Identification of a “cryptic” de novo deletion in *NKX2.1* in the Brain-lung-thyroid Syndrome using genome-wide arrays

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Introduction and objectives

Genetic defects in the *NKX2.1* gene, located in chromosome 14q13, are associated with hypothyroidism, choreo-athetotic movements and neonatal respiratory distress, known as the “Brain-Lung-Thyroid syndrome”.

The purpose of this study was to identify the genetic basis of the clinical phenotype of a young girl with features consistent with the “*NKX2.1* syndrome” but associating additional clinical characteristics not described in the syndrome.

Patient and Methods

A 10-year old girl was diagnosed with primary hypothyroidism (with negative CH neonatal screening) at 2,5 years of age and was under levo-thyroxine substitution since then. At birth, she presented generalized hypotonia and mild respiratory distress, followed by frequent episodes of bronchiolitis which required treatment until 5 years of age. Further features included developmental delay with late-onset walking, clumsiness, frequent falls and language delay. In early infancy (2 years) she presented subtle choreic movements in arms and limbs. Delayed teeth eruption (17 months) and partial absence of permanent teeth at age 10 were observed in orthopantomography. Recently, hypogammaglobulinemia was also detected through low titers of vaccine-related immunoglobulins. Marked joint hyper-extensibility of the arms was also present.

Thyroid ultrasounds showed hypoplastic gland (volume <P3 for girls her age). Brain MRI and electromyogram were normal. Her parents and brother were healthy.

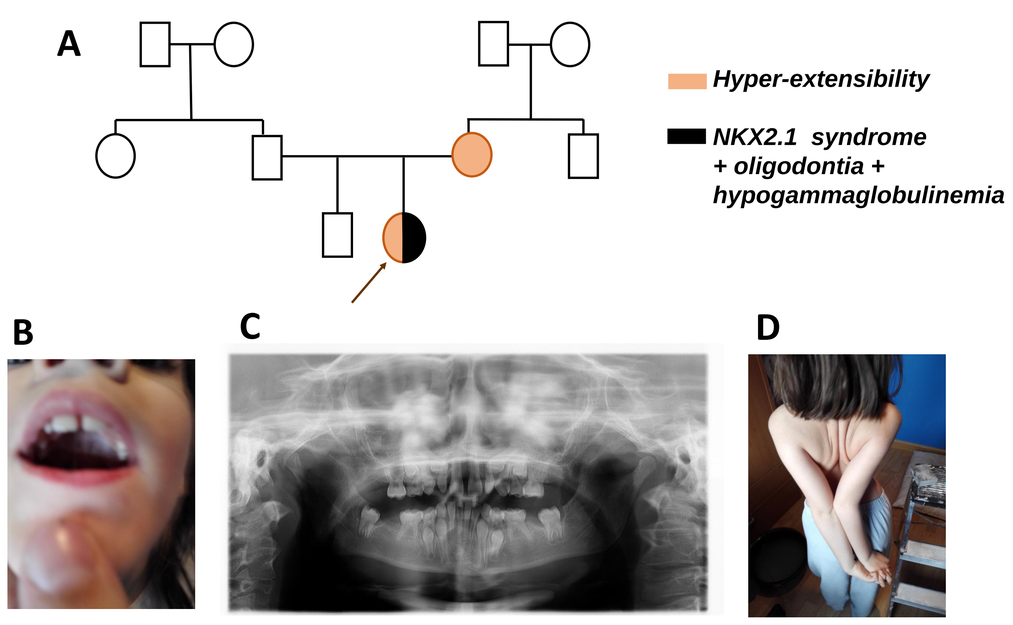

Fig 1. Family pedigree. (A y B) Delayed teeth eruption and orthopantomography. (C) Signs of hyperextensibility

PCR and direct sequencing of the whole coding region of *NKX2.1,* multiplex ligation-dependent probe amplification (MLPA, Kit Salsa P319 MRC-Holland, containing probes on *TPO, PAX8, FOXE1, NKX2.1* and *TSHR* genes) and Comparative Genomic Hybridization (CGH)-arrays (Karyo-Array R, 8X60 K, Agilent) were performed in an Illumina platform.

Results

No mutations were identified in the Sanger sequencing of the whole coding region of *NKX2.1.* Considering the strong consistency of the clinical phenotype, MLPA was performed showing heterozygous loss of gen dosage in the 3 probes corresponding to *NKX2.1* (Fig. 2A) The defect was *de novo* since it was absent in the parents. To identify the precise deletion size of the copy number variation (CNV) a CGH-array was performed showing a deletion of 3.44 Mb in the long arm of Chr. 14 encompassing 14q13.2-q21.1, including *NKX2.1* and 20 additional genes, including *PAX9,* which is related to oligodontia, and *NFKB1A* and *PPP2R3C,* as candidate genes for hypogammaglobulinemia. No candidate gen for hyperextensibility of joints was identified (Fig. 2B), suggesting this last feature does not form part of the phenotype caused by the deletion, but rather be maternally inherited.

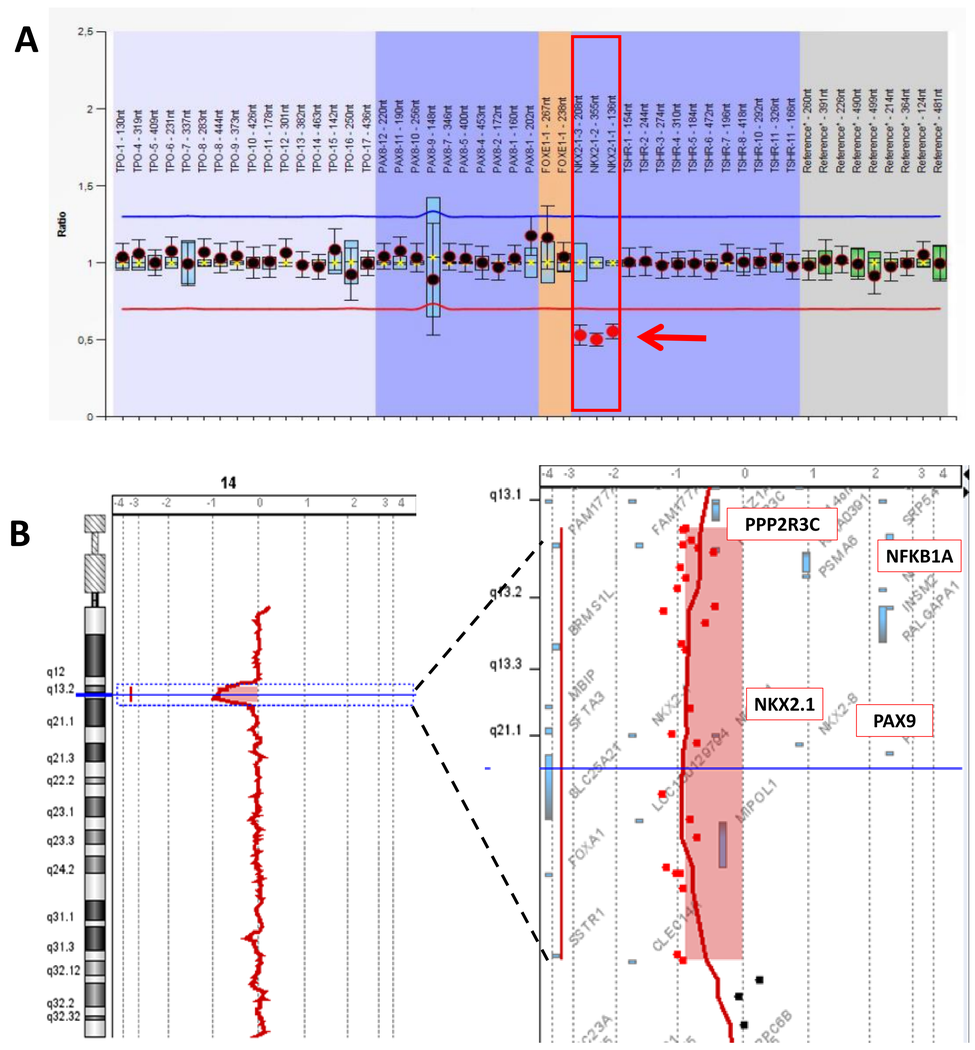

Fig 2A. MLPA: deletion of at least 4 Kb in the chromosomal region 14q13.3 completely affecting the NKX2.1 gene. 2B. CGH-Array: a deletion of about 3.32 Mb located in Cr14, q13.2-q21 showing that this novel CNV is mono-allelic.

Conclusion

A novel *de novo* deletion was identified as cause of the *NKX2.1* syndrome. When clinical suspicion is fully consistent, monoallelic deletions of Chr14q should be actively investigated in these patients through genomic techniques that detect gene-dosage variations. Haploinsufficiency of *PAX9* is responsible for oligodontia, and we propose for the first time, that hypogammaglobulinemia forms part of the phenotype of the identified deletion, since two plausible candidate genes linked to the phenotype are present in the deleted interval, namely genes *NFKB1A*(5) and *PPP2R3C* (6)

Disclosure Statement:
The authors report no conflicts of interest in this study

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