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Integrated analysis of clinical signs and literature data for the diagnosis and therapy of a previously undescribed 6p21.3 deletion syndrome.

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## SUMMARY

A de novo 0.3 Mb deletion on 6p21.3 was detected by array-CGH in a girl with mental retardation, drug-resistant seizures, facial dysmorphisms, gut malrotation and abnormal pancreas segmentation. Consistent with phenotypic manifestations is haploinsufficiency of *SYNGAP1*, that was recently demonstrated to cause nonsyndromic mental retardation, and of the flanking genes *CuTA*, a likely modulator of the processing and trafficking of secretory proteins in the human brain, and *hPHF1*, involved in *HOX* gene silencing. Mutations of both *CuTA* and *hPHF1* were never reported as causative of human diseases. Likewise, the present syndromic condition was not previously described and it can be regarded as a human model confirming the suggested biological properties of the genes included in the deletion interval. In addition, experimental evidence that SYNGAP1 and CuTA are involved in the secretory pathway in neurons, through glutamate and acetylcholinesterase signalling, prompted us to consider modulation of the glutamate pathway as target of a therapeutic strategy for the seizure control.

Chromosome abnormalities, including submicroscopic rearrangements detected by array-Comparative Genomic Hybridization (a-CGH), represent the most common cause of syndromic mental retardation. On the other hand, a consistent number of Copy Number Variations (CNVs), either inherited or de novo, are identified in normal individuals, complicating the interpretation of quantitative changes of several genomic regions. Current criteria in characterizing them as polymorphisms or pathogenic imbalances include evaluation of the CNV size, 1 Mb being considered a threshold for benign variants, and gene content analysis.<sup>1,2</sup> In some cases, based on the recurrence of chromosomal microdeletions in association with specific phenotypes, single genes have been identified as the underlying cause of syndromes with mental retardation and multiple congenital anomalies, such as the Mowat-Wilson<sup>3</sup> and Pitt-Hopkins<sup>4</sup> syndromes. In many instances, mental retardation is associated with morphological changes in dendritic spines, suggesting that altered synaptic plasticity acts as a common pathogenetic mechanism.<sup>5</sup> This evidence prompted Hamdan et al.<sup>6</sup> to look for mutations in *SYNGAP1*, an autosomal gene encoding a component of the N-methyl-D-aspartate (NMDA)-receptor complex which modulates synaptic plasticity, in nonsyndromic mental retardation. Truncating mutations were identified in three of 94 mentally retarded subjects, all lacking physical dysmorphisms and malformations.

By a-CGH, we detected a de novo 0.3 Mb deletion in 6p21.3 in a 3-year-old girl with mental retardation and multiple congenital anomalies, notably pancreas segmentation and intestinal malrotation. Included in the deletion interval are *SYNGAP1*, as well as the flanking genes *CuTA* and *hPHF1*. Both *SYNGAP1* and *CuTA* are likely involved in the secretory pathway in neurons.<sup>7,8</sup> *HPHF1* belongs to the polycomb group genes, and it was recently reported as a key regulator of homeobox and developmental gene

expression.<sup>9</sup> Because of the potential role of these genes in brain plasticity and organ morphogenesis, we hypothesize that their haploinsufficiency explains the clinical findings in our patient, allowing us to define a new contiguous gene syndrome.

#### CASE REPORT

The proposita is a 5-year-old girl, second child of healthy, non-consanguineous parents. Her older brother is healthy. She was born at 38 weeks by caesarean section after a pregnancy complicated by polyamnios. Birthweight was 3300 g (50<sup>th</sup>-90<sup>th</sup> centile), length 47 cm (10<sup>th</sup>-25<sup>th</sup> centile) and head circumference 35 cm (50<sup>th</sup> centile). The neonatal period was characterized by poor sucking, gastroesophageal reflux, and generalized hypotonia. Psychomotor delay was observed during the first months of life. Feeding difficulties due to constipation and aerophagy caused postnatal growth delay since the age of 10 months. She experienced an episode of acute pancreatitis at 12 months, and underwent surgery for a pancreatic cyst at 21 months. During the intervention, a malrotation of the upper gut was noted, and when examined with barium enema, a marked intestinal hypomobility was detected. At 18 months, a *pancreas divisum* was diagnosed by endoscopic retrograde cholangiopancreatography. Epilepsy started at the age of 3 months with myoclonic seizures, which were initially well controlled by Clobazam. In the following few months the patient experienced recurrent exacerbations of epilepsy, with polymorphic generalized seizures (myoclonic jerks, atonic attacks, atypical absences) that concurred with sleep abnormalities (derangement of sleep/wake rhythm, insomnia, sleep instability and fragmentation) and behavioural regression. EEG while awake and asleep showed multifocal abnormalities (slow spike-waves, spike- and polyspike-waves), which became sub-continuous during sleep and

were reminiscent of the EEG features of the Lennox-Gastaut syndrome. Sodium valproate associated with carnitine was effective in controlling seizures and led to remarkable improvement of the developmental and behavioral disorders. However, after a few months, seizures relapsed, which prompted the use of phenobarbital and topiramate. The use of topiramate resulted in a prolonged seizure-free period. At the age of 4 years the girl was able to utter a few words and to walk unsupported. Her DQ (Griffiths Mental Developmental Scale) was 32 (mental age 17 months). EEG alterations were only moderately influenced by treatment. Although transient exacerbations of seizures still occurred, the general clinical conditions markedly improved after topiramate therapy.

On physical examination at the age of 3 years, weight was 11.2 kg (3<sup>rd</sup> centile), length 86 cm (3<sup>rd</sup> centile) and OFC 48.4 cm (25<sup>th</sup> centile). A peculiar facial appearance was noted, consisting of frontal bossing, epicanthic folds, flat philtrum, tented upper lip and midface hypoplasia (Fig 1). Auricles were small and a lobular pit was noted, bilaterally. The midline furrow of the tongue was very deep, and a supernumerary nipple was present on the right thorax. Feet were small with crowding of toes. The girl was severely mental retarded: she could not walk unsupported, her social interaction was poor and inconsistent and the language essentially absent; she tended to withdraw and activate Rett-like stereotypies. Brain MRI and an extensive neurometabolic work-up were normal.

#### Molecular cytogenetics.

By R(RBG) banding method, an apparently normal 46,XX karyotype was detected.

Array-CGH was performed using Agilent oligonucleotide-array kit 44B (Human Genome CGH Microarray Kit 44B; Agilent Technologies, Santa Clara, CA, USA), with an average resolution of about 75 kb, following the manufacturer's instructions. A de novo interstitial deletion spanning about 0.3 Mb of chromosome region 6p21.3 was detected, with distal breakpoint (last deleted probe A\_14\_P100360, first preserved probe A\_14\_P122500) at about 33.4 Mb from the 6p telomere, and proximal breakpoint (last deleted probe A\_14\_P133907, first preserved probe A\_14\_P112116) at about 33.7 Mb from the 6p telomere. Included in the deletion interval are the genes *SYNGAP1*, *CuTA* and *hPHF1*.

Results were confirmed by FISH analysis on metaphase spreads with the BAC clone RP11.175A4 containing *SYNGAP1* (Sanger Institute). Results are summarized in Fig. 2. FISH analysis with the same probe was carried out in both parents, with normal results. Patient's karyotype was 46,XX.arr cgh (A\_14\_P160360→A\_14\_P133907) x 1 .ish del(6)(p21.3)(RP11.175A4 -)dn.

## DISCUSSION

We report on a previously undescribed association of severe mental retardation, hypotonia, seizures, facial dysmorphisms and abnormalities in gut rotation and pancreas segmentation. Causative of this complex disorder is a very small 6p21.3 deletion, spanning about 0.3 Mb, which was detected by a-CGH. Validation of the a-CGH results and genotype-phenotype correlations were possible on the basis of gene content analysis. Although a total of seven known genes are included in the deletion interval, we tentatively consider this condition a contiguous gene syndrome caused by haploinsufficiency of three of these genes, namely *SYNGAP1*, *CuTA* and *hPHF1*.

Known pathogenic mutations in humans are limited to *SYNGAP1*. Truncating mutations of this gene were recently described by Hamdan et al.<sup>6</sup> in three patients with mental retardation and language delay, two of whom were also epileptic. *SYNGAP1* is a component of the N-methyl-D-aspartate (NMDA)-receptor complex which regulates the plasticity of excitatory synapses through glutamate signalling.<sup>10</sup> Disruption of pathways modulating synaptic plasticity and spines morphogenesis appears to be a common mechanism in several forms of mental retardation.<sup>5,11</sup> Confirming the exclusive expression of *SYNGAP1* in the brain, Hamdan et al.'s patients lack physical anomalies. It is worth noting that the seizure disorder was reported to improve with valproate, which modulates the glutamate pathway.

In addition to mental retardation, language delay and epilepsy, our patient also presented with facial dysmorphisms and unusual abnormalities, including *pancreas divisum* and intestinal malrotation. We consider that *hPHF1* haploinsufficiency likely correlates with the altered morphogenesis. *hPHF1* encodes a polycomb group protein involved in histone methylation and Hox genes silencing.<sup>9</sup> It is the human homolog of the *Drosophila Pcl*, which acts in silencing Hox genes in a tissue-specific manner. Interestingly, *Pcl* mutants show a stronger misexpression of Hox genes in the viscera mesoderm, as can be hypothesized in our patient.<sup>12</sup>

With respect to *CuTA*, haploinsufficiency of this gene likely correlates with the seizure disorder, in addition to *SYNGAP1*. In fact, the mammalian protein CuTA seems to modulate the processing and trafficking of secretory proteins in the human brain. It was recently demonstrated that the mouse variants of CuTA affect the folding, oligomerization and secretion of acetylcholinesterase (AChE) into a catalytically active conformation, and several studies point to a role of AChE in the development of



epilepsy.<sup>8</sup> The inferred disruption, in our patient, of the glutamate pathway prompted us to consider anticonvulsant drugs modulating this pathway as prime drugs for seizure control. Indeed, valproate (transiently) and topiramate (more persistently) had a remarkable effect on seizure control and led to general improvement of the developmental and behavioural disorder.

The deletion interval includes other genes, i.e. *KIFC1*, *ZBTB9*, *BAK1* and *GGNBPI*, which could also be expected to play a role in clinical manifestations. *KIFC1* encodes a motor protein required for bipolar spindle formation. It may contribute to movement of early endocytic vesicles. The functional role of *ZBTB9* is unknown. *BAK1* (Bcl-2 homologous antagonist/killer) induces programmed cell death by binding to, and antagonizing the apoptosis repressor BCL2 and accelerates the opening of the mitochondrial voltage-dependent anion channel, which leads to a loss of membrane potential and release of cytochrome c. This protein also interacts with the tumor suppressor P53 after exposure to stress. *GGNBPI* (Gametogenin binding protein 1) is expressed specifically in germ cells from late primary spermatocytes to early round spermatids and it is likely involved in spermatogenesis. Based on their function and on clinical evidence, *KIFC1*, *ZBTB9*, *BAK1* and *GGNBPI* were considered not relevant for the observed phenotype.

In spite of extensive experimental investigations, the function of *CuTA* and *hPHF1* in humans is still unknown. Mutations or deletions of these genes have been described so far in association with human diseases. To the best of our knowledge, only one literature report deals with disruption of *SYNGAPI*, limited to three patients, as discussed above.<sup>6</sup>

Another patient with a 0.49 Mb deletion on 6p21.3, proximally contiguous , to the here reported deletion and including *SYNGAPI* only, is reported in the Decipher database (<https://decipher.Sanger.ac.uk>). This patient presented with mental retardation, hyperlaxity of joints and abnormal male genitalia. All together these reports, including the present one, confirm that *SYNGAPI* haploinsufficiency can cause mental retardation, through loss-of-function gene mutations, that can be detected by gene sequencing, or complete gene loss, that can be only detected by quantitative molecular cytogenetics techniques. We can speculate that the syndromic association we describe here represents a human model for down-regulation of *CuTA* and *hPHFI* as well. This report supports the notion that correlating gene content analysis of CNVs detected by a-CGH with clinical signs can suggest the pathogenesis, and possibly a targeted treatment of human diseases caused by chromosome imbalances.

**Conflict of interest:** the authors declare no conflict of interest

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## FIGURE LEGENDS

Fig.1. Frontal and lateral view of the patient.

Fig.2. *A*: Map of the deleted 300 kbp region within 6p21.3. Genes involved in the rearrangement are shown. Grey squares refer to genes not considered to be relevant for the phenotype. Black squares refer to pathogenic genes. *B*: Graphical overview of the results obtained by array-CGH analysis. Included in the deletion interval (grey rectangle) are 7 known genes. *C*: FISH results on metaphase chromosomes with BAC probe RP11.175A4 containing *SYNGAP1*. Only one signal is present.



