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

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25 **ABSTRACT**

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

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42 **Key Words:**

43 Syndactyly, *HOXD* cluster, 2q31.1q31.2 duplication

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49 **INTRODUCTION**

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

68 Animal models carrying internal duplications of part of the *HOXD* cluster and limb  
69 anomalies exist<sup>3,4</sup>. Indeed, mice with targeted disruptions of *Hoxd11* and *Hoxa11*  
70 genes showed marked zeugopod malformation<sup>10</sup>. A disconnection of 5' *Hoxd* genes  
71 from the regulator could result in a downregulation of 5' *Hoxd* genes in the distal limb  
72 (autopod) and an upregulation in the proximal limb, and it has been suggested that the

73 2q duplication could have the same effect, therefore explaining the mesomelic dysplasia  
74 recently reported<sup>11,4</sup>. Indeed, it has been recently reported that a 1Mb microduplication  
75 of *HOXD* gene cluster at 2q31.1 is associated with a dominant mesomelic dysplasia,  
76 Kantaputra type. The condition is mainly affecting the upper limbs, and is very variable  
77 among affected patients within the same family. This phenotype, linked with a small 2q  
78 duplication that contain the entire *HOXD* cluster, is far more severe than the one we  
79 report here, in which the duplicaton is larger and involving several other genes.

80 Indeed, we report on a father and his daughter referred to the genetic clinic for the  
81 association of bilateral 3-4 finger cutaneous syndactyly and nystagmus carrying a  
82 2q31.1q31.2 duplication involving 27 genes, among which the whole *HOXD* cluster,  
83 identified by array-CGH. We characterise this chromosomal anomaly and discuss the  
84 genotype-phenotype correlations.

#### 85 **PATIENTS AND METHODS**

86 The probands are a father and his daughter. The father presents an association of  
87 bilateral 3-4 hand cutaneous syndactyly and a pendular-resilient nystagmus which  
88 increases in up and right gaze and decreases in down gaze. Ophthalmologic examination  
89 and functional tests were normal (slit-lamp, fundoscopy, binocular visual field and  
90 electroretinogram), as well as a full neurological examination. Skeletal survey including  
91 hand x-rays was normal (Figure 1A). His six-year-old daughter is affected with the  
92 same hand malformations (Figure 1B). She was born after an uneventful pregnancy.  
93 Motor milestones were achieved normally and her psychomotor development is in  
94 correlation with her age. Her growth parameters are advanced (123 cm, +2.5 SD for the  
95 height; 29 kg, + 3 SD for the weight and 51 cm, -0.5 SD for the head circumference).  
96 No dysmorphic features were identified. She presents a slow pendular nystagmus which

97 causes amblyopia (confirmed by visual evoked potentials). A full neurological  
98 examination was normal as well as a skeletal survey including hand x-rays (Figure 1C).

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

107 Q-PCR was performed on genomic DNA extracted from the 3 members of the  
108 family. TaqMan analyses were performed in Fast Gene Quantification in 96-Well Pates.  
109 The final volume was 20µl and contained Genotyping master Mix (15µl), 4µl of DNA  
110 and 1µl of specific genes' primers and probes. Two exons per gene were studied:  
111 *HOXD13* (exon 1 and 2), *HOXD12* (exon 1 and 2), *HOXD10* (exon 1 and 2), *CHN1*  
112 (exon 1 and 13), *CHRNA1* (exon 2 and 10). All reactions were performed in triplicate.  
113 Thermal cycling conditions were as follows: denaturation at 95°C for 10 min and 40  
114 cycles of 95°C for 15 s; 60°C for 60 s; 72°C for 60 s. Analyses were carried out on a  
115 7900HT Sequence Detection System and interpreted with the comparative Ct methods.  
116 *RNASEP* was used as the reference gene control.

117 Total RNA was extracted from lymphoblasts cell cultures, using the RNeasy  
118 Mini Kit (Qiagen GmgH, Hilde, Germany) following the manufacturer's instructions. A  
119 total of 2µg of RNA was retrotranscribed into cDNA, with the High Capacity RNA-to-  
120 cDNA kit (Applied Biosystems, Foster City), for 1 hr at 37°C. TaqMan analyses were

121 performed in Fast Gene Quantification in 96-Well Plates. The final volume was 20µl and  
122 contained Gene Expression master Mix (10µl), 2µl of cDNA and 1µl of specific Gene  
123 Expression Assays for human *HOXD13*, *HOXD12*, *HOXD10*, *CHN1*, *CHRNA1* and  
124 *RPL13A* (primers and probes sequences Applied Biosystems trademarks), following  
125 manufacturers' instructions. All reactions were performed in quadruplicate. Thermal  
126 cycling conditions were as follows: denaturation at 95°C for 10 min and 40 cycles of  
127 95°C for 15 s; 60°C for 60 s; 72°C for 60 s. Analyses were carried out on a 7900HT  
128 Sequence Detection System and interpreted with the comparative Ct methods. *RPL13A*  
129 was used as the reference gene control.

130 Chromosomal analyses of peripheral blood lymphocytes according to routine  
131 procedures using GTG-banding (550 bands) and FISH analyses using bacterial artificial  
132 chromosome (BAC) clone RP11-483E17 localized at 2q31.1 (chr2:175,041,497-  
133 175,231,429) and clone RP11-250N10 localized at 2q31.2 (chr2:178,079,879-  
134 178,252,293) (hg18, NCBI Build 36) were performed in both patients.

## 135 **RESULTS**

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

140 We studied the level of cDNA in lymphoblasts to evaluate the impact of the duplication  
141 on the involved genes' expression. Q-PCR analysis confirmed the duplication of  
142 *HOXD13*, *HOXD12*, *HOXD10*, *CHN1* and *CHRNA1*. The analysed exons of each tested  
143 gene were double dosed in the duplicated patients comparatively to the unaffected  
144 mother (Figure 3). *HOXD13* and *HOXD10* were overexpressed in the father (3.7 and 2.9

145 fold respectively) and his daughter (3.0 and 6.2 fold respectively). The expression of  
146 *HOXD12* was diminished in the daughter (5 fold) but in the father no difference was  
147 shown (data not shown).

148 Chromosome analyses (550 bands) were normal and FISH analyses revealed direct  
149 2q31.1q32.2 duplication in both patients (Figure 4).

## 150 **DISCUSSION**

151 We report on a father and his daughter presenting a large 2q31.1 duplication involving  
152 the *HOXD* cluster, but also many other genes, and a very mild phenotype, namely a  
153 cutaneous syndactyly between two fingers and a nystagmus. Recently, two reports on a  
154 dominant mesomelic dysplasia type Kantaputra have been described in association with  
155 a 2q31.1 duplication involving the *HOXD* locus and other genes (*MTX2*, *EVX2*,  
156 *KIAA1715*) out of which some are also known to have important roles during digit  
157 development<sup>14,11</sup>. The patients presented severe shortening of the middle segments of  
158 the arm, relative shortening of the tibia and fibula and no ophthalmological associated  
159 anomaly. Since our cases had a normal full skeletal survey, their phenotype is very  
160 different and is restricted to a bilateral cutaneous syndactyly between the 3<sup>rd</sup> and 4<sup>th</sup>  
161 fingers. The 2q31.1 duplication in our cases was larger than that reported by the  
162 previous authors<sup>14,11</sup>. We do not know whether our cases' phenotype is linked with  
163 increased gene expression or dysregulation at the *HOXD* locus. *HOXD13*  
164 overexpression might explain the cutaneous syndactyly, although further expression  
165 studies in cells from the developing autopod rather than in lymphoblasts would be  
166 needed to ascertain this. It has been suggested that the ELCR could be needed to  
167 implement colinear expression of the *HOXD* cluster<sup>3</sup>. The duplication could disconnect  
168 the cluster from the ELCR and therefore explain the limb phenotype, although the



169 recent report from Kantaputra et al. did not identify the same 2q duplication in other  
170 affected individuals and suggested that a balanced structural chromosomal  
171 rearrangement affecting *HOXD* locus regulation could also explain the phenotype<sup>11</sup>.

172 A modification of the chromosomal environment due to the duplication could possibly  
173 be involved in the genesis of the finger phenotype. Such mechanisms have already been  
174 described by Dlugaszewska et al. and correspond to translocations and inversions with  
175 breakpoints near the *HOXD* cluster<sup>13</sup>. Patient 2 (as designated in the original article)  
176 carried a t(2;10)(q31.1;q23.33) translocation with a proximal breakpoint around 1050  
177 kb downstream to *HOXD13*. He harboured ulnar hypoplasia and absence of fingers 3 to  
178 5 and hypoplastic fingers 1 and 2. In this case, a first wave impairment could be  
179 suspected because of the zeugopod involvement and thus ELCR misregulation might be  
180 involved in the phenotype. Regarding our patients, karyotype and FISH analyses  
181 allowed to confirm that the duplication was tandem rather than being translocated to  
182 another chromosome.

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

189 Indeed, the overexpression of the most 5' located *HOXD* gene can, by itself, generate  
190 finger anomalies. It has been demonstrated that in presence of *GLI3*, *HOXD10* up  
191 regulation induces polydactyly, whereas up regulation of *HOXD13* and *HOXD12* leads  
192 to oligodactyly<sup>12</sup>. Moreover, the altered expression of *HOXD* genes probably modifies

193 their pattern of expression and impairs the digit shaping as described in an animal  
194 model presenting an internal duplication of the complex <sup>3,4</sup>. The overexpression of  
195 *HOXD10* and *HOXD13* modifies the ratio between 5' *HOXD* genes and *GLI3* products,  
196 probably mimicking a lack of *GLI3* products that corresponds to Greig syndrome in  
197 which cutaneous syndactyly occurs.

198 For the genes presumptively involved in the nystagmus, *CHNI* was over expressed in  
199 the duplicated patients (2.1 fold in the father and 1.6 fold in the daughter) as well as  
200 *CHRNA1* (1.3 fold in the father and 3.0 fold in the daughter) (data not shown).

201 Ocular motility depends on the precise innervation of ocular motor muscles. Abnormal  
202 innervation can give rise to nystagmus. The *CHNI* gene encodes two Rac-specific  
203 guanosine triphosphatase (GTPase)-activating  $\alpha$ -chimaerin isoforms. Miyake et al.  
204 recently identified missense mutations in *CHNI* which induce a gain of function of  
205  $\alpha$ 2-chimerin and cause aberrant innervation of oculomotor muscles in animal  
206 models <sup>15</sup>. Thus, overexpression of this gene in our patients might cause hyperactivation  
207 of  $\alpha$ -chimaerin and impair normal ocular motor innervation, although Duane syndrome  
208 is distinct from nystagmus and we can not prove this. The effect of this overexpression  
209 could possibly be modulated by the overexpression of *CHRNA1*. No other candidate  
210 gene seemed to be potentially associated with eye anomalies in the duplicated region.  
211 Another explanation could be the dysregulation of a gene, distant from the duplication,  
212 which we have not identified yet.

213 We show that duplication of the *HOXD* cluster disturbs, at least, *HOXD10*, *HOXD12*  
214 and *HOXD13* expression. This misregulation possibly gives rise to syndactyly through a  
215 direct effect of excessive *HOXD* genes' products, or because of ratio disequilibrium  
216 between 5' *HOXD* and *GLI3* products. In addition, the modification of chromosomal

217 environment could be involved in the complex dysregulation. Further experiments in  
218 animal models are needed to confirm these hypotheses. Although *CHNI* gene is the best  
219 candidate gene for the nystagmus, its over expression might not be the only explanation  
220 for this finding.

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222 Conflict of interest:

223 The authors declare no conflict of interest.

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289 **FIGURE LEGENDS**

290 **Figure 1**

291 A: Normal hands x-rays of the affected father.

292 B: Hands of the daughter after surgery. Note scars after surgery for 3-4 fingers' skin  
293 syndactyly

294 C: Normal hands x-rays of the affected daughter.

295 **Figure 2**

296 Array-CGH analysis and genes involved in the 3.8 Mb wide 2q31 duplication.

297 Comparison with the 2q31 duplication involved in the Kantaputra mesomelic dysplasia.

298 **Figure 3**

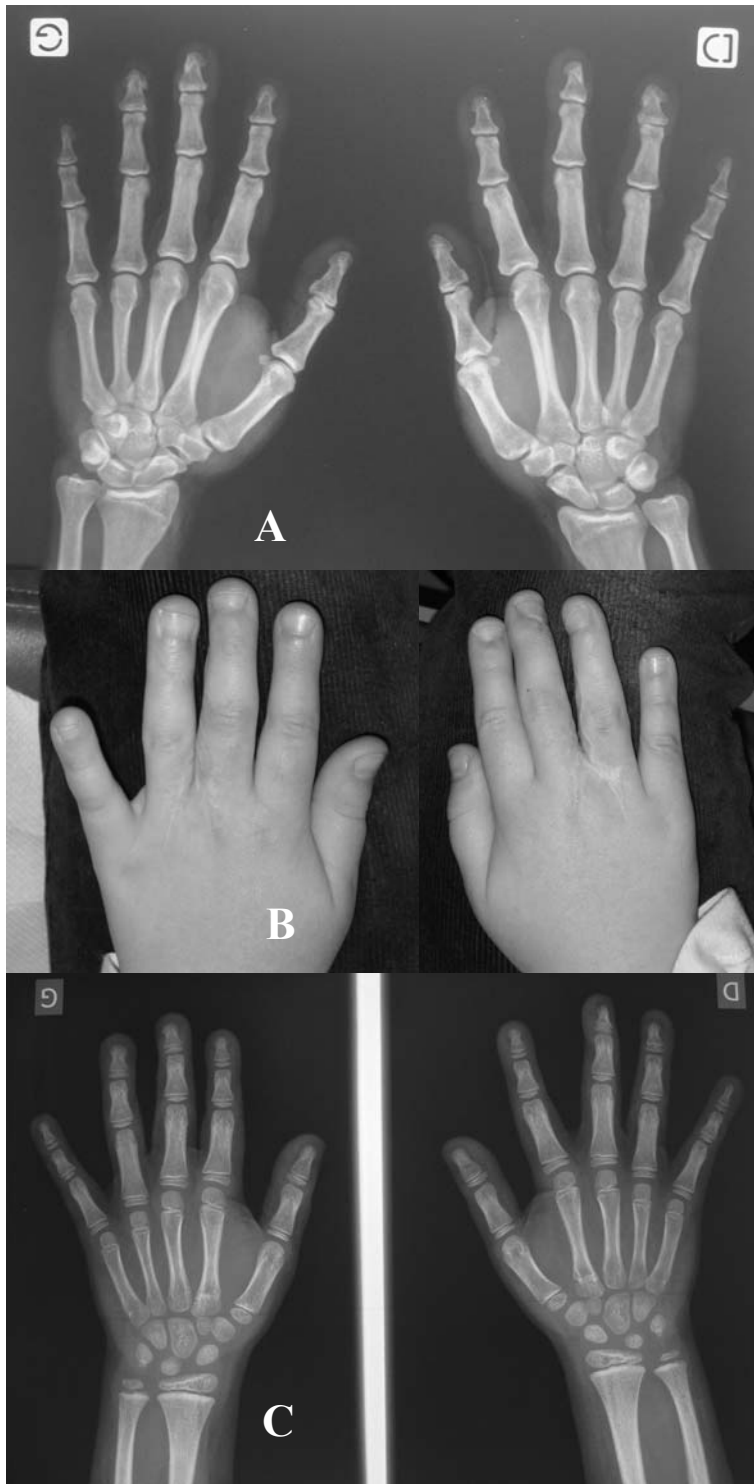
299 Q-PCR analysis. Estimated copy variations for the different analysed exons of *CHN1*,  
300 *HOXD13*, *HOXD12*, *HOXD10* and *CHRNA1*. The confidence interval is 95% with n=4.

301 There are 3 copies in the father and his daughter, whereas there are 2 in the mother and  
302 the control.

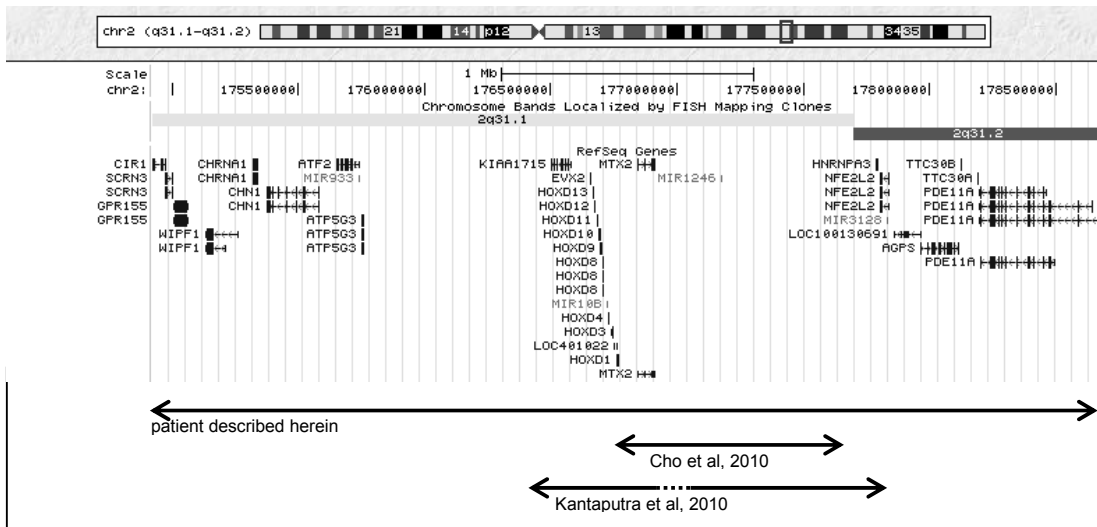
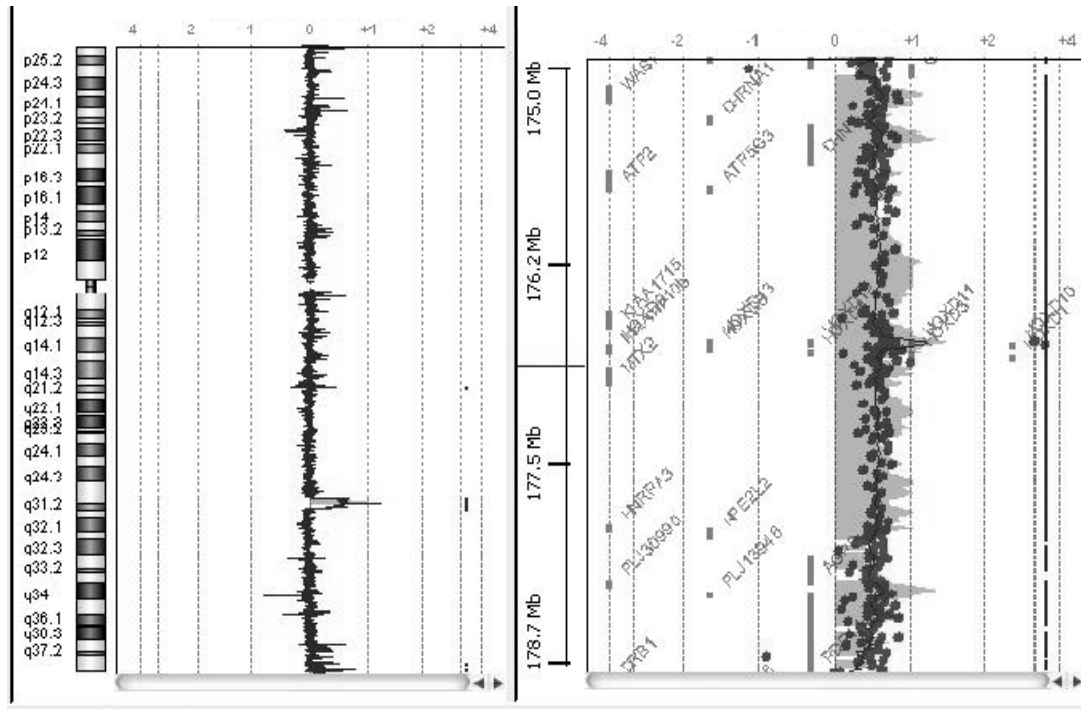
303 **Figure 4**

304 Karyotype and FISH analyses. Note tandem 2q duplication.

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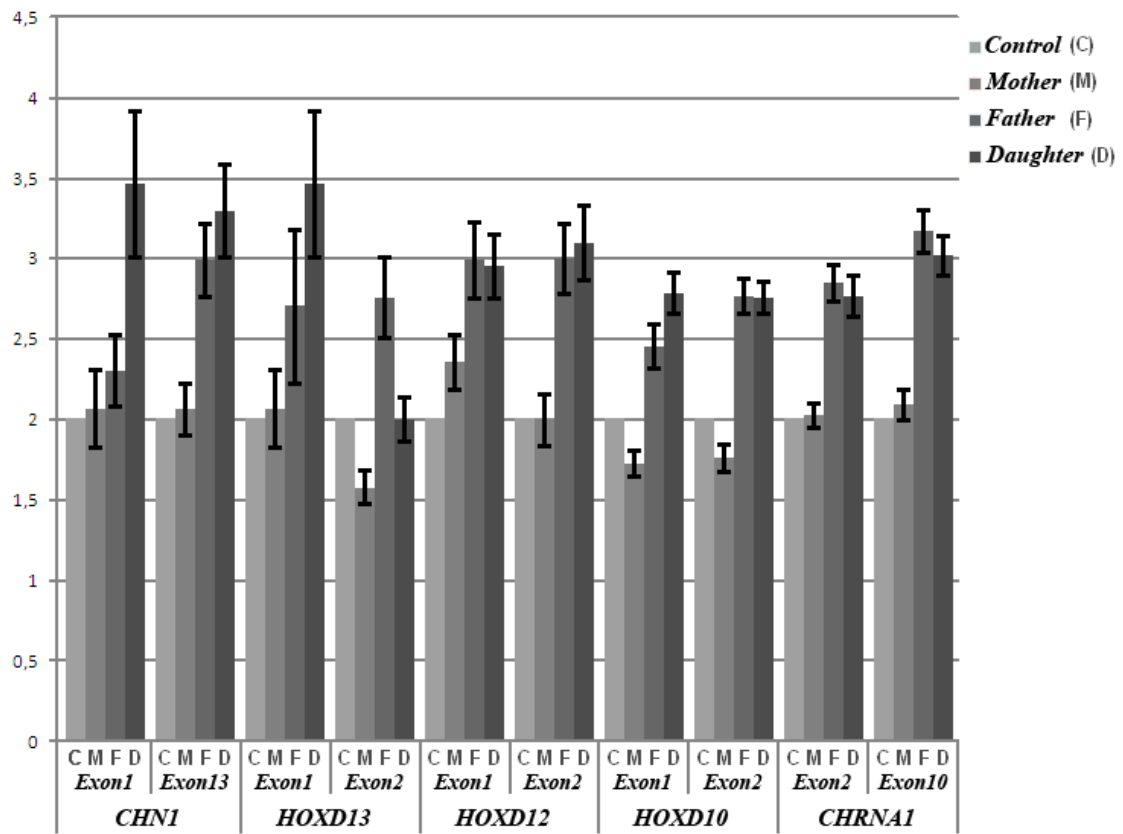


**Figure 1**





**Figure 3**



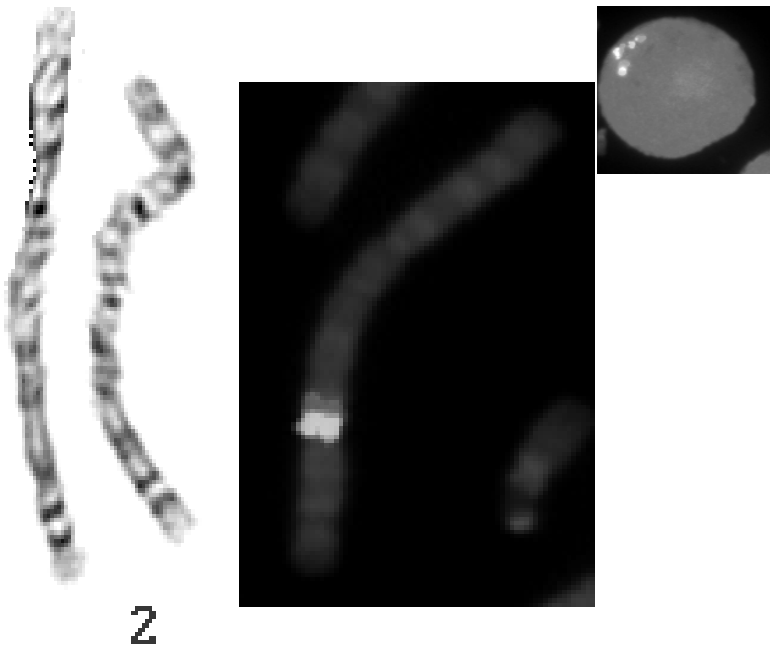


Figure 4