Novel *MECP2* Mutation c.1162_1172del; p.Pro388* in Two Patients with Symptoms of Atypical Rett Syndrome

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Established Facts

- *MECP2* mutations lead to Rett syndrome in females.
- Incomplete penetrance is observed.
- Clinical features include developmental arrest 6–18th month of life, epileptic seizures, intellectual disability, ataxia, microcephaly, and typical hand-washing stereotypic movements.

Novel Insights

- A de novo c.1162_1172del; p.Pro388* variant in *MECP2* leads to atypical Rett syndrome.
- Clinical features include intellectual disability, epileptic seizures, macrocephaly, precocious puberty, and adipositas.

Keywords

Atypical Rett syndrome · Frameshift · *MECP2* deletion · Premature stop codon · Truncated protein

Abstract

We report 2 cases of girls with *MECP2* gene variants who do not have typical clinical features of Rett syndrome except for intellectual disability and seizures. Both patients present with adipositas, macrocephalia, precocious puberty, and seizures. They have prominent eyebrows and a short neck as well as short and plump fingers. Sequencing by NGS revealed a novel variant c.1162_1172del; p.Pro388* in both patients.

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The *MECP2* gene is located in chromosome Xq28 and encodes for a chromatin-associated protein which binds methylated CpG dinucleotides in promoters. Therefore, it functions as a repressor as well as an activator of transcription and is needed in the process of maturing neurons. Mutations in *MECP2* are associated with Rett syndrome in females with a prevalence of 1:8,500. Furthermore, decreased expression of *MECP2* has also been detected in the cortex of autistic persons [Swanberg et al., 2009]. In male patients, mutations in *MECP2* lead to neurological disturbances ranging from mild intellectual disability to severe neonatal encephalopathy which can cause death in early infancy [Villard, 2007]. Thus far,

Fig. 1. Physical appearance of patient 1 at the age of 10 years.

3 major functional domains have been characterized in the MeCP2 protein: an amino-terminal methyl-binding domain (MBD), a nuclear localization domain, and a transcription repressor domain. Mutations in MBD are generally associated with more severe clinical Rett phenotypes. Truncating mutations in MBD tend to be associated with more severe Rett phenotypes [Weaving et al., 2003].

Rett syndrome has a prevalence of about 1:8,500 and in most cases, it occurs sporadically. Due to the possibility of unequal X inactivation in females, incomplete penetrance can be observed [Lesca et al., 2007]. Patients develop normally during their first months of life but present with an arrest as well as a regress between the 6th and 18th month of life. They lose already gained verbal capabilities, while cognitive and motor skills stop or regress. Moreover, patients often perform repetitive stereotypic hand movements, resembling hand washing. Epileptic seizures, intellectual disability, ataxia, and microcephaly have also been described [Ip et al., 2018].

Atypical Rett syndrome is characterized by only a subset of the symptom complex but includes both milder and more severe phenotypes. A period of regression is often followed by partial recovery or at least stabilization of patient skills. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM 5), the presence of 2 of the 4 main diagnostic criteria (partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language or language skills, gait abnormalities, or stereotypic hand movements) plus 5

out of the 11 supportive criteria (e.g., small cold hands and feet, scoliosis, abnormal muscle tone, bruxism when awake, or breathing disturbances) are required to diagnose atypical Rett syndrome. Females who present with all diagnostic criteria are classified as having typical or classic Rett syndrome. In addition, microcephaly is observed in nearly all cases of Rett syndrome. To date, only a single case of an adult woman with macrocephaly in association with a pathogenic *MECP2* variant, intellectual disability, and seizures was reported [Oexle et al., 2005]. The clinical variability may be due to the nature of the underlying *MECP2* mutation and skewed X inactivation. It should also be noted that mutations in several other genes have been associated with atypical Rett syndrome, e.g., *CDKL5*, *CACNA1A*, *GNAO1*, *GRIN2B*, or *FOXG1* [Villard, 2007; Epperson et al., 2018; Gerald et al., 2018; Harada et al., 2018; Kyriakopoulos et al., 2018].

Case Report

We report 2 girls with a *MECP2* deletion, c.1162_1172del; p.Pro388*. Both present with intellectual disability, obesity, metabolic syndrome, macrocephaly, and precocious puberty.

Our first patient is an 11-year-old female of healthy parents. She was born at 38 weeks of gestation after an uncomplicated pregnancy. Her weight was 3,390 g (P69), height 51 cm (P60), and her head circumference was 36 cm (P90). Developmental delay was noticed at ~6 months. She sat without support at 9 months and began to walk at 2 years. Later on, she presented with speech delay and behavioral disturbances, which were reduced by therapeutic administration of risperidone. Since early childhood, she has been

Fig. 2. Physical appearance of patient 2 at the age of 26 years.

obese and appeared to display hyperphagia. She developed a precocious puberty at the age of 8 years and had her first epileptic seizure at 10 years. Treatment with lamotrigine prevented further seizures. However, they became refractory to this treatment.

At the age of 10 years, she presented with a height of 160 cm (P>97) and a head circumference of 59 cm (P>97). She had hypertelorism and prominent eyebrows. Her nasal bridge was broad and auricles were fleshy. In addition, the girl's neck was short and the fingers were short and wide (Fig. 1). The head circumferences of her mother and father were 53 cm (P25) and 59 cm (P90), respectively. At the time of clinical examination, the parents had normal weight.

Our second patient is a 34-year-old female of healthy parents. Pregnancy was normal, and she was born during the 38th week of gestation. Her weight was 3,550 g (P81), height 53 cm (P87), and her head circumference was 33 cm (P16) at birth. She presented with delayed motor development, but normal speech development. She began to walk at 18 months, and fontanel closure was at 2 years of age. Obesity was noticeable since her early childhood without hyperphagia. Later on she developed severe intellectual disability and precocious puberty (menarche with 10 years) as well as grand mal epilepsy at 14 years. Seizures were therapy resistant.

At the age of 26 years, she presented with a height of 169 cm and macrocephaly with a head circumference of 57.5 cm (P>97). She had a deep anterior hairline, prominent eyebrows, low-set ears, short neck, and a slightly deep posterior hairline. Furthermore, she had plump and short hands and feet as well as a rudimentary 6th digit on her left hand (Fig. 2). A case of polydactyly has never been observed in any relative of the patient. The head circumferences of mother and father were 56 cm (P75) and 58 cm (P75), respectively. The parents' weight was in the normal range.

Methods and Results

In both cases, a conventional cytogenetic analysis was performed. The chromosomal analyses (46,XX) and array CGH results (BlueGnome CytoChip ISCA 4x180K v1.0; Agilent Human Genome CGH Microarray 180K) were

normal. In patient 1, we also performed a methylation-specific MLPA to exclude a Temple syndrome, which is also characterized by weight gain and precocious puberty. In addition, Prader-Willi syndrome was ruled out by methylation testing. This syndrome is another imprinting disease causing obesity and intellectual disability.

Subsequently, whole-exome sequencing analysis of the entire exome in patient 1 and targeted sequencing in patient 2 were performed which uncovered a *MECP2* deletion, specifically c.1162_1172del (p.Pro388*; NM_004992.3). Whole-genome sequencing showed heterozygosity in patient 1, which was confirmed by Sanger sequencing (Fig. 3). NGS revealed the mutation in patient 2 in about half of all reads, whereas Sanger sequencing suggested homozygosity in lymphocytes and buccal swab, respectively. Replicate sequencing in another laboratory also yielded mosaicism for this variant confirming our NGS results (Fig. 4). In both cases, the parents have been sequenced for this certain mutation, and the deletion was not detected. Therefore, the genetic alterations can be considered de novo in both cases.

The c.1162_1172del; p.Pro388* variant generates a frameshift, which is predicted to result in a premature stop codon and a truncated protein that lacks the carboxyl-terminal amino acid residues 388–486. This *MECP2* mutation has not been described before. The carboxyl-terminal deletion 388–486 falls outside of the 3 main functional MeCP2 domains, but it has been shown that an intact C-terminus of MeCP2 is required for interaction with microRNAs involved in transcriptional and translational regulation of other target genes [Cheng et al., 2014].

Furthermore, for patient 1, macrocephalic syndrome genes including *PTEN*, *NSD1*, *NFIX*, *SETBP1*, *RAI1*, and

Fig. 3. Sanger sequencing traces from patient 1 and her parents. The *MECP2* deletion c.1162_1172del (p.Pro388*) is shaded in orange, and the missing DNA sequence is indicated by red bars. The newly formed TGA stop codon is marked by a red dot.

PHF6 were analyzed, and no additional variants of interest (pathogenic, likely pathogenic, or variants of uncertain significance) were observed.

Discussion

More than 60% of the Rett syndrome cases arise from 8 hotspot mutations in *MECP2* (R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C). *MECP2* is expressed ubiquitously with augmentation in the brain. Brain-specific deletion in mice leads to a reduction in movements, an unusual way of walking, and abnormal respiration and death. It has also been described that the loss in different brain regions leads to different symptoms [Ip et al., 2018]. Thus, a variety of symptoms can be observed in *MECP2*-deleted mice. Cuppadah et al. [2014] performed a large-scale analysis of phenotypic variability and their associations with particular *MECP2* mutations in both typical and atypical Rett patients. Although their number of atypical Rett cases was relatively small (148 out of 1,052 total Rett patients), they observed an overrepresentation of C-terminal truncations among atypical Rett patients, relative to all other mutations. In general, C-ter-

минаl truncations were also associated with milder clinical phenotypes.

The novel variant c.1162_1172del; p.Pro388* presented in this report is not listed in the HGMD database. Some variants described as associated with Rett syndrome are found nearby (e.g., c.1163_1179del17; p.Pro388Argfs*11), and all are mentioned as disease causing. Many deletion, missense, and nonsense variants have been described within this region at neighboring amino acid residues and downstream amino acid residues, suggesting that this region may indeed be a hot spot for replication error. The novel *MECP2* variant is also not found in the ClinVar database and was predicted to be disease causing by MutationTaster. Three out of 5 algorithms used to analyze splice sites predict the mutation to cause altered RNA splicing. The C-terminal domain of MeCP2 appears to be necessary for inhibition of pre-microRNA processing; therefore this mechanism offers a parsimonious explanation for pathogenicity of various C-terminal truncation mutants [Cheng et al., 2014]. No further literature about this novel *MECP2* mutation was found in current databases.

Our problems to determine zygosity in patient 2 may be due to the very high GC content of this gene area. The deletion is located in a proline-rich domain (p.376–393; 11/18

Patient 2 (lymphocytes)

Patient 2 (buccal swab)

Mother Patient 2

Father Patient 2

Fig. 4. Sanger sequencing traces from patient 2 and her parents. The *MECP2* deletion c.1162_1172del (p.Pro388*) is shaded in orange, and the missing DNA sequence is indicated by red bars. The newly formed TGA stop codon is marked by a red dot.

amino acids are proline) with a GC content >70% that might favor PCR amplification of shorter products containing the deletion. Since we ruled out any pre-existing mutations in both parents of patient 2, we have to assume that c.1162_1172del; p.Pro388 occurred de novo in a mosaic status. Similar results were published by Karall et al. [2007].

Lesca et al. [2007] described two 7-year-old girls with *MECP2* mutations who do not show typical symptoms of Rett syndrome. The first patient presented with mental impairment and had a mutation (c.1162_1163delinsTA; p.Pro388*) resulting in a stop codon at the identical position as the 2 cases presented in this study. The second patient had a *MECP2* mutation in the transcription repression domain (c.679C>G; p.Gln227Glu) and showed intellectual disability and microcephaly. Her mother had been treated with valproate during pregnancy.

Oexle et al. [2005] reported a novel C-terminal frameshift deletion (c.1135_1142delCCCGTGCC; p.Pro379Thrfs*11) in a patient with macrocephaly, intellectual disability, and epileptic seizures and referred to it as atypical Rett syndrome. In addition, Bebbington et al. [2010] postulate, that C-terminal deletions in *MECP2* are more probable to develop a normal head circumference and weight as well as a later onset of stereotypic movements.

Summarizing, in this case report, we present 2 girls with the same deletion variant c.1162_1172del; p.Pro388* in the *MECP2* gene. Both patients did not show the full repertoire of Rett syndrome symptoms, but presented with intellectual disability and seizures. While micro-

cephaly has been described in Rett syndrome, both girls were macrocephalic. Moreover, both displayed precocious puberty and adipositas, one without hyperphagia. They did not present stereotypic movements typical for classical Rett syndrome.

To our knowledge, this is the first report describing the c.1162_1172del; p.Pro388* variant in *MECP2*. This mutation might lead to an atypical Rett syndrome. To substantiate this hypothesis, more clinical cases are needed. In addition, biochemical and functional studies of the C terminally truncated MeCP2 1–388 protein variant are needed to elucidate detailed physiological mechanisms that might produce aberrant gene regulation.

Statement of Ethics

Parents of the affected patients gave their written informed consent. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare that they have no conflicts of interest.

Author Contributions

U. Bernstein wrote the case report and did the research. S. Schulz and S. Demuth recruited the patients and provided patient information. B. Eichhorn and O. Puk performed molecular analysis. S. Schulz, B. Eichhorn, and O. Puk revised the manuscript.

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