European Journal of Medical Genetics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Benign hereditary chorea and deletions outside *NKX2-1*: What's the role of *MBIP?*

Federica Invernizzi[a], Giovanna Zorzi[b], Andrea Legati[a], Giovanni Coppola[c], Pio D'Adamo[d,e], Nardo Nardocci[b], Barbara Garavaglia[a], Daniele Ghezzi[a,f,*]

[a] *Molecular Neurogenetics, Foundation IRCCS Neurological Institute Besta, Milan, Italy*
[b] *Child Neurology Unit, Foundation IRCCS Neurological Institute Besta, Milan, Italy*
[c] *Departments of Psychiatry and Neurology, University of California, Los Angeles, CA, USA*
[d] *Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy*
[e] *Clinical Department of Medical, Surgical and Health Science, University of Trieste, Italy*
[f] *Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy*

ARTICLE INFO

Keywords:
NKX2-1
MBIP
Benign hereditary chorea
Brain-lung-thyroid syndrome

ABSTRACT

Heterozygous point mutations or deletions of the *NKX2-1* gene cause benign hereditary chorea (BHC) or a various combinations of primary hypothyroidism, respiratory distress and neurological disorders. Deletions proximal to, but not encompassing, *NKX2-1* have been described in few subjects with brain-lung-thyroid syndrome. We report on a three-generation Italian family, with 6 subjects presenting BHC and harboring a genomic deletion adjacent to *NKX2-1* and including the gene *MBIP*, recently proposed to be relevant for the pathogenesis of brain-lung-thyroid syndrome. We observed a clear reduction of *NKX2-1* transcript levels in fibroblasts from our patients compared to controls; this finding suggests that *MBIP* deletion affects *NKX2-1* expression, mimicking haploinsufficiency caused by classical *NKX2-1* related mutations.

1. Introduction

Several *NKX2-1* point mutations or deletions of chromosome 14 encompassing *NKX2-1* have been associated with Benign Hereditary Chorea (BHC) [Breedveld et al., 2002; Thorwarth et al., 2014; Peall and Kurian, 2015]. All patients were heterozygous for the mutation, therefore suggesting a dominant mode of inheritance. *NKX2-1* encodes a transcription factor, which is expressed during early development of thyroid, lung and forebrain regions. Accordingly, in addition to BHC, patients with *NKX2-1* mutations presented combinations of primary hypothyroidism, respiratory distress and neurological disorders. Few cases with BHC or brain-lung-thyroid syndrome have been reported to harbor deletions proximal to, but not disrupting, *NKX2-1* [Barnett et al., 2012; .Thorwarth et al., 2014; Kharbanda et al., 2017].

Here we describe a three-generation Italian family, with 6 subjects presenting with BHC and harboring a genomic deletion adjacent to *NKX2-1* and including the gene *MBIP*, recently proposed to be relevant for the pathogenesis of brain-lung-thyroid syndrome [Kharbanda et al., 2017].

2. Patients' data

The probands were two siblings (III-1 and III-2, pedigree in Fig. 1) with a very similar clinical picture (Table 1) consisting of hypotonia and motor delay, a hyperkinetic movement disorder noted since infancy and recurrent pulmonary infections. They both presented dysmorphic features (macrocephaly, prominent and large forehead, big round eyes, bilateral hallux valgus), oculomotor apraxia and generalized chorea with superimposed irregular myoclonic jerks. Cognitive functions were within normal limits. A younger half-sister (III-3) was referred for motor delay and unbalanced gait with frequent falls at age 4. The father (II-3) was affected by an early-onset, non progressive chorea and pulmonary pathology (severe asthma); after age 30, he suffered episodes of hypotension and hypoglycaemia. His brother (II-5) presented mild chorea associated with minor cognitive and psychiatric involvement; at age 35 he was diagnosed with lung carcinoma. Their grandmother (I-1) had only a mild early-onset chorea and she did not experience any pulmonary disease.

Thyroid function was measured in all cases during the follow-up and always resulted normal. The movement disorder spontaneously improved over time in all cases, causing little or no functional disability.

* Corresponding author. Molecular Neurogenetics Unit, Foundation IRCCS Neurological Institute "Besta", via Temolo 4, 20126, Milan, Italy.
E-mail address: daniele.ghezzi@istituto-besta.it (D. Ghezzi).

https://doi.org/10.1016/j.ejmg.2018.03.011
Received 23 November 2017; Received in revised form 9 February 2018; Accepted 27 March 2018

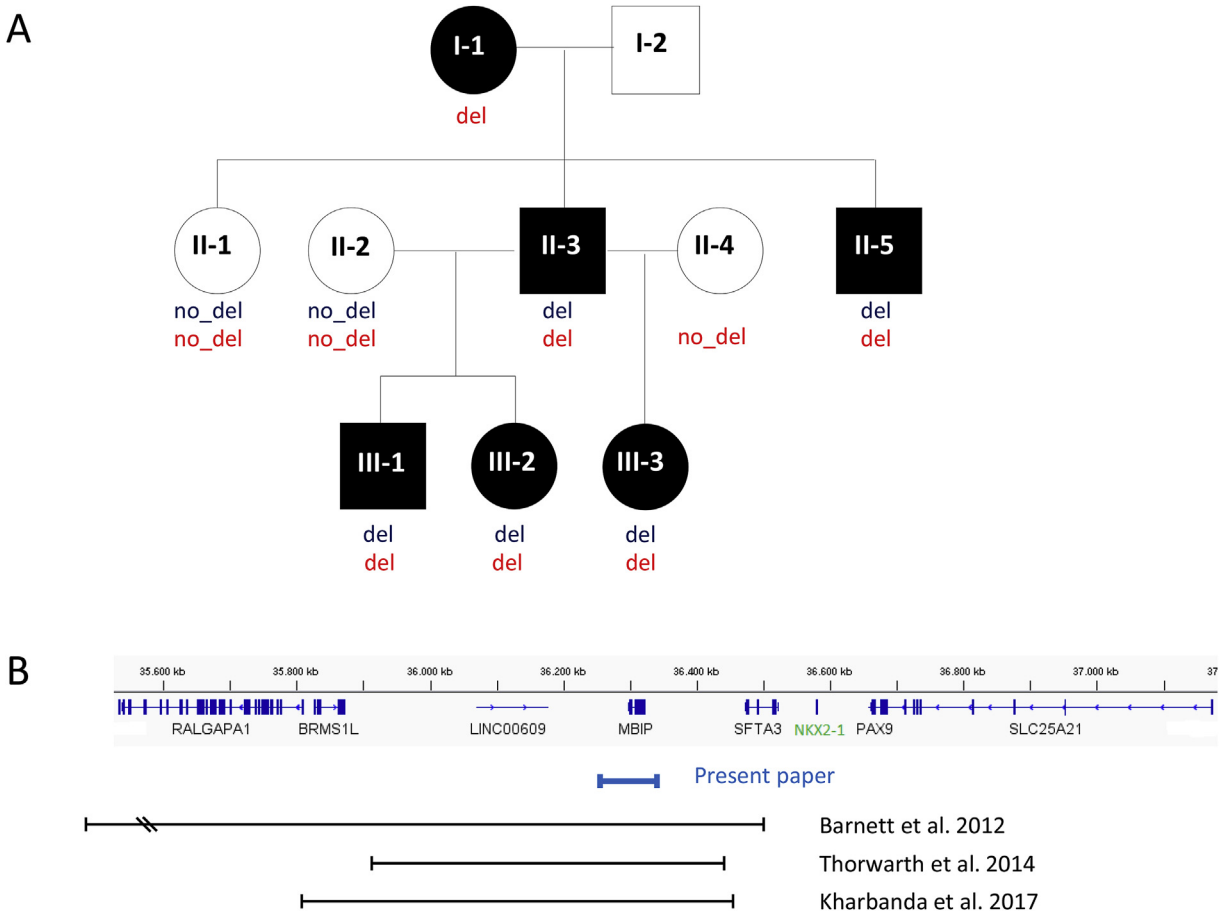

Fig. 1. Pedigree of the investigated family and genetic analyses

A. SNP-array analysis, performed on all available DNAs from the family (8 subjects: 6 affected and 2 healthy cases), revealed a genomic heterozygous deletion on chr14q13 in 5 affected family members, absent in two unaffected cases. The poor quality of the DNA from individual I-1 led to its exclusion from the analysis. Real-time quantitative PCR showed the heterozygous deletion of *MBIP* on genomic DNAs in all affected members of the family. Labels indicate the presence (del) or absence (no_del) of the 14q13 deletion by SNP-array analysis (in dark blue) or the *MBIP* deletion by RT-qPCR (in red).

B. Map of the Chr.14 genomic region surrounding *MBIP* and *NKX2-1* genes, with the deletions identified in our family and in previously published cases. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Clinical features of the affected family members.

Patient	Neurological	Respiratory	Thyroid	Psychiatric	Other features
III-1	Hypotonia, generalized chorea, myoclonic jerks	Recurrent pulmonary infections	/	Mild cognitive impairment	Dysmorphisms
III-2	Hypotonia, generalized chorea, myoclonic jerks, ataxia	Recurrent pulmonary infections	/	/	Dysmorphisms, macrocrania
III-3	Motor delay, unbalanced gait	/	/	/	/
II-3	Chorea	Severe asthma	/	/	Hypotension, hypoglycaemia
II-5	Mild chorea	Lung carcinoma	/	Minor cognitive and psychiatric involvement	/
I-1	Mild chorea	/	/	/	/

3. Methods

Informed consent for the genetic diagnosis was obtained from patients or parents according to the local regulations, DNA was extracted from venous peripheral blood lymphocytes according to standard procedures and was used as a template to amplify the 3 exons of the *NKX2-1* gene. PCR fragments were analyzed by automated nucleotide sequencing using the Big-Dye terminator Ready Reaction Kit version 2 on a 3100 Genetic Analyzer Automated Sequencer (Applied Biosystems).

Haplotype analysis was performed by SNPs array (ILLUMINA HumanCytoSNP-12 BeadChip).

For whole exome sequencing (WES), genomic DNA was processed for library enrichment according to the SeqCap EZ Exome v3.0 kit (Roche). Sequencing was performed on an Illumina® Genome Analyzer HiSeq2500. The sequencing reads were aligned to the National Center for Biotechnology Information human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA). Variant calling was performed using Genome Analysis Toolkit (GATK) and VariantStudio Data Analysis Software (Illumina®) was used for variants annotation and filtering. Coverage analysis was performed on WES data of individuals I-1 and III-2 using CANOES (http://www.columbia.edu/~ys2411/canoes). CANOES is a bioinformatics tool based on an