Short Report

Chromosome 2 deletion encompassing the MAP2 gene in a patient with autism and Rett-like features


We present here a unique case of a 14-year-old female with autism and some features similar to Rett syndrome (RTT). Genetic analysis demonstrated a large deletion of chromosome 2q instead of a MECP2 mutation. Like a Rett patient, she is dyspraxic and shows frequent hand-washing stereotypic activities, hyperpnea, and bruxism. Like a preserved speech variant (PSV) of RTT, she is obese, able to speak in second and third persons, frequently echolalic, and has final normal head circumference and autistic behavior. In addition, she has dysmorphic features such as down-slanting palpebral fissures, low set ears without lobuli, bilateral flat feet, and bilateral syndactyly of the second and third toes, which do not belong to the Rett spectrum. She has a de novo chromosomal deletion in 2q34 of paternal origin. Gene content analysis of the deleted region showed the presence of 47 genes (14 putative and 33 known genes). This region contains some interesting genes such as ADAM23/MDC3, CREB1, KLF7, and MAP2. Because alteration of neuronal maturation, dendritic anomalies, and a decrease in MAP2 immunoreactivity in white matter neurons are well documented in RTT patients, we propose MAP2 gene as a good candidate for the generation of PSV phenotype in this case.

Rett syndrome (RTT) (OMIM 312750) is a severe neurological disorder affecting almost exclusively girls. Classical RTT patients show regression of speech and purposeful hand movements, after a period of normal development. Among the typical clinical features of this condition are hand dyspraxia, stereotypic hand-washing activities, ataxia, abnormal breathing, and growth retardation. About 80% of classical RTT are due to mutations in MECP2 gene, encoding the methyl-CpG-binding protein 2. We also describe some patients affected by the preserved speech variant (PSV) of the RTT syndrome, who are characterized by recovery of the capability to speak. In these less severe cases, autistic behavior is more evident (1–4). Mutations in MECP2 gene are identified in the 50% of PSV cases.

Autism (OMIM 209850) is a defect in the communication, social interaction, and behavior areas. The causes are unknown, but there are strong evidences in favor of a genetic etiology. Recently, a region in 2q33 has been shown to be involved in autism (5, 6). A two-stage genomic screen analysis of autistic patients revealed suggestive evidence for linkage to markers D2S116 (LOD score 2.86) and D2S1384 (LOD score 0.80) (5). These linkage data focus the attention on the presence of a putative autism-causative gene on chromosome 2 and support the hypothesis that behavioral overlapping features of RTT and autism may have common molecular bases.

We present here a Rett-like patient with an intact MECP2 gene and an interstitial deletion on chromosome 2 (2q34) of paternal origin. She has phenotypic features similar to PSV and showed additional dysmorphic signs and an autistic behavior. Analysis of the gene content of the
deleted region allowed us to identify the presence of gene(s) with a role in neurobiological function or development, which could be good candidate for the generation of the Rett-like phenotype in the patient.

Clinical report

The proband, a 14-year-old girl, was the second child of second cousins once removed consanguineous parents (Fig. 1). The father was 35 years old and the mother, gravida 4, para 1, 31 years old. Pregnancy lasted 36 weeks. Her birth weight was 1920 g and head circumference was 31 cm. Morphologic and neurological abnormalities were noticed at birth: down-slanting palpebral fissures, low set ears without lobuli, syndactyly of the second and third toes in both feet, and simian creases. Her sucking was poor and she was hypotonic. In the subsequent months, her psychomotor development was slow and her ability to relate was poor. An electroencephalogram (EEG) showed no abnormalities. At 9 months, a subluxation of the hip was noticed and was treated accordingly with an orthopedic device that was kept for 4 months. At 18 months, she started a frequent clapping of both hands, which persisted in the following years. At 2 years, she was able to walk alone. At this age, her head circumference was below the norm (45 cm, less than the third percentile), she remained isolated from other people’s interactions, and she started to have frequent bouts of hyperpnea. A computerized tomography of the brain showed enlarged cisterna magna and peripontine spaces, which was confirmed by a subsequent magnetic resonance imaging. An EEG showed frequent spikes, occasionally, followed by slow waves in short sequences on both temporo-occipital leads. At 3 years, grand mal convulsions occurred and remained, in spite of a pharmacological treatment, relatively frequent up to 6 years. Afterwards, they remained well controlled with phenobarbital. At this age, lumbar lordosis, ataxic gait, and hand apraxia were noticed in a department of child neurology, and she had started to say numerous words and short phrases and had just attained sphincter control. She was initially seen by one of us (M.Z.) at 10 years. She had down-slanting palpebral fissures, low set ears without lobuli, bilateral flat feet, and bilateral syndactyly of the second and third toe. Head circumference (51.3 cm) was within the norm (25th percentile), her weight was 45 kg (90th percentile), and she was 149 cm tall (90–97th percentile). She was able to speak in second or third person and was frequently echolalic with an idiosyncratic, out of context speech, characterized by a special prosody, and she fulfilled all the criteria of autism at a DSM IV R evaluation [(1) Full positive, (2) Full positive, (3) Full positive]. She liked music and frequently sang popular songs. She was unable to use a spoon and was evidently dyspraxic. She, however, could build a tower of 10 cubes. In addition, frequent hand-washing stereotypic activities, hand clapping, and cold extremities were noticed. She would walk with an enlarged base and was unable to run. Bruxism was present, and her behavior was occasionally aggressive.

Cytogenetic and molecular genetic analysis

Analysis of MECP2 gene was performed by direct sequencing of the coding region of the gene, using PE Big dye terminator cycle sequencing kit on an ABI 310 Automated Sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed with the GENESCAN package software. Primers were described elsewhere (1).

Cytogenetic analysis was performed using standard techniques (7). Chromosomes were prepared from peripheral blood lymphocyte, treated with trypsin, and then stained with Giemsa stain to obtain a GTG-banded pattern.

Polymorphic markers located in the deleted region were identified using both the public database UCSC Genome Browser (http://genome.ucsc.edu) and the private Celera database (http://www.celera.com). Primer sequences are available at The Genome Database (http://www.gdb.org/gdb/). For marker analysis, a pre-cast gradient polyacrylamide gel (12.5–2%) using Fig. 1. Picture of the patient at age of 14 years.
the GenePhor electrophoresis unit (Amersham – Pharmacia – Biotech, Uppsala, Sweden) under a controlled temperature of 10°C was used.

The gene content analysis was performed using public and private databases (UCSC Genome Browser, http://genome.ucsc.edu; Celera database, http://www.celera.com).

Results

Analysis of MECP2 gene showed the presence of a silent variant (p.G273G, c.819G>T) inherited from the father. No additional changes were found, in spite of direct sequencing of the entire coding region.

Cytogenetic analysis using standard techniques showed the presence of an interstitial deletion of chromosome 2. In particular, the karyotype of the patient was 46,XX,del(2)(q34) (data not shown). Both the parents have a normal karyotype.

In order to define the extension and the origin of the deletion, we analyzed a series of polymorphic markers from 2q33.3 to 2q35. This analysis located the proximal breakpoint in a region of about 900 kb between AFM224zg5 (present) and D2S155 (deleted), and the distal breakpoint in a region of about 900 kb between D2S137 (deleted) and D2S301 (present) (Fig. 2). The deletion has an extension of about 9–11 Mb. Analysis of deleted markers showed the absence of paternal contribution, indicating that the deletion arose de novo in the paternal gamete (Fig. 2). Analysis of the gene content of the deleted region showed the presence of 47 genes (Fig. 3). Of these, 16 are putative genes, while 31 are known genes.

Discussion

We present here a unique case of a 14-year-old female who shows autism and some features similar to RTT syndrome. Genetic analysis demonstrated a large deletion of chromosome 2q instead of a MECP2 mutation. It is interesting to note that the deletion is very near to a region recently confirmed to be involved in autism (5, 6). D2S155, the first deleted marker in our patient, is located 5 Mb telomeric to marker D2S116 which has the highest LOD score (2.86) and 1.7 Mb telomeric to marker D2S1384, which is still included in the linked region (LOD score of 0.80). We cannot exclude that one of the deleted genes may be responsible for the high LOD score in the paper of Shao et al. (6) and may be responsible for autistic behavioral phenotype in the patient.

Direct sequencing of MECP2 has not shown the presence of a causative mutation in this gene. Although MECP2 intronic mutations may be missed and a mutated unknown recessive gene may be present in the patient, de novo origin of the deletion suggests the causal relationship between the deletion and the phenotype in the patient. The identification of this deletion on chromosome 2 opens the challenge of how to assign single phenotypic features to single genes present in the region. Gene content analysis of the deleted region showed the presence of 47 genes.
Thirty-three are known genes and 14 are putative genes. Some of them are of particular interest, due to their role in central nervous system development or to their preferential expression in brain. Some candidate genes with specific neurobiological functions are discussed below.

ADAM23 (a disintegrin and metalloproteinase)/MDC3 (metalloprotease, disintegrin, and cysteine-rich domain) interacts in brain with a specific integrin (αvβ3) and may mediate cell-adhesion mechanisms (8, 9). Low expression levels of ADAM23/MDC3 could cause defects in neuronal migration during brain development.

CREB1 (cAMP response element-binding protein 1) is involved in synaptic plasticity related to long-term memory (10) activating the cascade of proteins that participate in remodeling neuron processes (10, 11). The impairment of these remodeling events could be responsible for a reduction in dendritic outgrowth, necessary for the establishment of new synapses not only during brain development, but also during physiological reorganization of brain circuits that follows change in sensory stimulation and learning. KLF7 (Kruppel-like transcription factor 7) has a typical spatio-temporal pattern of expression in mouse. It is
highly expressed in spinal cord during the early embryonic period when motoneurons differentiate and in cortex in early postnatal period when a high rhythm of production of new synapses is observed (12). An impaired KLF7 expression could have a dramatic effect on the development of central nervous system, impairing correct differentiation of neuronal cells and production of new synapses.

Finally, the chromosome 2 deletion in our RTT-like patient encompasses MAP2 gene. This gene encodes for the microtubule-associated protein 2 (MAP2), a structural protein of developing brain dendrites. MAP2 reduces the critical concentration of tubulin required to polymerize microtubules and to maintain neuronal morphology (13). In RTT patients, alteration of neuronal maturation, dendritic anomalies (14–16), and decreased MAP2 immunoreactivity in white matter neurons are well documented (17). More recently, an expression profiling study performed on postmortem RTT brain showed decreased MAP2 expression. In particular, MAP2 expression is decreased more than 50% when compared with matched controls (18). The brain pathology in RTT reveals only subtle anomalies, in spite of the dramatic clinical deterioration observed in these patients (19). The neuropathological findings indicate that RTT may be a disorder due to an alteration of neuronal adaptive plasticity and to an arrest of neuronal development (20, 21). The dendritic alterations observed in postmortem tissue have been previously related to a reduction in the levels of MAP2 (22). MAP2, due to its role in maintaining neuronal morphology and in adaptive plasticity, could be a good candidate gene in the generation of features similar to RTT in our patient.

**Acknowledgments**

This work was supported by Telethon grants GGP02372A and GTF02006 to A.R. and PAR 2001 and 2003 from the University of Siena. We also thank Celera Genomics.

**References**