Saethre–Chotzen Phenotype With Learning Disability and Hyper IgE Phenotype in a Patient Due to Complex Chromosomal Rearrangement Involving Chromosomes 3 and 7

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The authors describe on a Brazilian girl with coronal synostosis, facial asymmetry, ptosis, brachydactyly, significant learning difficulties, recurrent scalp infections with marked hair loss, and elevated serum immunoglobulin E. Standard lymphocyte karyotype showed a small additional segment in 7p21[46,XX, add(7)(p21)]. Deletion of the TWIST1 gene, detected by Multiplex Ligation Probe-dependent Amplification (MPLA) and array-CGH, was consistent with phenotype of Saethre–Chotzen Chotzen syndrome. Array CGH also showed deletion of four other genes at 7p21.1 (SNX13, PRPS1L1, HD9C9, and FERD3L) and the deletion of six genes (CACNA2D2, C3orf18, HEMK1, CISH, MAPKAPK3, and DOCK3) at 3p21.31. Our case reinforces FERD3L as candidate gene for intellectual disability and suggested that genes located in 3p21.3 can be related to hyper IgE phenotype. © 2012 Wiley Periodicals, Inc.

Key words: TWIST1 gene microdeletion; Saethre–Chotzen syndrome; hyper-IgE syndrome; array-CGH; CISH gene; FERD3L gene; DOCK3 gene

INTRODUCTION

Saethre–Chotzen syndrome or acrocephalosyndactyly type III [OMIM 101400] is an autosomal dominant inheritance condition characterized by craniosynostosis, mainly of the coronal sutures, facial asymmetry, brachydactyly, partial soft tissue syndactyly, and other skeletal anomalies. There is a remarkable inter- and intra-familial phenotypic variability and mild cases can be misdiagnosed [Dollfus et al., 2002; Passos-Bueno et al., 2008]. It is caused by different heterozygous mutation in the TWIST1 gene (7p21) that results in haploinsuficiency [revised by Passos-Bueno et al., 2008]. In some instances, the phenotype is caused by microdeletion or deletion in chromosomal region 7p21 comprising the whole TWIST1 gene. Additional signs such as intellectual disability, learning difficulties, short stature, microcephaly, vertebral anomalies, respiratory, and circulatory tract pathology, and allergies had also been observed in these individuals with large TWIST1 gene deletions [Johnson et al., 1998; Chun et al., 2002; Cai et al., 2003; de Heer et al., 2005; Schluth-Bolard et al., 2008; Busche et al., 2011].

Hyper IgE syndrome [OMIM 147060 and 243700] is a rare primary immunodeficiency disorder, characterized by chronic eczema, recurrent staphylococcal skin and lung infections, and increased serum IgE. Non-immunological findings include coarse facial appearance with minor facial dysmorphism, skeletal involvement, and dental anomalies [Johnson et al., 1998; Domingo et al., 2008; Freeman and Holland, 2008]. Two types of Hyper IgE syndrome had been described: an autosomal dominant form (Job syndrome) [OMIM 147060], caused by heterozygous mutations in the STAT3 gene (17q21), and an autosomal recessive form [OMIM 243700], caused by mutations in TYK2 gene (19p13.2) and DOCK8 gene.
Here we present a Brazilian girl with 7p21 and 3p21.31 microdeletions, identified by array comparative genomic hybridization (CGH), who had Saethre–Chotzen phenotype, significant learning difficulties and hyper IgE syndrome. Clinical and molecular aspects were discussed.

**CLINICAL REPORT**

The patient (Fig. 1A–L), a girl born in 1997, is the only child of an unrelated 21-year-old mother and 20-year-old father. The mother was referred as having brachydactyly of fingers and toes, clinodactyly of the second and five fingers, and broad halluces. Clinodactyly of the second and five fingers were also referred in the maternal great-grandfather. The pregnancy was uncomplicated and delivery occurred at term. The birth weight was 3,350 g (50th centile) and length was 46 cm (3rd centile). Plagioccephaly and eyelids ptosis were noted at birth. After 3 years of age, she started to present recurrent scalp infections, which resulted in transient alopecia. Other chronic infections such as pneumonias, dermatitis, or abscesses were not observed. Motor development was normal.

Clinical examination at age 5 years (Fig. 1D–F) showed weight of 14.3 kg, height of 90 cm, and OFC of 45 cm (all below 3rd centile). She had microbrachycephaly, coronal synostosis, partial alopecia, low set ears with prominent crura and hypoplastic lobule, asymmetric face, hypoplastic supraorbital ridge at right, prominent eyes, eyelids ptosis at left, hypoplastic midface, anteverted nares, open mouth, brachydactyly of fingers, ulnar clinodactyly of fingers two, radial clinodactyly of fingers five, and toes brachydactyly. The halluces were broad and there were mild toes 2–3 and 4–5 syndactyly (Fig. 2A–C). Complementary investigations showed elevated serum IgE (355 IU/ml); normal brain CT scan and renal ultrasound. In the follow-up, at age 7 and 10 years, she presented behavior disturbance and marked learning disabilities. At this time, she had suffered various episodes of hair loss.

**CYTOGENETIC AND MOLECULAR ANALYSIS**

Cytogenetic analysis of peripheral blood lymphocytes from the patient, performed using standard techniques and processed by G-bandng chromosomes (550 bands), detected additional small segment in 7p21 [46,XX,add(7)(p21)]. FISH was not performed. To determine if TWIST1 gene was deleted in the patient, Multiplex Ligation Probe-dependent Amplification (MLPA) was performed with the commercially available kit SALSA P080 Craniofacial Disorders (www.mlpa.com—MRCHolland, Amsterdam). DNA denaturation and hybridization, probe ligation and PCR reactions were carried out according to the manufacturer’s instructions. Electrophoresis and analysis were performed using the MegaBACETM 1,000 DNA analysis system and Fragment Profiler software (GE Healthcare, Life Sciences, Cleveland, Ohio). Statistical analysis of the results was carried out upon the transfer of Fragment Profiler results to an excel spreadsheet (National Genetics Reference Laboratory Manchester, NGRL). Results of normalized relative peak heights of gene-specific and control probes show that probes of TWIST1 gene have relative peak heights of approximately 0.5 when compared to normal pool results, indicating that the patient is hemizygous for this region. Array-comparative genomic hybridization (aCGH) GeneChip® Human Mapping 500K Array Set platform (Affymetrix®, Santa Clara, CA) was performed according to the manufacturer’s recommendation. After DNA labeling, hybridization, washing and staining steps, the slide was scanned in GeneChip® Scanner 3000 7G (Affymetrix®). Raw data were generated using the GeneChip Command Console Software (Affymetrix®) and, after normalization, DNA copy number changes were assessed with the Genotyping Console™ Software (Affymetrix®), using default parameters. We detected on aCGH a deletion of band 7p21.1 between the SNP_A-2268171 to SNP_A-4272203 with a maximum size of 1,969 kb, spanning from positions 17518427 to 19486999 bp and encompassing five Reviewed RefSeq genes, including the TWIST1 gene [UCSC Genome Browser on Human March 2006 (NCBI36/hg18)]. We also detected on aCGH a deletion of band 3p21.31 between the probes SNP_A-1871167 and SNP_A-2044672 with a maximum size of 356K, spanning from positions 50382348 to 50738038 bp and encompassing six Reviewed RefSeq genes. The parents were not available to clinical, cytogentic, and molecular evaluation.

**DISCUSSION**

The girl here described presents facial appearance, coronal synostosis, facial asymmetry, ptosis, and extremities abnormalities, which are typical features of Saethre–Chotzen syndrome. In addition this patient also presents recurrent alopecia and increased serum IgE that fit in the diagnosis of hyper IgE syndrome. Non-immunological findings of hyper IgE syndrome/Job syndrome were not found. Overlapping of some clinical features between Saethre–Chotzen syndrome and hyper IgE syndrome had been observed in some instances. Craniosynostosis, typical sign of Saethre–Chotzen syndrome, without any immunodeficiency. The patient was observed only once in a boy reported by Boeck et al. [2001]. Molecular analysis of the TWIST1 gene, in this case, with an intragenic deletion of 11 bp on position 127 (c.127del11), confirmed the diagnosis of Saethre–Chotzen. This mutation was also detected in the patient’s mother, who had mild facial phenotype of Saethre–Chotzen syndrome, without any immunodeficiency. The authors commented that, probably, the 11bp deletion causes the Saethre–Chotzen phenotype and another unknown genetic defect would be responsible for the hyper IgE phenotype.

In our case, the conventional cytogenetic showed an unknown additional chromosome segment in 7p21.1 region and, the MLPA showed a reduction about 50% of the TWIST1 gene, when compared to normal control, indicating that the Saethre–Chotzen phenotype, in this patient, is caused by deletion of TWIST1 gene in one of the alleles. This deletion was confirmed by aCGH that showed an interstitial 7p21.1 deletion, spanning about 1.9 Mb and encompassing TWIST1 gene and four neighbor genes. However, as observed in previously published cases with Saethre–Chotzen
FIG. 1. A–L: Clinical aspects of the patient at age 2 years (A–C); 5 years (D–F); 6 years (G–I); and 7 years and 10 months (J–L).
phenotype caused by deletions in chromosome 7p21.1, our patient had also significantly learning disabilities [Johnson et al., 1998; Kosan and Kunz, 2002; Cai et al., 2003; Schluth-Bolard et al., 2008; Busche et al., 2011]. Some authors had suggested that 7p21.3 microdeletion results in a contiguous gene syndrome with TWIST1 gene being responsible for craniosynostosis and another gene(s) accounting for intellectual disability [Johnson et al., 1998; Schluth-Bolard et al., 2008; Busche et al., 2011]. The deletion observed in our patient was relatively small, encompassing four other genes. One of then, the FERD3L gene (RefSeq NM_152898), had been considered a potential candidate gene to intellectual disability in cases with microdeletion of 7p21.3 [Johnson et al., 1998; Schluth-Bolard et al., 2008; Busche et al., 2011]. This gene, also called N-TWIST, coding a basic helix-loop-helix transcription factor that binds the E-box, functioning as an inhibitor of transcription, being expressed in the developing central nervous system of mouse, chick and drosophila [Segev et al., 2001; Verzi et al., 2002; Mansour et al., 2011]. The influence of FERD3L in human nervous central system is yet unknown but the presence of marked microcephaly in patients with FERD3L deletion has been suggested that it is associated to brain growth and development [Busche et al., 2011]. Our patient reinforces the hypothesis that FERD3L gene is a potential candidate for intellectual disability. Besides the FERD3L gene, other deleted gene in 7p21.1 region, in our case, was the HDAC9 gene (RefSeq NM_014707) that encodes a protein of the histone deacetylase family. Histone deacetylases (HDACs) catalyze the removal of the acetyl group from the lysine residues in the N-terminal tails of nucleosomal core histones, resulting in a more compact chromatin structure, a configuration that is generally associated with repression of transcription. The HDAC9 gene has several alternative splice isoforms and the transcripts are expressed at high level in brain and skeletal muscle [Petrie et al., 2003]. It regulates a wide variety of normal and abnormal physiological functions, including cardiac growth, T-regulatory cell function, neuronal disorders, muscle differentiation, development, and cancer [Méjat et al., 2005; Yuan et al., 2010; Yan et al., 2011]. Like in our case, deletion of HDAC9 gene was observed in other patients with Saethre–Chotzen phenotype and mental retardation caused by 7p21 microdeletion [Johnson et al., 1998; Busche et al., 2011]. Therefore, it is possible that the HDAC9 gene may also contribute to the occurrence of intellectual disability.

In addition, the present case shows a 354 kb microdeletion in 3p21.31. Unfortunately, the DNA sample of the parents was not available to clarify if this is a de novo deletion, and we were also not able to perform a FISH test to clarify a possible complex rearrangement involving the chromosomes 3 and 7. This microdeletion encompass the CACNA2D2, C3orf18, HEMK1, CISH, and MAPKAPK3 genes and the upstream untranslated region, exon 1, and part of the intron1–2 of the DOCK3 gene. According to Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, from DECIPHER consortium, http://decipher.sanger.ac.uk/) deletions encompassing 3p21.31 result in abnormal phenotypes, characterized mainly by intellectual disability/developmental delay. Copy number variations, in normal control, were not observed in three (HEMK1, CISH, and MAPKAPK3) of these six genes (Database of Genomic Variants: http://projects.tcag.ca). Thus, in our patient, this deletion could be influencing the phenotype. One of these genes, CISH gene (NM_013324), is a member of suppressor of cytokine signaling
family proteins that controls interleukin-2 signaling [Khor et al., 2010]. The increase of CISH activity, in turn, leads to blocks the cytoplasmic docking and activation of signal transducer and activator of transcription 5 (STAT5), inhibiting downstream cytokine signaling. Variants of CISH gene were associated with susceptibility to diseases caused by diverse infectious pathogens [Khor et al., 2010]. Our patient had recurrent infection of the scalp leading to alopecia areata and, collapse of immune privilege in follicle hair has been considered as cause of alopecia areata [Gilhar, 2010; Petukhova et al., 2010]. The later authors showed some genomic regions that contribute to disease susceptibility, including one locus on chromosome 4p27, that contain the interleukin (IL)-2/IL-21 genes. Considering that CISH gene is a suppressor of cytokine, it is possible that deletion of the one CISH allele, in our patient, has deregulated cytokine signaling and influenced the immunological phenotype. Other interesting gene closed in 3p21.31 deleted region is the DOCK3 gene (RefSeq NM_004947), a member of the dedicator of cytokine protein (DOCK) family of guanine nucleotide exchange factors (GEFs), specifically expressed in the central nervous system. In our patient, the deletion encompassed the upstream regulation region, probably leading to haploinsufficiency of DOCK3 gene. Disruption of DOCK3 gene was observed in a family co-segregating an early-onset behavior/developmental condition with a pericentric inversion of chromosome 3 (p14q21) [de Silva et al., 2003]. These authors considered the possibility of the DOCK3 gene to be involved in pathway of this neuropsychological condition. Other interesting fact is that a member of the DOCK family, the DOCK8 gene, causes an autosomal recessive form of hyper IgE recurrent infection syndrome (OMIM 243700) [Zhang et al., 2010; Su, 2010]. If the haploinsufficiency of the DOCK3 gene could have influenced the behavior and immunological phenotype in our patient remains to be clarified.

In summary, our patient display clinical findings that fit into both diagnoses: Saethre–Chotzen syndrome and hyper IgE syndrome. The deletion of the TWIST1 gene is responsible for the Saethre–Chotzen phenotype and the deletion of the FERD3L gene reinforces it as a candidate for intellectual disability. Besides it is possible that the 3p21 deletion is causative of immunological findings in our patient, however, it is not possible to exclude this deletion as a fortuity finding, since the parents’ DNA samples were not available for study. Further studies are necessary to clarify if CISH and DOCK3 genes are related to hyper IgE syndrome.

REFERENCES


