yes	Usher	892
4	51	Male	No	Yes	Usher	727
5	18	Male	No	Yes	Usher	660
6	46	Female	No	Yes	Usher	511
7	46	Female	No	Yes	Usher	413
8	24	Female	No	Yes	Meinzer-Saldino	270
9	58	Male	Yes	No	LCA	761
10	17	Female	No	No	LCA	1046
11	26	Female	No	No	LCA	623
12	18	Male	No	Yes	Bardet-Biedl	1136
13	28	Male	No	Yes	Bardet-Biedl	400
14	65	Female	No	No	RP	229
15	30	Female	No	No	RP	1008
16	57	Female	No	No	RP	584
17	56	Male	No	No	RP	533
18	35	Male	No	No	RP	1722
19	77	Male	No	No	RP	577
20	43	Male	Yes	No	RP	758
21	55	Male	No	No	RP	784
22	30	Male	Yes	No	RP	549
23	36	Female	No	No	RP	727
24	49	Female	Yes	No	RP	818
25	51	Male	No	No	RP	1163
26	33	Male	Yes	No	RP	749
27	56	Female	No	No	RP	255
28	59	Female	Yes	No	RP	599
29	51	Male	No	No	RP	718
30	39	Male	No	No	RP	393
31	50	Male	No	No	RP	708
32	47	Male	Yes	No	RP	1016
33	45	Male	Yes	No	CRD	295
1	24	Male	No	N/A	HC	714
2	27	Female	No	N/A	HC	845
3	26	Female	No	N/A	HC	938
4	27	Female	No	N/A	HC	856
5	32	Female	No	N/A	HC	715
6	32	Female	No	N/A	HC	638
7	24	Male	No	N/A	HC	839
8	28	Female	No	N/A	HC	805
9	42	Male	No	N/A	HC	720
10	55	Male	No	N/A	HC	970
11	23	Female	No	N/A	HC	923
12	37	Female	No	N/A	HC	697
13	37	Female	No	N/A	HC	705
14	26	Female	No	N/A	HC	842
15	37	Male	No	N/A	HC	1082
16	57	Female	No	N/A	HC	950
17	43	Male	No	N/A	HC	980
18	28	Male	No	N/A	HC	1100
19	21	Male	No	N/A	HC	875
20	50	Male	No	N/A	HC	1050

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TABLE 1. *(Continued)*

Patient ID	Age, y	Sex	Atopy	Syndromic Inherited Retinal Dystrophies	Disease	nNO (ppb)
21	44	Male	No	N/A	HC	815
22	33	Female	No	N/A	HC	780
23	29	Female	No	N/A	HC	1150
24	48	Female	No	N/A	HC	720
25	42	Male	No	N/A	HC	777
26	28	Female	No	N/A	HC	797
27	23	Female	No	N/A	HC	1037
28	27	Female	No	N/A	HC	784
29	55	Male	No	N/A	HC	960
30	27	Female	No	N/A	HC	866

HC indicates Healthy Control.

Statistics

Comparisons between continuous variables were estimated using analysis of variance or the Mann-Whitney *U* test, depending on the distribution of the variables. Data were analyzed using a statistical software package (SPSS version 13.0 for Windows; SPSS Inc, Chicago, IL). All of the *P* values were 2-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Clinical and demographic characteristics are reported in Table 1.

Mean nNO was lower in all patients compared with controls (696 ± 319 vs 864 ± 134 ppb, respectively, $P = 0.007$) (Fig. 1). Seven patients were atopic, and the nNO levels were not different from the nonatopic patients.

No difference in mean nNO was observed between patients with syndromic compared with patients with nonsyndromic retinal ciliopathies.

Seven patients had nNO levels lower than 3 SD below the mean nNO value (<461 ppb) for healthy controls, wherein 3 of the 7 patients had syndromic, and 4 of the 7 had nonsyndromic disease.

DISCUSSION

Patients with inherited retinal dystrophies, both syndromic and nonsyndromic, had mean nNO levels that were lower than healthy controls. Moreover, the variation in nNO levels was much greater in the patient group compared with healthy subjects, which suggests greater heterogeneity in the patient, although not linked to a specific condition.

Seven patients had nNO levels below 3 SD of the mean value observed in healthy controls, wherein 4 of these patients had particularly low values, which were only slightly higher than the values commonly observed in patients with PCD. Common diseases that are caused by low nNO levels, such as cystic fibrosis[11] and nasal polyposis,[12,13] were clinically excluded. None of these patients reported symptoms suggestive of PCD, such as persistent cough, rhinosinusitis, otitis media, or infertility. Therefore, it is possible that defects underlying inherited retinal dystrophies may influence nasal cilia as well, leading to lower nNO levels. A variety of inherited retinal dystrophies was observed in the 7 patients with particularly low nNO levels: 3 patients had RP and 4 individual patients had either Mainzer-Saldino syndrome, Bardet-Biedl syndrome, Usher syndrome, or CRD.

Mainzer-Saldino syndrome is a rare ciliopathy considered part of the Joubert syndrome–related disorders and is characterized by RP, cone-shaped phalangeal epiphyses, and cystic dysplasia of the kidneys leading to early renal insufficiency.[14]

Bardet-Biedl syndrome, which is characterized by RP, mild mental retardation, obesity, dysmorphic facial features, and polydactyly, is genetically heterogeneous, and several genes known to be mutated in this syndrome are implied in cilia assembly or function.[15]

Usher syndrome is a recessive disorder that combines hearing loss with RP and is heterogeneous clinically and genetically. It is caused by the disruption of the Usher protein network in both the inner ear and the retina and thus leads to a wide variety of clinical and genetic phenotypes. The main colocalization sites of Usher proteins in the inner ear are the stereocilia and the synaptic regions of hair cells. The Usher proteins in the retina colocalize in this synaptic layer as well as in the ciliary region between the outer and inner segments of photoreceptors and, in particular, in the connecting cilium and in the calycal processes.[16]

Retinitis pigmentosa and CRDs are a clinically and genetically heterogeneous group of disorders characterized by photoreceptor degeneration. Vertebrate photoreceptor cells are ciliated sensory cells that specialize in light detection. Abnormal function of the proteins expressed in these cells leads to photoreceptor cell

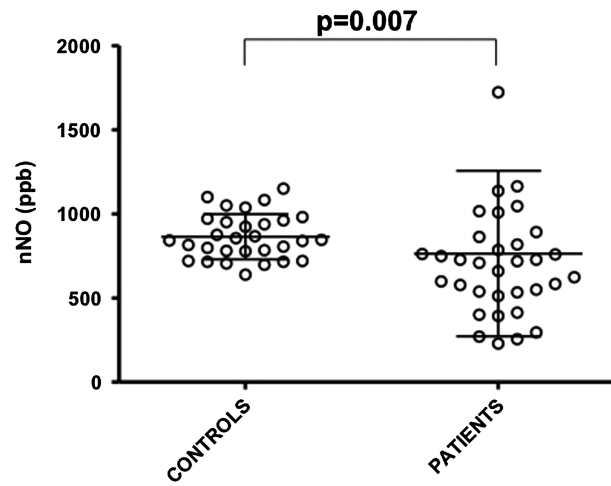

FIGURE 1. Nasal NO levels in patients with inherited retinal dystrophies and healthy controls. Nasal NO levels is lower in patients with inherited retinal dystrophies compared with healthy controls ($P = 0.007$).

death and retinal degeneration, and some inherited retinal disorders are now being classified as ciliopathies.[3,8]

In conclusion, in a selected group of patients with ciliopathies other than PCD, we observed nNO levels that were lower than normal controls, and 3 patients (one with Mainzer-Saldino syndrome and 2 with RP) had levels as low as those observed in PCD (<250 ppb).[4–7] Other diseases that are known to cause low nNO levels, such as cystic fibrosis[11] and nasal polyposis,[12,13] were unlikely to be present in these patients; therefore, this finding may allude to the presence of an underlying abnormal ciliary function involving nasal cilia as well.

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