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SHORT REPORT

Duplication at chromosome 2q31.1-q31.2 in a family presenting syndactyly and nystagmus

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HOXD **genes encode transcription factors involved in the antero-posterior patterning of the limb bud and in the specification of fingers. During the embryo development, *HOXD* genes are expressed, following a spatio-temporal colinearity that involves at least three regions, centrometric and telomeric to this cluster. Here, we describe a father and a daughter presenting a 3–4 hand bilateral syndactyly associated with a nystagmus. Array-comparative genomic hybridisation showed a 3.8 Mb duplication at 2q31.1-q31.2, comprising 27 genes including the entire *HOXD* cluster. We performed expression studies in lymphoblasts by reverse transcription-PCR and observed an *HOXD13* and *HOXD10* overexpression, whereas the *HOXD12* expression was decreased. *HOXD13* and *HOXD10* overexpression, associated with a misregulation of at least *HOXD12*, may therefore induce the syndactyly. Deletions of the *HOXD* cluster and its regulatory sequences induce hand malformations and, particularly, finger anomalies. Recently, smaller duplications of the same region have been reported in association with a mesomelic dysplasia, type Kantaputra. We discuss the variable phenotypes associated with such 2q duplications.**
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INTRODUCTION

HOXD genes encode a family of highly conserved transcription factors involved in the antero-posterior patterning of the limb bud and in the specification of fingers.[1,2] During embryo development, *HOXD* genes are expressed following a spatio-temporal colinearity involving at least three regulatory regions, centrometric (ELCR) and telomeric (POST and Global Central Region (GCR)-Prox) to the cluster.[3] Moreover, these genes are expressed through two waves[4] during the limb budding, and control the patterning of the stylopod and the zeugopod.[5] The width and the efficiency of the genes' expression depend on their rank in the cluster. Each gene presents a precise pattern of expression. *HOXD13*, the most 5′ end located gene, is highly expressed throughout the presumptive digits, whereas *HOXD10*, *HOXD11* and *HOXD12* are restricted to presumptive digit 2–5 and are underexpressed. In man, deletions of this cluster induce hand malformations and particularly finger anomalies.[5] Deletions of the whole cluster can cause severe defects, whereas deletions removing only *HOXD9–HOXD13* are responsible for a milder phenotype including fifth finger clinodactyly, variable cutaneous syndactyly of toes, hypoplastic middle phalanges of the feet and synpolydactyly.[5,6] Deletions removing GCR are deleterious too, but induce minor anomalies.[7–9] ELCR has not been localised so far. Its role is so critical that deletions would be lethal and thus there is no animal model.

Animal models carrying internal duplications of part of the *HOXD* cluster and limb anomalies exist.[3,4] Indeed, mice with targeted disruptions of *Hoxd11* and *Hoxa11* genes showed marked zeugopod malformation.[10] A disconnection of 5′ *Hoxd* genes from the regulator could result in a downregulation of 5′ *Hoxd* genes in the distal limb (autopod) and an upregulation in the proximal limb, and it has been suggested that the 2q duplication could have the same effect, therefore, explaining the mesomelic dysplasia recently reported.[11,4] Indeed, it has been recently reported that a 1 Mb microduplication of *HOXD* gene cluster at 2q31.1 is associated with a dominant mesomelic dysplasia, Kantaputra type. The condition is mainly affecting the upper limbs and is very variable among affected patients within the same family. This phenotype, linked with a small 2q duplication that contain the entire *HOXD* cluster, is far more severe than the one we report here, in which the duplicaton is larger and involving several other genes.

Indeed, we report on a father and his daughter referred to the genetic clinic for the association of bilateral 3–4 finger cutaneous syndactyly and nystagmus carrying a 2q31.1q31.2 duplication involving 27 genes, among which the whole *HOXD* cluster, identified by array-comparative genomic hybridisation (CGH). We characterise this chromosomal anomaly and discuss the genotype–phenotype correlations.

PATIENTS AND METHODS

The probands are a father and his daughter. The father presents an association of bilateral 3–4 hand cutaneous syndactyly and a pendular-resilient nystagmus, which increases in up and right gaze and decreases in down gaze. Ophthalmologic examination and functional tests were normal (slit-lamp, fundoscopy, binocular visual field and electroretinogram), as well as a full neurological examination. Skeletal survey including hand X-rays was normal (Figure 1a). His 6-year-old daughter is affected with the same hand malformations (Figure 1b). She was born after an uneventful pregnancy. Motor milestones were achieved normally and her psychomotor development is in correlation

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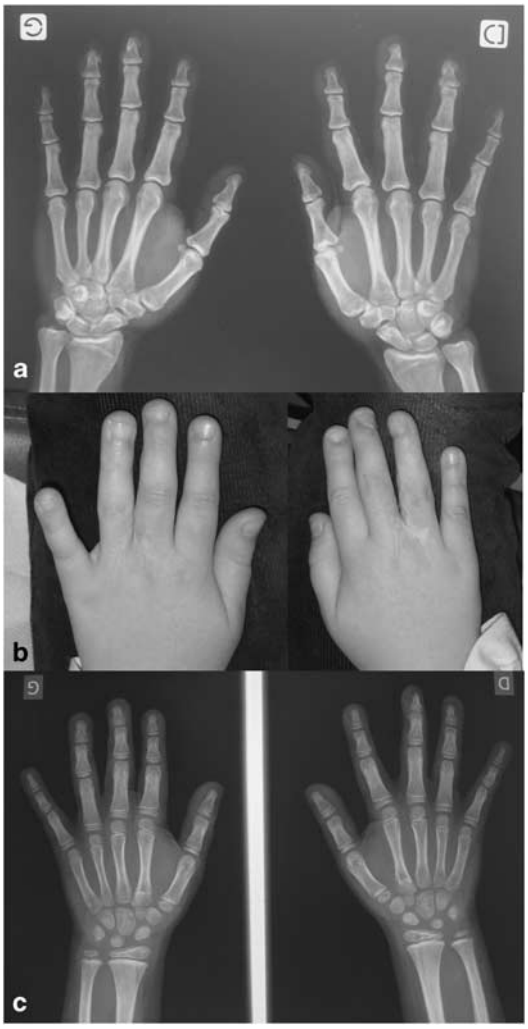

Figure 1 (**a**) Normal hands X-rays of the affected father. (**b**) Hands of the daughter after surgery. Note scars after surgery for 3–4 fingers' skin syndactyly. (**c**) Normal hands X-rays of the affected daughter.

with her age. Her growth parameters are advanced (123 cm, +2.5 SD for the height; 29 kg, +3 SD for the weight; 51 cm, −0.5 SD for the head circumference). No dysmorphic features were identified. She presents a slow pendular nystagmus, which causes amblyopy (confirmed by visual evoked potentials). A full neurological examination was normal, as well as a skeletal survey including hand X-rays (Figure 1c).

Genomic DNA was extracted from peripheral blood lymphocytes for both patients and the unaffected mother. Detection of gene copy number was performed by array-CGH (Agilent, Agilent Technologies, Santa Clara, CA, USA) using 44 000 oligo probes, approximately spaced at 40–100 kb intervals across the genome (Human Genome CGH microarray 44B kit, Agilent). Male and female genomic DNAs (Promega, Madison, WI, USA) were used as reference in hybridisations, which were analysed with the CGH-analytics software by applying a Z-score segmentation algorithm to identify chromosome aberrations.

Quantitative-PCR was performed on genomic DNA extracted from the three members of the family. TaqMan analyses were performed in Fast Gene Quantification (Applied Biosystems, Villebon-sur-Yvette, France) in 96-well plates. The final volume was 20 μl and contained Genotyping master Mix (Applied Biosystems) (15 μl), 4 μl of DNA and 1 μl of specific genes' primers and probes. Two exons per gene were studied: *HOXD13* (exon 1 and 2), *HOXD12* (exon 1 and 2), *HOXD10* (exon 1 and 2), *CHN1* (exon 1 and 13) and *CHRNA1* (exon 2 and 10). All reactions were performed in triplicate. Thermal cycling conditions were as follows: denaturation at 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 60 s and 72°C for 60 s. Analyses were carried out on a 7900HT Sequence Detection System (Applied Biosystems) and interpreted with the comparative Ct methods. *RNASEP* was used as the reference gene control.

Total RNA was extracted from lymphoblasts cell cultures, using the RNeasy Mini Kit (Qiagen GmgH, Hilde, Germany) following the manufacturer's instructions. A total of 2 μg of RNA was retrotranscripted into cDNA, with the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA), for 1 h at 37°C. TaqMan analyses were performed in Fast Gene Quantification in 96-well plates. The final volume was 20 µl and contained Gene Expression master Mix (Applied Biosystems) (10 μl), 2 μl of cDNA and 1 μl of specific Gene Expression Assays for human *HOXD13*, *HOXD12*, *HOXD10*, *CHN1*, *CHRNA1* and *RPL13A* (primers and probes sequences Applied Biostystems), following manufacturers' instructions. All reactions were performed in quadruplicate. Thermal cycling conditions were as follows: denaturation at 95°C for 10 min and 40 cycles of 95°C for 15 s, 60°C for 60 s and 72°C for 60 s. Analyses were carried out on a 7900HT Sequence Detection System and interpreted with the comparative Ct methods. *RPL13A* was used as the reference gene control.

Chromosomal analyses of peripheral blood lymphocytes, according to routine procedures, using GTG-banding (550 bands), and FISH analyses using bacterial artificial chromosome clone RP11-483E17 localised at 2q31.1 (chr2:175,041,497-175,231,429) and clone RP11-250N10 localised at 2q31.2 (chr2:178,079,879-178,252,293) (hg18, NCBI Build 36), were performed in both patients.

RESULTS

We report on a father and his daughter presenting a congenital nystagmus and a 3–4 hand bilateral syndactyly. Array-CGH identified a 3.8 Mb-wide 2q31.1q31.2 duplication, which comprises 27 genes and involves the whole *HOXD* cluster, *CHN1* and *CHRNA1*, and also a large portion of local chromosome environment (Figure 2).

We studied the level of cDNA in lymphoblasts to evaluate the impact of the duplication on the involved genes' expression. Quantitative-PCR analysis confirmed the duplication of *HOXD13*, *HOXD12*, *HOXD10*, *CHN1* and *CHRNA1*. The analysed exons of each tested gene were double dosed in the duplicated patients, comparatively to the unaffected mother (Figure 3). *HOXD13* and *HOXD10* were overexpressed in the father (3.7 and 2.9 fold, respectively) and his daughter (3.0 and 6.2 fold, respectively). The expression of *HOXD12* was diminished in the daughter (5 fold), but in the father, no difference was shown (data not shown).

Chromosome analyses (550 bands) were normal and FISH analyses revealed direct 2q31.1q32.2 duplication in both patients (Figure 4).

DISCUSSION

We report on a father and his daughter presenting a large 2q31. 1 duplication involving the *HOXD* cluster, but also many other genes, and a very mild phenotype, namely a cutaneous syndactyly between two fingers and a nystagmus. Recently, two reports on a dominant mesomelic dysplasia type Kantaputra have been described in association with a 2q31.1 duplication involving the *HOXD* locus and other genes (*MTX2, EVX2, KIAA1715*), out of which some are also known to have important roles during digit development.[14,11] The patients presented severe shortening of the middle segments of the arm, relative shortening of the tibia and fibula and no ophthalmological-associated anomaly. As our cases had a normal full skeletal survey, their phenotype is very different and is restricted to a bilateral cutaneous syndactyly between the third and fourth finger. The 2q31.1 duplication in our cases was larger than that reported by the previous authors.[14,11] We do not know whether our cases' phenotype is linked with increased gene expression or dysregulation at the *HOXD* locus. *HOXD13* overexpression might explain the cutaneous syndac-

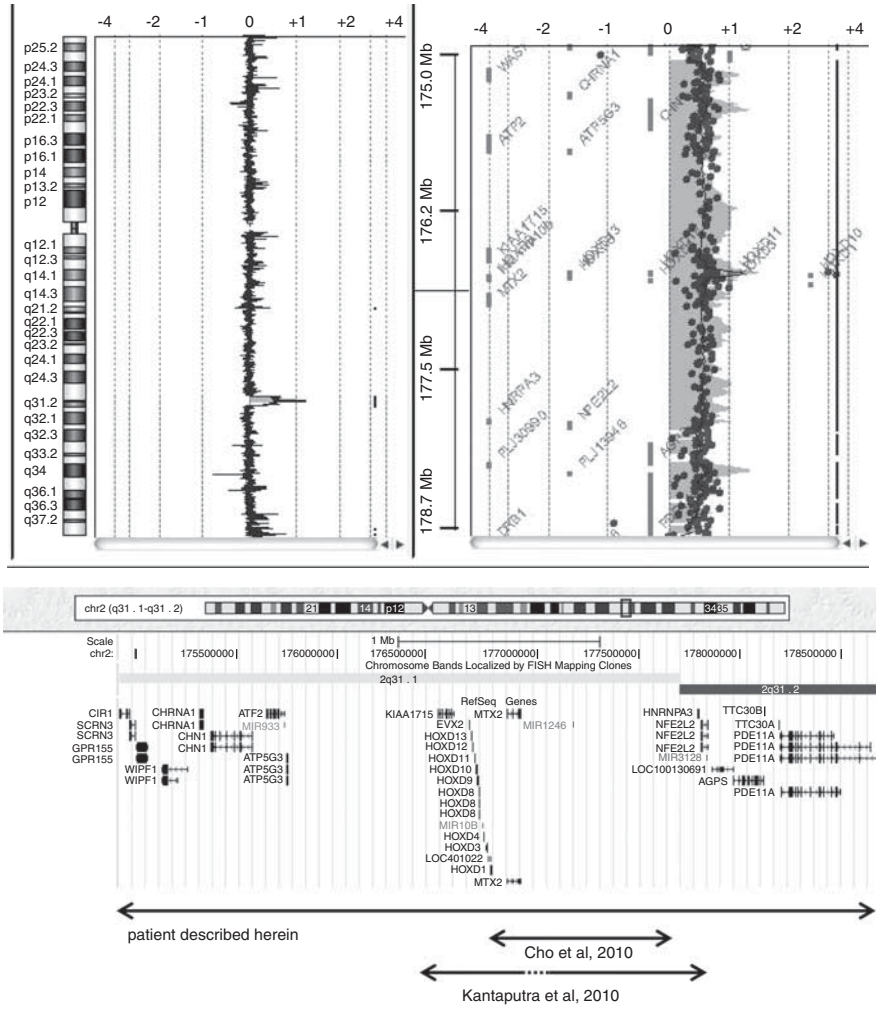

Figure 2 Array-CGH analysis and genes involved in the 3.8 Mb-wide 2q31 duplication. Comparison with the 2q31 duplication involved in the Kantaputra mesomelic dysplasia.

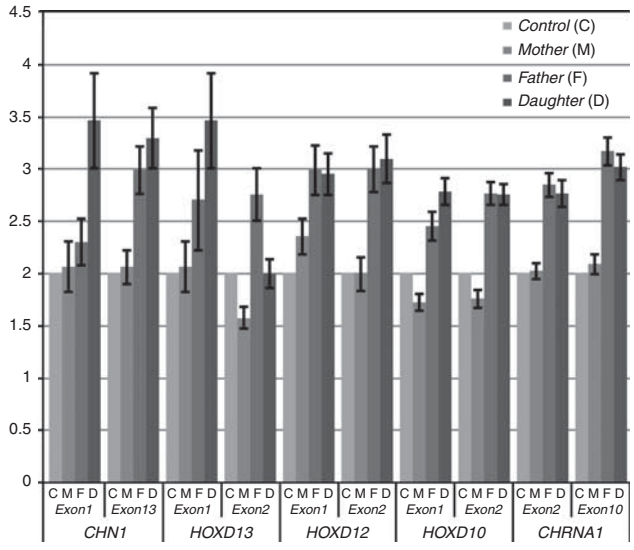

Figure 3 Quantative-PCR analysis. Estimated copy variatons for the different analysed exons of *CHN1, HOXD13, HOXD12, HOXD10* and *CHRNA1.* The confidence interval is 95% with n=4. There are three copies in the father and his daughter, whereas there are two in the mother and the control.

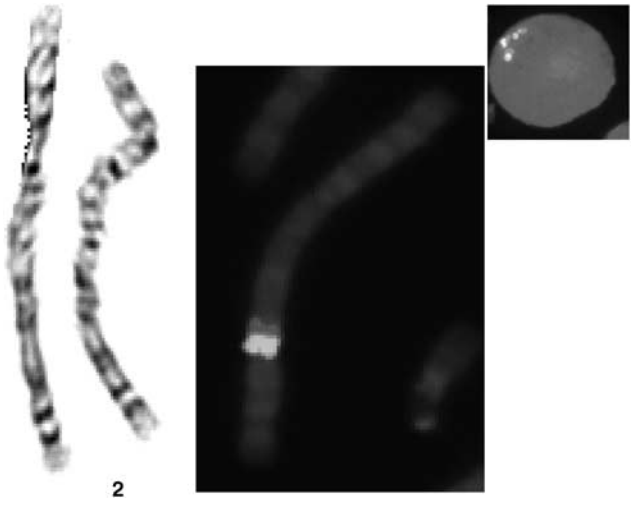

Figure 4 Karyotype and FISH analyses. Note tandem 2q duplication.

tyly, although further expression studies in cells from the developing autopod, rather than in lymphoblasts, would be needed to ascertain this. It has been suggested that the ELCR could be needed to

implement colinear expression of the *HOXD* cluster.[3] The duplication could disconnect the cluster from the ELCR and therefore explain the limb phenotype, although the recent report from Kantaputra *et al*[11] did not identify the same 2q duplication in other affected individuals and suggested that a balanced structural chromosomal rearrangement affecting *HOXD* locus regulation could also explain the phenotype.

A modification of the chromosomal environment due to the duplication could possibly be involved in the genesis of the finger phenotype. Such mechanisms have already been described by Dlugaszewska *et al*[13] and correspond to translocations and inversions with breakpoints near the *HOXD* cluster. Patient 2 (as designated in the original article) carried a t(2;10)(q31.1;q23.33) translocation with a proximal breakpoint around 1050 kb downstream to *HOXD13*. He harboured ulnar hypoplasia and absence of fingers 3 to 5 and hypoplastic fingers 1 and 2. In this case, a first wave impairment could be suspected because of the zeugopod involvement, and thus, ELCR misregulation might be involved in the phenotype. Regarding our patients, karyotype and FISH analyses allowed to confirm that the duplication was tandem, rather than being translocated to another chromosome.

It is known that *HOXD* products need to be adequately balanced for a normal digit pattern. Thus, we studied the expression of *HOXD13*, *HOXD12* and *HOXD10* in two affected patients carrying a 2q31.1q31.2 duplication. We showed that, in lymphobasts, the duplication was responsible for a complex modification of *HOXD* genes' expression, and we hypothesised that this may alter the limb bud development and cause the phenotype.

Indeed, the overexpression of the most 5′ located *HOXD* gene can, by itself, generate finger anomalies. It has been demonstrated that in presence of *GLI3*, *HOXD10* upregulation induces polydactyly, whereas upregulation of *HOXD13* and *HOXD12* leads to oligodactyly.[12] Moreover, the altered expression of *HOXD* genes probably modifies their pattern of expression and impairs the digit shaping as described in an animal model presenting an internal duplication of the complex.[3,4] The overexpression of *HOXD10* and *HOXD13* modifies the ratio between 5′ *HOXD* genes and *GLI3* products, probably mimicking a lack of *GLI3* products that corresponds to Greig syndrome in which cutaneous syndactyly occurs.

For the genes presumptively involved in the nystagmus, *CHN1* was overexpressed in the duplicated patients (2.1 fold in the father and 1.6 fold in the daughter), as well as *CHRNA1* (1.3 fold in the father and 3.0 fold in the daughter; data not shown).

Ocular motility depends on the precise innervation of ocular motor muscles. Abnormal innervation can give rise to nystagmus. The *CHN1* gene encodes two Rac-specific guanosine triphosphatase, activating α-chimaerin isoforms. Miyake *et al*[15] recently identified missense mutations in *CHN1*, which induce a gain-of-function of α2-chimaerin and cause aberrant innervation of oculomotor muscles in animal models. Thus, overexpression of this gene in our patients might cause hyperactivation of α-chimaerin and impair normal ocular motor innervation, although Duane syndrome is distinct from nystagmus and we cannot prove this. The effect of this overexpression could possibly be modulated by the overexpression of *CHRNA1*. No other candidate gene seemed to be potentially associated with eye anomalies in the duplicated region. Another explanation could be the dysregulation of a gene, distant from the duplication, which we have not identified yet.

We show that duplication of the *HOXD* cluster disturbs, at least, *HOXD10*, *HOXD12* and *HOXD13* expression. This misregulation possibly gives rise to syndactyly through a direct effect of excessive *HOXD* genes' products, or because of ratio disequilibrium between 5′ *HOXD* and *GLI3* products. In addition, the modification of chromosomal environment could be involved in the complex dysregulation. Further experiments in animal models are needed to confirm these hypotheses. Although *CHN1* gene is the best candidate gene for the nystagmus, its overexpression might not be the only explanation for this finding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 Johnson RL, Tabin CJ: Molecular models for vertebrate limb development. *Cell* 1997; **90**: 979–990.
2 Kessel M, Gruss P: Murine developmental control genes. *Science* 1990; **249**: 374–379.
3 Zakany J, Kmita M, Duboule D: A dual role for Hox genes in limb anterior-posterior asymmetry. *Science* 2004; **304**: 1669–1672.
4 Tarchini B, Duboule D: Control of Hoxd genes' collinearity during early limb development. *Dev Cell* 2006; **10**: 93–103.
5 Goodman FR, Majewski F, Collins AL, Scambler PJ: A 117-kb microdeletion removing HOXD9-HOXD13 and EVX2 causes synpolydactyly. *Am J Hum Genet* 2002; **70**: 547–555.
6 Del Campo M, Jones MC, Veraksa AN *et al*: Monodactylous limbs and abnormal genitalia are associated with hemizygosity for the human 2q31 region that includes the HOXD cluster. *Am J Hum Genet* 1999; **65**: 104–110.
7 Svensson AM, Curry CJ, South ST *et al*: Detection of a *de novo* interstitial 2q microdeletion by CGH microarray analysis in a patient with limb malformations, microcephaly and mental retardation. *Am J Med Genet A* 2007; **143A**: 1348–1353.
8 Prontera P, Bernardini L, Stangoni G *et al*: 2q31.2q32.3 deletion syndrome: report of an adult patient. *Am J Med Genet A* 2009; **149A**: 706–712.
9 Pescucci C, Caselli R, Grosso S *et al*: 2q24-q31 deletion: report of a case and review of the literature. *Eur J Med Genet* 2007; **50**: 21–32.
10 Boulet AM, Capecchi MR: Duplication of the Hoxd11 gene causes alterations in the axial and appendicular skeleton of the mouse. *Dev Biol* 2002; **249**: 96–107.
11 Kantaputra PN, Klopocki E, Hennig BP *et al*: Mesomelic dysplasia Kantaputra type is associated with duplications of the HOXD locus on chromosome 2q. *Eur J Hum Genet* 2010; **18**: 1310–1314.
12 Sheth R, Bastida MF, Ros M: Hoxd and Gli3 interactions modulate digit number in the amniote limb. *Dev Biol* 2007; **310**: 430–441.
13 Dlugaszewska B, Silahtaroglu A, Menzel C *et al*: Breakpoints around the HOXD cluster result in various limb malformations. *J Med Genet* 2006; **43**: 111–118.
14 Cho TJ, Kim OH, Choi IH *et al*: A dominant mesomelic dysplasia associated with a 1.0-Mb microduplication of HOXD gene cluster at 2q31.1. *J Med Genet* 2010; **47**: 638–639.
15 Miyake N, Chilton J, Psatha M *et al*: Human CHN1 mutations hyperactivate alpha2-chimaerin and cause Duane's retraction syndrome. *Science* 2008; **321**: 839–843.