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SHORT COMMUNICATION

Combined microdeletions and *CHD7* mutation causing severe CHARGE/DiGeorge syndrome: clinical presentation and molecular investigation by array-CGH

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Phenotypic variation in CHARGE syndrome remains unexplained. A subcategory of CHARGE patients show overlapping phenotypic characteristics with DiGeorge syndrome (thymic hypo/aplasia, hypocalcemia, T-cell immunodeficiency). Very few have been tested or reported to carry a mutation of the *CHD7* (chromodomain helicase DNA-binding domain) gene detected in two-thirds of CHARGE patients. In an attempt to explore the genetic background of a severe CHARGE/DiGeorge phenotype, we performed comparative genomic array hybridization in an infant carrier of a *CHD7* mutation. The high-resolution comparative genomic array hybridization revealed interesting findings, including a deletion distal to the DiGeorge region and disruptions in other chromosomal regions of genes implicated in immunological and other functions possibly contributing to the patient's severe phenotype and early death.
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Haploinsufficiency of the *CHD7* (chromodomain helicase DNA-binding domain) gene was recently identified as a cause of CHARGE syndrome,[1] with about two-thirds of patients having mutations in the *CHD7* gene.[2,3] A thorough literature review documented 17 patients with CHARGE and variable T-cell immunodeficiency,[4] with some cases having overlapping characteristics with DiGeorge syndrome, which in its classical presentation combines conotruncal heart defects, hypocalcemia and thymic hypoplasia.[4,5] No correlation between genotype and phenotype has been noted in CHARGE syndrome as shown from familial cases or large cohorts in which a *CHD7* mutation was found.[2,3,6–8] The role of possible modifying genetic or epigenetic factors in the phenotypic expression of T-cell immunodeficiency in CHARGE patients remains to be deciphered.

We report on a patient with overlapping characteristics of CHARGE and DiGeorge syndromes. The male proband, weighing 2600 g, was delivered by cesarean section at $39\frac{4}{7}$ weeks' gestation. His parents (28-years-old primiparous mother and 32-years-old father) were both healthy and unrelated. Because of increased nuchal translucency, amniocentesis was performed and was normal. Congenital anomalies were noted immediately after birth (Table 1). On the 10th day he developed persistent hypocalcemia, requiring calcium and 1-α vitamin D supplementation.

Generalized clonic seizures, proving refractory to different combinations of antiepileptic drugs, appeared on the 13th day and brain magnetic resonance imaging carried out on the 15th day showed no specific findings, while absence of thymus was noted by ultrasound. Microlaryngoscopy documented severe laryngomalacia causing repeated desaturation episodes, leading to a tracheotomy at the age of 1.5 months. A generalized erythroderma with a florid, scaly, icthyotic macular rash resembling that in Omenn's syndrome appeared at 2.5 months of age. He also developed total alopecia.

The infant, although under intravenous immunoglobulin replacement therapy and chemoprophylaxis, suffered from recurrent infections of the respiratory tract with frequent concurrent septicemia requiring aggressive antibiotic therapy. His respiratory function deteriorated and necessitated gradual increases of oxygen supply. Finally, the patient succumbed to respiratory insufficiency during the course of a new infection. No autopsy was performed because of parental refusal.

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Detailed testing to explore the proband's immune status[9] (Supplementary Table 1) revealed low number of T but normal B and natural killer lymphocyte subpopulations. There was no proliferative response of T lymphocytes to mitogens, and the white blood cell count showed eosinophilia (19%). At the age of 7 months, a second immune investigation noted marked lymphocytosis with increased T lymphocyte numbers together with low IgG and elevated IgE levels (Supplementary Table 1). T-cell receptor Vβ studies showed marked oligoclonality of T lymphocytes with Vβ3 of 26.2% (normal range 0.5–15.7%). There was no evidence of maternal engraftment by molecular studies.

Sequence analysis of the *CHD7* gene revealed a heterozygous pathogenic frameshift mutation in exon 16, c.3838_3851del (p.Phe1280fs).[3] The link of *CHD7* mutations with T–B+ severe combined immunodeficiency, and features of Omenn's syndrome has been confirmed in our patient and has been described in CHARGE syndrome.[10]

Table 1 Major clinical characteristics of the patient

Congenital anomalies/dysmorphisms	*Other abnormalities*
Arched eyebrows	Bilateral choroidal and disc coloboma
Micropthalmia	Bicuspid aortic valve
Micrognathia	Patent nasal choanae
Unilateral facial nerve palsy on the left	Absence of the semicircular canals
Low-set ears with abnormal pinnae	Absence of thymus
(cup-shaped)	Severe laryngomalacia
Broad nasal bridge	
Smooth philtum	
Flattened midface	
Short and webbed neck	
Pectus excavatum,	
Wide-spaced nipples	
Abnormal palmar creases	
Right unilateral cryptorchidism	
Hypoplastic scrotum, micropenis	
Syndactyly of the 2nd–3rd toes bilaterally	

To further characterize the infant's severe phenotype, a comparative genomic array hybridization was performed (Agilent Technologies, Santa Clara, CA, USA),[11] revealing interesting findings (Table 2). The *de novo* nature of the 15q22.31 deletion (0.077 Mb) with absence of known copy number variations makes it potentially pathogenic. *CILP-1* gene functions as an insulin-like growth factor 1 antagonist, especially in chondrocytes,[12] but we cannot speculate how it affects the proband's pathogenesis.

The second *de novo* Xp22.12 (0.052 Mb) microdeletion with no known copy number variations contains the *SH3KBP1* gene.[13] SH3KBP1 is a multiadaptor protein involved in different cellular functions, including downregulation of activated receptor tyrosine kinases, survival of neuronal cells and enhancement of tumor necrosis factor-mediated apoptotic cell death. *SH3KBP1* also controls post-membrane events, such as targeting receptors for degradation and regulation of gene transcription, possibly by binding to multiple adaptor proteins.[14] Similar to the *CHD7, SH3KBP1* appears to be involved through different networks and gene complexes on essential cell functions, and therefore may have contributed to the complex phenotype.

Microdeletion 7q21.11 (0.021 Mb) starts 15 bp into intron 9 of the *MAGI2* gene, and could therefore cause aberrant splicing of the gene. *MAGI2* is telomeric to the Williams–Beuren syndrome (WBS; OMIM# 194050) gene region (7q11.23). Findings on Williams–Beuren syndrome patients have implicated disruption of the *MAGI2* gene as a locus for infantile spasm.[15] Disruption of the *MAGI2* gene probably contributed to the infant's generalized clonic seizures, appearing on day 13 and refractory to different combinations of anti-epileptic drugs.

A paternally inherited aberration in 8p22 (0.074 Mb) included partial deletion of *MSR1*. A recent study strongly supports that genetic variants expressing different levels of *MSR1* show differing abilities to clear apoptotic cells, and eventually lead to the hyper-inflammatory stage characteristic of septic shock.[16] The loss of *MSR1* may not have been responsible for the infant's initial phenotype, but could have contributed to the recurrent infections of the respiratory tract with frequent concurrent septicemia, and was probably a major cause of the infant's early death.

Table 2 aCGH results

Abberation[a]	*Gene*[b]	*Inheritance*	*Presence of common CNVs/log$_2$ ratio of probes*
DEL2q35, 145.3 kb	*IGFBP5*: insulin-like growth factor binding protein 5	Paternal	No CNVs/−0.378
Nucleotides: 217072704–217217982	*RPL37A:* ribosomal protein, component of 60S subunit		
DEL 7q21.11; 20.55 kb	*MAGI2*: membrane-associated guanylate kinase inverted-2 gene	Paternal and maternal	No CNVs/−1.06
Nucleotides: 77703312–77723858			
Del 8p22; 74.33 kb	*MSR1:* macrophage scavenger receptor 1	Paternal	Yes/−0.657
Nucleotides: 15996382–16070713			
DEL 15q22.31; 76.62 kb;	*CILP:* cartilage intermediate layer protein	De novo	No CNVs/−0.626
Nucleotides: 63280700–63357315			
DEL 20q13.2; 31.31 kb;	*MATN4*: Matrillin 4	Paternal	No CNVs/−0.93
Nucleotides: 43343828–43375135	*RBPJL*: recombination signal binding protein for immunoglobulin kappa J region-like		
DEL 22q11.23; 249.5 kb;	*IGLL3*: immunoglobulin lambda-like polypeptide 3	Paternal	Yes/−0.454
Nucleotides: 23984069–24233543	*LRP5L*: low-density receptor-related lipoprotein		
DEL Xp22.12; 52.84 kb;	*SH3KBP1:* SH3-domain kinase binding protein 1	De novo	No CNVs/−0.953
Nucleotides: 19733082–19785919			

Abbreviations: aCGH, comparative genomic array hybridization; CNVs, copy number variations.
[a]Agilent 244K arrays with average resolution 6 kb.
[b]UCSC database (http://genome.ucsc.edu/), UCSC.

Another paternally inherited deletion 20q13.2 (0.031 Mb), lacking known copy number variations, contains two genes: *MATN4* and *RBPJL*. *RBPJL* has been implicated in the pancreatic acinar development through its association with a trimeric complex (PTF1-J).[17] The patient, however, was not investigated for pancreatic development and function.

Finally, microdeletion 22q11.23 located at a region distally to the one involved in DiGeorge and velocardiofacial syndromes is of interest because of its proximity to a recurrent genomic disorder, clinically distinct from the two aforementioned syndromes with clinical features similar to our proband, such as facial dysmorphism (arched eyebrows, flattened midface and smooth philtrum), a bicuspid aortic valve, and developmental and growth delay.[18] This more distal non-overlapping deletion probably contributes to the phenotype not only through the two genes: *IGLL3* and *LPR5L*,[19] but also through gene expression in the general area of the DiGeorge/velocardiofacial region by position effect possibly eliminating locus control regions from their targeted genes leading to this complex phenotype.[20]

The array comparative genomic hybridization method, carried out for the first time on a *CHD7* mutation carrier with severe CHARGE/DiGeorge and Ommen like-phenotypic overlap, detects genomic disruptions at regions that contain genes involved in immune and related functions that could also explain the mechanism of phenotypic discrepancies in CHARGE patients.

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