NR2F1 Mutations Cause Optic Atrophy with Intellectual Disability

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Optic nerve atrophy and hypoplasia can be primary disorders or can result from *trans*-synaptic degeneration arising from cerebral visual impairment (CVI). Here we report six individuals with CVI and/or optic nerve abnormalities, born after an uneventful pregnancy and delivery, who have either de novo heterozygous missense mutations in *NR2F1*, also known as *COUP-TFI*, or deletions encompassing *NR2F1*. All affected individuals show mild to moderate intellectual impairment. *NR2F1* encodes a nuclear receptor protein that regulates transcription. A reporter assay showed that missense mutations in the zinc-finger DNA-binding domain and the putative ligand-binding domain decrease NR2F1 transcriptional activity. These findings indicate that *NR2F1* plays an important role in the neurodevelopment of the visual system and that its disruption can lead to optic atrophy with intellectual disability.

Optic nerve abnormalities are increasingly recognized as a cause of poor vision. The most frequently identified abnormalities consist of optic nerve atrophy or hypoplasia, due to either genetic or acquired causes.[1] Optic nerve hypoplasia is often reported as part of a syndrome, for example, in conjunction with structural malformations of the brain or hypopituitarism, as observed in *SOX2*-related disorders (MIM 184429),[2] or combined with anterior segmental defects of the eye, such as iris coloboma in *PAX6*-related diseases (MIM 307108).[3] Optic nerve atrophy, however, can be part of a progressive syndromic or nonsyndromic disorder. The genetic defects underlying many syndromic forms of optic atrophy are known, but the molecular bases of nonsyndromic forms have been elucidated in only a few instances: mutations in *OPA1* (MIM 605290) and *OPA3* (MIM 606580) (autosomal-dominant inheritance),[4,5] in *TMEM126A* (MIM 612988) (autosomal-recessive inheritance),[6] and in mitochondrial DNA as part of the Leber Hereditary Optic Neuropathy (LHON) (MIM 535000).[7] Both optic atrophy and hypoplasia can be caused by *trans*-synaptic degeneration as a part of cerebral visual impairment (CVI), a class of disorders of the projection and/or interpretation of visual input in the brain.[8–10] Up to two-thirds of the cases of CVI are thought to be caused by perinatal damage, often the result of preterm birth, but genetic defects might play a significant role in the etiology of the remaining cases.[11–13] The majority of persons with CVI exhibit additional clinical features, including intellectual disability (ID).[11–14]

We collected 56 individuals with CVI, all born after an uneventful pregnancy and birth, without any identifiable causes, and performed clinical, ophthalmological, and copy number variant investigations (see Table S1 available online). This study was approved by the Ethics Committee of the Radboud university medical center (Commissie Mensgebonden Onderzoek, regio Arnhem-Nijmegen), and written informed consent was obtained for all enrolled subjects. For 12 individuals and their parents, we performed exome sequencing by using a trio approach.[15,16] Exome sequencing was done in ten of these trios through the Baylor-Hopkins Center for Mendelian Genomics; methods have been reported previously.[17] In brief, 1 μg of genomic DNA from each individual was fragmented in a Covaris sonication system. Whole-exome targeted capture was performed in solution with the BCM-HGSC Core design. Sequencing was performed on the Illumina

HiSeq 2000 platform (Illumina), producing an average of 9–10 Gb of raw data per sample. The sequence reads were mapped and aligned to the Human Genome Reference Assembly GRCh37/hg19 with the BCM-HGSC Mercury pipeline[18] at an average depth of coverage of 114×. Variant calling was performed with the Atlas2[19] and SAMtools[20] algorithms; variant annotation was performed with an intramurally developed annotation pipeline[21] based on ANNOVAR[22] and custom scripts to incorporate multiple databases to inform on identified variants. In the other two trios, we performed exome sequencing on a SOLiD 5500XL platform (Life Technologies) with the Agilent SureSelect All Exon V4 reagent for target enrichment (Agilent Technologies). The 50 bp paired-end reads were mapped to USCS Genome Browser GRCh37/hg19 Human Genome Reference Assembly, and variants and indels were called with Lifescope v2.1 (Life Technologies). There was an average coverage of 76×. After quality filtering (variant reads >15%) and exclusion of local and global variants (<1% occurrence), a de novo analysis was performed for all trios. Synonymous variants with no effect on splicing were excluded.

In two persons (individuals 1 and 2) de novo missense mutations predicted to be pathogenic were identified in nuclear receptor subfamily 2, group F, member 1 gene, *NR2F1* (chr5: 92921073 G>C; c.344G>C; p.Arg115Pro and chr5: 92921068 C>A; c.339C>A; p.Ser113Arg, respectively; Figure 1, Table S2) (MIM 132890, RefSeq accession number NM_005654.4). Both were situated in the DNA-binding domain. In addition to CVI, individual 1 had small optic discs with large excavations and ID; individual 2 manifested pale optic discs, small optic nerves on MRI, and developmental delay (Table 1; Table S3). We screened the remaining 44 CVI persons for mutations in *NR2F1* by Sanger sequencing and identified a third case (individual 3) with a de novo missense mutation, predicted to be pathogenic, in the ligand-binding domain, chr5: 92923914 T>C; c.755T>C; p.Leu252Pro. She had excavated, pale optic discs and ID.

The Poisson probability of identifying three de novo mutations by sequencing a cohort of 56 persons was calculated for *NR2F1*. Based on a de novo mutation rate of 1.18×10^{-8} per position[23] and accounting for GC-content and gene size,[24–26] the Poisson probability is $p = 1.40 \times 10^{-10}$, indicating that this is highly unlikely to have occurred by chance. This is further strengthened by the consistent clinical phenotype observed in the affected individuals.

Point mutations in *NR2F1* have not yet been reported, but large deletions spanning *NR2F1* and other genes have been associated recently with optic atrophy, visual motor integration deficit, visual perceptual disorder, and ID.[27] Therefore, we searched for deletions of *NR2F1* in both the CVI cohort and the intramural database of the Radboud university medical center, Nijmegen, Netherlands, that includes more than 10,000 array analyses from persons with intellectual disability and/or multiple congenital abnormalities. We identified a 5q15 deletion, arr 5q15(92845157–93679748)x1 (Human Genome Reference Assembly GRCh37/hg19) comprising *NR2F1* with the CytoScan HD array (Affymetrix) in individual 4, part of the CVI cohort, who had pale optic discs and mild ID (Figure 1). The inheritance of the deletion is unknown, because the deletion was on the paternal allele, but the reportedly phenotypically normal father was not available for testing. We also identified a de novo deletion on chr5: 91064110–93896378 (Human Genome Reference Assembly CRCh37/hg19), arr SNP 5q14.3q15(SNP_A-1810903–>SNP_A-1788922)x1 in the intramural cohort (individual 5) with the 250k SNP array (Affymetrix). Individual 5 had developmental delay and ophthalmological examination showed poor visual acuity, CVI, in the right eye a small pale optic disc, and in the left eye a pale optic disc with a large excavation.

In parallel, a single additional individual with congenital optic atrophy and ID was enrolled through the Baylor-Hopkins Center for Mendelian Genomics study (individual 6, BAB3468, Figure 1; Table 1). This 35-year-old female also had autism spectrum disorder with marked obsessive-compulsive behaviors. The pregnancy and delivery had been uneventful (Table S1). Family history was negative for optic nerve atrophy, ID, developmental delay, and autism. The family was consented through a research protocol approved by the Institutional Review Board (IRB) of Baylor College of Medicine. DNA samples from the proband and her unaffected parents were submitted for exome sequencing by using a trio approach as described above at an average depth of coverage of 107× and with >91% of the target bases covered at a minimum of 20×. Inheritance of the identified variants was examined with the DeNovoCheck algorithm.[16] Analysis of the variants identified in the proband under a sporadic de novo model of inheritance revealed a missense mutation (chr5: 92921064 G>A; c.335G>A; p.Arg112Lys) in *NR2F1*. This variant was situated in the DNA-binding domain and was predicted to be pathogenic.

As individual 6 manifested optic nerve atrophy without CVI, a cohort of mainly nonsyndromic persons (n = 37) with optic nerve abnormalities was screened for *NR2F1* variants by Sanger sequencing (Table S4). In a 68-year-old male with optic atrophy, a missense mutation was identified, chr5:92924068 G>C; c.909G>C; p.Gln303His. However, this variant was also present in his unaffected sister and son and was interpreted as a nonpathogenic variant.

NR2F1 encodes a conserved orphan nuclear receptor protein (nuclear receptor subfamily 2, group F, member 1) that, activated by a yet unknown ligand, regulates transcription.[28] It belongs to the nuclear receptor superfamily, of which several members already have been implicated in human disease: *NR2E3* (MIM 604485) in retinitis pigmentosa,[29] *ESRRB* (MIM 602167) in deafness,[30] *NR0B1* (MIM 300473) in congenital adrenal hypoplasia,[31] and *NR2F2* (MIM 107773) in congenital diaphragmatic hernia.[32] NR2F1 has a classic nuclear receptor structure with two main domains, a functional DNA-binding domain (DBD)
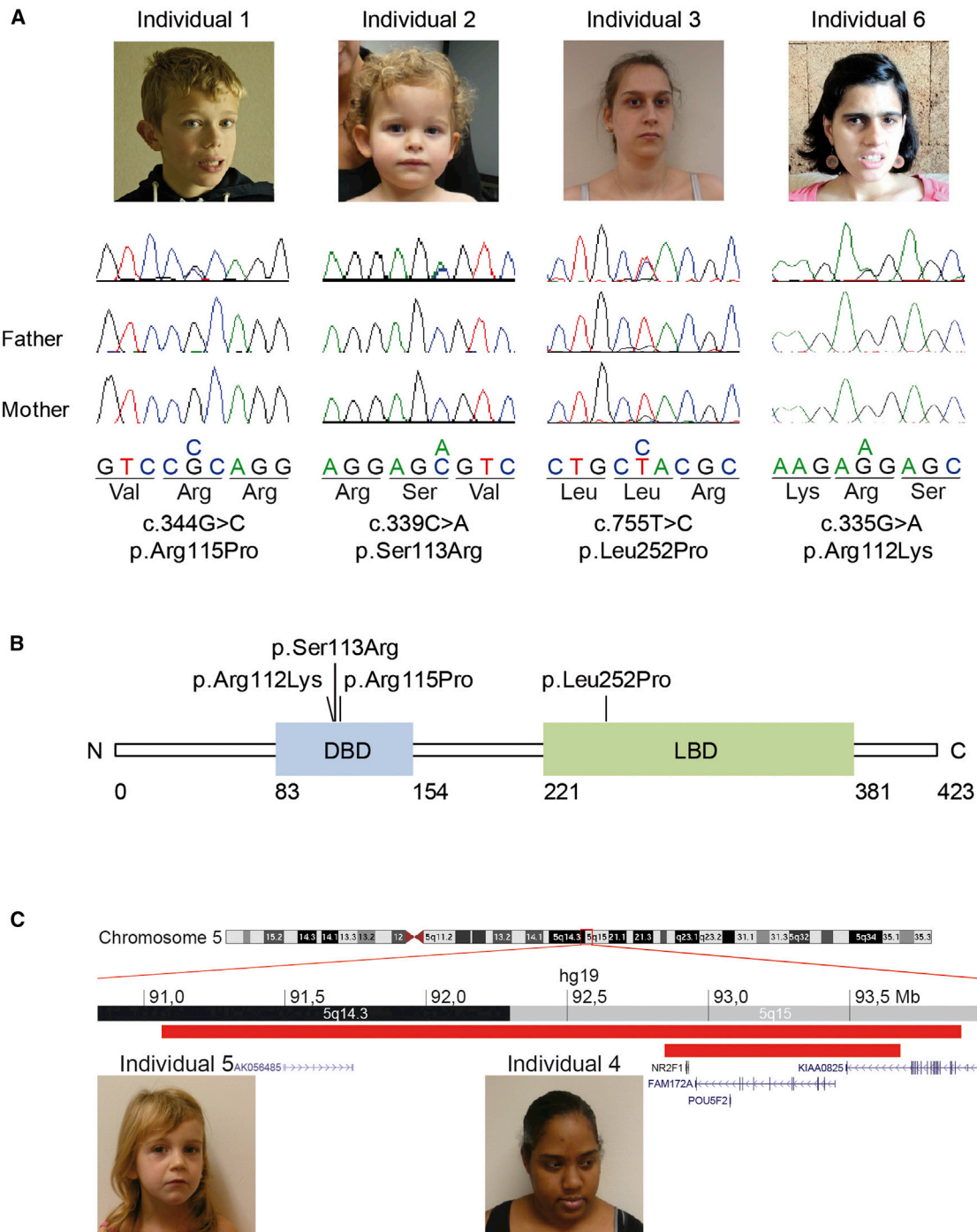
Figure 1. Phenotype and Genotype of the Individuals with Optic Atrophy

(A) Confirmation by Sanger sequencing of the de novo mutations in *NR2F1* [NM_005654.4] found by exome sequencing (individuals 1, 2, and 6), the de novo mutation in *NR2F1* found by cohort screening (individual 3), and segregation analyses. The pictures of the individuals show nonspecific facial dysmorphisms.

(B) Schematic representation of *NR2F1*, containing a DNA-binding domain (DBD) and a ligand-binding domain (LBD). The positions of the mutations identified in the affected individuals are indicated.

(C) Overview of the 5q14-q15 region encompassing the two overlapping microdeletions of individual 4 (lower red bar) and 5 (upper red bar) comprising *NR2F1*. The inheritance of the deletion in individual 4 was unknown, because the father was not available for testing and the deletion was on the paternal allele. The deletion of individual 5 was de novo. The pictures of the affected individuals show nonspecific facial dysmorphisms.

formed by two zinc-finger structural domains and a ligand-binding domain (LBD) with two highly conserved sequence regions.[33] Interestingly, three of the four point mutations that we identified localize to the DBD of NR2F1, probably affecting the binding of NR2F1 to its transcriptional targets.

Table 1. Genotype and Phenotype of the Affected Individuals

	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5	Individual 6
Defect in NR2F1	c.344G>C p.Arg115Pro	c.339C>A p.Ser113Arg	c.755T>C p.Leu252Pro	Deletion (0.83 Mb)	Deletion (2.85 Mb)	c.335G>A p.Arg112Lys
Gender	Male	Female	Female	Female	Female	Female
Age	12y	2^{+4}y	18y	24y	4y	35 y
Development	IQ 48	DD	IQ 55–65	IQ 61–74	DD	IQ 52
Behavioral abnormalities	-	-	-	-	-	OCD, autism
OFC (centile)	98th	55th	55th	99th	50th	13th
CVI	+	+	+	+	+	-
Optic disc abnormalities	Small and large excavation	Pale	Pale and large excavation	Partial pale	Small, pale and large excavation	Pale
MRI brain	Normal	Small optic nerve and chiasm	ND	ND	Normal	Normal

DD, developmental delay; ND, not done; OCD, obsessive-compulsive disorder; OFC, occipital frontal head circumference.

To investigate the functional effects of our identified point mutations in NR2F1, we established a reporter assay. In this assay, luciferase is under control of an NR2F1 activated promoter and cells are cotransfected with this plasmid and either wild-type (WT) or mutant Nr2f1 constructs. The luciferase activity reflects the transcriptional activity of the constructs. The expression plasmids of the mouse Nr2f1 - COUP-TFI, and the NGFI-A (−168/+33) promoter luciferase reporter in pXP2 were previously described.[34,35] All point mutations were generated with the QuickChange site-directed mutagenesis kit (Agilent). For luciferase assays, HEK293T cells were plated in 24-well plates (1 × 10^5 cells per well) 24 hr prior to transfection. Cells were transfected with 5 ng of reporter per well with Lipofectamine 2000 following the manufacturer's instructions. Forty-eight hr after transfection, cells were harvested and luciferase activity was measured by the luciferase assay system (Promega). As indicated in Figure 2, all DNA-binding domain mutants (p.Arg112Lys, p.Ser113Arg, and p.Arg115Pro) and the ligand-binding domain mutant (p.Leu252Pro) lost their ability to fully activate NR2F1 - COUP-TFI reporter. This result supports our contention that persons who have these mutations do not have fully functional NR2F1 - COUP-TFI product.

Additionally, the function of NR2F1 has been investigated in mice. The mouse ortholog, Nr2f1, is expressed in various tissues throughout development, with important functions in cell-fate determination, differentiation, migration, and survival, and an apparently important role in organogenesis.[33] In the developing nervous system, it is mainly expressed at high levels in the optic nerve, thalamus, and pallium (future cortex).[36–38] Studies of Nr2f1 knockout mice indicate that the protein is important for neurodevelopment, including oligodendrocyte differentiation (partly in vitro experiment),[36] cortical patterning,[39–41] and guidance of thalamocortical axons,[42] as well as eye and optic nerve development.[35,43] Furthermore, over-expression of Nr2f1 in mouse neuronal differentiating teratocarcinoma PCC7 cells antagonized neuronal differentiation induced by retinoic acid signaling.[44] Given our results from the in vitro reporter assays and because deletions of NR2F1 give rise to a similar phenotype, we hypothesize that the heterozygous mutations identified in our affected individuals cause loss of function and that the observed phenotypes are due to haploinsufficiency. Interestingly, it appears that the latter are restricted to brain and optic nerves, because no other major organ abnormalities are observed in our cohort of patients with either NR2F1 point mutations or deletions. This might be due directly to levels of NR2F1 reduced below a threshold necessary during brain development or indirectly via the target genes it regulates for specific expression in brain development. Brown et al. proposed haploinsufficiency of NR2F1 as the cause of deafness and multiple congenital anomalies in an individual with NR2F1 deletion and paracentric inversion inv(5)(q15q33.2).[45] The absence of deafness and multiple congenital anomalies in the affected individuals 4 and 5 suggests that these might be caused by the more complex chromosomal rearrangement identified in the reported person, although phenotypic variability cannot be ruled out at this time.

In addition to the optic nerve abnormalities, five out of six individuals were diagnosed with CVI. This potentially could reflect thalamocortical axon projection disorders, as reported in Nr2f1 knockout mice.[42] Until now, optic atrophy in combination with optic disc excavation was thought to be the result of perinatal damage.[46–48] We showed, however, that optic disc excavations can also be present in individuals with a genetic cause of CVI (individuals 1, 3, and 5). NR2F1 investigation should, therefore, also be considered in preterm children with CVI.

In conclusion, four individuals with optic atrophy and CVI of unknown cause had heterozygous de novo point mutations in NR2F1, and two persons with a similar
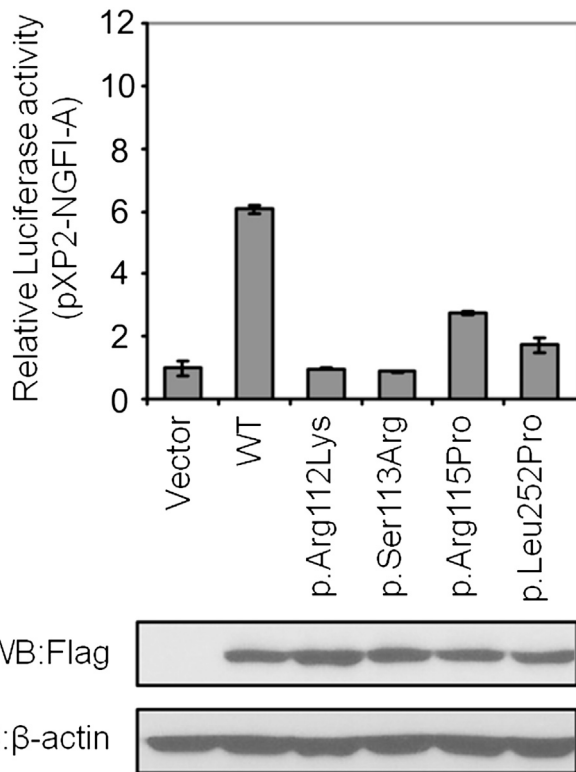
Figure 2. In Vitro Luciferase Reporter Assay for *NR2F1* Point Mutations

Effects of the *Nr2f1* (*COUP-TFI*) mutations on activation of an NGFI-A promoter-driven reporter. Top shows that pXP2-NGFI-A was cotransfected with 5 ng FLAG-tagged *Nr2f1* (*COUP-TFI*) WT, FLAG-tagged *Nr2f1* (*COUP-TFI*) mutations or empty vector into HEK293T cells as indicated. The cell sample transfected with pXP2-NGFI-A and vector served as the control, and its value (relative luciferase activity) was set as 1. The relative luciferase activities of the other samples are normalized to this control. Data are means ± SD (n = 3). Bottom shows immunoblot analysis of *Nr2f1* (*COUP-TFI*) expression in transfection. Results indicate similar expression of WT and mutant *Nr2f1* (*COUP-TFI*) constructs.

phenotype had deletions comprising *NR2F1*. Our findings indicate that *NR2F1* has an important role in the development of the visual system and that haploinsuffiency can lead to optic atrophy with intellectual impairment.

Supplemental Data

Supplemental Data include four tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

Web Resources

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/

RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq

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