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

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**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 \textit{CHD7} have been shown to cause CHARGE syndrome. Here, we report the identification of a novel \textit{de novo} p.Asp2119_\_Pro2120ins6 duplication variant in a conserved region of \textit{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, \textit{CHD7}, \textit{de novo} variant, WES

**Introduction**

CHARGE syndrome (MIM 214800) is a multifaceted condition affecting between 1 in 8500 and 1 in 17 000 individuals.¹ 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.²³ 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.⁴⁵ 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),⁶⁻¹³ and inheritance patterns including autosomal-recessive, X-linked, and \textit{de novo}/autosomal dominant using the Varapp filtering software.¹⁴ 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 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.
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

Table 1. Major and minor diagnostic characteristics of CHARGE syndrome and phenotype of the proband.3

| CHARACTERISTICS           | MANIFESTATIONS                                                                                                                                                                                                 | FREQUENCY       | PROBAND                                                                 |
|---------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------|------------------------------------------------------------------------|
| Major diagnostic characteristics |                                                                                                                                                                                                                                                                   |                 |                                                                         |
| Ocular coloboma           | Coloboma of the iris, retina, choroid, disc; microphthalmia                                                                                                                                                                                                         | 80%–90%         | Bilateral choroid coloboma involving optic nerve                      |
| 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%         | Swallowing problems                                                   |
| 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%        | Smaller and wide right ear; Bilateral sensorineural hearing loss      |
| Minor diagnostic characteristics |                                                                                                                                                                                                                                                                   |                 |                                                                         |
| Genital hypoplasia        | Males: micropenis, cryptorchidism                                                                                                                                                                                                                                   | 50%–60%         | Micropenis, cryptorchidism                                            |
|                           | Females: hypoplastic labia                                                                                                                                                                                                                                         |                 |                                                                         |
|                           | Males and females: delayed puberty secondary to hypogonadotrophic hypogonadism                                                                                                                                                                                      | Frequent        |                                                                         |
| Developmental delay       | Delayed milestones, hypotonia                                                                                                                                                                                                                                       | Almost 100%     | Delayed psychomotor development; Autism spectrum disorder            |
| Cardiovascular malformation | Including conotruncal defects (eg, tetralogy of Fallot), AV canal defects, and aortic arch anomalies                                                                                                                                                                | 75%–85%         | Small atrial septal defect                                           |
| Growth deficiency         | Short stature, usually postnatal with or without growth hormone deficiency                                                                                                                                                                                        | 70%–80%         | Weight and height <3rd centile                                     |
| 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%         | Prominent forehead, prominent metopic ridge                         |
| Other features            |                                                                                                                                                                                                                                                                   |                 |                                                                         |
|                           | Congenital umbilical hernia and congenital hernia at the linea alba; Mildly hypoplastic inferior vermis and slight dilatation of the lateral ventricles; Hypoglycemic episodes                                                                                   |                 |                                                                         |

Abbreviation: AV, atroventricular.
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 (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-thirds of all children, who fulfil the clinical diagnostic criteria for CHARGE syndrome.16–18 Truncating variants including nonsense, frameshift, and splice variants (89%) that typically result in haploinsufficiency are the most frequently encountered followed by missense (8%) variants.17,19,20 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.21

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 acid.
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 | GGGCGGCGGCGGC | C11orf180 | G/GAAAA | Inframe insertion | Heterozygous |
| chr2       | 153476066 | G | GGGCGGCGGCGGC | FMNL2 | −/PPPPP | Inframe insertion | Heterozygous |
| chr6       | 30996720 | C | CAGGCTCTGAGACCAC-CACAGCCTCTACTGA | 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 | | Homozygous |
| chr4       | 87615711 | AATAGTAGTGACACGACG | − | DSPP | Non-frameshift deletion | Heterozygous |
| chr8       | 60853065 | − | ATCACAATCCTAATGACC | 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 semicircular canal hypoplasia.22 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 sensorineuronal deafness, dysorphic ears, hypoplastic semicircular canals, and bifid uvula. 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,17 and duplications/insertions.

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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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