Two interstitial rearrangements (16q deletion and 17p duplication) in a child with MR/MCA

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Key Clinical Message

Patients with rare deletions in 16q12 and a duplication of 17p, both interstitial and de novo. Only seven cases have been described with these deletions and none of them presented other chromosomal abnormalities. The proband showed a complex phenotype with features found in patients with dup17p11.2 syndrome, deletions in 16q12.

Keywords
16q Deletion CGH array, 17p duplication, interstitial rearrangements, mental retardation, MR/MCA syndromes, Potocki syndrome, Townes-Brocks syndrome.

Introduction

Cytogenetic imbalances are the most frequently identified cause of mental retardation (MR) associated with congenital malformations (MR/MCA syndrome). The number of recognizable imbalances has increased with the improvement of techniques, including higher resolution chromosomal banding, FISH (Fluorescence in situ hybridization), MLPA (Multiplex ligation-dependent probe amplification) and recently array-CGH (Comparative genomic hybridization). While FISH analyses with subtelomeric probes have proved the implication of submicroscopic terminal rearrangements in 2.6% of developmental delay and MR cases [6], array-CGH has expanded the detection rate in 4.4% [8]. These techniques also allow an accurate delineation of the chromosomal breakpoints, which might help to establish the associated genes to specific phenotypes as well as to understand the mechanism involved in the formation of chromosomal rearrangements. Finding rearrangements is sometimes more complicated to analyze than previously thought [1]. Simultaneous occurrence of two independent and unrelated cytogenetic events affecting interstitial regions remains rare and the possible mechanisms implicated unknown [7].

A patient with two apparently independent cytogenetic imbalances both interstitial and de novo is presented: a duplication of 17p detected by high-resolution karyotype analysis and a deletion of 16q detected by array-CGH. In the complex phenotype of the patient, we could recognize some features found in dup17p11.2 syndrome [5] as well as some features found in patients with deletions in 16q12 [3] in accordance to the Townes–Brocks syndrome (TBS), whose responsible gene SALL1 [1] was deleted in the patient.

Case History/Examination

A 18-year-old boy was referred for genetic evaluation because of severe MR and multiple congenital anomalies of unknown origin. Conventional karyotype during the perinatal period did not reveal any abnormality. The patient was the second child of a 36-year-old mother and 32-year-old father. Pregnancy history was unremarkable. The patient was born in the 40th week. Birth weight was 2600 g. Clinical examination at birth revealed characteristic face with retromicrognathia, wide nasal bridge, dysplastic, and low-set implanted ears (Fig. 1A and B). He showed also left peripheral facial palsy. Upper limbs were abnormal, with a triphalangeal thumb in the right hand and a hypoplastic thumb in the left hand (Fig. 1C and D). A congenital luxation of the right hip, umbilical hernia, and right inguinal hernia were observed that were
surgically corrected later. At the age of 7 years, esophagitis due to gastroesophageal reflux was diagnosed, and was also surgically corrected.

During clinical follow up, he showed hypotonia, psychomotor retardation, and failure to thrive. Some musculoskeletal abnormalities, such as cubital deviation of the fingers, clubfeet or scoliosis became more evident with age. At 18 years of age, in addition to these abnormalities and previous ones observed at birth, he showed short stature and was severely mentally retarded with walking impairment and absent speech. No specific evaluation with autism diagnostic test was done but his parents referred a good contact considering his severe MR. The protocol for the study of this case was reviewed and approved by the Ethics Committee of Fundacion Jimenez Diaz Hospital (Madrid, Spain) and was performed according to the tenets of the Declaration of Helsinki and further reviews (general assembly in Seoul, 2008). Prior to molecular study, an informed consent was signed by every participant and his/her anonymity was preserved.

**Genetic Diagnosis/Outcome**

**Cytogenetic and FISH**

High-resolution chromosome analysis was performed on peripheral blood samples using standard protocols. An extra band on 17p11.2 was observed (Fig. 2). In addition, FISH analysis using the Smith–Magenis probe (Qbiogene, Canada, United States) was performed according to the manufacturer’s protocol, showing a duplicated signal on one of the short arms of chromosome 17.

Cytogenetic analysis of blood samples of parents and the patient’s sister revealed normal karyotypes.

**Molecular analysis**

In order to establish if the duplication included the Charcot–Marie–Tooth region (type 1A CMT1A), MLPA was performed using salsa P033B probe mix (MRC-HOLLAND Amsterdam, The Netherlands). The results showed...
no duplication of the \textit{PMP22} gene but a duplication of the genes \textit{TNFRSF13B} and \textit{DKFZP586M1120}, both proximal to \textit{PMP22} and located on 17p11.2 in the Smith–Magenis syndrome (SMS) deletion region. Further analysis using some specific microsatellites of the region around \textit{PMP22} gen (D17S799, D17S955, D17S1356, D17S1357, D17S261, D17S1843, D17S1857, D17S1794, D17S1871) revealed that the duplication occurred in the maternally inherited chromosome 16. D17S1843 and D17S1871 STRs (short tandem repeats) were observed duplicate in the patient (Fig. 3A). The presence of a double dosage for these markers inherited from one of the maternally derived chromosomes in the patient suggested that the duplication emerged from a maternal intrachromosomal rearrangement (Fig. 3A).

Finally, for a better delimitation of the duplication, DNA from the patient was analyzed by 180K-resolution array CGH (Agilent Oligonucleotide Array-Based CGH). An interstitial duplication of approximately 5.89 Mb affecting band 17p12.2 was confirmed. The duplication
on 17p spanned from 10,561,251 base to 26,447,543 base into chromosome 17. Thus, he showed the common duplication seen in the duplication 17p11.2 syndrome, similar to the deleted region seen in SMS patients.

In addition, an unexpected deletion of 6.29 Mb affecting a pericentromeric band of chromosome 16 (16q12.1–q12.2) was found (Fig. 2). A posterior revision of the karyotype showed that the deletion had taken place in 16qh+ chromosome. According to the size of the deleted region it could have been seen by G-banding. However the presence of the large heterochromatic region might have hindered its possible deletion by conventional karyotyping. (Fig. 2). The proximity of the heterochromatin to the deleted region might have hindered its detection. Further studies of the parental origin of the 16q deletion using specific microsatellites for the region (D16S3044, D16S3136, D16S3117, D16S408) revealed that deletion originates in paternal-derived chromosome (Fig. 3). The deletion of 16q spans from 45,347,560 base to 61,641,943 base into chromosome 16. The array shows that SALL1 gene was deleted. This deletion is associated with TBS [1].

**Discussion**

Simultaneous occurrence of two independent and apparently unrelated cytogenetic events has rarely been reported. In addition, interstitial rearrangements have been less often reported than terminal ones. Moreover, two independent interstitial rearrangements have not been reported in the same patient until now. The application of array-CGH (180 kb resolution) to the study of patients with MR/MCA syndromes has improved the detection of these rearrangements, suggesting that their frequency might be higher than previously thought. In fact, the present case illustrates the limits of conventional cytogenetic techniques. Conventional karyotyping in the perinatal period was reported normal. A later high-resolution chromosome analysis detected only the duplication on chromosome 17, while the deletion of 16q12 could be only seen by re-investigation of G-banded analysis after its detection by array-CGH. The detection of such de novo rearrangements as a cause of MR/MCA cases represents an important factor for a correct genetic counselling, so the estimated risk could change, as in the present
Chromosomal imbalances affecting the short arm of chromosome 17 are relatively common and result in various well-characterized clinical conditions, such as Charcot–Marie–Tooth type 1 (CMT1A), Hereditary neuropathy with liability to pressure palsies (HNPP), SMS and duplication dup(17)(p11.2p1.2) syndrome. They are associated with deletions or duplications of proximal 17p, whose formation is mediated by LCRs (low copy repeats) [11]. This common duplication and reciprocal deletion results from unequal crossing over due to homologous recombination between the proximal and distal SMS LCRs, called SMS-REPs. The present patient showed an uncommon duplication seen in duplication 17p11.2 syndrome. The common duplication in this syndrome is the 3.7 Mb, which also represents the same region deleted in patients with SMS. However, this

Table 1. Clinical features of the patient, 17p+ and del16q12/Townes–Brocks syndromes.

| Feature                                              | Patient | 17p+ syndrome | Del 16q12/Townes Brocks syndrome |
|------------------------------------------------------|---------|---------------|----------------------------------|
| Low birth weight                                     | +       | +             |                                  |
| Failure to thrive                                     | +       | +             | +                                |
| Short stature                                         | +       | +             | +                                |
| Hypotonia                                             | +       | ++            | +                                |
| Microcephaly                                          | +       | +             | +                                |
| Broad nasal bridge                                    | +       | +             | +                                |
| High arched palate                                    |         |               |                                  |
| Broad forehead                                        |         |               |                                  |
| Down-slanted palpebral fissures                      | +       | +             | +                                |
| Long nasal tip                                        | +       | +             | +                                |
| Triangular face                                       |         |               |                                  |
| Micromax                                               | +       | +             | +                                |
| Low-set dysplastic ears                               | +       | +             | +                                |
| Congenital heart defects                              |         |               |                                  |
| Renal anomalies                                       | +       | +             | +                                |
| Inguinal hernia                                       | +       | +             | +                                |
| Umbilical hernia                                      | +       | +             | +                                |
| Gastroesophageal reflux                               | +       | ++            | +                                |
| Anal stenosis/imperforate anus                        |         |               | +                                |
| Anomalies of internal/external genitalia              |         |               | +                                |
| Scoliosis                                             | +       | +             |                                  |
| Broad/bifid thumb                                     |         |               |                                  |
| Triphalangeal thumb                                   | +       | +             | +                                |
| Hypoplastic thumb                                     | +       | +             | +                                |
| Preaxial polydactyly                                  | +       | +             | +                                |
| Finger/toes syndactyly                                | +       | +             | +                                |
| Metatarsal anomalies                                  |         |               | +                                |
| Congenital dislocation of the hips                    | +       | +             | +                                |
| Cubital deviation of the fingers                      | +       | +             | +                                |
| Clubfoot                                              | +       | +             | +                                |
| Left facial nerve paralysis                           | +       | [+]           |                                  |
| Oral–pharyngeal dysphagia                             | +       | +             |                                  |
| Mental retardation                                    | +       | ++            | +                                |
| Language/cognitive impairment                         | +       | +             | +                                |
| Epilepsy/EEG abnormalities                            | +       | +             | +                                |
| Autistic features                                     |         |               | +                                |
| Sleep apnea                                           | ?       | +             | +                                |
| Hypermetropia                                          | ?       | +             | +                                |
| Hearing impairment                                    | ?       | +             | ++                               |

?, not specifically explored; ++, features observed in more than 70% of the patients with the common duplication; [+], Though not strictly described as facial palsy, an asymmetric smile is seen in many photographs of these patients. ++, those features found also in Townes–Brocks syndrome.

1Features described in duplications 17p11.2 syndrome [5].
2Features found in the patient.
3Features described in deletions 16q12 [2, 3].
patient shows a duplication of 5.89 Mb, larger than that common duplication. The size of duplication in the patient does not appear to be important because it has seen that alteration in copy number of RAI1 gene is predominantly responsible for the 17p duplication phenotype [11]. Although in first reports [4], a preferentially paternal origin of the duplications was observed, further analysis confirmed that there is no substantial bias for parental origin or mechanism (intra vs. interchromosomal) of the duplications [5]. In the present case, the duplication was maternal in origin and due to an intrachromosomal crossing over. In contrast, the deletion revealed by array-CGH was shown to be on the paternally derived chromosome 16. No mosaicism was observed, although only one tissue of the patient had been studied (peripheral blood). Thus, two independent events, one in the maternal and one in the paternal meiosis, seem to have coincided in the patient by chance. Nevertheless, an early somatic event, independent or related to a previous meiotic event cannot be discarded. So far only 7 cases with deletion 16q12 have been described [9]. Our patient has in addition another chromosomal rearrangement (duplication 17p11.2). To date, both rearrangements are not described together in the same individual.

In the complex phenotype of the patient, we could recognize some of the characteristic features found in dup (17p11.2p11.2) syndrome [5] as well as some found in patients with deletions in 16q12 [2, 3] (Table 1). Some of these features like ear and limb malformations are similar to those found in TBS, an autosomal dominantly inherited disorder caused by mutations and haploinsufficiency in the SALL1 gene (Table 1). Deletions of this gene were found in three families with clinical diagnosis of TBS, confirming that SALL1 haploinsufficiency causes TBS.

Actual cytogenetic and molecular techniques have increased the number of cases described with small imbalances. They also have allowed more refined comparative analysis of the different cases with apparently the same deletion or duplication, which implicates a better delineation of the critical region of different syndromes. In some cases, the study of such imbalances revealed dosage sensitivity for genes included within the aberrations. That is the case of two genes implicated in the present case, the SALL1 gene located on 16q12 and RAI1 located on 17p12 [6]. Thus, an increased use of new techniques in complex cases might contribute to better knowledge of the genes involved, as well as to a better understanding of the mechanisms involved in these rearrangements.

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Conflict of Interest

None declared.

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