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Sequencing Analysis of the ATOH7 Gene in Individuals with Optic Nerve Hypoplasia

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Abstract

Purpose—The Atonal Homolog 7 (ATOH7) gene has been implicated in association studies with optic nerve head diameter size. Hence, we screened optic nerve hypoplasia (ONH) patient DNA samples from Australia, France, and the United States for sequence variants in the ATOH7 gene using Sanger sequencing.

Methods—Sanger sequencing of the ATOH7 gene was performed on 34 affected individual DNA samples. Sequencing was also carried out in 3 unaffected family members to confirm segregation of identified single nucleotide variations.

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Results—Seven sequence variations were identified in ATOH7. No disease-causing sequence changes in the ATOH7 gene was discovered in the ONH patient samples.

Conclusions—Mutations within the ATOH7 gene are not implicated in the pathogenesis of optic nerve hypoplasia in our patient cohort.

INTRODUCTION

Optic nerve hypoplasia (ONH) is a non-progressive congenital abnormality of the peripapillary retina and optic nerve development in one or both eyes. It is characterized by regression of optic nerve axons, leading to small optic discs with decreased retinal ganglion cells and centripetal fibers projecting through the optic nerve to the lateral geniculate body.(1, 2) Other clinical features include a hypoplastic optic disc surrounded by a yellow halo bordered by a pigmented ring (described as a double-ring sign), and tortuous retinal blood vessels with anomalous branching patterns.(3) ONH is the most common congenital optic nerve anomaly and is the third leading cause of blindness in the United States.(4, 5) The prevalence of ONH in North America is unknown, although several studies have noted an increase in recent birth cohorts.[3] The prevalence rate of 5.7% to 12.9% is seen in blind students in the U.S.(3) In 1997, ONH was reported to be the single leading cause of infant blindness in Sweden, with a prevalence rate of 6.3 per 100,000 live births.(3)

ONH patients typically present with decreased visual acuity and visual field defects. Visual function can vary from 20/20 to no light perception.(1) Nasal visual field defects are the most common visual field defects.(6) ONH can be present in one or both eyes. In its unilateral form, ONH is usually accompanied by unilateral loss of vision, infantile strabismus, and abnormal pupillary responses to light.(1) Bilateral ONH commonly presents with nystagmus, which appears at 1 to 3 months of age.(3) ONH can occur in isolation or may be associated with other systemic anomalies, namely cerebral malformations and endocrine disturbances. Septo-optic dysplasia, with abnormal or absent septum pellucidum, is the most common syndrome associated with ONH.(1) Common endocrine disturbances associated with ONH include growth hormone deficiency, adrenocorticotropic hormone insufficiency, hypothyroidism, disturbances of antidiuretic hormone production, precocious puberty and hypogonadism.(1) In general, visual prognosis related to ONH depends upon its degree of severity and association with other systemic abnormalities.(1) In the majority of cases, ONH is idiopathic and occurs sporadically.(7) It has rarely been reported in siblings, identical twins, or presents with familial inheritance.(8–10)

Multiple genetic studies of the ONH phenotype propose that the key proteins guiding the development of the retina are nuclear transcription factors.(11–13) Basic helix-loop-helix (bHLH) transcription factors are involved in regulating retinal neuron formation in both vertebrates and invertebrates.(14–16) In *Drosophila*, the key proneural *bHLH* gene that guides photoreceptor development is atonal.(17) In particular, the ATH5/7 subclass is highly expressed by retinal progenitors during the early stages of eye development in several vertebrate models.(18–21) Mutations in the gene counterparts in *Drosophila* (*atonal*), zebrafish (*Ath5*), and mouse *Atoh7* (*Math5*) cause agenesis of the initial neuron class in the eyes of these organisms, including R8 photoreceptors in *Drosophila* and retinal ganglion

cells in vertebrates.(12, 17, 22, 23) The atonal homolog in humans is Atonal homolog 7 (ATOH7), located in chromosome 10q21.3 (http://genome.cse.ucsc.edu- GRCh37). ATOH7 comprises a single exon that encodes a 152-amino acid protein with a basic helix-loop-helix (bHLH) domain spanning residues 41–96.(24)

In a recent study, autozygosity mapping and next generation sequencing were used in analyzing two consanguineous families with multiple ocular developmental defects, including optic nerve hypoplasia.(24) The paper identified two homozygous mutations in ATOH7, p.Glu49Val and p.Pro18Rfs*69, thought to be causal.(24) Also, a genome-wide association (GWA) study of mean optic disc area identified a significant associated single nucleotide polymorphism (SNP) (rs3858145, $P = 3.4 \times 10^{-10}$) in Australian and United Kingdom twin cohorts 20 kilobase pairs (kb) downstream of ATOH7.(25) Another GWA study of two Rotterdam cohorts determined a significant SNP (rs1900004, $P = 2.67 \times 10^{-33}$) within 10kb of the ATOH7 gene associated with optic disc area.(26)

Therefore, mutations in the ATOH7 gene may be causal for congenital malformations of the optic nerve, including optic nerve hypoplasia and aplasia.(27) In this study we screened the DNA samples of a cohort of 34 patients with optic nerve hypoplasia from Australia, France, and the United States for sequence variations in the ATOH7 gene using Sanger sequencing.

METHODS

Subject information

Informed consent was obtained from all participants prior to entering the study, with approval by the respective Institutional Review Board of all sites according to the principles of the Declaration of Helsinki. The diagnosis of ONH was made by ophthalmoscopic inspection demonstrating the presence of some or all of the following clinical features: 1) small optic nerve disc head of less than 4mm in diameter, 2) double ring sign defining the putative scleral canal surrounding the optic disc, 3) peri-papillary abnormal nerve fiber layer, 4) tilting of the optic nerve head, and 5) variance from age-appropriate ratio changes of the horizontal disc diameter (DD) relative to the distance from the macula to the temporal edge of the disc (DM), with ratios less than 0.35 defined generally as ONH.(3) Brain imaging studies were conducted to identify midline brain abnormalities, and to visualize the hypothalamus, pituitary gland, and optic nerves. Patients manifesting ONH as part of a myriad of functional and anatomical abnormalities of the central nervous system, such as septo-optic dysplasia, were included in this study. Patients with other ocular conditions involving the optic nerve, including optic nerve atrophy, were excluded. A questionnaire regarding family and medical history was completed by the subject and/or subject's parents.

A total of 37 participants were screened in this study, with the cohort comprised of 34 individuals diagnosed with ONH and 3 additional unaffected family members from unrelated cases. Venous blood samples were collected and genomic DNA was extracted using AutoPure LS® DNA Extractor and PUREGENE™ reagents (Gentra Systems Inc., Minneapolis, MN/USA). DNA was collected in individuals from four geographic locations and encompassing three countries; Australia, France, and United States.

Polymerase Chain Reaction and Sequence Analysis

The ExonPrimer program (Helmholtz Center, Munich, Germany; http://ihg.helmholtz-muenchen.de/cgi-bin/primer/ExonPrimerUCSC.pl?db=hg19&acc=uc003esw.3) was used to design primers for polymerase chain reaction (PCR) and sequencing to cover the entire ATOH7 exon. Primers were designed not to exceed 700 base pairs for optimal sequence analysis; three primer sets were needed for full coverage of the coding and untranslated regions, including the intron/exon boundaries. (Table 1)

A standard PCR protocol was used to amplify the samples, and amplicons were visualized by electrophoresis on a 2% agarose gel. The Applied Biosystems ABI3730×l DNA Sequencher was used to perform Sanger sequencing of the amplicons, utilizing BigDye Terminator 3.1 technology (Applied Biosystems, Inc., Foster City, CA/USA). Analyses of sequences were completed using Sequencher 5.0™ software (Gene Codes, Ann Arbor, MI/USA), where the sequence files were compared against the known reference sequence from the UCSC Genome Browser website (http://genome.cse.ucsc.edu- GRCh37) and sequence variants were identified.

RESULTS

A total of 34 unrelated affected individuals were sequenced along with 3 unrelated unaffected family members. Appendix 1 illustrates the clinical data of the participants with ONH. Affected males (18/37, 48.6%) and females (19/37, 51.4%) were equally represented. The majority of subjects were Caucasian, 89.2% (33/37), while the remaining were of African American background (3/37, 8.1%) or unknown race (1/37, 2.7%). With respect to geographic location, 2.70% (1/37) were Australian, 21.6% (8/37) were French, and 75.7% (28/37) were from two sites in the United States (24 individuals from Cleveland, OH and 4 from Durham, NC).

Of the 34 individuals with ONH, 79.4% (27/34) presented with bilateral optic nerve involvement. Less than half (14/34, 41.2%) of affected individuals were diagnosed with septo-optic dysplasia underlying the cause of ONH. Other intracranial features common among the participants included pituitary insufficiency, cerebellar hypoplasia and microcephaly. All affected individuals were diagnosed during infancy or before 1 year of age.

Seven sequence variants were identified within the ATOH7 gene, consisting of six untranslated region (UTR) variants and one intronic variant. (Table 2) Of the six UTR variants identified, five were previously reported in the dbSNP135 database and one was considered to be novel. Five of the UTR variants were found in the 5’ region of the ATOH7 gene, while one was located at the 3’ end. The intronic variant identified was also previously reported in the dbSNP135 database. Of the seven variants, two variants in the 5’ UTR region (rs7916697, rs61854782) were previously reported in a GWA study of Australian and UK combined twin cohorts involving sequencing of the ATOH7 gene in 12 affected individuals with ONH.(25) The variant rs61854782 was present in 5 affected individuals, while the variant rs7916697 was present in 31 affected and unaffected individuals in our cohort.

DISCUSSION

In our cohort of 34 ONH patients and 3 unaffected family members, 26 cases (76.5%) had the bilateral optic nerve hypoplasia, which is the more common manifestation.(2) Almost half (41.2%) of the affected cohort had septo-optic dysplasia, with partial and complete agenesis of the septum pellucidum. Nineteen of the 34 cases (55.9%) presented with pituitary insufficiency and additional intracranial abnormalities, such as cerebellar hypoplasia, corpus callosum hypoplasia and cerebral atrophy.

With Sanger sequencing, we identified 7sequence variants within the ATOH7 gene in our cohort. Of the 7 variants, 2 variants in the 5' UTR region (rs7916697, rs61854782) were previously noted in a GWA study of Australian and UK combined twin cohorts, with ATOH7 sequence screening of 12 ONH individuals.(25) The variant rs7916697 had a P value of 1.3×10^{-10} in the GWA study and was observed in 30 of 34 ONH individuals in our cohort.(25) Although this variant has a strong association with optic nerve hypoplasia, it was also present in unaffected family members. The variant rs61854782 was found in 5 ONH individuals from Australia, Cleveland, and France, but had no association value in the GWA study.(25)

Although the SNVs are located in the UTR region, they may play an indirect role in regulating gene expression via translation. Translational regulation of gene expression is controlled by both trans-acting factors and cis-regulatory elements, and the latter are located in the 5' and 3' UTR regions. Modifications to the UTR regions can potentially dysregulate mRNA production. This can affect cellular function and metabolism, ultimately causing diseases including diabetes mellitus, Burkitt lymphoma, and breast cancer. (28–30)

UTR variants found in association studies with statistically significant P values may serve as regulators of the ATOH7 gene, and influence gene expression and its translational activity. As a result, the altered quantity of the ATOH7 protein may adversely affect proper optic nerve development. These include the top GWA study variants rs1900004 ($P = 4.2 \times 10^{-11}$) and rs3858145 ($P = 6.2 \times 10^{-10}$), which are 5kb and 20kb downstream of the ATOH7 gene respectively.(25) Therefore, investigations screening the UTR variants among ONH cohorts should be explored. This was not completed in our study, where the main aim was to screen for potential coding variants within the ATOH7 gene that could be causative for ONH.

In summary, the ATOH7 gene protein is known to critically influence ocular development of model organisms, and association studies have identified the gene as a major determinant in human optic disc size. In this study, we did not discover pathogenic variants within the coding region of the ATOH7 gene in our ONH cohort. Since no variants found in the ATOH7 gene proved to be causal for ONH, there are likely other factors involved in human optic nerve development.

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Appendix 1. Clinical Data of Individuals with Optic Nerve Hypoplasia

Patient ID	Sex	Location	Ethnicity	Unilateral /Bilateral	Septo-optic Dysplasia	Other Intracranial/ Ocular Features
1	F	Australia	Caucasian	Bilateral	No	Not reported
2	F	France	Caucasian	Bilateral	Yes	Small corpus callosum and hypophysis
3	F	France	Caucasian	Bilateral	Yes	Congenital pituitary insufficiency
4	F	France	Caucasian	Bilateral	No	Not reported
5	F	France	Caucasian	Bilateral	Yes	Septal agenesis, partial agenesis of the corpus callosum, hypoplasia of the lower cerebellar vermis, pituitary hypoplasia intrasellar and of the pituitary stalk with ectopic post-hypophysis
6	M	France	Caucasian	Bilateral	No	Severe hypoplasia of the anterior optic chiasm
7	M	France	Caucasian	Bilateral	Yes	Severe hypoplasia of the optic chiasm, pituitary hypoplasia with absent pituitary stalk and post-

Patient ID	Sex	Location	Ethnicity	Unilateral /Bilateral	Septo-optic Dysplasia	Other Intracranial/ Ocular Features
						hypophysis, and absent interventricular septum
8	M	France	Caucasian	Bilateral	Yes	Hypoplasia of the optic chiasm and De Morsier anomalies
9	M	France	Caucasian	Bilateral	Yes	Pituitary hypoplasia
10	F	USA-Cleveland	Caucasian	Unilateral	No	None
11	F	USA-Cleveland	Caucasian	Unilateral	No	None
12	F	USA-Cleveland	Caucasian	Bilateral	No	Not reported
13	F	USA-Cleveland	Caucasian	Unilateral	Yes	Bilateral 3rd nerve palsy, aplasia of infundibulum and posterior pituitary, and hypoplasia of anterior lobe of the pituitary gland
14	F	USA-Cleveland	Caucasian	Bilateral	No	None
15	F	USA-Cleveland	Caucasian	Bilateral	No	None
16	F	USA-Cleveland	Caucasian	Unilateral	Yes	Absent septum pellucidum, focal gyral abnormalities and bilateral temporal perisylvian regions
17	F	USA-Cleveland	Caucasian	Bilateral	No	None
18	F	USA-Cleveland	Unknown	Bilateral	Yes	Absent septum pellucidum, and schizencephaly/holoprosencephaly
19	M	USA-Cleveland	Caucasian	Bilateral	No	Mild cerebral atrophy
20	M	USA-Cleveland	Caucasian	Unilateral	No	Microcephaly, absent septum pellucidum, parenchymal defect, extensive malformation of cortical defect, predominately in the left cerebral hemisphere
21	M	USA-Cleveland	Caucasian	Unilateral	No	Mild globe hypoplasia bilaterally and mild deformity of the dorsal aspect
22	M	USA-Cleveland	Caucasian	Bilateral	Yes	Partial agenesis and hypoplasia of corpus callosum
23	M	USA-Cleveland	Caucasian	Unilateral	No	Incidental stable cisterna magna
24	M	USA-Cleveland	Caucasian	Bilateral	Yes	Ectopic posterior pituitary
25	M	USA-Cleveland	Caucasian	Bilateral	No	Thinning of corpus callosum
26	M	USA-Cleveland	Caucasian	Bilateral	Yes	None
27	M	USA-Cleveland	Caucasian	Bilateral	No	Diffuse disorder of cortical formation with subependymal heterotopia, absent corpus callosum and right cerebellar hypoplasia
28	M	USA-Cleveland	Caucasian	Bilateral	No	Hypoplasia of optic chiasm and pituitary gland, delayed myelination within

Patient ID	Sex	Location	Ethnicity	Unilateral /Bilateral	Septo-optic Dysplasia	Other Intracranial/ Ocular Features
						subcortical white matter of occipital lobe and exterior parietal lobe
29	M	USA-Cleveland	African American	Bilateral	No	Absent septum pellucidum
30	M	USA-Cleveland	African American	Bilateral	Yes	Not reported
31	M	USA-Cleveland	African American	Bilateral	Yes	None
32	F	USA-Durham	Caucasian	Bilateral	No	Nystagmus, exotropia, mild myopia, astigmatism
33	F	USA-Durham	Caucasian	Bilateral	No	Nystagmus, nasal lacrimal duct obstruction, hyperopia, astigmatism, accomodative esotropia
34	M	USA-Durham,	Caucasian	Bilateral	No	Nystagmus, foveal hypoplasia, consecutive intermittent exotropia and amblyopia OS

ID = Identification; F = Female; M = Male

Table 1

ATOH7 Primers

Gene Primer	Forward Primer Sequence	Reverse Primer Sequence	Product Size (base pair)
ATOH7-1.1	TTCCCTCCTTCAGCTCTTTG	GAAGGCAGTGTTGAGCCC	666
ATOH7-1.2	TCTGAGGACTGGAACAGAATAGC	AGTGGGGCCAGGATAAAAAG	600
ATOH7-1.3	AAGCGGCACATTCGTTTATT	CAGACCTATGGACGCAATCA	94

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Table 2

ATOH7 Sequence Variants Identified

Chr 10 location (base pair)	Rs number (dbSNP135)	Variant Type	Allele Change	Homozygous /Heterozygous Allele Change	MAF (%)*	Patient ID	Affection Status
69990685	rs73269167	5' UTR	C>G	Heterozygous	5.9	31	Affected
69991474	novel	5' UTR	C>T	Heterozygous	N/A	12	Affected
69991619	rs6480319	5' UTR	A>G	Homozygous	24.6	29	Affected
69991668	rs6480320	5' UTR	C>T	Homozygous	0	29	Affected
69991749	rs61854782	5' UTR	A>C	Both	9.2	Multiple (x5)	Affected
69991853	rs7916697	3' UTR	T>C	Both	21.2	Multiple (x31)	Both affected and unaffected
69991909	rs144172027	Intronic	T>C	Heterozygous	-	15	Affected

Chr = chromosome; UTR = untranslated region; CNS = coding non-synonymous; N/A = not applicable; MAF = minor allele frequency in European Standard Population(ESP);

*dbSNP135 database (http://www.ncbi.nlm.nih.gov/snp/); ID = Identification