

***Corresponding author**

*Antonella Peduto, MD et PhD,
Pediatric Unit, S. Croce and Carle
Hospital, Via M. Coppino 26, 12100
Cuneo- Italy.

*Eleonora Barabesi, MD, Child Neu-
ropsychiatrist, Maternal and Child
Department, Child Neuropsychiatric
Unit, ASL CN 1, Cuneo- Italy.

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**Identification of a new mutation in kmt2c causing
Kleefstra syndrome TYPE 2: A very rare disorder
characterized by autism and development delay**

Antonella Peduto¹, MD et PhD, Eleonora Barabesi², MD, Maria Chi-
ara Giraudo², MD, Franco Fioretto², MD, Davide Colavito³, MS, Alberta
Leon³, MS, Lorena Sorasio¹, MD

¹Pediatric Unit, S. Croce and Carle Hospital, Via M. Coppino 26, Cuneo, Italy.

²Child Neuropsychiatrist, Maternal and Child Department, Child Neuropsychiat-
ric Unit, ASL CN 1, Cuneo, Italy.

³R&I Genetics srl, C.so Stati Uniti 4. Int.F, Padova, Italy.

Abstract

The prevalence of autism spectrum disorders (ASD) has risen over the last few decades from 2-4 in 10,000 to an estimate of 1 in 100. This is mostly due to changes in the broadening of investigation and diagnostic criteria. There are several rare monogenic diseases in which autism is a trait of neurodevelopment disorder, characterized by cognitive and motor disabilities, language impairment, in association with epilepsy, as well as other psychiatric disorders and distinctive physical features or multiorgan malformations. Some consider these disorders as “syndromic autism” and, among those, Kleefstra syndrome is a rare condition with a heterogenous clinical phenotype which includes autistic- like features. Kleefstra syndrome is caused by haploinsufficiency of EHMT1 (euchromatin histone methyltransferase 1). The disorder is characterized by moderate-severe development delay, absent or limited language, hypotonia, distinctive facial features, brachycephaly. In addition, de novo variants in four additional genes (MBD5, SMARCB1, NR1/3 and KMT2C) are known to be correlated with clinical aspects overlapping with those found in Kleefstra syndrome, known as Kleefstra syndrome type 2 (KLEFS2). To date, only about ten cases of KLEFS2 are reported in literature. Both syndromes are inherited by an autosomal dominant way. As for other genetic disorders diagnosis is difficult and often years delayed after many medical consultations, principally due to high clinical phenotypic heterogeneity.

We present the case of a Caucasian child referred to neurologist and pediatric genetist for development delay and autism. Clinical exome analysis identified a novel de novo c.10420C>T (p.Gln3474Ter) mutation in the KMT2C gene associated with Kleefstra syndrome type 2. In addition to KLEFS2 typical clinical signs the patient showed also orthopedic anomalies like hip dysplasia, pectus excavatum, hammer toe, valgus rearfeet, gastroesophageal reflux, partial empty sella and corpus callosum anomaly. We report also his neurological follow up from two since six years old, his actual age, by using standardized neurological tests.

Introduction

Autism spectrum disorders (ASD) DSM-5 (APA American Psychiatric Academy 2013) 1 include a large broad of clinical conditions mostly characterized by difficulties in social communication, repetitive behaviors and limited interests. ASD manifestations can vary significantly ranging from mild forms with relatively preserved communicative abilities to forms characterized by more severe clinical features with significant social impairments.

Neurodevelopment disorders are a heterogeneous group of medical

conditions affecting the proper global development of cognitive, motor, linguistic and social functioning during childhood. 2. Autism and neurodevelopment disorders can often be present in association in congenital disorders. During the last ten years, the new and most up-to-date techniques of genetic sequencing (NGS) have allowed the identification of an increasing number of genes associated with orphan diseases³. This, in the context of highly heterogeneous diseases such as neurodevelopment disorders, has significantly enhanced the diagnostic yield of genetic investigation. Despite this, many genes and molecular mechanisms still remain unknown.

Kleefstra syndrome (OMIM 610253) is characterized by a clinical recognizable phenotype that includes intellectual disability, from moderate to severe, childhood hypotonia, autistic features, and physical dysmorphic features such as broad forehead, arched eyebrows or synophrys, anteverted nares, coarse face, that becomes more evident with age, together with obesity in adulthood. Most children show delayed motor development and severe expressive speech delay. Several other systemic findings can be viewed as epilepsy, heart defects, brain anomalies, genitourinary malformations and severe respiratory infections^{4,5}. Kleefstra syndrome is known to be correlated with EHTM1 gene loss of function mutations, almost all cases to date reported are carrier of de novo mutations⁶.

More recently, Kleefstra et al reported a patient with intellectual impairment, autism, absence of language and suggestive dysmorphic features in which a de novo truncating mutation in the KMT2C gene (Type 2 lysine methyl transferase) was identified⁷. Since then, other few patients were reported with clinical phenotypes overlapping with Kleefstra syndrome, carrier of significant mutations in four genes, including KMT2C, MBD5, SMARCB1, NR 113 8, 9. These patients were designated as having Kleefstra syndrome-type 2 (KLEF2) (OMIM 617768).

Case Presentation

The child was firstly referred at the age of two years to the neurological service and genetic consultant for global development delay. He is the only child of healthy non-consanguineous Caucasian parents. Pregnancy was uneventful and he was born at 35+4 weeks through spontaneous delivery. Normal growth parameters were reported at birth: weight 2680g (65th percentile), length 47cm (55th p) head circumference 32.6 cm (49th p). At the clinical examination, hip dysplasia was identified and, after radiological confirmation, a hip retractor was used in the first two months of life. At one month of age the infant was admitted to neonatal intensive care for ALTE (apparent life-threatening event) and anemia that were caused by gastroesophageal reflux. In that occasion axial hypotonia

and occipital plagiocephaly was noted too by clinicians.

Neurological examination, performed two years old, showed global development delay, ambulation was still not acquired, language delay and behavioral anomalies. He was evaluated using the Bayley III scale¹⁰ that showed cognitive development in the borderline range and motor skills in the low to medium- low range. A psychodiagnostic evaluation was performed with ADOS-2 (Autism Diagnostic Observation Scale)¹¹ to assess the presence of clinical signs related to autism spectrum disorders. At the time of evaluation, the patient could walk with support, verbal language was delayed, with mainly vocalizations and sporadic babbling. Limited relational interest was observed, while he was attracted by all sound and flashing toys. Stereotyped behaviors like rocking were present together with hypersensitivity to loud noises. Adequate eating habits and regular sleep/wake rhythm were reported. The ADOS-2 total score was 20, that is related to a moderate to severe risk of presenting symptoms of autistic disorders.

At the age of three years the patient was referred to pediatric genetic consultation. The clinical examination showed growth parameters in normal range (weight, length and head circumference at 50th percentile). Facial minor dysmorphic features were noted like saddle nose, bushy and horizontal eyebrows and hint of coarse face, occipital plagiocephaly, foot third hammer toe, pectus excavatum. The autonomous walking was possible, but it was yet uncertain, with bilateral valgus rearfoot and externally rotated lower limbs. Ligamentous hyperlaxity was present. No hepatomegaly was found. Due to facial appearance, neurodevelopment delay and orthopedic aspects some lysosomal storage disorders were ruled out as mucopolysaccharidoses and oligosaccharidoses. Blood tests showed CK within range. At the same time first level genetic tests were performed. FRAXA analysis and Array-CGH (Array- Comparative Genomic Hybridation) resulted with normal profiles. Moreover, in the suspicion of a syndromic disease several instrumental investigations were performed to evaluate possible other organ involvement, including abdominal ultrasound, video electroencephalogram during sleep, ophthalmological and audiological examination, all resulted normal. Brain MRI showed a slightly thinning appearance of the posterior septum of the corpus callosum and a partial empty sella aspect.

Considering the moderate- severe global development delay, the autistic and dysmorphic features whole exome TRIO analysis was performed, at the age of three years. NGS sequencing and data interpretation highlighted the presence of a novel heterozygous nucleotidic variant c.10420C>T in the KMT2C gene predicted to result in the premature nonsense STOP codon mutation p.Gln3474Ter. TRIO analysis

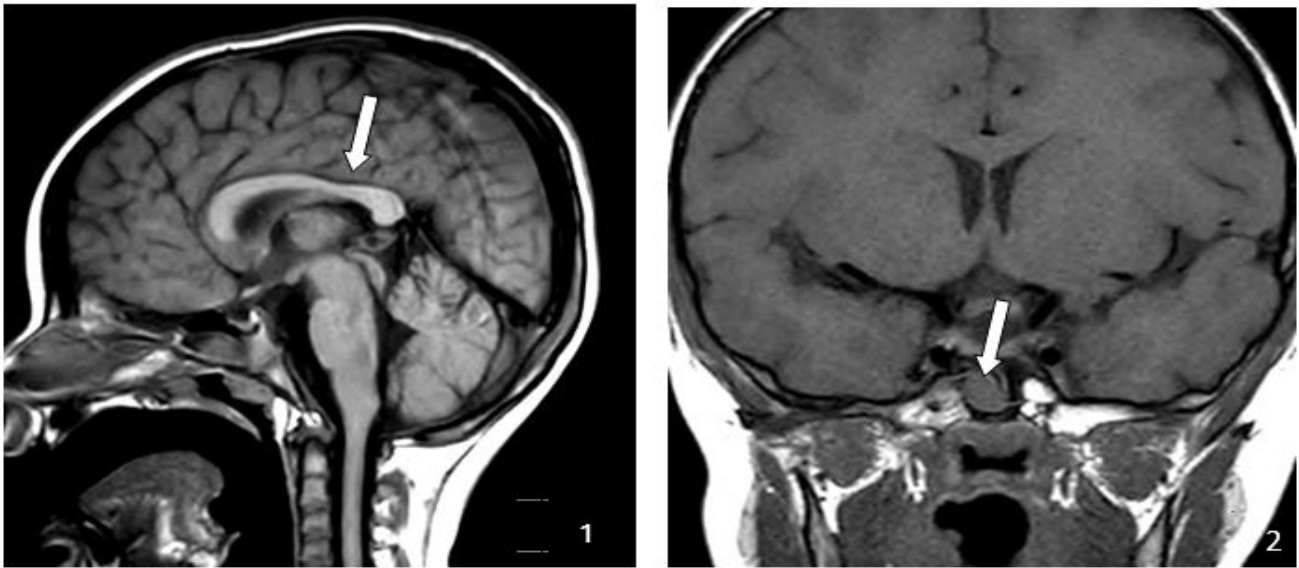


Figure: Patient's brain IMR showing slight thinning

Figure 1: patient's brain IMR showing slight thinning of the isthmus between the corpus callosum and the splenium

Figure 2: patient's brain IMR showing partial empty sella



Figure 3,4,5: Patient's pictures respectively at 6 months (3), 2 years (4) and 6 years old (5). We observe widely spaced eyes, saddle nose, thick eyebrows and wide forehead, slightly hinting coarse face, more evident with age.

Table 1: Patient's neurodevelopment scores resulted by Griffiths III scale performer at 72 months of age (6 years old). Scores from 50 to 70 are extremely below for the norm requiring specific support and therapy, from 70 to 80 they are below the norm needing to be reinforced, from 90 to 115 they are perfectly in line with age.

	Raw Score	Age Equivalent in Months	Developmental Quotient	95% Confidence Interval of the DQ	Percentiles
Learning Foundations	15	50	<20	20-22	<1°
Language and communication	0	45	<20	20	<1°
Eye-Hand Coordination	32	51	<20	20-21	<1°
Personal - Social - Emotional	22	37	<20	20-23	<1°
Gross Motor Skills	25	48	<20	20-23	<1°
Overall Development Score	18.8	47	<20	20-21	<1°

showed that both parents were negative for this mutation suggesting its de novo occurrence. Accordingly to the ACMG guidelines the mutation here found was classified as likely pathogenic (class 4).

At 4 years of age, development delay and autistic features were confirmed during patient's follow up. The PEP-3 (Psycho- Educational Profile)¹² was administered to the patient. The evaluation highlighted the following adaptive/developmental delays divided by areas: verbal/preverbal cognitive skills corresponding to 1 year and 6 months, expressive language below 12 months, receptive language 1 year, fine motor skills: 1 year and 10 months, gross motor skills 1 year and 5 months, motor vision imitation 1 year and 1 months. Moreover, problematic behaviors and poor social interests were noted too.

Currently at 6 years of age, Griffiths III scale showed a globally delayed developmental profile, corresponding to a developmental quotient < 20, severely below the normal range, equivalent to an age of 47 (< 1st percentile). Neurodevelopment scores are summarized in table 1.

Currently the child has making some progress in language using visual AAC (Augmentative and Alternative Communication) through photographs. Regarding motor skills, the child presents difficulties in executive control and in planning both gross and fine motor gestures. Walking still follows a wide-based pattern with feet rotated outward. He shows global hypotonia and joint laxity, difficulties in coordinating simple and complex voluntary movements. Stereotyped hand and arm movements and head shaking are observed in response to displays joy or excitement. No self/hetero-aggressiveness. Non-constant gastroesophageal reflux was reported anymore, neither food selectivity. Sleep/wake rhythm has always been regular. The child regularly undergoes medical check-ups. Logopedic and neuropsychomotor care continues with treatment cycles and rehabilitation goals periodically defined ensuing follow-up evaluations. Inclusive school practices have been initiated with a supporter teacher. Considering the neuromotor profile, the possibility of using orthotic insoles to improve gait motor organization and balance is under evaluation. Psychological support has been offered to the parents following the diagnosis, as it is known that parents of individuals with autism spectrum disorder and KS experience high levels of stress¹³.

Methods

Previously informed consensus the patient and their parents underwent a blood draw to perform clinical whole exome sequencing. Briefly, genomic nucleic acids underwent DNA library preparation and whole exome enrichment employing Agilent All Exon V.6 kit (Agilent

Technologies, Inc., Santa Clara CA, USA). Library sequences were obtained using the HiSeq2500 Illumina Sequencer (150-bp paired end). Bioinformatics analysis included the following: next-generation sequencing (NGS) reads mapping to whole genomes using the Burrows-Wheeler Alignment tool with default parameters, polymerase chain reaction (PCR) duplicate removal using Picard (<http://picard.sourceforge.net>), single nucleotide polymorphisms and indel calling using the Genome Analysis Toolkit (GATK) UnifiedGenotyper, variant annotation using snpEff (<http://snpeff.sourceforge.net>) and false positive variant filtration using the GATK VariantFiltration module. Exome sequencing data and reads alignment analysis were checked for coverage depth and alignment quality employing Bedtools software package. CNV calling was performed by Varseq software. This algorithm uses changes in coverage depth relative to a collection of reference samples (30 or more reference samples recommended, having on average 100X across all regions, and derived from the same library prep methods) as evidence of CNV events. High-quality CNVs were then annotated and filtered against CNV and gene annotation tracks like OMIM, Orphanet, RefSeq Genes, ClinVar and ClinGen.

Phenotype driven analysis coupled with the employment of in silico multigene panels specific for different neurodevelopment diseases was used to filter, select and interpret genetic variants. Variant analysis was performed employing bioinformatic prediction tools (Polyphen2, SIFT, MutationTaster, PhyloP, CADD-Phred) and classification was conducted in accordance with the guidelines from the American College of Medical Genetics and Genomics. In brief, variants were classified as follows: 1) benign variant, not considered to be the cause of the tested disease, 2) likely benign variant, not likely to be the cause, 3) variant of uncertain significant (VUS), it is unclear whether it is connected to a health condition, 4) likely pathogenic variants, it is often a mutation not previously reported in literature, that results in premature truncation 5) pathogenic variant, it is well established as disease cause by databases and literature.

Discussion

Neurodevelopment disorders like intellectual disability and autism spectrum disorders represent a significant challenge for healthcare professionals, their families and society as whole¹⁴. Timely diagnosis and appropriate management are essential to ensure adequate support to children and their families, promoting improvements in their motor, cognitive and communicative skills and quality of life¹⁵. Among these conditions a large number of affected patients are, to date, without a clinical and molecular diagnosis or they wait years for it (diagnostic odyssey).

Rare disorders often characterized by broad clinical heterogeneity with possible clinical overlap make diagnosis process difficult. In these last years the availability of new diagnostic technologies, such as next new generation DNA sequencing techniques provided valuable information about the biological basis of these conditions and enabled increasingly early and accurate diagnosis¹⁶.

In this report we present the clinical features and the diagnosis walk of a six years old child affected by Kleefstra syndrome 2 (KLEF2), in which a novel mutation c.10420C>T was identified in the KMT2C gene. This mutation, arising de novo, it is predicted to result in a loss of function variant, leading to a premature stop codon (variant class 4).

KLEF2 phenotype mainly overlaps with Kleefstra syndrome, that is caused by EHMT1 gene mutations. Both KMT2C and EHMT1 genes encode for a histone methyltransferase which regulates gene transcription through modification of chromatin structure⁷. Dysregulation of this epigenetic mechanism are associated with a wide range of human diseases, including cancer, immune dysfunction and multiorgan congenital syndromes as well as neurodevelopment disorders¹⁷. KMT2C is a evolutionarily conserved protein that forms part of a nuclear structure known as KMT2C/D COMPASS which is implicated in the central nervous system development. Mutations in Key COMPASS complex genes have been linked to three human congenital syndrome: Kleefstra syndrome type 2, Kabuki and Rubinstein-Taybi syndrome¹⁸.

Even though the KLEF2 syndrome is currently believed to be very rare, with very few reported cases, it is crucial to acknowledge the possibility of a greater prevalence. The implementation of emerging, up to date and highly processing sequencing techniques, have the capability to more easily identify new affected patients, possibly giving new information concerning the incidence of this syndrome in the context of neurodevelopment disorders.

In conclusion, we here report the 6 year clinical follow-up of a patient affected by type 2 Kleefstra syndrome, for which the diagnosis was performed at 3 years old. In addition to typical clinical signs of KLEF2 our patient showed also orthopedic anomalies like hip dysplasia, pectus excavatum, hammer toe, valgus rearfeet, severe gastroesophageal reflux in the first year, brain anomalies like partial empty sella and thinning appearance of the posterior septum of the corpus callosum. The child was first referred for diagnostic investigations for neurodevelopment delay and autism. We emphasize that ASD is often in comorbidity and we suggest looking also for KLEF2 in those cases of patients showing autistic traits. Actually genetic analyses are directive in the context of diseases characterized by

high phenotypic heterogeneity. Moreover, it highlights how clinical diagnosis, supported by cutting-edge genetic analysis, can drastically reduce the time required to achieve an accurate diagnosis.

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