Case Report

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Intragenic Deletion in the *LIFR* Gene in a Long-Term Survivor with Stüve-Wiedemann Syndrome

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LIFR · Next-generation sequencing · Stüve-Wiedemann syndrome

Abstract
Stüve-Wiedemann syndrome (SWS, OMIM 601559) is a rare autosomal recessive bent-bone dysplasia, caused by loss-of-function mutations in the leukemia inhibitory factor receptor *(LIFR)* gene, which usually leads to early death. Only few patients with long-term survival have been described in the literature. We report on a 5-year-old boy from a consanguineous marriage with molecular analysis for the *LIFR* gene. Sanger and next-generation sequencing (NGS) of *LIFR* were performed. Copy number variation analysis with NGS showed a novel mutation as the cause for the syndrome: an intragenic homozygous deletion in *LIFR*, involving exons 15–20. Bridging PCR was carried out to confirm the intragenic deletion. This is the first description of a large deletion in *LIFR*, broadening the spectrum of mutations in SWS. Besides the reported allelic heterogeneity, further studies such as exome sequencing are required to identify a novel gene in order to confirm the locus heterogeneity in SWS.

Stüve-Wiedemann syndrome (SWS, OMIM 601559) is a rare autosomal recessive skeletal dysplasia, characterized by bowing of the long bones, contractures of large joints and camptodactyly, respiratory and feeding difficulties, dysautonomia including hyperthermic episodes, and high lethality in the first year of life [Akawi et al., 2012]. Only 22 patients have been reported in the literature with a long-term survival [Jung et al., 2010]. Molecular analysis of 19 families showed that the syndrome is caused by loss-of-function mutations in the leukemia inhibitory factor receptor *(LIFR)* gene, which is able to bind to several cytokines and induces signaling through JAK/STAT and MAPK pathways [Dagoneau et al., 2004]. A recent study suggested genetic heterogeneity, by reporting 4 patients with a typical SWS phenotype, but without *LIFR* mutations [Jung et al., 2010].

Here, we report on the follow-up of a Brazilian patient with long-term survival as well as his clinical and radiologic features. Molecular analysis by Sanger sequencing and next-generation sequencing (NGS) revealed a homozygous deletion of the final exons of the *LIFR* as the cause for SWS in our patient.

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Fig. 1. Clinical pictures and X-rays of the patient at the age of 5 years. **A**, **B** Frontal and dorsal views of the patient showing large joint restrictions, bowing of the lower limbs, and scoliosis. **C** Bilateral corneal opacity. **D** Smooth tongue. **E** Hand X-rays showing camptodactyly. **F**, **G** Frontal and lateral views of the spine showing scoliosis. **H**, **I** Bowing and shortening of the long bones in the lower and upper limbs, especially the femurs with metaphyseal widening.

Case Report

The proband is a 5-year-old boy, the second child of consanguineous, first-cousin parents. He has an older healthy brother. Pregnancy was uneventful, but the mother reports that fetal movements started during the fifth month and were weak. The boy was born at term, by vaginal delivery, with a birth weight of 2,955 g and a length of 43 cm (<3rd centile). Bilateral clubfoot deformities were observed. He evolved with respiratory distress, requiring orotracheal intubation, and seizures, controlled with phenobarbital. Moreover, feeding difficulties and the inability to swallow were also disclosed, requiring prolonged hospitalization and leading to the placement of a gastrostomy tube at 5 months of age. Several episodes of unexplained hyperthermia were observed since birth.

The boy was first seen at the genetics unit at the age of 8 months, and physical examination showed: a weight of 7,000 g (3rd centile), a length of 59 cm (<3rd centile) and an OFC of 46 cm (>85th centile). He had bilateral corneal opacities, a short nose with upturned nostrils, shortening and bowing of the limbs, contractures in the elbows and knees, camptodactyly, and a single left palmar crease. Skeletal survey showed bowed long bones (fig. 1), widened metaphyses with decreased density and an abnormal trabecular pattern, elongation of the epiphyseal plates, and anterior elongation of the ribs and wormian bones in the skull. Ophthalmologic evaluation disclosed leukocoria (fig. 1), probably related to corneal ulcers, and normal fundoscopy. Developmental milestones showed delay: he sat unsupported by 8 months of age, walked and spoke his first words at 24 months, but presented apparently normal cognitive function. At 2 years and 5 months, he fractured his right arm after falling. At 3 years and 11 months, he presented a smooth tongue without fungiform papillae (fig. 1), absent patellar reflexes and progressive scoliosis that required bracing. He was still partially fed by the gastrostomy tube and in use of ibuprofen for his episodes of hyperthermia and phenobarbital for seizures. At the age of 5 years, the gastrostomy was removed and his height remained <3rd centile.

As the clinical findings were highly suggestive of SWS, molecular analysis of *LIFR* (coding exons 2–20 and their flanking regions NM_001127671.1) was carried out in our patient. This study was approved by the Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, and informed consent was obtained prior to the collection of the samples. After DNA was extracted by salting-out protocol, exons 2–14 were amplified, and no pathogenic mutations were identified by Sanger sequencing. The amplification of exons 15–20 did not occur (fig. 2A), leading to the hypothesis of a homozygous deletion at the end of the gene. The analysis of the parents showed amplification of all exons similarly to the positive controls. NGS with capturing probes for all exons of *LIFR* was performed, which demonstrated the deletion involving exons 15–20 (fig. 2B). To confirm the intragenic deletion by gold standard methods, bridging PCR was carried out using a pair of primers for the initial portion of intron 14 and UTR region of exon 20. The sequencing of this amplification showed that these regions were adjacent, confirming the diagnosis of SWS by a large homozygous intragenic deletion in our patient.

Discussion

All patients described so far with SWS have a very homogeneous pattern of clinical findings [Jung et al., 2010], and our patient follows the same model. The syndrome was originally described as a lethal disorder, and it took more than 20 years for reports of patients surviving in-

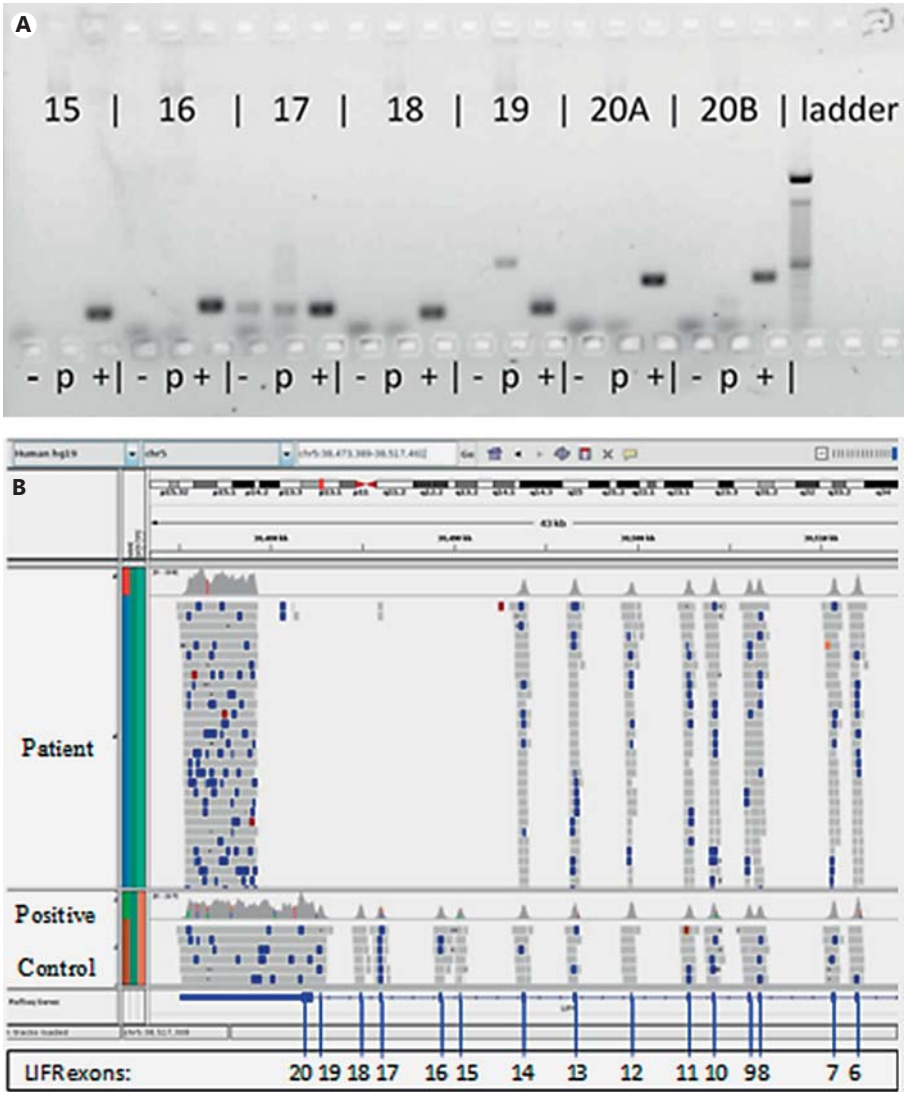

Fig. 2. A Amplification of *LIFR* exons 15–20. Proband's DNA did not amplify, similarly to the negative control. The unspecific band in exon 19 was verified by sequencing and BLAST. The ladder shows DNA size markers. **B** NGS. The capture was performed with homology probes for all exon regions of *LIFR*. Note that all exons are represented in the positive control, while in our patient exons 15–19 are completely absent as well as the coding portion of exon 20. This indicates a homozygous deletion of these exons. – = Negative control; p = proband; + = positive control. Image obtained from the Integrative Genome Viewer (IGV) software.

fancy to appear in the literature. Episodes of hyperthermia and feeding/swallowing difficulties with pulmonary aspirations and apnea are still the leading causes of death either in the neonatal period or in infancy and, therefore, require proper managements. The survivors also present lack of pain, which is responsible for several injuries that may lead to secondary infection and sequelae, such as corneal opacity, dental loss, and a smooth and deformed tongue [Chen et al., 2001; Di Rocco et al., 2003].

The mutations in *LIFR* previously described in the literature (fig. 3) have been either missense or nonsense, almost universally predicting the expression of a truncated or damaged protein and consequently null alleles, abolishing the LIF-mediated JAK-STAT3 signaling. These mutations are spread throughout the exons encoding the extracellular domain, even within the patients showing long-term survival [Mikelonis et al., 2014]. The loss-of-function mutations could explain the homogeneity of the clinical findings, but do not seem to be the mechanism underlying the longer survival presented by some patients. It is possible that an earlier recognition of the disorder and a more aggressive and proper management in the first months of life could play an important role in survival.

The patient described here presents a new class of mutations in *LIFR*, i.e. a large gene deletion. In this case, the mutation was in homozygosity. This raises the possibility that this type of mutation could be missed when it is in a heterozygous status. In this particular scenario, NGS technique was capable of identifying the mutation responsible

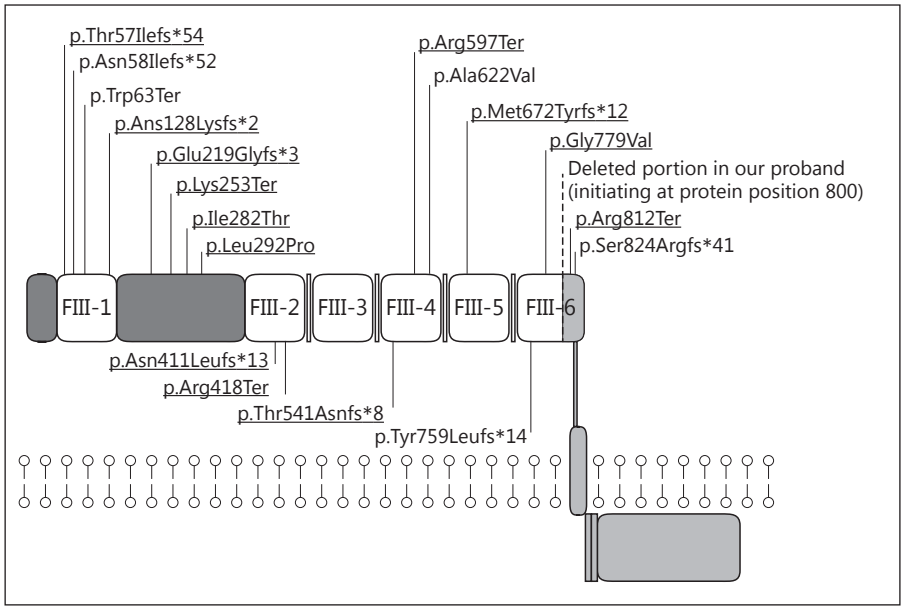

Fig. 3. LIFR protein structure based on the database Universal Protein Resourse (UniProt) with previously described exon mutations and the intragenic deletion found in our patient: the extracellular portion is composed of 6 domains of FNIII and a WSXWS motif (in the third domain of FNIII); transmembrane portion; intracellular portion containing a BOX1 motif (second citoplasmic portion). The deletion found in our proband is represented in the light grey area. Underlined mutations represent the ones previously described in long-term survivors [Dagoneau et al., 2004; Morava et al., 2006; Gaspar et al., 2008; Corona-Rivera et al., 2009; Jung et al., 2010; Catavorello et al., 2013].

for the SWS phenotype in our patient which was further confirmed by bridging PCR. All techniques have limitations in their own capacity of detecting a gene alteration, but NGS could combine the identification of both sequence and copy number variants which other methods cannot.

This report broadens the mutational spectrum in SWS, and therefore, this type of mutation should be sought when only one or no mutations in *LIFR* are identified in an individual with SWS suspicion, either by Sanger or NGS.

Previously, 4 individuals with a clinical phenotype of this disorder did not present mutations in *LIFR*, and Western blot analysis of STAT3 phosphorylation in response to LIF in cultured skin fibroblasts from these patients showed normal results [Jung et al., 2010], suggesting that a different gene could also play a role in SWS. Further studies comprising these *LIFR*-negative individuals, such as exome sequencing and segregation analysis in familial cases, might be able to identify this new gene, confirming locus heterogeneity in SWS.

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