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ARTICLE

Novel genetic causes for cerebral visual impairment

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Cerebral visual impairment (CVI) is a major cause of low vision in children due to impairment in projection and/or interpretation of the visual input in the brain. Although acquired causes for CVI are well known, genetic causes underlying CVI are largely unidentified. DNAs of 25 patients with CVI and intellectual disability, but without acquired (eg, perinatal) damage, were investigated by whole-exome sequencing. The data were analyzed for *de novo*, autosomal-recessive, and X-linked variants, and subsequently classified into known, candidate, or unlikely to be associated with CVI. This classification was based on the Online Mendelian Inheritance in Man database, literature reports, variant characteristics, and functional relevance of the gene. After classification, variants in four genes known to be associated with CVI (*AHDC1*, *NGLY1*, *NR2F1*, *PGAP1*) in 5 patients (20%) were identified, establishing a conclusive genetic diagnosis for CVI. In addition, in 11 patients (44%) with CVI, variants in one or more candidate genes were identified (*ACP6*, *AMOT*, *ARHGEF10L*, *ATP6V1A*, *DCAF6*, *DLG4*, *GABRB2*, *GRIN1*, *GRIN2B*, *KCNQ3*, *KCTD19*, *RERE*, *SLC1A1*, *SLC25A16*, *SLC35A2*, *SOX5*, *UFSP2*, *UHMK1*, *ZFP30*). Our findings show that diverse genetic causes underlie CVI, some of which will provide insight into the biology underlying this disease process.
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INTRODUCTION

Cerebral visual impairment (CVI) is one of the major causes of visual impairment in Western countries, as it accounts for 27% of low vision in childhood.[1] CVI is a collective term of visual disorders, resulting from damage or malfunctioning of cerebral parts of the visual system, such as the optic tracts, optic radiations, and the visual cortex. It is diagnosed when no ocular abnormality can explain the impairment in vision, which can consist of a reduced visual acuity and/or visual field defects.[2] In addition, abnormal visual behavior, such as staring into light or delayed fixation, can be present. Deficits in higher perceptual functions, for example, difficulties with recognition of objects and faces, or visio-spatial disorders can occur and are sometimes the only features of CVI.[3–5] CVI can occur in isolation, but more often additional features are present, such as intellectual disability (ID), epilepsy and/or deafness.[6–8] An important cause of CVI is acquired damage to the brain, mainly the result of perinatal problems (eg, cerebral hemorrhage or periventricular leukomalacia), but also other types of acquired damage, such as congenital infection, hypoglycemia, meningitis, or head trauma, can be causal.[2] Furthermore, West syndrome and hydrocephalus can result in CVI.[9,10] So far, less attention has been paid to genetic causes of CVI, although associations with several neurodegenerative causes and chromosomal aberrations have been described.[11] Recently, we reported in 7% of CVI patients associations with copy number variants, among others trisomy 21, 1p36 deletion, and 22q13.3 deletion (Phelan–McDermid syndrome).[12] Moreover, CVI was recently shown to be caused by *de novo* variants in *NR2F1*, leading to the Bosch–Boonstra–Schaaf optic atrophy syndrome (#615722, http://www.NR2F1gene.com).[13] In other neurological disorders, such as ID, epileptic encephalopathies, or autism, a high rate of (probably) disease-causing *de novo* variants were identified by whole-exome sequencing (WES) by using a child–parents trio approach.[14–20] In addition, WES has also shown to be a powerful tool for identifying autosomal-recessive and X-linked variants in persons with ID.[19–24] Here we used WES to identify underlying genetic causes for CVI.

SUBJECTS AND METHODS

Twenty-five patients with CVI and a visual acuity ≤ 0.3 were included, and WES was performed in the patients and their parents (detailed methods are presented in the Supplementary Methods). After performing quality filtering, the common variants ($>1\%$) were excluded, and the data were analyzed for variants following a *de novo*, X-linked, and autosomal-recessive inheritance pattern. Truncating variants and variants predicted to affect function were validated by Sanger sequencing. The genes in which the variants were identified were further classified partly based on the method reported by de Ligt *et al*[15] and Gilissen *et al*.[25] (Figure 1). A second, more stringent filtering was used for the *de novo* and X-linked variants (frequency $\leq 0.1\%$ in controls) and truncating variants (frequency of truncating variants in controls across the whole gene ≤ 0.1 or $\leq 1.0\%$ (autosomal-recessive) in controls). 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. Three patients (12, 13, and 23) were part of previous reports.[13,26] The variants identified have been submitted to the Leiden Open Variation Databases (LOVDs) (http://databases.lovd.nl/, patient IDs #00025011 and #00039389–#000394012).

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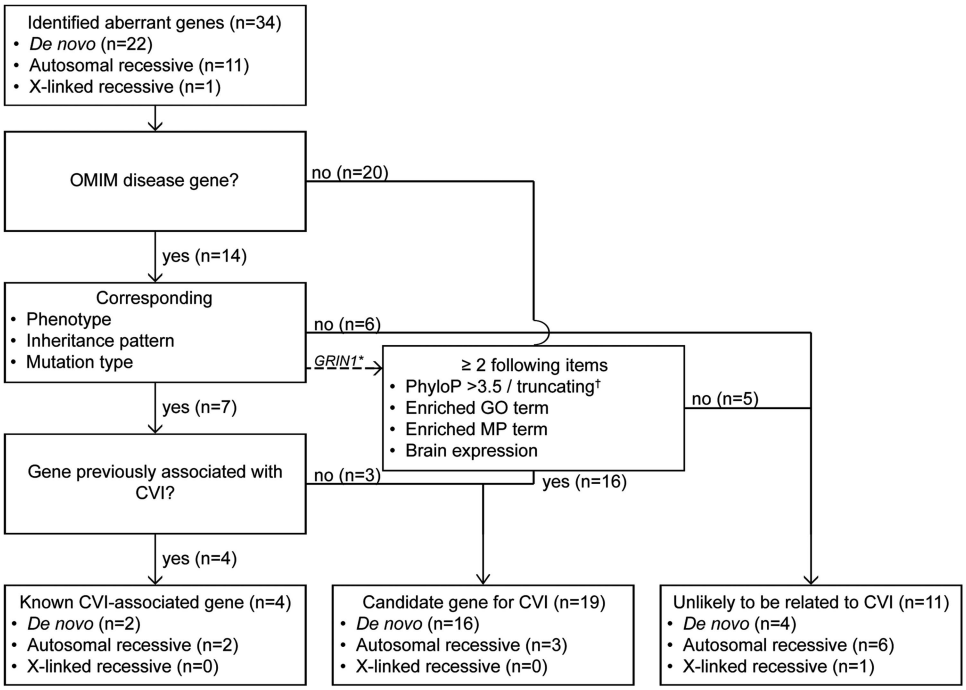

Figure 1 Flow chart of gene classification. *Inheritance does not fit with the reported OMIM disease (autosomal-recessive instead of the reported *de novo* autosomal dominant). However, this variant is further classified according to de Ligt *et al*[15] and Gilissen *et al*[25]; see Results section. †Frameshift, nonsense or splice site variant.

RESULTS

The clinical characteristics of the 25 patients analyzed were as described in Table 1 and in more detail per patient in Supplementary Table S1. The mean age was 12 years (range 1–33 years) and one patient had a visual acuity <0.05, which is defined as blindness by the WHO (http://apps.who.int/classifications/icd10/browse/2015/en). In addition to CVI, all patients had ID, ranging from mild to severe. In all patients, WES was performed by trio approach with an average read depth of 120× (Illumina Hiseq 2000, Illumina, San Diego, CA, USA), 115× (Illumina HiSeq, Illumina), or 72× (Solid 5500XL, Life Technologies, Carlsbad, CA, USA). A *de novo* ratio could not be established, because only protein truncating variants and missense variants predicted to affect function were validated. After prioritization and validation of the identified variants in a patient and its parents, further segregation analysis for the autosomal-recessive and X-linked variants was performed in the families for whom this was possible. The variants in one autosomal-recessive gene, *ITPRIPL1*, and five variants in X-linked genes, *CACNA1F*, *CNGA2*, *FLNA*, *PCDH11X*, and *ZMAT1*, could be discarded because of their presence in healthy (male) family members (Supplementary Table S2).

In total, 52 variants fulfilling the prioritization criteria in 45 genes were identified: 28 *de novo* variants in 27 genes, 19 autosomal-recessive variants in 13 genes, and 5 X-linked recessive variants in 5 genes (Supplementary Tables S3 and S4). Two of the *de novo* variants were, based on the exome data and Sanger sequencing results, probably mosaic variants: one stop variant in *PPFIA4* and one missense variant in *SLC1A1*. In addition, five *de novo* frameshift variants were identified in *AHDC1*, *AKAP9*, *DLG4*, *RAB11FIP1*, and *TRIOBP*, one *de novo* variant in *AMOT* with a possible splice effect and 20 *de novo* missense variants. The autosomal-recessive variants consisted of 7 homozygous and 12 compound heterozygous variants of several variant types. The X-linked recessive variants consisted of four missense and one variant in the translation-initiating Methionine. The latter variant was identified in *ALAS2*, and other transcripts of this gene have alternate start codons (Biosoftware Alamut, version 2.3 rev2, Interactive Biosoftware, Rouen, France).

All 45 genes were classified, but 11 genes (*AKAP9*, *ALAS2*, *FAM166B*, *KAL1*, *MAP3K15*, *MUT*, *POF1B*, *PPFIA4*, *SLC6A13*, *SPTBN5*, and *TRIOBP*), which were excluded based on the more stringent criteria (Supplementary Table S4), are not further discussed. Of the remaining 34 genes, 14 have previously been indicated in OMIM diseases (Figure 1).

For seven genes, the phenotype of the patients was in line with the reported phenotype: CVI was reported previously in four genes, *AHDC1*, *NGLY1*, *NR2F1*, and *PGAP1*, whereas the other three genes were classified as candidate genes for CVI, *GRIN2B*, *KCNQ3*, and *SLC35A2*.[13,27–29]

For five genes, *HSPG2*, *PHKB*, *SRP72*, *SYNE1*, and *TENM3*, the reported phenotype in literature was not in line with the phenotype of the patient, and those variants were classified as unlikely to be causative for CVI in those patients (#224410, #255800, #261750, #614675, #612998, #610743, #615145). In *APOPT1*, the phenotype was also not in line with the reported phenotype (#220110). Moreover, we identified a *de novo* heterozygous variant in this gene, whereas the related OMIM disease has an autosomal-recessive inheritance pattern and no second variant could be identified in the raw exome data. For another OMIM disease gene, *GRIN1* (#614254), *de novo* heterozygous variants in this gene have been reported to cause ID; however, we identified a homozygous variant. Therefore, this variant was further classified according to the method by de Ligt *et al*[15] and Gilissen *et al*[25] as a candidate gene for CVI. This method was also used for the non-OMIM disease-related genes ($n=20$) and consisted of the assessment of the variant characteristics (PhyloP/truncating variant) in combination with the functional relevance of the gene (GO-terms, MP-terms,

Table 1 Cohort characteristics

Characteristic	*Number of patients*
Gender	
Male	17
Female	8
Age group	
<4 years	4
4–10 years	9
11–20 years	6
>20 years	6
Visual acuity	
≤0.3 and ≥0.05	24
<0.05	1
Fixation abnormalities	
Yes	13
No	12
Visual field defects	
Yes	17
No	8
Developmental delay and/or intellectual disability	
Yes	25
No	0
Able to walk independently	
Yes	15
No	10
Speak words	
Yes	12
No	13
Microcephaly (<3rd centile)	
Yes	4
No	20
Unknown	1
Macrocephaly (>97th centile)	
Yes	3
No	21
Unknown	1
Abnormality on brain MRI	
Yes	14
No	8
Not assessed	3
Epilepsy	
Yes	9
No	16
Hearing loss	
Yes	1
No	23
Unknown	1

Table 2 Identified known and candidate genes for CVI per patient

Patient	*Dominant de novo*	*Autosomal recessive*
1	*DLG4*	
2		*NGLY1*[a]
3		
4		
5		
6	*SLC35A2*	
7	*AHDC1*[a]	
8	*GABRB2, ARHGEF10L*	
9	*AMOT*	
10	*UHMK1*	
11	*SLC25A16*	*GRIN1, DCAF6*
12		*PGAP1*[a]
13	*NR2F1*[a]	
14		
15		
16		
17	*SOX5, KCTD19*	
18		
19	*GRIN2B, ZFP30*	
20	*ATP6V1A, UFSP2*	
21		
22	*RERE, SLC1A1*[b]	
23	*NR2F1*[a]	*ACP6*
24	*KCNQ3*	
25		

[a]Identified known CVI-associated gene.
[b]Probably mosaic mutation.

and brain expression), leading to a classification of 15 candidate genes for CVI and 5 genes as unlikely to be related with CVI (Figure 1).

In total, 4 known CVI-associated genes, 19 candidate CVI genes, and 11 genes unlikely to be related to CVI were identified (Table 2 and Supplementary Table S3), and in five of the 25 patients (20%), a genetic diagnosis for the CVI could be established. In another 11 patients (44%), one or more candidate genes for CVI could be identified. Photographs of the patients in whom variants in known or candidate genes were identified are shown in Figure 2 and their clinical features are summarized in Table 1 and Supplementary Table S1. Pictures of patients 12, 13, and 23 have previously been published.[13,26]

DISCUSSION

WES was performed in 25 patients with CVI and a visual acuity of ≤0.3, without acquired risk factors for CVI nor pathogenic copy number variants. We identified variants in four known CVI-associated genes, namely *AHDC1*, *NGLY1*, *NR2F1*, and *PGAP1*, and 19 candidate genes for CVI. In some patients, multiple variants in more than one gene were found. In addition, *de novo* variants in *NR2F1* were identified in two patients (13 and 23). The identification of variants in known CVI-associated genes strengthened our hypothesis that WES is the right approach to identify the underlying genetic causes in patients with CVI. Moreover, several identified candidate genes have a functional link with genes known to be associated with CVI.

Three genes, in which variants were identified, have been implicated in glycosylation: *NGLY1*, *SLC35A2*, and *PGAP1*. Previously, CVI has been reported as part of congenital disorders of glycosylation (CDG) type 1a (*PMM2*), type 1q (*SRD5A3*), and type 1v (*NGLY1*).[8,28,30,31] The phenotype of patient 2 with *NGLY1* variants is similar to the previously reported patients, including the microcephaly, hypotonia,

Figure 2 Photographs of the patients in whom known or candidate genes for CVI were identified, except for patient 11 (consent to publish could not be obtained).

movement disorder, and alacrima.[28,32,33] Variants in *SLC35A2* lead to CDG type 2m, featured by ID, epilepsy, facial dysmorphisms, and transient abnormalities in transferin testing.[34–36] In the seven reported patients with CDG type 2m, CVI has not been mentioned, but other features, including the facial dysmorphism, epilepsy and severe ID were present in patient 6. The third glycosylation gene in which a variant was identified, *PGAP1* (patient 12, reported elsewhere), is important in the GPI-anchor synthesis pathway.[26] Several other genes, *PIGA*, *PIGN*, and *PIGT*, implicated in this pathway are known to be implicated in CVI and ID as well,[29,37–40] and recently, also *PGAP1* variants were found to be associated with CVI and ID.[29,41]

RERE is another gene with a functional link with a gene known to be aberrant in CVI. A *de novo* variant in this arginine-glutamic acid repeats-encoding gene was identified in patient 22. RERE binds directly to NR2F1, which has recently been identified to be aberrant in Bosch–Boonstra–Schaaf optic atrophy syndrome, of which one of the features is CVI.[13,42] In addition, *RERE* null mice show severe central nervous system abnormalities and defects of the optic vesicles.[43] Furthermore, RERE forms a complex with NR2F2 and EP300 and positively regulates retinoic acid signaling in mice.[44] Retinoic acid signaling induces optic vesicle and brain development and Nr2f1 and Nr2f2 transcription in mice stem cells.[45] These findings indicate that *RERE* is a likely candidate gene for CVI (http://www.REREgene.com).

Several other candidate genes, *GRIN2B*, *GRIN1*, *KCNQ3*, *GABRB2*, and *SOX5*, have been implicated in neurological diseases other than CVI. In *GRIN2B*, a *de novo* missense variant was identified in patient 19. Variants in *GRIN2B* have previously been found in individuals with ID.[46] *GRIN2B* encodes the subunit NR2B of the NMDA receptor, which is present during development. In the first decade, during the critical period of developing cerebral visual cortex, NR2B is replaced by NR2A, encoded by *GRIN2A*.[47] For *GRIN2A*, one 4-year-old girl has been reported with low vision.[48] So it might be expected that variants in *GRIN2B* can lead to a disturbed development and subsequently CVI. So far, 18 patients with *GRIN2B* variants have been reported.[17,18,46,49–52] In two patients with West syndrome, poor eye contact was reported,[50] whereas in the other patients no assessment for CVI or visual acuity measurement was mentioned.

In patient 11, a homozygous missense variant in *GRIN1* was identified. *GRIN1* encodes for NR1, which, together with NR2B or

NR2A, forms the NMDA receptor. NR1 is an essential subunit for the NMDA receptor, and full *Nr1* knockout mice are not viable.[53] So far, only four patients with *de novo* heterozygous variants in *GRIN1* have been reported with ID with or without epilepsy.[18,54,55] Those variants are located in or nearby the transmembrane domains,[54] in contrast to the homozygous variant identified in patient 11, which is situated in the extracellular ligand-binding domain of *GRIN1* (http://www.ebi.ac.uk/interpro).[56] Whether the here identified variant might be considered as a hypomorphic variant, giving rise to an autosomal-recessive disorder, awaits functional proof.

In patient 24, with ID and absence seizures, a *de novo* missense variant in *KNCQ3* was identified. The same variant was previously reported in a patient with severe ID and multifocal abnormalities on EEG.[16] *KNCQ3* encodes a potassium channel subunit and has been implicated in benign epileptic seizures. Several families have been reported with seizures that resolve before the age of 6 years without CVI or ID.[57–65] However, recently two additional families with seizures and variants in *KCNQ3* have been reported in which family members had various IQ levels from severe ID to normal.[66,67] Therefore, the phenotypic spectrum of *KCNQ3* variants appeared to be broader than benign epilepsy only and might well include CVI.

A *de novo GABRB2* variant in the transmembrane domain was identified in patient 8. In addition to CVI and ID, by EEG he had continuous spike and wave during slow wave sleep epilepsy, a severe epileptic encephalopathy, from the age of 6 years. One *de novo GABRB2* variant, in the N-terminal extracellular domain implicated in GABA-binding, has been reported in a patient with ID and febrile seizures, tonic clonic convulsions, and partial seizures.[68] Variants in other genes encoding GABA type A-receptor subunits have been identified in different epilepsy syndromes, making *GABRB2* a likely explanation for the phenotype in our patient.

Finally, in patient 17 a *de novo* missense variant in *SOX5* was identified, located in the HMG-domain, which is important for DNA and protein binding. Intragenic deletions in *SOX5* have been reported as a cause for ID.[69–71] In one patient, optic nerve hypoplasia was reported, which is in agreement with the slightly pale optic discs in our patient. However, several intragenic deletions are reported in healthy individuals in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home).[72] Without functional assays, it is difficult to ascertain whether the here identified missense variant leads to loss or gain of function.

In literature, several genes, for example, *PAX6* and *SOX2*, are reported to influence the structural development of eye and brain.[73] These genes may affect other parts of the visual system as well. However, whether aberrant genes lead to a vision disorder exclusively owing to structural eye defects, or whether an additional cerebral component is present, is difficult to distinguish. In the here presented patients without structural eye abnormalities, no rare variants have been identified in these genes.

In total, in 5 out of the 25 patients (20%) a genetic diagnosis for the CVI could be established. The proportion of genetically solved cases is lower than previously reported for ID, but this is probably due to the fact that only a few CVI genes are yet known.[15,16,19,20] In another 11 patients (44%), variants in one or more candidate genes for CVI could be identified with several showing a functional link with known genes for CVI. In addition, several candidate genes have been implicated in a neurological disorder, such as ID and epilepsy. In those reported patients, ophthalmological investigation has not always been performed or mentioned, and especially in patients with ID CVI can easily remain unnoticed.[74] In addition, for some disorders only few patients have been reported, and the full clinical spectrum and its variability is probably not yet clear. Nevertheless, for the identified candidate genes reported here it is of importance to find more patients with CVI and a variant in the same gene to establish a causal relationship. In combination with additional functional studies, this will increase our insight into the development of the visual system. So far, CVI has been mainly investigated in the light of acquired brain damage. Previously, we associated several chromosomal aberrations and *NR2F1* with CVI.[12,13] Here we show the importance of monogenetic disorders in the pathogenesis of CVI and the necessity to test for genetic defects using genome-wide diagnostic tools.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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