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

Jamal Ghoumid (1), Joris Andrieux (2), Bernard Sablonnière (1), Sylvie Odent (3), Nathalie Philippe (1), Xavier Zanlonghi (4), Pascale Saugier-veber (5), Thomas Bardyn (1), Sylvie Manouvrier-Hanu (6) , Muriel Holder-Espinasse (6)

(1) Department of Molecular Biology, University Hospital, Lille, France

(2) Department of Cytogenetics, University Hospital, Lille, France

(3) Department of Clinical Genetics, University Hospital, Rennes, France

(4) Department of Ophthalmology, Nantes, France

(5) Department of Molecular Biology, Rouen, France

(6) Department of Clinical Genetics, University Hospital, Lille, France

Correspondance to:

Muriel Holder-Espinasse

Service de Génétique Clinique

Hôpital Jeanne de Flandre

CHRU

59037 Lille Cedex, France

Tel : 0033320444911

Fax : 0033320444901

muriel.holder@chru-lille.fr

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, centrometric 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^{1,2}. 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 cluster³. Moreover, these genes
55 are expressed through two waves⁴ during the limb budding and control the patterning
56 of the stylopod and the zeugopod⁵. 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 anomalies⁵. 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^{6,5}. Deletions removing GCR are deleterious too but induce minor
66 anomalies⁷⁻⁹. 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^{3,4}. Indeed, mice with targeted disruptions of *Hoxd11* and *Hoxa11*
70 genes showed marked zeugopod malformation¹⁰. 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 ^{11,4}. 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 duplication 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 Plates.
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^{14,11}. 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 3rd and 4th
161 fingers. The 2q31.1 duplication in our cases was larger than that reported by the
162 previous authors^{14,11}. 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* cluster³. 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 phenotype¹¹.
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* cluster¹³. 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 oligodactyly¹². 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 ^{3,4}. 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 α -chimaerin isoforms. Miyake et al.
204 recently identified missense mutations in *CHNI* which induce a gain of function of
205 α 2-chimerin and cause aberrant innervation of oculomotor muscles in animal
206 models ¹⁵. Thus, overexpression of this gene in our patients might cause hyperactivation
207 of α -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 *CHN1* 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.

305

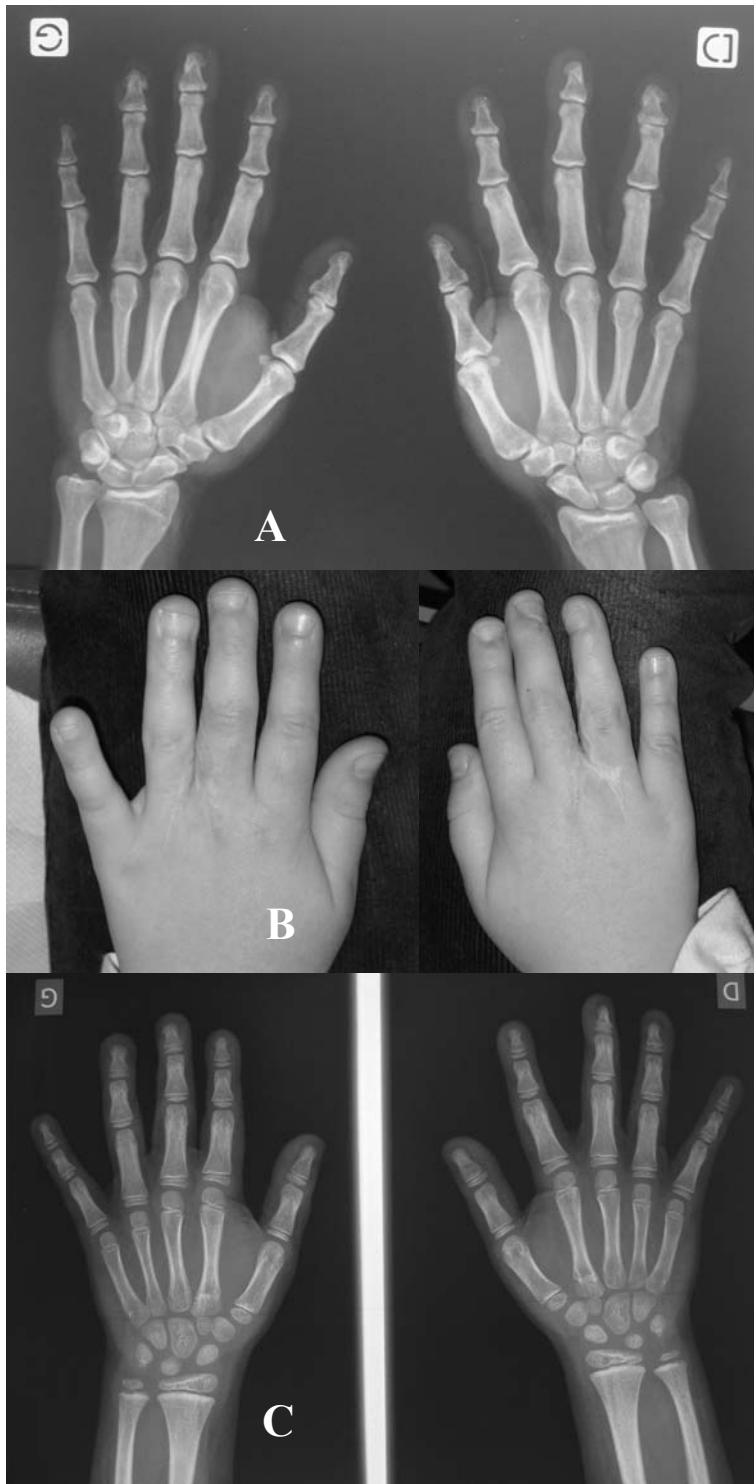


Figure 1

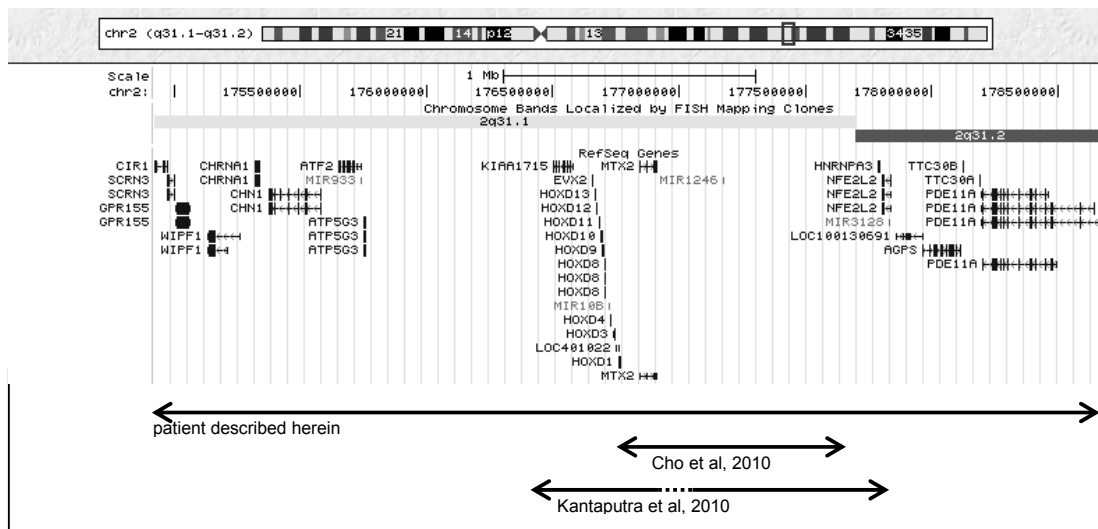
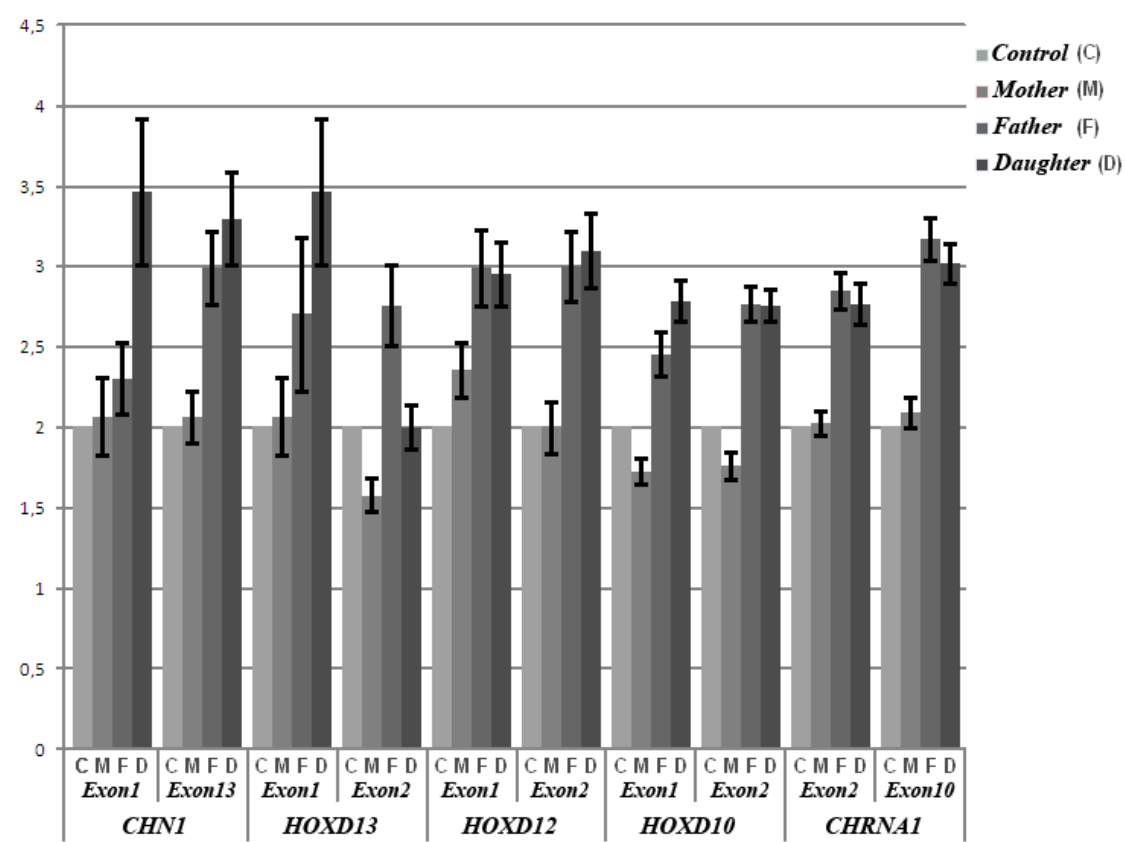


Figure 3



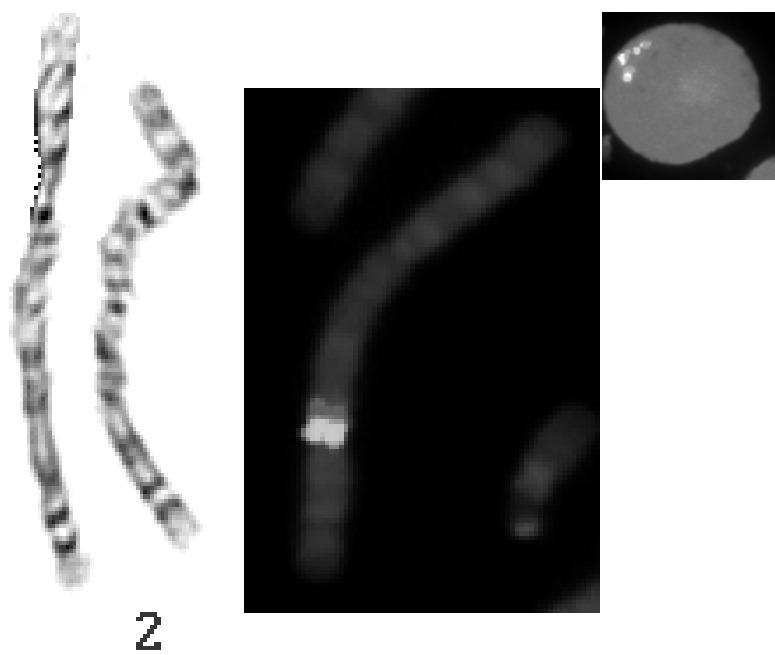


Figure 4