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 *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

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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.[2,3] 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.[4–8] 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),[9–13] and inheritance patterns including autosomal-recessive, X-linked, and *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 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.[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 Females: hypoplastic labia	50%–60%	• Micropenis, cryptorchidism
	Males and females: delayed puberty secondary to hypogonadotropic 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, atreoventricular.

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

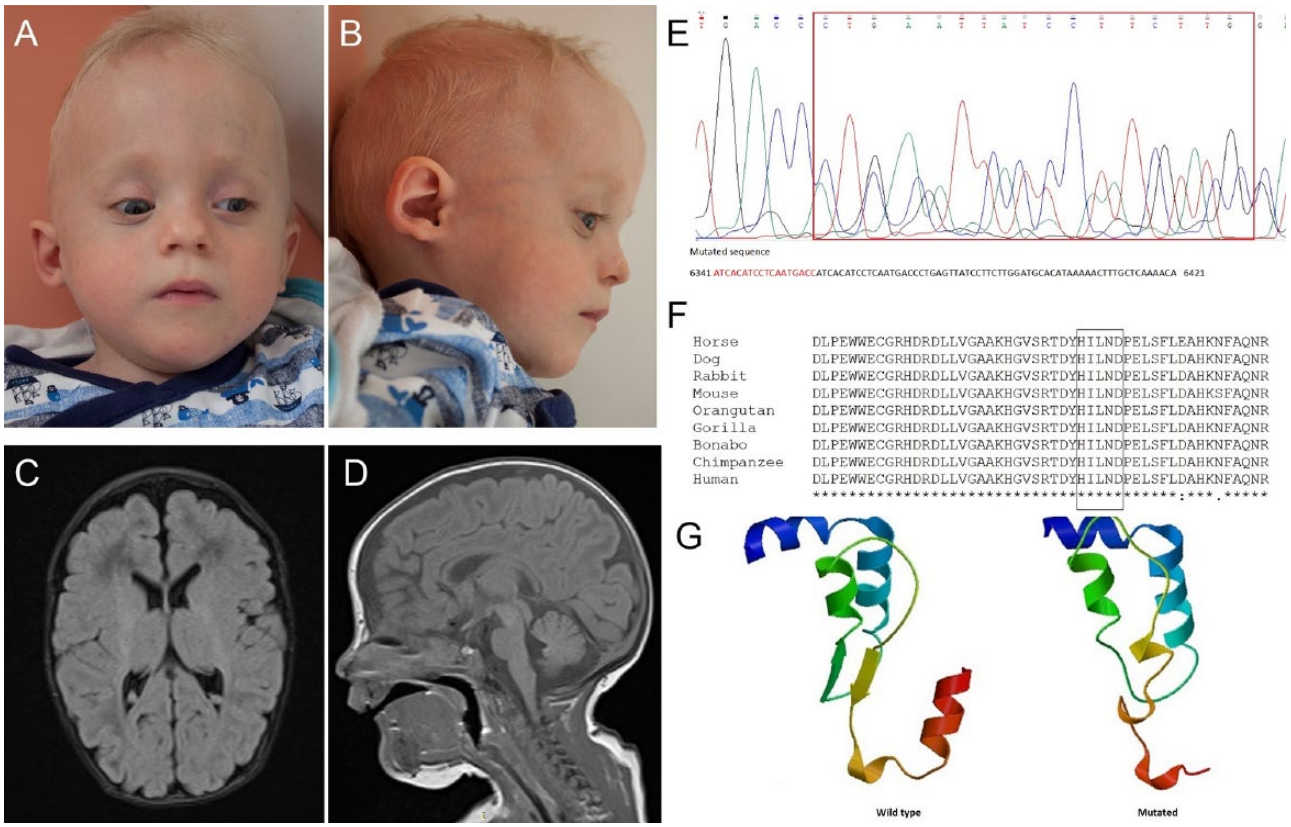

Figure 1. (A) Front and (B) side views of the patient at 1 year and 9 months of age. Brain MRI at 1.5 months of age showed (C) slight dilatation of the lateral ventricles, mildly hypoplastic inferior vermis and (D) dilated fourth ventricle. (E) An electropherogram of the genomic DNA sequence of the patient 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.[15] 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.[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

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	*C11orf80*	G/GAAAA	Inframe insertion	Heterozygous
chr2	153476066	G	GCCACCCCCCCCCCCA	*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	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 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 sensorineural deafness, dysmorphic ears, hypoplastic semicircular canals, and bifid uvula.[23] 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.

REFERENCES

1. Issekutz KA, Graham JM Jr, Prasad C, Smith IM, Blake KD. An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study. *Am J Med Genet A*. 2005;133A:309–317.
2. Blake K, MacCuspie J, Hartshorne TS, Roy M, Davenport SL, Corsten G. Postoperative airway events of individuals with CHARGE syndrome. *Int J Pediatr Otorhinolaryngol*. 2009;73:219–226.
3. Vuorela PE, Penttinen MT, Hietala MH, Laine JO, Huoponen KA, Kääriäinen HA. A familial CHARGE syndrome with a CHD7 nonsense mutation and new clinical features. *Clin Dysmorphol*. 2008;17:249–253.
4. Borck G, Hög F, Dentici ML, et al. BRF1 mutations alter RNA polymerase III-dependent transcription and cause neurodevelopmental anomalies. *Genome Res*. 2015;25:155–166.
5. Alfaiz AA, Micale L, Mandriani B, et al. TBC1D7 mutations are associated with intellectual disability, macrocrania, patellar dislocation, and celiac disease. *Hum Mutat*. 2014;35:447–451.
6. Lodder EM, De Nittis P, Koopman CD, et al. GNB5 mutations cause an autosomal-recessive multisystem syndrome with sinus bradycardia and cognitive disability. *Am J Hum Genet*. 2016;99:704–710.
7. Tumienė B, Voisin N, Preikšaitienė E, et al. Inflammatory myopathy in a patient with Aicardi-Goutières syndrome. *Eur J Med Genet*. 2017;60:154–158.
8. Gueneau L, Fish R, Shamseddin HE, et al. KIAA1109 variants are associated with a severe disorder of brain development and arthrogryposis. *Am J Hum Genet*. 2018;102:116–132.
9. Petrovski S, Wang QL, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet*. 2013;9:e1003709.
10. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods*. 2010;7:575–576.
11. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res*. 2001;11:863–874.
12. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods*. 2010;7:248–249.
13. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016;536:285–291.
14. Delafontaine J, Masselot A, Liechti R, Kuznetsov D, Xenarios I, Pradervand S. Varapp: a reactive web-application for variants filtering [published online ahead of print June 28, 2016]. *bioRxiv*. doi:https://doi.org/10.1101/060806.
15. Biasini M, Bienert S, Waterhouse A, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res*. 2014;42:W252–W258.
16. Bergman JE, Janssen N, Hoefsloot LH, Jongmans MC, Hofstra RM, van Ravenswaaij-Arts CM. CHD7 mutations and CHARGE syndrome: the clinical implications of an expanding phenotype. *J Med Genet*. 2011;48:334–342.
17. Katoh-Fukui Y, Yatsuga S, Shima H, et al. An unclassified variant of CHD7 activates a cryptic splice site in a patient with CHARGE syndrome. *Hum Genome Var*. 2018;5:18006.
18. Martin DM. Epigenetic developmental disorders: CHARGE syndrome, a case study. *Curr Genet Med Rep*. 2015;3:1–7.
19. Vissers LE, van Ravenswaaij CM, Admiraal R, et al. Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet*. 2004;36:955–957.
20. Zentner GE, Layman WS, Martin DM, Scacheri PC. Molecular and phenotypic aspects of CHD7 mutation in CHARGE syndrome. *Am J Med Genet A*. 2010;152A:674–686.
21. Sanlaville D, Etchevers HC, Gonzales M, et al. Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 truncating mutations correlates with expression during human development. *J Med Genet*. 2006;43:211–217.
22. Jongmans MC, van Ravenswaaij-Arts CM, Pitteloud N, et al. CHD7 mutations in patients initially diagnosed with Kallmann syndrome – the clinical overlap with CHARGE syndrome. *Clin Genet*. 2009;75:65–71.
23. Jongmans MC, Hoefsloot LH, van der Donk KP, et al. Familial CHARGE syndrome and the CHD7 gene: a recurrent missense mutation, intrafamilial recurrence and variability. *Am J Med Genet A*. 2008;146A:43–50.