Case study

Coinheritance of mutated SMN1 and MECP2 genes in a child with phenotypic features of spinal muscular atrophy (SMA) type II and Rett syndrome

S. Voutoufianakis\textsuperscript{a}, S. Psoni\textsuperscript{c,*}, P. Vorgia\textsuperscript{b}, F. Tsekoura\textsuperscript{a}, K. Kekou\textsuperscript{c}, J. Traeger-Synodinos\textsuperscript{c}, S. Kitsiou\textsuperscript{c}, E. Kanavakis\textsuperscript{c}, H. Fryssira\textsuperscript{c}

\textsuperscript{a}Pediatric Department of Venizelion General Hospital Iraklion, Crete, Greece
\textsuperscript{b}Medical School, University of Crete, Iraklion, Greece
\textsuperscript{c}Department of Medical Genetics, University of Athens, “St. Sophia” Children’s Hospital, Thivon and Levadias St., 11527 Athens, Greece

\textbf{A B S T R A C T}

Spinal muscular atrophy (SMA) is a neuromuscular autosomal recessive disease characterized by progressive muscle weakness and atrophy combined with motor neuron degeneration caused by mutations in the SMN 1 gene locus (5q11.2–13.2). Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder caused by mutations in MECP2 (Xq28) and characterized by normal development until 6–12 months of age, followed by regression with loss of acquired skills, gradual onset of microcephaly, stereotypic hand movements and psychomotor delay. We report a 6-year-old girl who, at 2 years of age, presented with hypotonia, psychomotor delay, amyotrophy and areflexia of the lower extremities. Molecular DNA analysis (PCR-RFLP’s) for SMA type II revealed that both exons 7 and 8 of SMN 1 gene were deleted. Over the past 4 years, onset of stereotypic hand-washing movements, epileptic seizures, microcephaly, hyperventilation/breath-holding attacks and severe psychomotor delay raised the suspicion of the coexistence of RS. DNA analysis (DGGE and sequencing) identified the hotspot missense mutation R306C (c.916C\textsuperscript{44}T) in exon 4 of the MECP2 gene. The coinheritance of SMA and RS, two rare monogenic syndromes in the same patient, has not been previously reported. Thorough clinical evaluation in combination with DNA analysis, allowed accurate diagnosis, providing valuable information for the genetic counseling of the family.

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1. \textbf{Introduction}

The spinal muscular atrophies (SMAs) constitute a heterogeneous group of disorders of infancy or early childhood characterized mainly by progressive degeneration of the anterior horn cells in the spinal cord. SMAs are clinically classified into four types: acute, intermediate, mild and adult (SMA types I-IV, respectively) and the incidence is estimated approximately 1–10,000 live births. SMA I (Werdnig–Hoffmann disease) is the early-onset type with pronounced muscle weakness and hypotonia. The other 3 types SMA II-IV are the chronic forms with later-onset of muscle weakness symp-
toms and secondary complications.1 SMAs are transmitted by an autosomal recessive gene, the SMN gene which was mapped in 1999 to a locus on chromosome 5q and is considered to be the primary SMA disease-causing gene.2–4 It is present in two almost identical copies (one centromeric and one telomeric), and it is the telomeric copy (SMN1) that is functional. Homozygous deletions of exons 7 and 8 of SMN1 are detected in approximately 95% of patients with SMA, although deletions within the SMN region may also include other genes such as the neuronal apoptosis inhibitory protein (NAIP) gene.5,6

Rett syndrome (RS) is a severe neurodevelopmental disorder characterized by progressive loss of intellectual functioning, fine and gross motor skills and communicative abilities, deceleration of head growth and also development of stereotypic hand movements, occurring in the majority of cases after a period of normal development of 6–18 months. Girls with RS often develop seizures, disturbed breathing pattern with hypertventilation and periodic apnea, scoliosis, growth retardation and gait apraxia. Elaborate clinical scoring systems and guidelines along with well established diagnostic criteria are being currently used in order to describe the full clinical course of the disease.7–10 The association of RS with mutations in the methyl-CpG binding protein 2 gene (MECP2) was recognized in 1999.11 The mechanisms by which MeCP2 dysfunction causes RS have not yet been totally clarified.

A case of a 6-year-old girl who initially fulfilled the clinical criteria for SMA type II and subsequently also RS is reported. The clinical diagnosis of both diseases was confirmed by DNA analysis.

2. Case report

A case of a 6-year-old girl, offspring of unrelated and phenotypically healthy parents is reported. The mother had a normal full term pregnancy and an uncomplicated delivery. At birth, the patient weighed 2950 g, with a length of 53 cm and a head circumference of 33 cm. The postnatal period was also uneventful. Mild delay of psychomotor development was also observed during the following years. The head control and the sitting position were acquired at the age of 8 and 13 months, respectively. The parents reported that the child made the first sounds at 12 months, while the first words were spoken between 13 and 15 months.

At the age of 17 months, the proband underwent her first developmental evaluation by Denver II Developmental Test.12 Gross and fine motor skills were equivalent to those of a 7 and 10 months old infant, respectively. Language and social milestones were estimated at an age of 7 months. Two successive evaluations at the age of 23 and 29 months revealed developmental stagnation and autistic like behavior. Hypotonia of the lower extremities with absence of tendon reflexes comprised the main clinical presentation of the patient. Standard hematological and biochemical testing (CPK included) and brain MRI were normal. The neurophysiologic study showed a diffused neurogenic pattern and normal motor and sensory conduction velocities.

The disturbance of the tendon reflexes prompted the DNA analysis for spinal muscular atrophy (SMA). DNA was extracted from peripheral venous blood of the patient using a DNA extraction kit (Qiamp DNA blood Mini kit, Cat. No. 51104). The patient was tested for the presence of the exon 7 and 8 deletion in the SMN1 gene by PCR-RFLP. The PCR products were digested with restriction enzymes (HinfI and Ddel for exon 7 and 8, respectively) as described previously, but with a slight modification using internal controls from dystrophin gene (exon 44 and 48 sequence primers).6,12 NAIP homozygous deletion of exon 5 was also tested by PCR. The analysis revealed a homozygous deletion for exon 7 and 8 of SMN1 gene but not for the NAIP gene (Fig. 1).

Despite the diagnosis of SMA type II, the clinical progress of the child during the following years was also strongly suggestive of an additional neurodevelopmental disorder, whereby at the age of 2 years she presented hand stereotypy, loss of purposeful hand movements, loss of acquired language skills, communication dysfunction and deceleration of head growth and onset of epileptic seizures. The subsequent EEG study showed background slowing with multiple spike wave complexes in the occipital derivations (Fig. 2). The child also manifested breathing disturbances with hyperventilation interchanging with breath holding crises. The sleeping pattern was also abnormal. At the age of 6, the child fulfilled all the clinical criteria compatible with a diagnosis of RS.9,10

The patient underwent subsequent molecular analysis of the MECP2 gene in order to identify mutations associated with RS. Exons 3 and 4 were divided into two and three fragments, respectively, and were amplified by PCR using specifically designed sets of primers. The PCR products were then subjected to denaturant gradient gel electrophoresis (DGGE) (Fig. 3a). The middle fragment of exon 4 presented an abnormal pattern of migration in DGGE analysis and was sequenced directly. Direct sequencing was performed using fluorescently labeled M13 primers (forward Cy5.0: 5′-GTTAAAAACGACGGCCAGT-3′; reverse Cy5.0: 5′-CAGGAAAACCACCTATGAC-3′), and the visible genetics sequencing kit (Visible Genetics, Ont., Canada). The sequencing reactions were analyzed on the Opengene™ automated sequencing system (Visible Genetics, Ont., Canada). The hotspot missense mutation R306C (c.916C>T) in exon 4 of MECP2 gene was identified (Fig. 3b). Subsequent analysis of the mother's DNA sample was negative.

3. Discussion

We report a case of a 6-year-old girl who at the age of 2 had generalized hypotonia with areflexia of lower extremities and psychomotor delay. However the absence of tendon reflexes, accompanied by a diffuse neurogenic pattern in the neurophysiologic study, directed the diagnosis towards SMA type II. Psychomotor retardation was the only feature which was not consistent with the SMA clinical diagnosis. However, the results of the molecular analysis confirmed the SMA. Homozygous deletions of the SMN1 gene, as in our proband, are detected in 95% of SMA cases and larger deletions extending
to other genes, such as the \textit{NAIP} gene, are usually associated with severe phenotypes.\textsuperscript{13}

Further evaluations until the age of 6 recognized additional clinical symptoms that were consistent with the criteria for diagnosis of RS.\textsuperscript{9,10} The subsequent molecular analysis was also positive for RS, as the identified R306C missense mutation is one of the eight most recurrent mutations associated with classic RS phenotype.\textsuperscript{15,16} The R306C mutation is considered to lead to lower clinical severity scores in comparison with early and late truncating mutations, although all the cardinal RS criteria are usually fulfilled.\textsuperscript{17} The milder phenotype includes late onset of regression and seizures, approximately after 30 and 60 months of age, respectively, and preservation of some language skills and ambulation.\textsuperscript{15–17} Indeed, in our patient the characteristic stereotyped hand movements, the loss of speech ability and the onset of seizures became evident after the age of 2 years.

At the molecular level, the substitution of the arginine residue at position 306 (R306C) affects the transcriptional repression domain (TRD) of the \textit{MECP2} gene. Functional studies nevertheless have demonstrated no significant effect of this mutation in the transcriptional repression capacity of the \textit{MECP2} protein.\textsuperscript{18}

The concomitant presence of SMA and RS in a single patient is logically assumed to be coincidental. To our knowledge, this is the first reported case of such an occurrence. In the literature, RS has been associated with certain chromosomal abnormalities, such as Klinefelter syndrome and others.
syndrome, trisomy 21 and triple-X syndrome, while SMA has been reported in combination with rhabdomyosarcoma in only one case.\cite{19-22}

However, given the fact that SMA and RS are both neurological diseases, they show some clinical overlap. The first observation is that both diseases have cumulative effect regarding the motor skills of the patient. In fact, the absence of tendon reflexes, amyotrophy and trophic changes of the lower extremities that were established by the age of 2 years, initially directed the diagnosis towards SMA. However, according to post hoc evaluations of the clinical symptoms of RS patients’ cohorts, muscle tone disturbances can also occur early in the course of RS, before the age of 12 months, even from birth.\cite{23} As a result, the girl never acquired a standing position and has always been wheelchair dependent. However, no signs of truncal ataxia or scoliosis have been observed yet. On the other hand, the mental development with profound mental retardation appears to be attributed exclusively to RS and according to recent studies may also include early and non-well recognizable patterns of impairment.\cite{23} Indeed, apparent developmental delay was documented since birth also in our patient.

At the present age of 6 years, the girl demonstrates absence of language skills, presence of repetitive stereotyped hand movements and apraxia. Intermittent episodes of apnea and hyperpnea also occur. The eye contact, social smile and autistic features are not as severe as usually observed in RS, while the seizures also remain well-controlled due to proper medical treatment. The encouraging signs may be partly justified due to the relatively mild phenotypic effect produced by the R306C mutation. However, complete genotype–phenotype correlation in the context of this complex diagnosis is difficult and since two different molecular defects are present, a more devastating phenotype should be expected. On the contrary, the girl presents a relatively favorable clinical outcome, as she has not yet developed scoliosis and cardiorespiratory complications which later characterize both diseases.

In conclusion, this case represents the first observation of the co-inheritance of RS and SMA. It illustrates the value of thorough clinical evaluation and sufficient clinical experience to recognize the coexistence of two rare monogenic syndromes in the same patient. The most significant point of this case is that a more extended differential diagnosis should be taken into account in a patient with a molecularly documented genetic condition, but whose phenotype is highly atypical. In addition, the ability to perform DNA analysis facilitates the definitive diagnosis, which provides valuable information for the genetic counseling of the family and the clinical course of the patient.

**Fig. 3 – (a) Denaturing gradient gel electrophoresis (DGGE) analysis.** The sequences of the GC-clamped (underlined bases) primers which were used to amplify the regions of exons 3 and 4 of the MECP2 gene for subsequent DGGE analysis were the following:

*Exon 3.1:* F: 5’-51GC-GCTCACCTTGGCTGGAGACT-3’, R: 5’-CTGACCCCTCTCTGTGTCTCT-3’,

*Exon 3.2:* F: 5’-CTCTGCTGAGCTCGCAGA-3’, R: 5’-57GC-TCCATGAGGGATCCTTGT-3’,

*Exon 4.1:* F: 5’-45GC-TGACATGCTATGGAGAGCC-3’, R: 5’-GTGGTGATCAGATCACTGCG-3’,

*Exon 4.2:* F: 5’-57GC-AGGGGGAAGCTGAGGGGG-3’, R: 5’-GTTGCTCCTTCTTGAGG-3’,

*Exon 4.3:* F: 5’-AGGACTGAAGACCTGTAAGACG-3’, R: 5’-45GC-CTTCTCTTGGCAATCGGCTCC-3’.

The amplification product of the exon 4.2 showed an abnormal migration pattern in the DGGE analysis, while the analysis of the mother was normal. The electrophoretic conditions were chosen according to the Meltmap software.\cite{14} The 6% polyacrylamide gel contained a linearly increasing gradient from 20 to 80% denaturant (100% denaturant is 7 M urea and 40% formamide) and was run at 65 V and 60 °C for 18 h. (b) Sequencing analysis of the middle fragment of exon 4 of the MECP2 gene, which identified the R306C missense mutation. The PCR template was generated using M13-tailed (underlined bases) MECP2 gene-specific primers:

*Exon 4.2F*: 5’-GTAAAACGACGGCCAGTTCCTGGGAAGCTCCTTGTCAAGAT-3’,

*Exon 4.2R*: 5’-CAGGAAACAGCTATGACTCCTCTGTCTCCT-3’.
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REFERENCES