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Case Report

A New Heterozygous Duplication Variant c.953G>A p.(Arg318His) in MECP2 Gene in a 6 Years-Old Female Confirming Rett Syndrome

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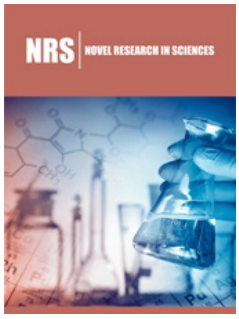

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Abstract

Variants in MECP2 are associated with X-linked Rett syndrome, a neurological developmental disorder that almost exclusively affects females. It is characterized by regression of acquired skills, movement loss, loss of speech, stereotypical movements, intellectual disability, microcephaly, seizures, and other features. Our rare variant is not listed in the genom database and has not been detected in other cohorts before. The pathogenicity of the variant is considered possible by in silico programs. Two other missense variants rated as likely pathogenic and pathogenic at the same amino acid position have been reported in relation to Rett syndrome. In summary, we currently classify this variant as pathogenic according to the guidelines in a 6 years-old female with epilepsy, developmental delay and spastic dystonic movement disorder. The duplication identified in Chr 10:46,959,968-48,277,445 (approximately 1.3 Mb, appearing as partial duplications) was confirmed through NGS-based CNV analysis. This duplication is located in a polymorphic region and does not involve clearly disease-associated genes [1-3].

Keywords: Variant-child-Rett syndrome-MECP2

Introduction

Rett syndrome is a profound developmental disorder caused by an encephalopathy following an X-chromosomal dominant inheritance pattern. X-chromosomal mutations occur at the time of conception in both male and female embryos. However, in male embryos, mutations almost always lead to intrauterine fetal death due to hemizygosity. Therefore, Rett syndrome is predominantly observed in girls. The syndrome was first described in 1966 by Viennese pediatrician Andreas Rett (1924–1997). In Germany, the frequency is estimated to be 1:15,000 to 1:10,000. Affected children initially appear to develop normally. However, between the seventh month and the second year of life, the child experiences a variable phase of developmental stagnation, followed by a partial loss of previously acquired skills, especially speech and hand use. The children's condition then stabilizes [4-8], and reaching a normal age is possible. Individuals with Rett syndrome typically exhibit symptoms of autism and movement coordination disorders. Some affected individuals have intellectual disabilities, many can speak a few words, and follow simple commands. Additionally, epileptic seizures and hand stereotypies resembling handwashing movements are characteristic of Rett syndrome. In the diagnostic code DSM-IV, Rett syndrome, Asperger syndrome, and "unspecified pervasive developmental disorder" are grouped under code 299.80. In the ICD-10, Rett syndrome has its own code F84.2. In 1998, the cause of Rett syndrome was localized to a mutation in the MECP2 gene at Baylor College of Medicine in Houston and Stanford University. Since October 1999, Rett syndrome can be diagnosed early in a child's development using a genetic test. In about 80 to 90% of cases, dominant de novo mutations of the X chromosome, a germline

neomutation, are responsible, mainly originating in the male germline and primarily passed from father to daughter, sons inherit the Y chromosome from the father. The MeCP2 gene (Methyl-CpG-Binding Protein 2) is located in the chromosomal region Xq28. MeCP2 is a transcription factor that selectively binds to methylated CpG islands and represses the transcription of various genes, disrupting cholesterol metabolism [9-13].

Rett discovered the typical hand movements, washing movements, in 1965 when two young girls were sitting on their mothers' laps in his practice, and their mothers accidentally released their daughters' hands simultaneously. These hand stereotypies are now considered the most typical criterion for Rett syndrome. Over time, additional diagnostic criteria have been added. Main criteria include Initially normal development followed by a regression between the 6th and 18th month, with a loss of acquired skills and hand use with normal head circumference at birth, with a slowdown in skull growth between the 5th month and 4th year. A delayed or absent speech development is found. Hand stereotypies like washing movements at chest level or mouth level and rhythmic upper body movements are often present. Severe cognitive impairment, making actual intelligence difficult to assess is also often present. An unsteady, wide-legged gait, often with no ability to walk without assistance. The suspected diagnosis up to 2 to 5 years of age is typical. There is currently no cure for Rett syndrome [14-20], but supportive therapies can positively impact various aspects of the multiple disabilities associated with the syndrome. Physiotherapy, hippotherapy, and occupational therapy are among the treatments that can help improve mobility, balance, and motor functions in affected individuals. All therapies are suitable for every child with Rett syndrome, and a combination of therapies should be tailored to the individual's needs. It is essential to consider the family situation and seek professional advice when determining the most appropriate treatment plan. The exact size and chromosomal boundaries of the duplication make it difficult to determine. A case of duplication in a similar chromosomal region described in the literature is larger than the duplication detected here and includes the region 10q11.22-q11.23. Whether the duplication detected in the region 10q11.22 has an impact on the disease observed in the female patient cannot currently be definitively stated. This anomaly is currently classified as unclear. To better characterize this anomaly, an array analysis is currently being conducted. We will report separately on the results of this analysis. The heterozygous MECP2 variant is currently considered the most likely cause of the patient's syndromic disease based on current knowledge. Whether it was inherited or occurred de novo, genetic counseling for family members is possible.

Methods of Analysis

Genomic DNA sequencing of a 6 years-old female in detail:

Sequencing of genomic DNA using Next-Generation Sequencing (NGS) on an Illumina NovaSeq 6000: After DNA fragmentation, the regions of interest (protein-coding exons and adjacent intron regions) are amplified and enriched using the Twist Bioscience Exom NGS Workflow, then converted into sequenceable libraries and sequenced. Variants are identified using the Illumina Dragen-IT platform. Limitations of the assay: No short-read technology can achieve 100% coverage of protein-coding regions. Methodologically, repeat expansions, variants in homopolymer regions, paralogs/pseudogenes, and inadequately covered regions may not be reliably detected or excluded. The same applies to somatic mosaics and variants in mitochondrial DNA with low heteroplasmy levels [21-23]. If no disease-relevant variants are found in the analyzed gene regions, it does not rule out the presence of variants in other genes or unanalyzed regions (e.g., regulatory regions outside the examined areas). It is also possible that individual sequence variants may not be identified by the bioinformatic algorithms used and therefore may not be reported even if they are disease-relevant.

NGS-based analysis of Copy Number Variants (CNVs): CNVs (deletions and duplications) are detected using the Illumina Dragen platform with sensitivity/specificity comparable to direct quantification methods like MLPA. Copy-neutral structural aberrations (e.g., balanced translocations or inversions) cannot be detected with this method. Mosaics may only be partially detected. A normal CNV analysis does not definitively rule out the presence of deletions and/or duplications. If technically feasible, CNV variants identified in the analysis are verified using an independent second method (MLPA, long-range PCR, SNP array). An in-house developed analysis of the samples is performed, based on multiplex PCR directly from the blood sample, followed by sequencing and comparison with the reference genome Homo sapiens GRCh37.75. Evaluation of known and potentially clinically relevant variants is based on a comparison with databases and clinical experience. Only variants in protein-coding exons and exon-intron boundaries +6/-20 bp are evaluated. Allele frequency and various software tools are used for interpretation. Pathogenic variants are primarily considered for reporting, while variants of uncertain clinical significance (VUS) may be reported depending on clinical symptoms and data. Single heterozygous (likely) pathogenic variants in autosomal recessive genes are usually not reported if they alone do not explain the phenotype or only indicate carrier status unrelated to the clinical question [24-27]. Regarding mitochondrial changes, pathogenic variants and duplications in the mtDNA are reported. The Mitomap database is used for assessing mitochondrial changes. It is possible that sequence variants initially considered non-pathogenic may be reclassified as non-clinically relevant in the future. Non-significant findings are reported based on the consent provided in the consent form. It is important to note that in silico predictions have limitations and should be interpreted cautiously.

Discussion

First described in 1966, Rett syndrome is one of the most common causes of severe mental retardation in girls. It is characterized by a period of normal development in the first 6 to 18 months of life followed by a developmental plateau and loss of acquired skills, such as language and purposeful hand use (stereotypical hand movements, so-called "washing movements"). The central nervous system developmental disorder leads to

progressive microcephaly with severe mental retardation and social withdrawal. Ataxia and sleep disturbances may occur. Many children exhibit autonomic symptoms that can precede the manifestation of typical symptoms, including constipation, cold hands and feet, hypo- or hyperventilation. In the later stages of the disease, episodes of screaming due to pain symptoms without organic findings may occur. Approximately one-third of affected individuals develop epilepsy, and almost all patients show interictal EEG changes. The onset, course, and severity of individual symptoms can vary greatly. The cause of Rett syndrome are mutations and deletions in the MECP2 gene (Methyl-CpG-binding protein 2) on Xq28. The inheritance pattern is X-linked dominant, meaning a mutation or deletion on one of the parental X chromosomes leads to the development of Rett syndrome in girls. It was previously believed that mutations in males were intrauterine lethal. Recently, mutations in MECP2 have been found in boys with a highly variable symptomatology, ranging from mental retardation without typical Rett syndrome symptoms to severe, early lethal encephalopathy [27-29].

The CRISPR-Cas9 technology holds promise for treating diseases like Rett syndrome by correcting genetic mutations in patient cells. However, challenges such as low delivery efficiency and genome-editing precision need to be overcome for widespread use. A Magnetic Nanoparticle-Assisted Genome Editing (MAGE) platform addresses these challenges by enhancing transfection efficiency, biocompatibility, and editing accuracy. MAGE successfully corrects the mutated MeCP2 gene in Rett syndrome patient-derived neural progenitor cells. By combining magnetofection and magnetic-activated cell sorting, MAGE achieves high delivery and repair efficiencies with shorter incubation times compared to conventional methods. The repaired cells exhibit normal neuronal characteristics, demonstrating MAGE's potential for clinical applications in genetic disease therapies. This nanobio-combined CRISPR-Cas9 technology shows promise for diverse clinical uses, especially in stem cell therapies for genetic disorders.

Variations in the FOXG1 gene cause FOXG1 syndrome spectrum, a condition similar to Rett syndrome. This syndrome is characterized by early regression, jerky movements, and visual impairment. The pathophysiological mechanisms underlying this condition are not fully understood, making specific treatment challenging (15). Both haploinsufficiency and overexpression of FOXG1 can lead to disease, suggesting that gene editing may be a safer approach than introducing a new gene under nonnative regulatory sequences. In a study published in 2024, we used an adeno-associated virus (AAV)-based CRISPR/Cas9 system to target and correct FOXG1 variants in patient-derived cells (15). Researchers selected variant-specific guide RNAs and donor DNAs and cloned them with a reporter system. They found that AAV serotypes varied in efficiency depending on the cell type, with AAV9 being most effective in fibroblasts and neurons, and AAV2 in induced pluripotent stem cells (iPSCs) (15). Next-generation sequencing of transfected cells showed high efficiency and precision in repairing mutated alleles (20-35% reversion) with minimal off-target effects (16). This genome editing strategy shows promise for precise FOXG1 repair, and AAV9 delivery could be a step towards developing a therapy for Rett syndrome.

Recent research explored a nuclease-free genome editing approach using homologous recombination to correct mutations in the MECP2 gene (17). The study focused on correcting mutations in Exons 3 and 4 of the MECP2 gene to restore the wild-type sequence while maintaining regulatory elements (17). The approach aimed to provide a durable correction at the genomic level. The study showed successful correction of mutations in Rett cell lines, leading to the restoration of MECP2 gene expression (17). The editing vector used in this study was designed to be fully homologous to the target region and included a promoterless Venus reporter. Analysis of edited cells confirmed accurate correction of mutations in Exons 3 and 4. Successful correction was achieved when crossover events flanked the mutations at both ends. This important study demonstrates the therapeutic potential of homologous recombination-based genome editing for correcting pathogenic mutations in Rett syndrome (17).

In another interesting study, researchers investigated the role of the c-Jun N-terminal kinase (JNK) stress pathway in RTT pathogenesis using animal and cell models. Results showed that the JNK pathway is activated in RTT models, and inhibiting JNK with D-JNKI1 improves body weight, locomotor function, and dendritic spine alterations in RTT mice (20). D-JNKI1 also reduces breathing dysfunction in RTT mice and prevents neuronal death in human MECP2-mutated neurons (20). These findings suggest that targeting the JNK pathway could be a promising therapeutic approach for RTT (20). The study demonstrates the potential of JNK inhibition in alleviating RTT symptoms at both clinical and cellular levels (20). This highlights the importance of targeting downstream signaling pathways in developing effective therapies for RTT.

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