

# A 14q distal chromoanagenesis elucidated by whole genome sequencing

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## **A 14q Distal Chromoanagenesis elucidated by whole genome**

## 2 sequencing.

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#### 20 ABSTRACT

21 Chromoanagenesis represents an extreme form of genomic rearrangements involving multiple breaks occurring on a single or multiple chromosomes. It has been recently 22 described in both acquired and rare constitutional genetic disorders. Constitutional 23 chromoanagenesis could lead abnormal phenotypes 24 events to including developmental delay and congenital anomalies, and have also been implicated in 25 some specific syndromic disorders. We report on a girl presenting with growth 26 27 retardation, hypotonia, microcephaly, dysmorphic features, coloboma, and hypoplastic corpus callosum. Karyotype showed a *de novo* structurally abnormal chromosome 28 14q31qter region. Molecular characterization using SNP-array revealed a complex 29 unbalanced rearrangement in 14q31.1-q32.2, on the paternal chromosome 14, 30 including thirteen interstitial deletions ranging from 33kb to 1.56Mb in size, with a total 31 of 4.1 Mb in size, thus suggesting that a single event like chromoanagenesis occurred. 32 To our knowledge, this is one of the first case of 14g distal deletion due to a germline 33 34 chromoanagenesis. Genome sequencing allowed the characterization of 50 35 breakpoints, leading to interruption of 10 genes including YY1 which fit with the patient's phenotype. This precise genotyping of breaking junction allowed better 36 definition of genotype-phenotype correlations. 37

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#### 40 KEY WORDS

chromoanagenesis; constitutionnal; intellectual disability; microcephaly; 14q deletion;
coloboma; hypoplastic corpus callosum; genome sequencing

#### 44 Introduction

Constitutional complex chromosome rearrangements (CCRs) are rare structural 45 anomalies characterized by three or more breakpoints, involving at least 46 a 47 chromosome <sup>1</sup>. They are usually identified by standard karyotyping and further characterized by array-based analysis. Recently, highly complex changes termed 48 49 chromoanagenesis, arising from shattering mechanisms, were observed in cancer 50 genomes, and have also been described in rare patients with developmental disorders. 51 Genomic imbalances included deletions, duplications and /or triplications, translocations and inversions, concentrated on a single or few chromosomes <sup>2, 3, 4, 5</sup>. 52

53 Depending of the mechanism of chromoanagenesis, several terms are used,

54 chromotripsis referred to a mitotic event with chromosome shattering and non-

55 homologous end joining repair; and chromoanasynthesis referred to a local defective

56 DNA replication with microhomology-mediated template switching that produces local

57 rearrangements with altered gene copy numbers. The presence of chromoanagenesis

in healthy individuals affecting reproduction has also recently been reported <sup>6, 7</sup>.

59 Here, we describe a complex 14g distal deletion consistent with a constitutional chromoanagenesis process, in a girl with developmental delay and congenital 60 61 anomalies. Our patient shows clinical findings described in patients with 14g terminal deletion syndrome (developmental delay, hypotonia, growth retardation, microcephaly, 62 a characteristic facial appearance and a variety of ocular anomalies, including retinal 63 pigmentary changes, microphthalmia and coloboma)<sup>8</sup>, especially ocular and brain 64 malformations. The complexity of this case of chromoanagenesis, as well as the 65 66 possibility to identify new genotype-phenotype relationships, has been elucidated by whole genome sequencing. 67

68

69 Clinical Report

70 The proband, an 8 month-old-girl, is the third child of healthy unrelated 42 years old mother and 38 years old father. The family history was negative for birth defects or 71 developmental delay. The pregnancy had been complicated by maternal hypertension 72 and intrauterine growth retardation (IUGR). Third trimester ultrasound examination 73 revealed hypoplasia of the corpus callosum and reduction of the biparietal diameter. 74 Cytogenetic analysis from amniotic cells performed in another institution showed an 75 apparent normal 46,XX karyotype. She was born at 33+2 weeks of gestation after a 76 77 caesarian section. Birth weight was 2050 g (< 3rd centile), birth length 43 cm (< 3rd centile) and head circumference 32 cm (3rd centile). After birth, she was hospitalized 78 for one month because of hypotonia, feeding difficulties and growth retardation. 79 Subsequently, hypertonic movements of the limbs were noted. At the age of 8 months, 80 81 the neurological examination revealed axial hypotonia and peripheral hypertonia. She 82 was unable to hold her head and to sit without support. She was not able to vocalize. 83 She had a height of 60 cm (-3SD), a weight of 5100 g (-3SD), a microcephaly with a 84 head circumference of 39 cm (-3SD) and a large anterior fontanel. Facial dysmorphic 85 features included a broad and flat nasal bridge, epicanthus, a short nose, a broad philtrum, a small mouth with thin upper lip, and small and low-set ears. An 86 electroencephalogram (EEG) gave normal results. Brain Magnetic resonance imaging 87 88 (MRI) confirmed the corpus callosum hypoplasia. Ophthalmologic examination 89 revealed bilateral chorioretinal coloboma. Echocardiography and renal ultrasound 90 examination gave normal results. The patient died after a severe rhabdomyolysis event associated with multiorgan failure at the age of 2 years. 91

#### 92 Investigations and Results

93 Cytogenetic analysis was performed again in our laboratory on GTG an RHG 94 banded metaphases from lymphocytes at a resolution of approximately 500 bands

95 according to the standard cytogenetic protocol (Fig. 1.A). FISH analyses using WCP 14 Probe (Metasystem, Altlussheim, Germany), tel 14g probe (Vysis, Illinois, Unated 96 97 States) and bacterial artificial chromosome (BAC) probes targeting the rearranged 14g region, were realized according to standard procedures (Fig.1.B,C,D). Single 98 99 nucleotide polymorphism (SNP) microarray analysis was performed using a Human 100 OmniExpress24, which contains 713,599 markers including 395,094 SNPs (Illumina, 101 San Diego, United States), according to the manufacturer's protocol. Results were 102 analyzed with Illumina GenomeStudio software. SNP profiles were analyzed by 103 comparing the Log R ratio, i.e., In (sample copy number/ reference copy number), and 104 the B allele frequency (BAF) (Fig 1.E,F). Data analysis was based on information from 105 the UCSC Genome Browser (NCBI37 hg19).

106 G-banded chromosomes analysis revealed an abnormal female karyotype with 107 structural changes spanning the terminal region of the long arm of one chromosome 14, in all the cells examined (Fig.1). Parental karyotypes were normal. Methylation 108 109 specific PCR of the differentially methylated region at 14q32 showed normal biparental 110 inheritance and no abnormality of methylation at the GTL2/DKL1 region, confirming 111 that the patient does not have chromosome 14 uniparental disomy (UPD). SNP array 112 analysis revealed thirteen interstitial deletions of chromosome 14, between bands 113 14g31.1 and 14g32.2 (chr14: 83088298 100452660), ranged from 33 kb to 1.56 Mb in size, with a total of 4.1 Mb in size (Fig.1, supplemental data 1). These deletions were 114 115 separated by normal copy number segments. The distal breakpoint was located at 740kb from the imprinted gene cluster. FISH analysis using BACs RP11-300J18 116 117 88484949 88502663) RP11-543C4 (14q31.3: (Fig.1), (14q32.2: 118 100070893 100092077) confirmed the deletions. The BAC RP11-433J8 (14q32.2: 119 97059105 97061097) probe was not deleted. SNP-array analysis of deletions intervals in the parents and patient revealed that the rearrangement derived from the paternalchromosome 14 (supplemental data 2).

122 For a better understanding of the mechanism of this event, whole genome sequencing (WGS) with break point analysis has been performed. A 350-bp fragments library was 123 124 prepared following the Illumina TruSeg DNA PCR-free protocol (Illumina, San Diego, 125 California, USA) with 3µg genomic DNA, according to manufacturer instructions. DNA 126 library was sequenced on an Illumina NextSeg 500 as paired-end 101 bp reads using 127 the High Output (300 cycles) NextSeg500 kit, yielding a mean sequencing depth of 128 14.32X. Image analysis and base calling were performed using Illumina Real Time Analysis Pipeline 2 with default parameters. Alignment of the reads against the hg19 129 version of the human genome was done using BWA-MEM v 0.7.10<sup>9</sup>. The reads were 130 sorted using Samtools v 1.3.1<sup>10</sup>, and the duplicates removed by PicardTools v 1.138 131 132 (picard.sourceforge.net). Structural variants (SV) were detected using BreakDancer v 1.4.5<sup>11</sup> and annotated using an in-house program in order to filter out recurrent 133 variants. Integrative Genomics Viewer v 2.3<sup>12</sup> was used for the SV visualization and 134 135 validation. Junction sequences were determined using split-read sequences when at 136 least 2 split-reads were available. If not possible, junction fragments were amplified by PCR (junctions n°2, 6, 16, 20, 27, 28, 30, 41 and 46), in the patient and a control with 137 138 no chromosomal rearrangement, using Tag DNA Core kit 10 (MP Biomedicals, Solon, Ohio), according manufacturer instructions followed by Sanger sequencing. Junctions 139 140 sequences were then aligned using BLAT tool (UCSC) against hg19 reference in order to obtain the breakpoint at the base-pair level. WGS identified a highly complex 141 142 rearrangement including 50 breakpoints on chromosome 14, clustered in the 143 14g31.1g32.33 region. In 8 breakpoints (n°2, 6, 16, 20, 27, 28, 41 and 46), we observed insertions of 3 to 9 small fragments (19-177 bp) derived from various 144

145 chromosomes (1,2,3,4,5,6,7,8,11,12,14,15,16,17 and 22) (Fig.2 and 3, supplemental data 1 and 3), resulting in 35 small inserted fragments. Taken these small insertions 146 into account, this rearrangement resulted in 85 junctions. Twenty-three fragments of 147 chromosome 14 were in an inverted orientation, as well as 18/35 small inserted 148 fragments. We observed 2 to 7 bp microhomology (≥ 2bp) in 25/85 junctions. There 149 150 were twenty-one deletions on chromosome 14 (from 723 bp to 1565077 bp) 151 concordant with microarray analysis results (Fig.2). Besides these deletions, the 152 rearrangement disrupted 10 OMIM genes (FLRT2, EML5, FOXN3, RPS6KA5, 153 CCDC88C, UNC79, SERPINA3, CLMN, GSKIP, YY1).

154

#### 155 **Discussion**

156 This case highlights the increasing complexity in genetic analysis and the fact that the different steps complement each other. Although karyotype is not considered any more 157 158 as the first line laboratory examination in children presenting with developmental 159 defects and/or intellectual disability, many laboratories continue to perform standard 160 chromosome analysis, in parallel with molecular techniques like SNP-Array or next-161 generation sequencing, since it allows the rapid diagnosis of most balanced 162 chromosomal rearrangements. When associated with an abnormal phenotype, these 163 latter are of importance in the identification of new genes responsible for these 164 pathologies. This strategy, from karyotype to NGS, is still justified since whole genome 165 sequencing is not vet widely available in current diagnosis.

166 In our patient, karyotyping revealed that one chromosome 14 showed an unusual 167 banding pattern on the long arm, thus leading to further investigations by SNP array. It 168 allowed to detect 13 deletions and to confirm the *de novo* occurrence of the event on 169 the paternal chromosome. However, deleted and interrupted genes, as identified by this technique, didn't explain fully the phenotype of the patient suggesting that the
situation was more complex than expected and needing more sensitive methods like
Next Generation Sequencing (NGS).

Therefore, whole genome sequencing (WGS) was conducted and breakpoint cloning identified 50 breakpoints and insertions of fragments involving 15 different chromosomes, thus allowing a better characterization of this rearrangement and new relationships between genes and the patient's phenotype.

177 Usually, chromoanagenesis in the context of congenital disorders involves one or few chromosomes <sup>5</sup>. In our case, NGS revealed dramatically complex chromosomal 178 179 events since it involved 15 different chromosomes. Regarding to the chromotripsis 180 mechanism, firstly by analyzing ten constitutional CCRs, Kloosterman et al. (2012) 181 concluded that chromosome shattering by multiple DSBs and non-homologous repair 182 may be a common mechanism underlying chromothripsis rearrangements <sup>13</sup>. More recently, Zhang et al.<sup>14</sup> demonstrated that the mechanism for chromothripsis can 183 184 involve the fragmentation and subsequent reassembly of a single chromatid from a 185 micronucleus, providing an explanation for the restriction of chromothriptic 186 rearrangements to a single chromosome.

We showed that the rearrangement had occurred *de novo* on the paternal chromosome 14. This is in agreement with previous reports that demonstrated the occurrence of this kind of events in the germline, as well as a paternal bias for CNV or CCR formation <sup>17,18</sup>. The deletions, insertions and inversion pattern identified in this case approached the pattern reported by Nazaryan-Petersen et al, and reinforced the probability of a chromotripsis mechanism.<sup>19</sup>

Owing to data obtained from both SNP-array and WGS in our patient, we were able to
establish valuable genotype-phenotype correlations. In the literature, few patients with

357 pure terminal deletions of 14g, with or without ring chromosome formation, and characterized by molecular cytogenetic methods have been reported <sup>20,21,8</sup>. A recurrent 358 359 1.11 Mb microdeletion of 14q32.2 including the *DLK1/GTL2* imprinted gene cluster, mediated by expanded TGG repeats, has also been described in two unrelated 360 361 patients presenting with clinical features compatible with UPD (14)mat <sup>22</sup>. In our report, 362 the imprinted region on 14g was not involved, as determined by SNP-array and this finding was confirmed by direct analysis of the methylation profile of the DLK1/GTL2 363 364 region (data not shown). Typical clinical features of the 14g terminal deletion syndrome include developmental delay, hypotonia, growth retardation, microcephaly, a 365 characteristic facial appearance and a variety of ocular anomalies, including retinal 366 pigmentary changes, microphthalmia and coloboma<sup>8</sup>. Similar phenotypic features 367 were present in our patient, including chorioretinal coloboma. The structural eye 368 369 defects, microphthalmia, anophthalmia and coloboma (MAC) have a large genetic component with several loci of genes located in the proximal and central parts of the 370 371 14q arm<sup>8</sup>. Review of the literature suggests that an additional locus for coloboma may map to the terminal region of 14q32.31<sup>8 21</sup>. In our case, the deleted regions, although 372 373 in the vicinity of the MAC spectrum locus, do not overlap with it but could have position 374 effects altering the expression of intact genes near the breakpoints. Alternatively, as 375 suggested by Salter et al. (2016), the possibility of an underlying multigenic mechanism could to be considered. Indeed, our patient was also presenting with a hypoplastic 376 377 corpus callosum. Agenesis or dysgenesis of the corpus callosum is a heterogeneous condition, for which several different genetic causes are known. Interestingly, in the 378 379 literature, an abnormal corpus callosum had previously been reported in four patients 380 with a 14q32 deletion, suggesting that a gene involved in corpus callosum development may map to this region <sup>23</sup>. A chromoanagenesis on chr 14 has been 381

382 reported in a patient with developmental delay, characterized by the deletion of 2.7 Mb in 14g32.33 and the insertion of 4g32.3 into the long arm of chromosome 14.24 383 384 Developmental delay fit with our case, but no other phenotypical features were fitting 385 between the two cases. 386 In our patient, the deleted regions contain genes like *EML1*, encoding for Echinoderm 387 microtubule-associated protein-like 1 which is expressed in eye and associated to the 388 photoreceptor <sup>25</sup> or *CYP46A1*, encoding for cytochrom oxydase 46A1 which is involved 389 in brain cholesterol metabolism <sup>26</sup>. Loss of *CYP46A1*, which may play an important role 390 in brain tissue, may be responsible for the neurologicals disorders observed in patients with 14g deletion <sup>27</sup>. In the inserted fragments, no argument (content, localization or 391 orientation) was found to explain the patient's phenotype. In addition, breakpoint 392 393 analysis by genome sequencing revealed the interruption of 10 genes: EML5, FOXN3, 394 RPS6KA5, c14orf159, CCDC88C, UNC79, SERPINA3, CLMN, GSKIP, YY1. This 395 latter encodes for a zinc-finger transcription factor involved normal development and 396 malignancy. Deletions of YY1 have been associated with cognitive impairments, 397 behavioral alterations, intrauterine growth restrictions, feeding problems, and various 398 congenital malformations <sup>28</sup>. These features could correspond to the phenotype in our 399 patient but with less severe issues. However, although eyes abnormalities (strabism 400 and hypermetropia) have been reported in 3 patients in the cohort of Gabriele-de 401 Vriess syndrome, coloboma has not been associated with this syndrome.

402

In conclusion, this report highlights the complementarity of different genomic
investigation techniques leading to a better comprehension of the mechanism in CCR
events, therefore leading to a better genetic counselling to the families.

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408 We thank the technical team of the cytogenetic laboratory for their excellent assistance.

### 410 Legend

411 **FIGURE 1** 

### 412 **G-Banding, FISH and SNP array analyses.**

413 A: Representative pair of G-banded chromosome 14. The distal region of the g arm 414 showed an abnormal banding pattern on one chromosome (arrow). **B**: FISH using the 415 Whole Chromosome Painting Probe 14 (wcp14 spectrum green, Metasystem). C: FISH 416 using subtelomere 14q probe (spectrum orange, hg19, chr14:107,159,193-417 107,268,415) and LSI TCR 14g11.2 probe (spectrum agua) (Vysis), showing signals 418 on both 14 chromosomes. D: FISH using BAC RP11-300J18 (14q31.3) (spectrum red, 419 hg19, chromosome14:88,484,949-88,502,663) spanning one deleted region (308 K) and 14gtel probe (spectrum green, hg19, chromosome 14:107,159,193-107,268,415, 420 421 Vysis) do not show red signal on the der(14g) chromosome (arrow). **E**: SNP-array 422 profile of the terminal region of the long arm of chromosome 14 showing 13 deletions 423 clustered at 14g31.1-g32.2. B allele frequency and logR ratio are shown in the upper 424 panel. Orange bands indicate deleted regions. F: Genome studio representation of the 425 rearranged region between 14q31.1 and 14q32.2 bands (chr14: 83,088,298-426 100,452,660), and the OMIM deleted genes.

427

## 428 **FIGURE 2**

429 Circos plot issue from the WGS data, showing clustering of the breakpoints on 430 chromosome 14 and the involvement of 15 other chromosomes.

431

FIGURE 3: Example of breakpoint junction number 41 structure. On the left, schematic
representation of the recombined region, with the different chromosomes' regions
represented by different colors. On the right, the details of the junction, the underlined
nucleotides correspond to homology region.

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## Figure 1







Figure 3



\_chr14:86439890::chr11:17722368\_17722396::chr7:11546442\_11546482::chr1:2271621 65\_227162125::chr16:87703254\_87703427::chr14:95084795\_