Complex Phenotype of a Boy With De Novo 16p13.3-13.2 Interstitial Deletion

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Abstract
Interstitial deletions encompassing chromosome 16p13.3-13.2 are rarely described in the literature, whereas terminal deletions or duplications involving this region are slightly more frequently described. The authors describe a boy harboring a de novo 16p13.3-13.2 interstitial deletion, with intellectual disability, verbal dyspraxia, epilepsy, and a distinctive brain magnetic resonance finding, namely a nodular heterotopia. The authors found partial genotype-phenotype correspondences regarding epilepsy and intellectual disability, which have been associated with 16p1 region. Conversely, nodular heterotopia and verbal dyspraxia have not been clearly related to this region. These data are in agreement with the emerging concept that similar copy number variants may be the general risk factors for distinct disorders. Verbal dyspraxia, which has not responded to speech therapy, is the child’s most disabling trait. In view of the above, genetic studies should be appraised in cases of serious speech difficulties, especially if they are associated with intellectual disability and epilepsy.

Keywords
verbal dyspraxia, continuous spike-waves during sleep, nodular heterotopia, microdeletion, 16p13.3-13.2

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confirmed by fetal magnetic resonance performed at 31 weeks of gestation, which revealed an asymmetry between temporal horns, with the right one larger than the left one. Delivery was uncomplicated. Birth weight was 2820 g (25th percentile), length 49 cm (25th percentile), and head circumference 34 cm (25th percentile). Apgar score was 8 at 1 minute and 9 at 5 minutes. Breastfeeding was not possible because of suction difficulties, and after weaning, he showed some difficulties in chewing.

Developmental milestones were delayed; he attained unsupported sitting at 12 months and independent walking at about 30 months.

At the age of 4 years, the child was admitted to the department for a developmental examination. His height has been in the 5th percentile consistently throughout his childhood, his weight between the 25th and the 50th percentile, and head circumference above the 50th percentile. Physical and neurological examinations revealed mild dysmorphic signs (long nasal philtrum, frontal bone crest, right cryptorchidism), diverging strabismus, drooling, hyperreflexia, and muscular hypotonia.

He displayed difficulties in coordination and maintaining equilibrium, especially when movement tasks were required, such as jumping (dynamic equilibrium). There were deficiencies in eye–hand and motor coordination and constructional praxis. Right upper limb exhibited a mild tremor.

At this time, he had never had seizures. He presented severe speech delay. Verbal comprehension was possible for simple messages, not necessarily linked to context. Verbal production was limited to a few single words, which were not completely intelligible. He was more effective using simple gestures and facial expressions to convey messages. The child could express a sense of humor through nonverbal language.

Execution of simple tongue praxias appeared to be very difficult, particularly the action of blowing or inflating the cheeks, with a noticeable automatic–voluntary dissociation. Difficulty in chewing was also present. In conclusion, there was a relevant praxic deficit defining verbal and oral dyspraxia.

Cognitive level regarding nonverbal quotient, measured with Leiter-R, was in mild delay (Brief IQ 68). Verbal aspects were greatly influenced by oral–verbal dyspraxia. The child was able to show his age with his fingers, to classify according to color criterion and recognize colors. He was not able to maintain a sequence requiring alternation but seemed good at identifying some situations even if a cognitive conflict was present. Slowness in execution was the distinguishing sign of performance.

Behavior was characterized by anxiety and alertness toward any unfamiliar environment, constantly looking for reassurance from his parents and having difficulty in accepting strangers. He underwent multidisciplinary rehabilitation at the institute. In particular, he followed speech therapy and psychomotor and psychoeducational treatment. During treatment, it was observed that the patient had better emotional control and was more receptive toward the therapists; oral and verbal dyspraxia, however, did not improve, despite intensive speech therapy focused on both pragmatic and formal aspects of speech. Treatment was oriented toward improving his intention to communicate, planning, sequencing, and coordination of muscle movements for speech production and oral motor control of chewing and speech. This treatment was thus integrated with augmentative alternative communication that allowed the child to begin to interact with other people, showing them objects he liked and performing activities, and asking for approval, despite verbal difficulties. Some separation anxiety traits occasionally emerged and it was necessary to reassure him about proximity of reference figures and subsequent reunification.

At 6 years, the propositus began to have absence seizures. The first video polygraphic electroencephalography (EEG) during wakefulness and sleep showed a mild slowing of background activity, focal spikes prevalent in right parietal–occipital temporal areas, and generalized spike-and-wave complexes (Figure 1). Atypical absence seizures were usually associated with longer discharges. Drowsiness and sleep markedly activated these abnormalities, configuring a continuous activity of spikes and waves prevalent in frontal regions during slow sleep (Figure 2). The patient started antiepileptic therapy with valproate, but only partial seizure control was obtained. Ethosuximide was not tolerated. Valproate was then combined with clonazepam, resulting in a reduction in atypical absences. At the follow-up evaluation (2 years later), continuous EEG abnormalities during slow sleep and sporadic focal seizures during sleep were persistent.

The last cerebral magnetic resonance imaging (MRI) was performed at 8 years. It displayed 2 heterotopic gray matter nodules, the one on the lateral face of the right ventricular trigonum, protruding into ventricular lumen, and the second on the right temporal lobe. Ventriculomegaly, already described in previous brain imaging studies, was confirmed. Pericerebellar subarachnoid spaces appeared prominent. Corpus callosum and especially the posterior third and splenium were thin (Figure 3).

Routine biochemical blood examinations, thyroid function, celiac disease tests, ammonemia, acylcarnitines, redox state, very long-chain fatty acids, urinary organic acids, amino acids, and biotinidase were normal. Abdominal ultrasounds, ophthalmologic, and audiological evaluations did not reveal anomalies. Spine radiography showed lumbar hyperlordosis, hooked coccyx, and scoliosis, without vertebral malformations. Cardiac function and anatomy, evaluated by echocardiography and electocardiogram, were normal, thus excluding congenital heart defects.

Materials and Methods

Array-based comparative genome hybridization with a genomic resolution of about 100 kb was performed using the Agilent 60K platform. Data were analyzed through Genomics Workbench software (Agilent, version 7.4, Agilent Technologies, Italy). Genomic DNA was extracted from peripheral blood.

Results

One hundred-kilobyte array comparative genomic hybridization analysis disclosed a de novo interstitial deletion of about
3.100 Mb in the region 13.3-13.2 of short arm of chromosome 16 (16p13.3-13.2 deletion), ranging from position 6 520 292 to position 9 656 507,4 hg 19.

This region involves 9 known genes (A2BP1, TMEM114, C16orf68, ABAT, TMEM186, PMM2, CARHSP1, USP7, C16orf72), according to the Genome Assembly February 2009 (GRCh37) hg 19.4

Discussion

The current challenge when approaching copy number variants is to try to give meaning to genetic information and find genotype–phenotype correlations. Moreover, expanding phenotype descriptions related to genotype could support clinical practice both in diagnostic pathways and in providing proper counseling from a prognostic and rehabilitative perspective.

The authors report on this child in order to describe the possible presentation of genetic anomalies that, until now, have not been sufficiently described in the literature. His clinical presentation deserves attention because of its complexity, since it includes neurodevelopmental disorders, neurological disturbances, and neuronal migration anomalies. His phenotype is thus quite different from other cases present in the literature.

Among genes involved in the deletion, C16orf68, TMEM186, and CARHSP1 have not been related to human disorders; USP7 has been associated with cancer development.
while chromosomal aberrations involving TMEM114 can cause congenital and juvenile cataracts.\textsuperscript{5} $A2BP1/RBFOX1$, $ABAT$, $PMM2$, and $C16orf72$ could instead be considered as possible candidate genes in determining the patient’s phenotype. $A2BP1$ gene, one of the largest genes in the human genome (1.7 Mb)\textsuperscript{5,6} and mapped to chromosome 16p13.3, is transmitted through dominant inheritance and regulates positively or negatively tissue-specific splicing by binding to messenger RNA precursors.\textsuperscript{6,7} It is specifically expressed in heart, muscle, and neuronal tissues,\textsuperscript{8} and its deletions are related to ataxia, epilepsy, neurobehavioral disorders, such as autism and schizophrenia, intellectual disability, and nonspecific dysmorphic features,\textsuperscript{9-12} suggesting a strong role for aberrant RNA processing in neurodevelopmental disorders.\textsuperscript{12}

In considering $A2BP1$ involvement in the deletion, it can be assumed that it plays a role in causing mild ataxia (disequilibrium, tremor) and difficulties in movement organization and coordination. It may also contribute, together with other genes, to intellectual disability, mild dysmorphic signs, and behavioral peculiarities characterized in this patient.

The propositus did not display congenital heart or urinary tract defects or hearing impairment, otherwise described in other $A2BP1$ deletion cases, while he did exhibit hypotonia and light vertebral anomalies,\textsuperscript{12} common in this condition.

More recently, impaired splicing events depending on $A2BP1$ gene aberrations have been also related to defects in neuronal migration in mouse models and in vitro analyses.\textsuperscript{13} In light of these findings, the authors can hypothesize the effects of this gene deletion in determining nodular heterotopias and this report could be the first to link the role of $A2BP1$ in humans.

$ABAT$ gene encodes for 4-aminobutyrate aminotransferase,\textsuperscript{14} and patients with mutations or deletions in the $ABAT$ gene were described as having severe psychomotor retardation/intellectual disability, refractory seizures, hypotonia, strabismus, and hyperekplexia.\textsuperscript{14,15} It should be emphasized that this gene has been frequently related to recessive disorders, even though in some cases it was not possible to identify a mutation.

Figure 3. Axial T1-weighted image (A) and coronal T2-weighted (B) image show nodule of heterotopic gray matter protruding into trigone of the right lateral ventricle. Wider than usual lateral ventricles. Axial T1-weighted (C), coronal T2-weighted (D), and coronal T1-weighted images show a further small nodule of gray matter into white matter lateral to ventricular temporal horn. Sagittal T1-weighted image (F) shows a mild thinning of corpus callosum and enlarged pericerebellar cisterns.
in the second allele.\textsuperscript{15} Autistic traits described in other studies seem to be solely dependent on \textit{ABAT} gene deletion.\textsuperscript{16,17} Another gene to consider in light of emotive–affective peculiarities characterized by the patient and his consequent difficulties in relationships is \textit{C1orf72}, since it has been related to both autism and schizoaffective disorders.\textsuperscript{4,18} Deletion in 16p13.2 chromosomal region encompassing \textit{ABAT} gene was reported in one patient with intellectual disability, atypical absences, electrical status epilepticus in sleep, and dysmorphic features. However, it would be necessary to investigate the role of \textit{GRIN2A} in determining patient phenotype.\textsuperscript{19}

In this case, epilepsy could be dependent both on \textit{A2BP1} and \textit{ABAT} genes, enrolled in the deletion, and on heterotopia, which to the authors’ knowledge has never been indicated in other studies and may represent a unique feature. Particularly, EEG anomalies prevailed in the right hemisphere, where heterotopia is localized, thus determining a possible focus from which paroxysmal activity could find an origin.

Ultrasonography and sequential brain MRI performed before and after birth detected moreover not specific cerebral anomalies, which could be attributable to a congenital malformative origin, probably related to the role of genes encompassed in the deletion during ontogenesis of the central nervous system. \textit{PMM2} gene encodes phosphomannomutase, an enzyme necessary for the synthesis of guanidine diphosphate mannose. It is mapped on chromosome 16p13.2. Deletions involving this gene have been described in cases of hypotonia and intellectual disability.\textsuperscript{20} \textit{PMM2} is a candidate for congenital deficiency in glycosylation,\textsuperscript{20} a recessive metabolic disorder. Some previously described patients might not exhibit some of the symptoms that are suggestive for the diagnosis, such as inverted nipples and abnormal fat deposition; conversely, they may show mild intellectual disability, hypotonia, cerebellar hypoplasia, mild dysmorphisms, and strabismus.\textsuperscript{20}

\textit{PMM2} gene deletion could determine a state of imbalanced heterozygosis, interfering with \textit{PMM2} activity, and even if this condition is different from a full-blown congenital disorder of glycosylation, it can contribute in provoking some clinical signs expressed by the propositus. Transferrin profile was however normal as expected since the disease is fully expressed in a homozygosity state.

Recently, reciprocal 16p13 microduplication has been described\textsuperscript{21} in a female patient who shared with this propositus some genes enrolled in the aberration (\textit{A2BP1/ RBFOX1, TMEM114, ABAT, PMM2}), even if their interpretation should be different, being a duplication. In contrast to this patient, she showed a complete agenesis of corpus callosum, while this patient had a thin one (particularly in the posterior third and splenium). Both had ventriculomegaly.

This girl had focal epilepsy, which responded well to antiepileptic drugs. The propositus’ epileptic picture was more complex, since focal seizures have been associated with atypical absence seizures and continuous spikes and waves during slow sleep. This difference can be explained by considering the cerebral vulnerability in developing epilepsy in this patient is due to both the genetic basis and the heterotopic areas.

Verbal dyspraxia was not clearly highlighted in previous studies regarding 16p13 interstitial deletion even if, in a study considering candidate genes for verbal dyspraxia, 2 cases harbored an overlapping deletion at 16p13.2, also including \textit{ABAT} and \textit{PMM2} genes. As hypothesized in this study, haploinsufficiency of these genes by themselves, however, would not be sufficient in determining verbal dyspraxia.\textsuperscript{22} Lack of opercular involvement excludes a “macrostructural” origin of dyspraxia and could validate its genetic basis, accounting for the lack of response to intensive speech therapy described in this case. A fascinating hypothesis could regard the interference of genetic imbalance provoked by 16p13.3-13.2 deletion on the expression of other genes localized in neighboring regions, more directly involved in verbal dyspraxia, that is, \textit{CNTNPA2} gene, and more generally genes encompassed in 16p11.2, whose deletion syndrome extends to possible causes of verbal dyspraxia. Particularly, \textit{A2BP1} gene, which is involved in splicing regulation and is localized upstream from the 16p11.2 region, could hypothetically have downstream effects.

In light of these considerations, genetic studies should be appraised in cases of serious speech difficulties, especially if they are linked to intellectual disability and epilepsy.

\section*{Conclusion}

In this clinical description, the authors tried to find phenotype–genotype correlations and to expand data regarding 16p13.3-13.2 deletion. Four of the genes encompassed in the deletion were analyzed in order to formulate a hypothesis on their pathogenic role in determining intellectual disability, epilepsy, ataxic characteristics, and behavioral peculiarities. Further studies are necessary to determine possible effects of the deletion on verbal dyspraxia and nodular heterotopias, though the contribution of some other enrolled genes cannot be excluded.

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\section*{Author Contributions}

RM prepared the first draft and subsequent revisions under the guidance of SB and ARF. RM, SB, and ARF evaluated the patient and reviewed patient history. ARF evaluated epilepsy, analyzed electroencephalography, and prescribed pharmacological therapy. RP analyzed brain magnetic resonance imaging and individuated nodular heterotopias. RM, SB, and ARF interpreted genetic data. All authors approved the final draft.

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Ethical Approval
The patient’s parents provided informed consent to publish all clinical information.

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