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## **Chromosomal imbalance letters**

### **Mosaic 15q13.3 deletion including *CHRNA7* gene in monozygotic twins**

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**Running title:** Mosaic 15q13.3 deletion including *CHRNA7* gene in MZ twins

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## **Abstract**

Deletions in 15q13.3 belong to the most frequently identified recurrent CNVs, and lead to mental retardation, seizures and minor dysmorphism. We report on two monozygotic twin boys with a mosaic 1.5 Mb deletion in 15q13.3, including *CHRNA7*. The growth parameters were in the normal range for both twins. Both had language delay with hyperactivity, temper tantrums and poor social interaction but attended regular school. The percentage of abnormal cells was 40% on lymphocytes, and 25 and 35% on buccal smear in the first and second twins, respectively. The mosaicism for the 15q13.3 deletion can explain the milder phenotype observed in these two boys.

## Introduction

The use of array-CGH in large populations of individuals with intellectual disability and/or autism has demonstrated that clinically relevant CNVs accounted for a significant proportion of cases [1]. In the great majority of cases, these CNVs originate prezygotically and are identified in homogenous state in the affected individuals. However, in rare cases, the microrearrangements occur postzygotically, during the first mitotic divisions of the fertilized oocyte. These divisions are chromosomally unstable and can generate blastomeres with various numerical as well as structural chromosome abnormalities [2]. Depending on the distribution of blastomeres in the developing fetus, the chromosomal anomaly may be identified in homogenous or mosaic state. To date, evidence for mosaic microdeletions has been obtained pre or postnatally only for a very limited number of microrearrangement, including microdeletions in 16p11.2, 17p11.2p12, 22q11.2 or 22q13 [3-7].

Among recurrent CNVs, 15q13.3 deletion is one of the most frequently identified microrearrangements. Classically, the 1.5 Mb deleted region is located between the previously characterized breakpoints BP4–BP5 and contains 6 genes, including *CHRNA7* (alpha-7 neuronal nicotinic acetylcholine receptor), but larger (between BP3–BP5) or smaller (including only the *CHRNA7* gene) deletions were reported [8]. The phenotype associates mental retardation, seizures and minor dysmorphism. In most cases, the deletion is inherited from a carrier parent with a mild or no phenotype. To the best of our knowledge, this rearrangement has never been reported in a mosaic state. In this paper we report the first couple of twin boys presenting with a mosaic 15q13.3-q13.3 microdeletion involving the *CHRNA7* gene.

## **Clinical description**

These MZ twin boys, born from the first pregnancy of a healthy 23 year-old mother and a 32 year-old unrelated father, were referred to our clinical genetics service for developmental delay. Family history was unremarkable. Delivery took place at 37 week gestation. Birth weights were 2.3 and 2.6 kg, lengths 46.5 and 48 cm and head circumference 32.5 and 33 cm. Both twins had a hypospadias. Motor milestones were in the normal range with walking achieved at 18 months. Language was delayed. Parents reported behavioral disturbances with hyperactivity, temper tantrums and poor social interaction. Boys attended regular school. On examination at age 6 years, growth parameters were in the normal range for both twins: height: 113 cm (M); weight: 19 and 20 kg; head circumference: 51.5 and 52 cm (between M and -1SD). They exhibited striking facial resemblance with high and prominent forehead, deep set eyes, thin upper lip and large ears.

## **Materials and Methods**

Conventional and molecular cytogenetic analyses were done on peripheral blood lymphocytes using standard techniques. The array-CGH analysis was performed on the high resolution whole-genome oligo-array SurePrint G3 Human CGH 4x180K Oligo Microarray Kit G4449A (Agilent Technologies, Massy, France) according to Agilent's protocol using DNA extracted from peripheral whole blood using a saline precipitation method. The data were processed using Agilent Feature Extraction and DNA Analytics softwares (both from Agilent Technologies), with the statistical algorithm ADM-2, sensitivity threshold 5 and at least 3 consecutive aberrant probes.

The zygoty was assessed by genotyping of eleven microsatellite markers located on chromosomes 14 and 15, and by analysis of the 26 polymorphic CNVs identified by array-CGH in the two twins.

### **Genomic rearrangement**

Conventional cytogenetic analysis by RHG banding showed a normal karyotype at the 550 bands resolution for both twins. The array-CGH analysis revealed a 1.5 Mb microdeletion at 15q13.2 – q13.3 (Fig. 1A and B), ranging from genomic position 28,758,822 to 30,226,176 (first and last deleted probes, hg 18) and covering the *CHRNA7* gene. The average value of the  $\log_2$  ratio suggested the presence of a mosaic deletion, the proportion of cells with deletion being estimated between 30 and 40%. The mosaicism was confirmed by metaphase FISH on cultured lymphocytes with BAC clone CTD-3242G24 (chr15:24,949,159-25,084,127, hg 18) covering the *CHRNA7* gene, 40% of examined metaphases showing the deletion. To exclude a twin-to-twin blood transfusion, interphase FISH was performed on buccal epithelial cells, showing 35% and 25% cells with the deletion in the first and second twin, respectively. All identified structural polymorphisms and all analyzed microsatellite polymorphisms were shared by both twins, thus confirming the monozygoty.

### **Discussion**

In this paper we report a couple of monozygotic twin boys with classical 15q13.2-q13.3 deletion between BP4–BP5 involving the *CHRNA7* gene in a mosaic state.

*CHRNA7* encodes the subunit alpha 7 of the neuronal cholinergic receptors, which are involved in synaptic transmission throughout the peripheral and central nervous system [9].

Our report provides new data concerning the mechanism and the timing of occurrence of the 15q13.3-q13.3 microdeletion. Regarding the mechanism, initially it was postulated that chromosomal rearrangements at the origin of recurrent microdeletion/microduplication syndromes occur in meiosis, mainly by a meiotic nonallelic homologous recombination (NAHR) mechanism. Later, the identification of mosaic cases in some microrearrangements pointed to another alternate mechanism, the postzygotic, mitotic NAHR. The frequency of mitotic NAHR is variable among different rearrangements, from very rare or anecdotal in some recurrent rearrangements such as microdeletions 16p11.2, 17p11.2p12, 22q11.2 or 22q13 [3-7], to very frequent in the type 2 *NF1* microdeletions, which are predominantly of postzygotic origin [10]. A survey of the literature using the key words “mosaic”, “deletion” and “15q13” or “*CHRNA7*” failed to return any item. Thus, to the best of our knowledge, the present report is the first description of patients with mosaic 15q13.3 deletion resulting by postzygotic NAHR.

Regarding the timing of occurrence of the 15q13.3-q13.3 microdeletion by the postzygotic NAHR, the identification of the same mosaic state in the monozygotic twins allows placing the NAHR occurrence on the embryonic developmental time scale. For years, it has been postulated that MZ twins are genetically identical and that phenotypic differences between twins are mainly due to environmental factors. There is an increasing body of evidence pointing to genetic differences between MZ twins, due to somatic mosaicism. These differences may explain at least in part the phenotypic differences. CNV differences are more frequent in phenotypically

discordant twins but occur in concordant twins pairs [11]. At the opposite, in the present report both twins are clinically concordant and exhibit the same mosaic pattern, suggesting that the somatic recombination of polymorphic regions might have occurred at a relatively early stage in embryonic development. The twinning process occurs early in the development, the pattern of placentation being a reference mark for the time of cell mass splitting: if separation occurs before the first three days post fertilization the twins are dichorionic diamniotic, between three to nine days post fertilization monochorionic diamniotic, and, if splitting occurs after nine days post fertilization, monochorionic monoamniotic. The subsequent distribution of cellular clones among different tissues and organs in the twins may be similar or different, the twins being phenotypically concordant or discordant. In both twins the deletion was present in blood lymphocytes in a similar proportion, around of 40% of examined metaphases, but slight differences were observed in buccal smear. Unfortunately, we know neither the placentation pattern, nor the distribution of the abnormal cellular clone among the fetal annexes. Thus, to the best we only can suppose the occurrence of somatic NAHR before the eleventh-twelfth days post fertilization.

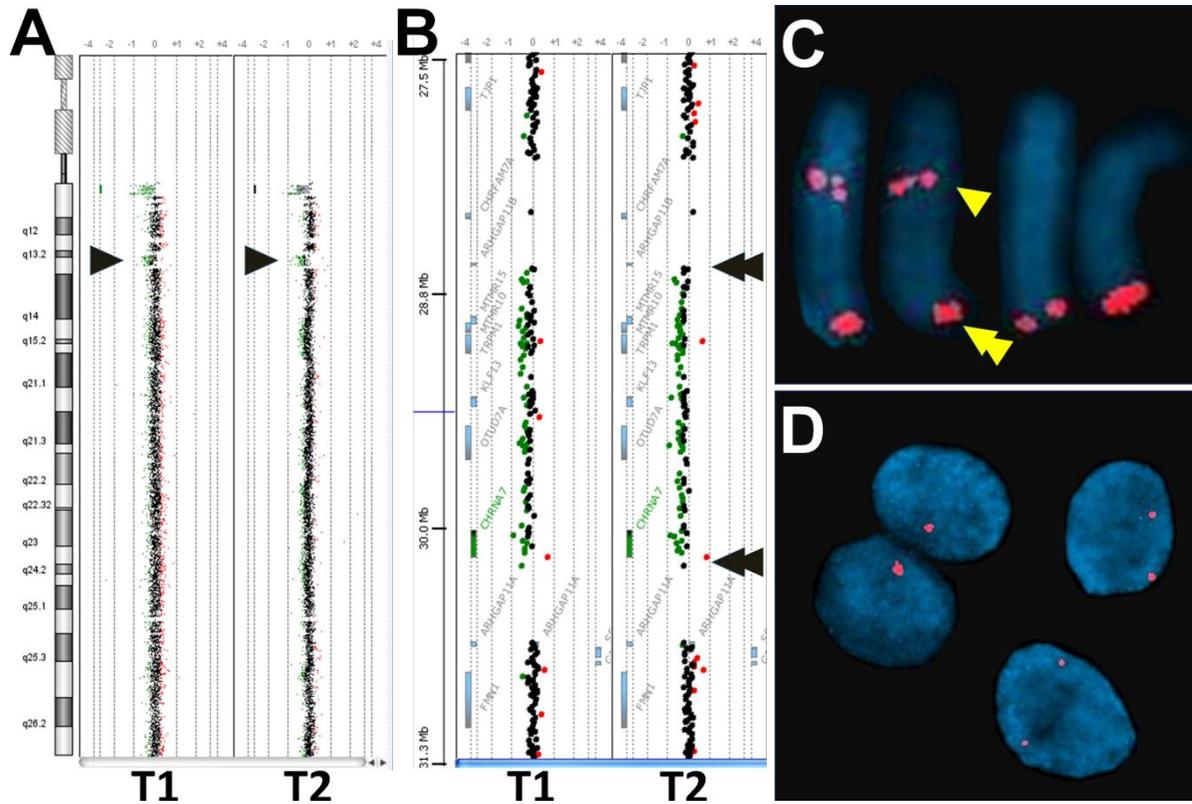
The heterozygous 15q13.3 microdeletion is characterized by phenotypic variability ranging from a full phenotype, with seizures, developmental delay, and subtle facial dysmorphism, to an incomplete penetrance [12]. Homozygous deletion gives rise to a much more severe clinical picture, even in the case of the small deletion containing only the *CHRNA7* gene, confirming the major role of this gene's haploinsufficiency in the phenotype. The case under report illustrates the milder end of the phenotypic spectrum, the two boys presenting the CNV in 40% of lymphocytes and showing a milder phenotype compared with the one usually observed in children carrying this

microdeletion. However, the proportion of deleted cells may be different in neurons and any correlation between somatic mosaicism identified in lymphocytes and the severity of phenotype must be made with caution. In a case of 50% mosaic 7q31 deletion involving *FOXP2*, the phenotype did not appear to be milder than in other reported cases [13]. Conversely, somatic mosaicism has also been reported in asymptomatic parents of children carrying deletions, such as in Kleefstra syndrome [14]. An increasing body of literature demonstrates the role of somatic mosaicism in sporadic diseases. Recently, in patients with segmental overgrowth disorders like Proteus syndrome, hemimegalencephaly and fibroadipose hyperplasia, somatic gain-of-function mutations were identified in genes acting in the PI3K-AKT-mTOR pathway [15]. Although it is still too early to attempt to draw conclusions, at least in some situations it would seem that a relationship exists between the tissue mutation burden and the phenotype. In the same way, tissue specific presence of *de novo* somatic CNVs or large variations in the proportion of cell lines with or without a CNV among different tissues can explain the phenotypic discrepancies observed in some patients. Provided that the tissue material is available, array-CGH analysis is a powerful technique for identifying tissue-specific mosaicism for genomic imbalances from 10% abnormal cells [16].

In conclusion, this is the first report of mosaic 15q13.2-q13.3 deletion in monozygotic twins. The percentage of abnormal cells, between 25 and 40% in different tissues, can explain the milder phenotype observed in these two boys. However, due to the phenotypic variability observed among 15q13 carriers, we cannot exclude that the milder phenotype observed in twins denotes the mild end of the clinical spectrum.

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**Conflict of interest:** None declared

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**Figure Titles and Legends**

**Figure 1.** Array-CGH analysis in monozygotic twin boys (T1 and T2) showing (A) whole chromosome 15 log<sub>2</sub> plot with the mosaic 15q13.2-q13.3 deletion (arrowhead) and (B) zoom in of the 15q13.2-q13.3 deleted region (the limits of the deleted region are marked by double arrowheads). The imbalance was confirmed by metaphase FISH analysis on lymphocytes with BAC probe CTD-3242G24 corresponding to the *CHRNA7* gene (arrowhead) and TelVysion 15q probe (double arrowhead) (C) and by interphase FISH analysis on buccal smear with BAC probe CTD-3242G24 (D).

