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Case Report

Microduplication of Xp22.31 and *MECP2* Pathogenic Variant in a Girl with Rett Syndrome: A Case Report

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Abstract

Rett syndrome (RS) is a neurodevelopmental infantile disease characterized by an early normal psychomotor development followed by a regression in the acquisition of normal developmental stages. In the majority of cases, it leads to a sporadic mutation in the *MECP2* gene, which is located on the X chromosome. However, this syndrome has also been associated with microdeletions, gene translocations, and other gene mutations. A 12-year-old female Colombian patient was presented with refractory epilepsy and regression in skill acquisition (especially language with motor and verbal stereotypies, hyperactivity, and autistic spectrum disorder criteria). The patient was born to non-consanguineous parents and had an early normal development until the age of 36 months. Comparative genomic hybridization array-CGH (750K) was performed and Xp22.31 duplication was detected (6866889-8115153) with a size of 1.248 Mb associated with developmental delay, epilepsy, and autistic traits. Given the clinical criteria of RS, *MECP2* sequencing was performed which showed a de novo pathogenic variant c.338C>G (p.Pro113Arg). The features of RS include intellectual disability, developmental delay, and autism. These features are associated with copy number variations (CNVs) on the X chromosome (Xp22.31 microduplication). Here we present the first reported case of simultaneous CNV and *MECP2* pathogenic mutation in a patient with RS. We propose that both DNA alterations might have a synergistic effect and could lead to variable expressivity of the phenotype.

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Keywords ● X-linked genetic disease ● DNA copy number variations ● Autism ● Rett syndrome ● Exome sequencing

What's Known

- Rett syndrome is a neurodevelopmental infantile disease characterized by an early normal psychomotor development followed by a regression in milestones. In the majority of cases, it leads to a sporadic mutation in the *MECP2* gene located on the X chromosome.
- Xp22.31 microduplication is associated with developmental delay, epilepsy, and autistic traits.

What's New

- For the first time, we clarified the pathogenicity of Xp22.31 microduplication. We propose that microduplication is an additional injury caused by a genetic factor or environmental disturbance.
- The first reported case of simultaneous *MECP2* gene mutation and a microduplication of the Xp22.31 region.

Introduction

RS (MIM: #312750) is a severe, progressive, neurodevelopmental, X-linked dominant disorder.[1] It is characterized by an early normal growth and neurodevelopment in the first 6 to 18 months of life with subsequent deceleration of growth and developmental regression involving loss of purposeful use of hands and verbal speech. Other common features are gait abnormalities, stereotypic movements, seizures, postnatal microcephaly, and intellectual disability (ID).[2] RS is diagnosed in accordance with the revised diagnostic criteria.[1] In most cases, it occurs in sporadic form and is the second genetic etiology of severe ID in women with a prevalence of 1:10,000-15,000 live female births.[2]

Based on clinical criteria, there are two forms of RS, namely typical Rett and atypical Rett. Patients that meet all diagnostic criteria have typical Rett and a mutation in the *MECP2* gene is found in 97% of these cases. Those patients who do not meet all diagnostic criteria belong to the atypical Rett category in which only 50-70% of the cases are associated with mutations in the *MECP2* gene.[1] It is known that *MECP2* protein binds to chromatin and could activate and inhibit gene transcription.[3, 4] However, to date, the role of *MECP2* is not completely elucidated.

RS is a disease with a broad phenotypic spectrum and heterogeneous genetic components. Around 30 types of mutations in RS have been reported, including CDKL5/STK9 and NTNG genes.[2] The transcription factor *FOXG1* has also been associated as part of the genotype spectrum of this syndrome.[5] Duplication and deletion of *FOXG1* have also been reported in individuals with Rett-like features, justifying chromosomal analysis in patients with probable RS.[6]

Xp22.31 duplication (from 6.4 to 8.1 Mb, hg19) has been described in various studies since 2004.[7-10] However, its clinical significance is currently unknown. Some studies have reported Xp22.31 duplication as a risk factor for ID.[7-9] It is found in 0.37%-0.46% of the cases and in 0.15%-0.41% of the controls or unaffected parents of the affected carriers.[9, 10] This chromosomal rearrangement has been proposed as part of a clearly defined syndrome with the following features: ID, seizures, autism spectrum disorders (ASD), feeding problems, talipes, and hypotonia.[7]

We present a case of a 12-year-old girl with clinical criteria of typical RS and *MECP2* gene mutation associated with a microduplication of the Xp22.31 region. Such simultaneous mutations in a patient with RS have not been reported previously.

Case Presentation

A 12-year-old female patient was the fourth gestation of a 38-year-old mother and a non-consanguineous 31-year-old father, both without any significant family history. There were no known exposures during pregnancy. The pregnancy was uncomplicated and antenatal ultrasounds were normal. Vaginal delivery at the 37th week of gestation was without complications and the birth weight was 3.500 g (50th centile), length 57 cm (98th centile), and the head circumference was 34 cm (50th centile) with an unremarkable neonatal period. Until the age of 36 months, her psychomotor development was normal, but then her mother noticed that she was losing her acquired milestones. She stopped speaking and lost her purposeful hand skills around the age of 44 months. Additionally, she displayed inappropriate laughing spells, a diminished response to pain, and episodes of hyperventilation. She started independent walking at the age of 11 months and never lost that ability.

The patient was referred from the Neurology Department to the Genetics Department for refractory epilepsy. At the age of 3, she had a simple untreated febrile seizure followed by a spontaneous generalized tonic-clonic seizure. Occasionally she experienced focal crisis as well as absence or tonic seizures. She underwent daily treatment with carbamazepine 10 mg/kg/d and clobazam 1 mg/kg without control. The last electroencephalogram (EEG) was performed at the age of 12 and it showed slowing background with spikes and slow waves in the central region during wakefulness. Furthermore, magnetic resonance imaging (MRI) of the brain revealed dilatation of the perivascular basal space without additional abnormalities. Chromatography of amino acids in the blood and organic acids in urine, hemogram, hepatic function test, and abdominal ultrasound were normal.

The physical examination of the patient at the age of 12 revealed a normal head circumference and ID. She was unable to speak and the use of her hands was preserved, but hampered by stereotypic hand movements including mouthing, washing automatisms, and involuntary clapping. She had a slight dyspraxic gait, inappropriate visual contact, exhibited hyperactivity as well as hyperkeratosis in hands and fingers secondary to self-harm.

High-resolution chromosomal microarray was performed using a 750K Affymetrix GeneChip 6.0 array. The results demonstrated a de novo 1.28 Mb microduplication in Xp22.31 (chrX:6866889-8115153) without inherited origin (figure 1). This chromosomal rearrangement has not been associated with a genetic syndrome. Her medical records were reviewed and RS was diagnosed (table 1).[1] A complete sequencing of *MECP2* (NM_004992.3) was performed and it showed a de novo variant c.338C>G (p.Pro113Arg) as previously reported in ClinVar and HGMD databases and classified as likely pathogenic. Variant functional prediction software VARSOME (Human Genomic Variant Search Engine: https://varsome.com/) classified it as a damaging variant. This finding confirmed the clinical suspicion of RS. The carrier status of her parents was not confirmed due to the absence of symptoms.

A written informed consent was obtained from the patient's parents for the publication of her images and clinical data for scientific purposes.

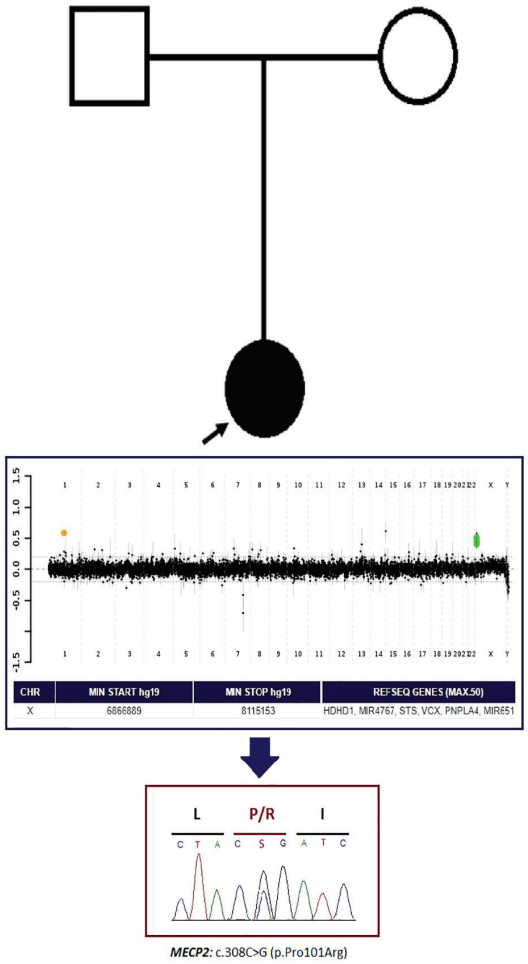

Figure 1: Genetic findings in the patient. Array-CGH results showed a microduplication (green letters) in the Xp22.31 region and RefSeq genes. The *MECP2* sequencing showed a pathological change that led to a change in protein position 101 of proline-to-arginine (red letters). None of these changes were inherited from her parents.

Discussion

For the first time, we report a case of a simultaneous presence of the *MECP2* gene mutation associated with a CNV. As illustrated in figure 2, there is a clinical overlap between patients with RS and with Xp22.31 microduplication. Mutations in the *MECP2* gene have the capacity to alter the chromatin. The Murine *MECP2* knockout model has shown an abnormal organization of heterochromatin during neural differentiation and an altered neurons morphology (fewer dendritic spines and relative less arborization).[4, 11] An overload of a de novo high intragenic mutation has also been reported and was suggested to be related to CNVs. The regulatory function of the *MECP2* could modulate this phenotypic expression.[3, 11]

Several studies, mostly single case reports and retrospective microarray analyses, have described microduplications of Xp22.31 (table 2). Some studies have reported it as a benign variant[9, 10] and some others have described it as a risk factor for developmental delay.[7-9] The role of Xp22.31 microduplications in causing an abnormal phenotype is controversial. Reduced penetrance, expression variability, involvement of multiple genes in the region, positional effect, X-inactivation (21% of the genes on the X chromosome), and the presence of other CNVs or variants in the affected individuals may all contribute to phenotype variability. Additionally, a benign variant can also behave differently in populations with different genetic backgrounds and could cause a pathological phenotype under certain conditions.[9] A previous study

Table 1: A summary of clinical features in our patient in accordance with the revised diagnostic criteria (Neul 2010)

Clinical criteria*		Our patient
Required	A period of regression followed by recovery or stabilization	Positive
Main criteria	Partial or complete loss of acquired purposeful hand skills	Positive
	Partial or complete loss of acquired spoken language	Positive
	Gait abnormalities: Impaired (dyspraxia) or absence of ability	Positive
	Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing, and washing/rubbing automatisms	Positive
Supportive criteria	Breathing disturbances when awake	Negative
	Bruxism when awake	Positive
	Impaired sleep pattern	Negative
	Abnormal muscle tone	Positive
	Peripheral vasomotor disturbances	Negative
	Scoliosis/kyphosis	Negative
	Growth retardation	Negative
	Small cold hands and feet	Negative
	Inappropriate laughing/screaming spells	Positive
	Diminished response to pain (supportive)	Positive
	Intense eye communication-"eye pointing" (supportive)	Negative

*Required for typical or classic RS: A period of regression followed by recovery or stabilization and the presence of all main criteria (excluding brain injury secondary to trauma, neurometabolic disease, or infection). Supportive criteria are not required, although often present in typical RS

Table 2: Reported cases with Xp22.31 microduplication in comparison with our patients

	Xp22.31 microduplication							Another mutation+Xp22.31 microduplication	
Literature	**Shafer et al.[13] (2007)**	**Wagenstaller et al.[10] (2007)**	**Mencarelli et al. (2008)[14]**	**Li et al.[9] (2010)**	**Liu et al.[15] (2011)**	**Faletra et al.[8] (2012)**	**Esplin et al.[7] (2014)**	**Qiao et al.[12] (2018)**	**Present case**
Sample	n=5	n=2	n=2	n=35	n=14	n=1	n=9	n=1	n=1
Age (years)	NA	NA	20.5 (19-22)	NA	From 14 months to 10 years	12	13.2 (3-19.5)	19	12
Abnormal perinatal period	NA	NA	NA	NA	NA	0/1	1/9	0/1	0/1
Diagnosed at age (years)	NA	NA	NA	NA	NA	4	9	9	12
Microcephaly	NA	1/2	NA	4/11	1/14	0/1	1/8	0/1	1/1
Speech deficits	NA	2/2	NA	NA	12/14	1/1	5/6	1/1	1/1
Developmental delay	5/5	1/2	NA	24/35	11/14	1/1	3/9	1/1	1/1
Intellectual disability	NA	2/2	NA	24/35	8/14	1/1	9/9	1/1	1/1
Seizures	1/5	NA	NA	4/35	2/14	1/1	4/9	0/1	1/1
Sitting	NA	NA	NA	NA	NA	NA	3/3	NA	1/1
Walking disability	NA	NA	NA	NA	NA	NA	2/3	1/1	1/1
Regression of developmental milestones	NA	NA	NA	NA	2/14	0/1	1/4	0/1	1/1
Feeding difficulty	NA	NA	NA	7/35	3/14	NA	2/9	1/1	1/1
Autism spectrum disorder	1/5	NA	1/2	9/35	7/14	NA	2/9	0/1 (anxiety behavior)	1/1
Hypotonia	NA	NA	NA	7/35	4/14	1/1	2/9	1/1	
MRI abnormalities	NA	NA	1/2	NA	3/14	0/1	1/3	0/1	1/1
Talipes Anomalies	NA	NA	NA	NA	NA	1/1	4/9	0/1	0/1
Sex									
Male	NA	2/2	0/2	11/27	11/14	1/1	5/9	1/1	0/1
Female	NA	0/2	2/2	14/27	3/14	0/1	4/9	0/1	1/1
Dysmorphic features: Face	2/5	NA	2/2	17/35	NA	1/1	7/9	1/1	1/1
Short stature	NA	NA	1/2	2/6	2/14	NA	4/8	0/1	1/1
Abnormal karyotype	NA	NA	0/2	3/11	NA	0/1	NA	0/1	0/1
Affected genes	STS	Between the VCX3A and VCX2 genes	STS, KAL1	VCX3A, HDHD1A, STS, VCX, PNPLA4	VCX3A, HDHD1A, STS, VCX, PNPLA4, VCX2, VCX3B	STS	VCX3A, HDHD1A, STS, VCX, PNPLA4, KAL1	VCX-A, PNLPA4	(chrX:6866889-8115153) PUDP, STS, VCX, VCX2, PNLPA4
Size (Mb)	NA	0.87 (0.6-1.13)	NA	0.15 to 1.9	0.35-1.9	1.5	0.77 (0.56-0.6)	0.6	1.24
Status of inherited origin									
Parents (maternal)	NA	1/2	NA	19/27	3/14	0/1	4/9	1/1	0/1
De novo	NA	1/2	NA	1/27	11/14	1/1	5/9	0/1	1/1
Additional mutation	NA							PURA c.563T>C; p.Ile188Thr	*MECP2* c.338C>G; p.Pro113Arg

NA: Not available

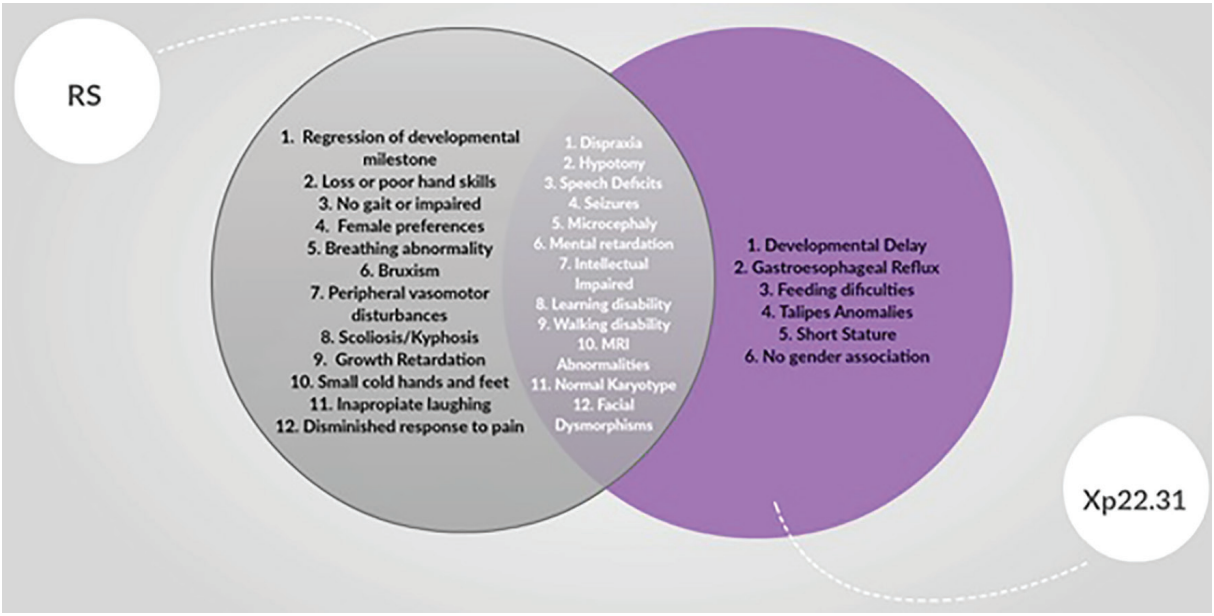

Figure 2: The overlap between clinical features reported in patients with Xp22.31 microduplication and those diagnosed with RS.

and one recent case report[12] have suggested that Xp22.31 duplication might be a risk factor predisposing to a disease, but clinical manifestation requires additional genetic changes having an additive effect.[9, 12]

CNVs of genes on the X chromosome can contribute to the deregulation of normal cognitive development.[16] Position effect resulting in altered long-range regulation of genes in proximity to the area of duplication, as a possible mechanism for the described phenotypes, can account for the lack of a minimum common region of duplication among patients.[16] In conclusion, the absence or low frequency of Xp22.31 microduplication in a healthy population increases the confidence about its pathogenicity (table 2) related to ID and minor facial dimorphisms.[7, 9]

Li and colleagues reported 29 patients with developmental delay, autism, and ID with Xp22.31 microduplication.[9] In all cases, duplication included the STS gene, which encodes steroid sulfatase, an enzyme that hydrolyzes the 3-beta-hydroxysteroid sulfate neurosteroids precursor, which affects normal neurophysiologic function[16] and behavioral processes linked with ID. The association between STS deficiency and susceptibility to attention deficit and hyperactivity disorder (ADHD), autism, and social communication deficits is known.[16] VCX3A is another well-studied gene in this region. It is associated with an abnormal neurocognitive phenotype, which plays a role in neurogenesis regulation.[11] PNPLA4, another gene in this region, is related to human obesity.[17] Both the PNPLA4 and STS genes have been shown to escape X-inactivation.[18] Considering the above, this mechanism could be significant in the phenotypic expression of this duplication.

The affected region in our patient (figure 1) also contained two microRNA (MIR651 and MIR4767) and PUDP genes, which codifies for pseudouridine-5'-phosphatas;[17] non-disease associated until now. In addition, the distal portion of the short arm of the human X chromosome (Xp22.3) is a region that undergoes frequent genomic rearrangements. In the pseudoautosomal region PAR1, an obligatory recombination occurs in every male meiosis to maintain the homology between the X and Y chromosomes.[18] It is possible that this genomic instability is a feature of the human Xp22.31 region and suggests that the microduplication might serve as a risk factor for neurological diseases.

Conclusion

Additional genetic events should be considered when CNVs of uncertain clinical relevance are noted in diagnostic investigations. In particular, those with severe phenotypes associated with Xp22.31 duplication should be investigated to rule out more likely genetic causes of ID. Further studies are required to elucidate the specific role of the Xp22.31 region in neurogenetic disorders and to clarify its pathogenicity. We propose that microduplication can have an additive or synergistic effect and may generate a worse phenotype in the presence of other genomic alterations like the *MECP2* mutation. Disruption of regulatory regions and chromatin condensation induced by the *MECP2* gene could explain DNA breakage.

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Conflict of Interest: None declared.

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