

# De Novo Duplication in the *CHD7* Gene Associated With Severe CHARGE Syndrome

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Genomics Insights  
Volume 12: 1–5  
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DOI: 10.1177/1178631019839010



**ABSTRACT:** CHARGE syndrome is an autosomal dominant developmental disorder associated with a constellation of traits involving almost every organ and sensory system, in particular congenital anomalies, including choanal atresia and malformations of the heart, inner ear, and retina. Variants in *CHD7* have been shown to cause CHARGE syndrome. Here, we report the identification of a novel *de novo* p.Asp2119\_Pro2120ins6 duplication variant in a conserved region of *CHD7* in a severely affected boy presenting with 3 and 5 of the CHARGE cardinal major and minor signs, respectively, combined with congenital umbilical hernia, congenital hernia at the linea alba, mildly hypoplastic inferior vermis, slight dilatation of the lateral ventricles, prominent metopic ridge, and hypoglycemic episodes.

**KEYWORDS:** Intellectual disability, CHARGE syndrome, *CHD7*, *de novo* variant, WES

**RECEIVED:** January 22, 2019. **ACCEPTED:** February 28, 2019.

**TYPE:** Case Report

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This study was supported by the Lithuanian-Swiss Cooperation Programme under UNIGENE project agreement no. CH-3-SMM-01/04 and a grant from the Swiss National Science Foundation (31003A\_160203) to AR.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

CHARGE syndrome (MIM 214800) is a multifaceted condition affecting between 1 in 8500 and 1 in 17000 individuals.<sup>1</sup> The acronym CHARGE was established based on the combination of patients' phenotypes, such as coloboma, heart defect, atresia choanae (also known as choanal atresia), retardation of growth and/or development, genital defects, ear anomalies, and/or deafness in patients. All malformations related to CHARGE syndrome occur early in the first trimester of pregnancy, while growth and developmental retardation become more obvious as the child matures. In some patients, clinical phenotype overlaps with those described for other syndromes, such as DiGeorge syndrome, velocardiofacial, oculo-auriculo-vertebral, and Kallmann syndromes.<sup>2,3</sup> Clinical diagnosis is made with a set of criteria (Table 1) and CHARGE patients typically have all 4 major or 3 major and 3 minor features, while individuals suspected to have CHARGE syndrome may only have 1 or 2 major and several minor characteristics. Subsequent molecular confirmation enables genetic counselling concerning recurrence risk.

## Methods

### Whole exome sequencing

DNA was extracted from peripheral blood lymphocytes using the phenol-chloroform extraction method. The proband and his healthy parents were assessed by whole exome sequencing (WES). We followed the procedure we routinely and successfully used to identify the cause of Mendelian diseases.<sup>4–8</sup> Briefly, exomes were captured using the Agilent SureSelect Human All Exon V5 enrichment kit and multiplex sequenced (6-plex) on an

Illumina HiSeq 2500 platform to reach about 100-fold coverage on average and were mapped according to the human reference genome build 38. Variants were filtered based on allele frequency in ExAC and in the Lithuanian population, pathogenicity predictions scores (SIFT, PolyPhen, MutationTaster, CADD),<sup>9–13</sup> and inheritance patterns including autosomal-recessive, X-linked, and *de novo*/autosomal dominant using the Varapp filtering software.<sup>14</sup> Sanger sequencing confirmed the anticipated segregation of the potentially causative variant.

## Results

The proband (3 years 7 months old; Figure 1A and B) is the first-born male child of healthy non-consanguineous parents. Family history was unremarkable, with no undue exposure to teratogens reported. The pregnancy was complicated by imminent preterm labour at 32 weeks of gestation. The patient was born at 34 weeks of gestation with the umbilical cord wrapped around his neck and green amniotic fluid. The propositus head circumference, weight, and length at birth were 34 cm (90th centile), 2275 g (50th centile), and 49 cm (90th centile), respectively. On delivery, his Apgar scores at 1 minute and 5 minutes were 8 and 8, respectively. He was apneic at birth and was intubated for several days. He also received nasogastric tube feeding to circumvent swallowing issues. A brain magnetic resonance imaging (MRI) examination performed at 1.5 months of age showed mildly hypoplastic inferior vermis, slight dilatation of the lateral ventricles, and dilated fourth ventricle (Figure 1C and D). Brain MRI examination has not revealed anomalies of olfactory bulb, optic nerves, or pituitary gland. At 4 months of age, a gastrostomy tube was placed. Multiple congenital birth defects were observed soon after birth.



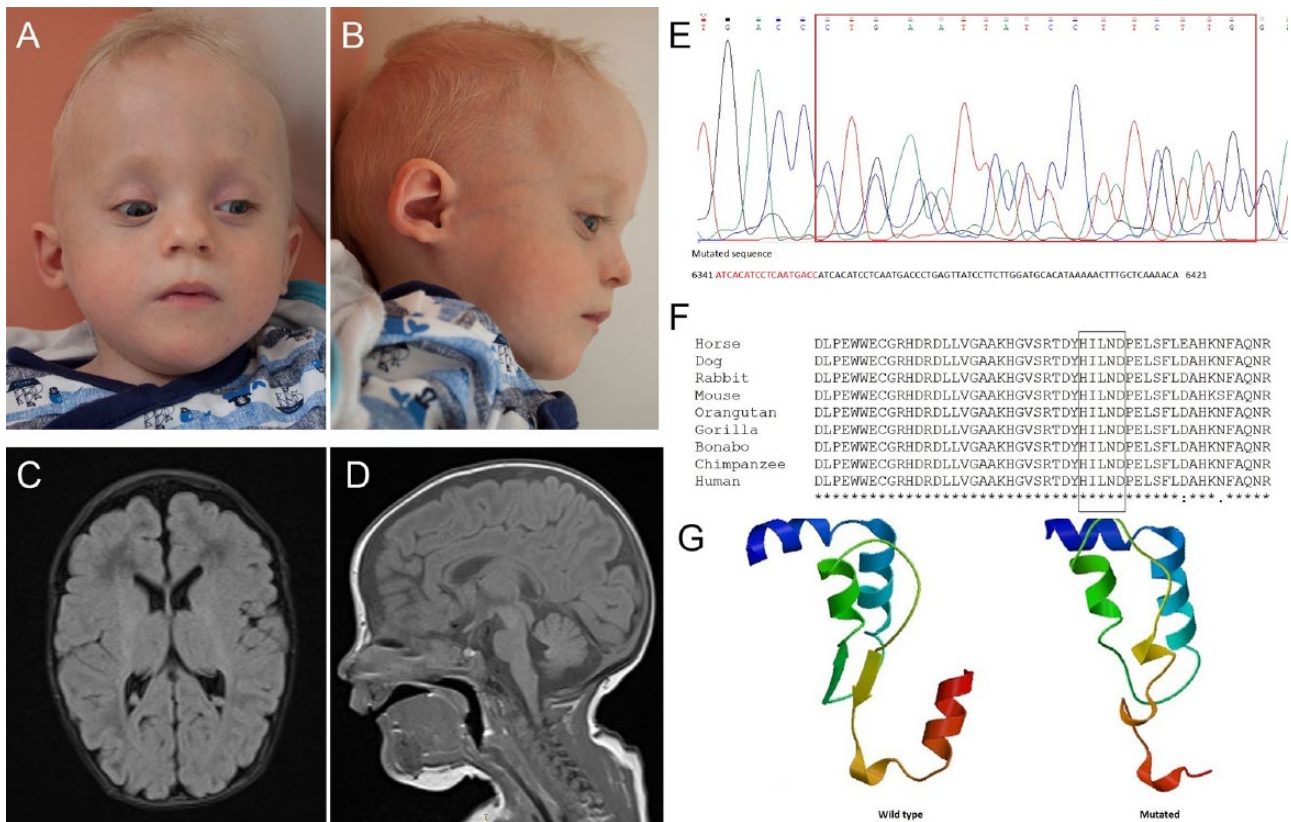
**Table 1.** Major and minor diagnostic characteristics of CHARGE syndrome and phenotype of the proband.<sup>3</sup>

CHARACTERISTICS	MANIFESTATIONS	FREQUENCY	PROBAND
<i>Major diagnostic characteristics</i>			
Ocular coloboma	Coloboma of the iris, retina, choroid, disc; microphthalmia	80%–90%	<ul style="list-style-type: none"> <li>Bilateral choroid coloboma involving optic nerve</li> </ul>
Choanal atresia or stenosis	Unilateral/bilateral: bony or membranous atresia/stenosis	50%–60%	
Cranial nerve dysfunction or anomaly	I: hyposmia or anosmia	Frequent	
	VII: facial palsy (unilateral or bilateral)	>40%	
	VIII: hypoplasia of auditory nerve	Frequent	
	IX/X: swallowing problems with aspiration	70%–90%	<ul style="list-style-type: none"> <li>Swallowing problems</li> </ul>
Ear	Outer ear: short, wide ear with little or no lobe, ‘snipped off’ helix, prominent antihelix that is often discontinuous with tragus, triangular concha, decreased cartilage; often protruding and usually asymmetric; Middle ear: ossicular malformations; Mondini defect of the cochlea; Temporal bone abnormalities; absent or hypoplastic semicircular canals	80%–100%	<ul style="list-style-type: none"> <li>Smaller and wide right ear</li> <li>Bilateral sensorineural hearing loss</li> </ul>
<i>Minor diagnostic characteristics</i>			
Genital hypoplasia	Males: micropenis, cryptorchidism Females: hypoplastic labia	50%–60%	<ul style="list-style-type: none"> <li>Micropenis, cryptorchidism</li> </ul>
	Males and females: delayed puberty secondary to hypogonadotropic hypogonadism	Frequent	
Developmental delay	Delayed milestones, hypotonia	Almost 100%	<ul style="list-style-type: none"> <li>Delayed psychomotor development</li> <li>Autism spectrum disorder</li> </ul>
Cardiovascular malformation	Including conotruncal defects (eg, tetralogy of Fallot), AV canal defects, and aortic arch anomalies	75%–85%	<ul style="list-style-type: none"> <li>Small atrial septal defect</li> </ul>
Growth deficiency	Short stature, usually postnatal with or without growth hormone deficiency	70%–80%	<ul style="list-style-type: none"> <li>Weight and height &lt;3rd centile</li> </ul>
Orofacial cleft	Cleft lip and/or palate	15%–20%	
Tracheoesophageal (TE) fistula	TE defects of all types	15%–20%	
Distinctive facial features	Square face with broad prominent forehead, prominent nasal bridge and columella, flat midface	70%–80%	<ul style="list-style-type: none"> <li>Prominent forehead, prominent metopic ridge</li> </ul>
Other features			<ul style="list-style-type: none"> <li>Congenital umbilical hernia and congenital hernia at the linea alba;</li> <li>Mildly hypoplastic inferior vermis and slight dilatation of the lateral ventricles;</li> <li>Hypoglycemic episodes</li> </ul>

Abbreviation: AV, atreioventricular.

An echocardiogram identified a small atrial septal defect, while a brain ultrasonographic assessment revealed inferior *vermian hypoplasia*. Bilateral choroid coloboma involving the optic nerves was diagnosed by an ophthalmologist at 13 months. Surgical correction was performed for a congenital umbilical hernia and a congenital hernia at the *linea alba*. Bilateral sensorineural hearing

loss was managed with hearing aids. Hypoglycemic episodes started to manifest at approximately 9 months of age, and therapy with slow release cornstarch was initiated. Notably, there has also been clear psychomotor delay as the proband could sit without support by 15 months of age and walk independently at 37 months of age. His first teeth erupted at 14 months of age. The boy has no



**Figure 1.** (A) Front and (B) side views of the patient at 1 year and 9 months of age. Brain MRI at 1.5 months of age showed (C) slight dilatation of the lateral ventricles, mildly hypoplastic inferior vermis and (D) dilated fourth ventricle. (E) An electropherogram of the genomic DNA sequence of the patient showed a *de novo* duplication in *CHD7*: c.6341\_6358dup (Asp2119\_Pro2120ins6) A mutated sequence with duplication in red written beneath. (F) Alignment of *CHD7* protein fragment for 9 different placental mammal species confirmed that the Asp2119\_Pro2120ins6 non-frameshift insertion affects an evolutionary conserved region. Secondary structures of the peptide encoded by the wild type (left) and Asp2119\_Pro2120ins6 mutated (right) exon 31 of *CHD7* are shown as rainbow ribbons. (G) The new short alpha helix that is formed by the inserted amino acid residues is in yellow. Models of wild-type and mutated protein structure were formed using SWISS-MODEL software.<sup>15</sup> MRI indicates magnetic resonance imaging.

expressive language. At 2 years 8 months old, his psychomotor development was evaluated to be in the range of 3 (self-help) to 10 (fine motor) months according the Diagnostic Inventory for Screening Children (DISC) scale. In addition, he was diagnosed with autism spectrum disorder at 2 years 9 months of age. During his last examination, at the age of 3 years 7 months, his weight was 11.5 kg (<3rd centile) and his height was 88 cm (<3rd centile). He had a prominent metopic ridge, smaller and wide right ear, sacral dimple, clinodactyly of the 5th fingers, micropenis, and cryptorchidism. He was still suffering from laryngomalacia and severe feeding problems necessitating gastrostomy tube feeding. Frequent feeds and uncooked cornstarch every 6 hours was used to prevent hypoglycemic episodes. In summary, the proband presents with 3 major and 5 minor features of CHARGE syndrome (Table 1) combined with additional features.

As no cytogenetic alterations were identified, we assessed the genome of the proband and his parents by WES. We identified a *de novo* 18 nucleotides duplication in exon 31 of the *CHD7* gene (c.6341\_6358dup, p.Asp2119\_Pro2120ins6); NM\_017780; NP\_060250; MIM 214800) confirmed by Sanger sequencing (Figure 1E), which affects an evolutionary constrained region (Figure 1F). Modelization of the putatively

encoded mutated protein suggests that the insertion of this chain of 6 amino acid residues potentially substitutes a linear chain into a short alpha helix (Figure 1G). The other deleterious *de novo* variants, which contribute to the current patient phenotype, were not identified. All unique *de novo* variants identified for this proband are listed in Table 2.

## Discussion

Alterations in *CHD7* have been identified in more than two-third of all children, who fulfil the clinical diagnostic criteria for CHARGE syndrome.<sup>16–18</sup> Truncating variants including nonsense, frameshift, and splice variants (89%) that typically result in haploinsufficiency are the most frequently encountered followed by missense (8%) variants.<sup>17,19,20</sup> *CHD7* encodes a chromodomain helicase DNA-binding protein, which plays a significant role in early embryonic development and controls gene expression via chromatin remodelling during the cell cycle.<sup>21</sup>

Besides the duplication described here, other variants affecting the same region in the protein have been identified, for example, the *de novo* missense variant c.6347T>A; p.Ile2116Asn (CM090041) that changes a hydrophobic into a polar amino

**Table 2.** List of *de novo* variants identified for the proband.

CHROMOSOME	START	REFERENCE ALLELE	ALTERNATIVE ALLELE	GENE SYMBOL	AA CHANGE	IMPACT	GENOTYPE
chr20	52557934	GGT	G	AC005220.3		Splice donor variant	Heterozygous
chr7	150811966	G	A	AGAP3	G/S	Missense variant	Heterozygous
chr7	150811967	G	A	AGAP3	G/D	Missense variant	Heterozygous
chr11	13435092	T	G	BTBD10	K/Q	Missense variant	Heterozygous
chr11	66512290	G	GGCGGCGGCGGC	C11orf80	G/GAAAA	Inframe insertion	Heterozygous
chr2	153476066	G	GCCACCCCCCCCCCA	FMNL2	-/PPPPP	Inframe insertion	Heterozygous
chr6	30996720	C	CAGGCTCTGAGACCAC-CACAGCCCTACTGA	MUC22	T/TGSETTTASTE	Inframe insertion	Heterozygous
chr19	2015540	GGGCGGCGGCGGC	G	BTBD2	AAAA/-	Inframe deletion	Homozygous
chr3	42251577	C	CGGA	TRAK1	T/TE	Inframe insertion	Heterozygous
chr14	73874252	G	-	PTGR2		NA	Heterozygous
chr4	87615711	AATAGTAGTGACAGCAGC	-	DSPP		Non-frameshift deletion	Heterozygous
chr8	60853065	-	ATCACATCCTCAATGACC	CHD7	-/HILNDH	Non-frameshift insertion	Heterozygous

*De novo* variant in *CHD7* gene associated with CHARGE syndrome is marked in grey.

acid. The associated patient presented with a milder phenotype than our proband characterized by cleft palate, auricular dysplasia, nystagmus, bilateral perceptive deafness, and semi-circular canal hypoplasia.<sup>22</sup> Similarly, the c.6322G>A; p.Gly2108Arg (CM080142) missense variant has been detected in 3 patients with mild CHARGE syndrome features, which include unilateral optic nerve coloboma and microphthalmia, bilateral sensorineural deafness, dysmorphic ears, hypoplastic semicircular canals, and bifid uvula.<sup>23</sup> The association between the insertion of the HILNDH peptide and a severe phenotype is not surprising as it is predicted to form a novel alpha helix within a highly conserved region of the protein.

While additional work is necessary to understand this complex disease, this study further expands the understanding of CHARGE syndrome pathogenesis and suggests that severe CHARGE syndrome phenotypes can be caused by deletions, point mutations,<sup>17</sup> and duplications/insertions.

### Acknowledgements

We extend our sincere appreciation to the patient's family for their support.

### Author Contributions

LP performed data analysis. LP and EP prepared the manuscript. Sequencing of trios exomes was performed by LG. AR and VK contributed to conception and design and critically revised the manuscript.

### Ethical Approval

The patient's parents provided written informed consent to publish all clinical information, including photographs of the patient.

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