Disruption of the *IQSEC2* transcript in a female with X;autosome translocation t(X;20)(p11.2;q11.2) and a phenotype resembling X-linked infantile spasms (ISSX) syndrome

MANUELA MORLEO[1], DANIELA IACONIS[1], DAVID CHITAYAT[2,3], IVANA PELUSO[1], ROSALIA MARZELLA[4], ALESSANDRA RENIERI[5], FRANCESCA MARI[5] and BRUNELLA FRANCO[1,6]

[1]Telethon Institute of Genetics and Medicine (TIGEM), Via Pietro Castellino 111, Naples, Italy; [2]Department of Pediatrics, Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto; [3]The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; [4]Department of Genetics and Microbiology (DiGeMi), University of Bari, Bari; [5]Medical Genetics, Department of Molecular Biology, University of Siena, Siena; [6]Medical Genetics, Department of Pediatrics, Federico II University, Naples, Italy

Received October 25, 2007; Accepted November 14, 2007

Abstract. We report on a female patient with severe infantile spasms, profound global developmental arrest, hypsarrhythmia and severe mental retardation, associated with a *de novo* apparently balanced X;autosome translocation. Her neurological phenotype resembles that of X-linked infantile spasms (ISSX). Molecular study showed that the translocation disrupts a transcript involved in GTPases signalling, *IQSEC2*, mapped to the Xp11.22 region. Several genes involved in intracellular signalling pathways via Ras-homologous small GTPase have been implicated in X-linked neurological disorders. Expression studies revealed that the murine homolog of this transcript, *Iqsec2*, is highly expressed in the nervous system from the early stages of development. These data suggest that *IQSEC2* could be considered a candidate gene for X-linked neurological disorders.

Introduction

Infantile spasm syndrome (ISS), also called West syndrome, is an etiologically heterogenous disorder with an incidence of 2-5 per 10,000 live births. It is characterized by seizures that usually start early in life with an electroencephalogram pattern of hypsarrhythmia and subsequent mental retardation. Most cases are sporadic, but familial cases have been reported as an isolated finding or in association with a variety of abnormalities. An X-linked form of West syndrome has been reported (ISSX, MIM 308350) and some of these patients have been shown to carry mutations in the *ARX* (aristaless-related homeobox) gene, which maps to Xp21.3-p22.1 (1). *ARX* was also found mutated in patients with mental retardation, lissencephaly and abnormal genitalia (2). Recently a second gene, *CDKL5* (cyclin-dependent kinase-like 5), which maps to Xp22.3, was found mutated in patients with X-linked infantile spasms (ISSX) and in patients with the early seizure variant of Rett syndrome (RTT; reviewed in ref. 3). RTT (MIM 312750), an X-linked dominant male lethal neurodevelopmental disorder caused by a mutation in the *MECP2* gene and characterized by a wide spectrum of clinical manifestations, was also found to be associated with West syndrome (4). Mutation analysis suggests the existence of additional loci possibly involved in both ISS and RTT (3,5-9).

Here, we report on a female patient, named AD, with a phenotype resembling ISSX. Chromosome analysis showed a *de novo* apparently balanced X;autosome translocation involving the short arm of the X chromosome and the long arm of chromosome 20. FISH analysis allowed us to map the X chromosome breakpoint inside the first intron of the *IQSEC2* transcript in the Xp11.22 region. Expression studies revealed that the *Iqsec2* murine homolog is highly expressed in the central and peripheral nervous systems.

These results led us to hypothesise that the phenotype observed in the patient could be the result of a disruption of the *IQSEC2* gene in Xp11.22, thus rendering *IQSEC2* a candidate gene for ISSX and RTT-like cases characterized by the early onset of seizures and other neurological phenotypes.

Materials and methods

In silico analysis. BAC contigs necessary for the characterization of the patient's breakpoints were established *in silico* using the UCSC Genome Bioinformatics web site (http://genome.ucsc.edu/cgi-bin/hgGateway).

Correspondence to: Dr Brunella Franco, Telethon Institute of Genetics and Medicine (TIGEM), Via Pietro Castellino 111, I-80131 Naples, Italy
E-mail: franco@tigem.it

Key words: ISSX, RTT, X-linked disorders, X-chromosome, *IQSEC2*

Table I. Primer sequences used in the study.

Exon	Forward primer	Reverse primer	Ta (˚C)	PCR product length (bp)
IQSEC2				
1	tcctcccgagcagtggctg	ctctcgatgatgttgttcagctcc	60	333
	ggatccgagagcccaa atc	tgtaaccggtagtgtcctggag	60	416
	gaggctgtgtatcgggacaag	tactctatcagctggacaagttggagag	60	400
2	gcagagggagaagagagcgtatac	tggatcctgcagttacaaaccag	58	175
3	agcaaagtttccag ttgcaatctg	ctgctgaatcagtagcccaggg	58	264
4	actcactctcaccttcttgccc	tggcaatgacactgtatatttgtctgg	60	263
5	gccctggataggaaagaagg	tccagcccctgaattaaagag	57	525
	aagctctgcttctcccacattttag	tgggtgtgaccctggataatc	60	508
6	ccactctcagtatggcccagtag	ttgcatcccgactcccattc	58	377
7	catgactttctgaccttgttccc	taggtaaaaagtagcaggagtagccag	60	514
8	ttgctgacctaccaggaagactc	gagtgccttcctcacggctacc	60	450
	aagacggtagccgtgaggaag	atctccaccatcagagttctcgc	58	450
	gaggttggggaagtgcgag	gttctgtcccaggaacccaag	58	335
9	aatgagacagagagcacagttccag	gctgtccttttccaggaatccctg	60	300
10	catttgagcaggtccaggtg	cttataaacacatactacgtcgatgcc	58	270
11	gtgccagactgggtggatg	ggatctcctgcatccttccc	58	320
12	tggtggagcacccctacc	gaggtgcagtgacttaccacttagtg	58	285
13	caggaggcctgaagctgatg	tacatcaatatggttcacaccagacttag	58	265
14	taggtgccacgtggaaatcc	aactggagctggtatagatgacgg	58	250
15	gtatgagccaccttgcaccag	gagattctctgaggataggattcttgg	58	300
16	ctagggtcagtcagatgggctc	agtgacaggaggcaaagaggac	60	315
17	cagctctggagcccagcag	tgggtctccatggccg	60	185
18	cagggacaggggtggaatttag	tggaatggagcccagctg	60	500
	ccccagcagccctctcttc	tggctgaagatgaagtgcttagg	58	320
	ccctccagcccacaaacag	agcaaagagggtgcaaggtc	60	400
ARX				
1	caacacacacccatccatcc	caattccaggccactggcc	58	380
2	ctgatagctctcccttgccc	cccctgcgccgtccggccgttc	60	258
	aggcccctccgccgccaccgccaa	cttcttcctcgtcctcatcttctt	62	354
	aggaggagctgctggagg	tccaggagccaagcgtcc	60	540
3	catagaggaggaaatagctgagag	ggttttgtgaaggggatctcac	60	250
4	cccagacgcgtccgaaaac	tgtaaccctgtttgactcctgcc	58	570
5	gtaccgcgcccctcagc	agtggatgttggagttggagcg	58	630

Fluorescence in situ hybridisation (FISH). BAC clones used for FISH studies were derived from the RPCI-11 Human Male BAC Library (10). FISH analysis was performed on chromosome metaphases of fibroblastoid cell line of the patient. FISH studies were performed as described (11).

X chromosome inactivation studies. Primers were designed in the (CAG)n flanking sequences of the androgen receptor (HUMARA) gene intron 1 (12). Forward primer AR-P1 was labelled (Fam) and the reverse primer was unlabelled. Primer sequences were: AR-P1: Fam 5'TCC AGA ATC TGT TCC AGA GCG TGC 3'; AR-P2: 5'GCT GTG AAG GTT GCT GTT CCT CAT3'. A total of 800 ng of DNA was digested by *Hpa*II and ethanol precipitated. PCR reactions were performed with 100 ng of DNA on both *Hpa*II digested and undigested DNA from the mother and the daughter. PCR conditions were as follows: 1X PCR buffer, 0.2 mmol/l dNTP, 1.25 mmol/l Mg^{++} and 0.5 U AmpliTaq Gold (Applied Biosystems), in a 20-μl final volume. Annealing temperature was 60˚C for 28 cycles. PCR products were electrophoresed on an ABI PRISM 3100 Genetic Analyzer, and peak heights were analyzed using GeneScan software (Applied Biosystem).

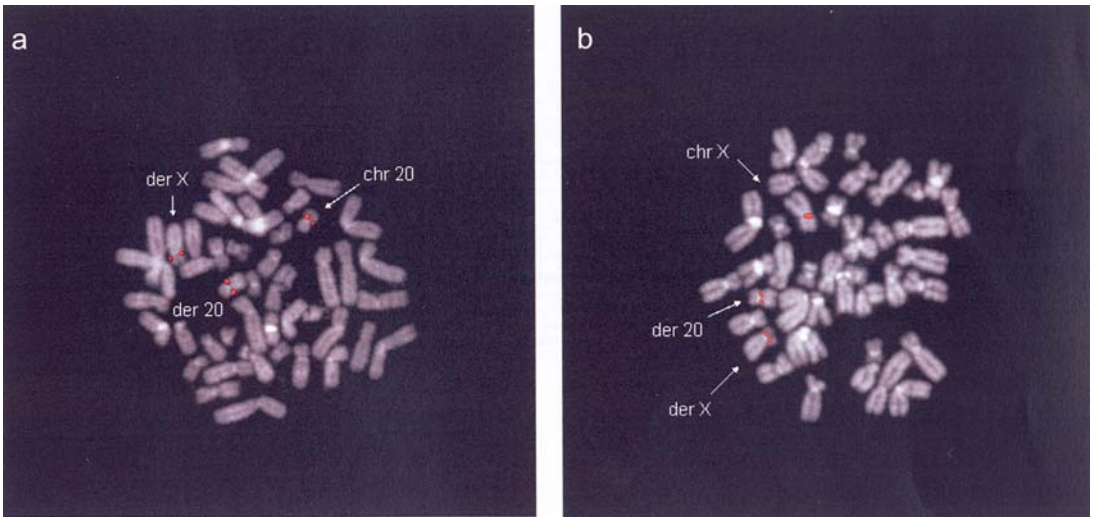

Figure 1. Fluorescent *in situ* hybridizations of the two BACs spanning the 20 and X chromosomes translocation breakpoints. (a) Hybridisation of clone RP11-957H19 (20q11.22) on a metaphase of female patient AD. The BAC clone hybridises with the normal chromosome 20 and the two translocated chromosomes, indicated by the arrows, and indicates that this genomic clone spans the patient's breakpoint. (b) Hybridisation of clone RP11-473J14 (Xp11.22) on a metaphase of female patient AD. The normal chromosome X and the chromosomes carrying the translocation are marked with arrows. This result indicates that this clone spans the breakpoint on Xp11.

RNA in situ hybridization. The expression pattern of *Iqsec2* was examined by RNA *in situ* hybridization on murine sections from E14.5 and E16.5, and adult brain. Two different riboprobes were used. One, corresponding to a 826-bp sequence spanning exons 4-5 of *Iqsec2*, was transcribed from the cDNA clone IMAGE:803296 (acc. AA396309). The second was obtained by PCR amplification of the cDNA clone IMAGE:6530479 (acc. BU523772) and is specific for the 1010-bp sequence of the 3'UTR of *IQSEC2* (primer sequences available upon request). RNA *in situ* hybridization experiments were performed as described (13).

Mutation screening. For mutation analysis of the *MECP2* and *CDKL5* genes, primers and conditions were as described (8,14). In order to perform mutation analysis for *ARX* and *IQSEC2*, primers were designed to amplify exons and exon-intron boundaries. The boundaries were determined by the alignment of the consensus cDNA sequences with available genomic sequences. PCRs were carried out on genomic DNA extracted from peripheral blood leukocytes. Mutation analysis was performed by denaturing high performance liquid chromatography using the Wave DNA fragment analysis system (Transgenomic, Inc.) according to the manufacturer's instructions. Table I shows the primer sequences, PCR product length and annealing temperatures. Full ethical approval was obtained for the study.

Results

Case report. AD was the second female child born to unrelated parents. She was born by induced vaginal delivery at 37 weeks gestation following an uncomplicated pregnancy. Delivery was induced for premature rupture of membranes. She was born with a left talipes equinovarus, treated with casting and later surgery, and congenital hip dislocation, which was corrected with a Pavlik harness. She was also noted to have esotropia requiring surgical correction. Development was apparently normal for the first 6-8 months, but subsequently slowed and regressed. She did not sit until 13 months of age, and never learned to crawl or walk. She said 'mama' and 'papa' at 12-13 months of age. At 15 months, she developed severe myoclonic seizures involving both arms, which have been refractory to therapy. Since the development of the seizures she has not gained any new skills, has stopped speaking and has regressed to babbling again. She cannot eat solid foods and has remained on liquids.

She is severely hypotonic, with full range of motion in all extremities. Her growth, regarding weight and height, and head circumference have been normal. Her features are not dysmorphic. Her EEG was consistent with hypsarrhythmia and a brain MRI showed non-specific volume loss and periventricular white matter changes, but no structural brain abnormalities. She is now 12 years old. Chromosome analysis showed a *de novo* apparently balanced translocation 46,X, t(X;20)(p11.2;q11.2), absent in both parents' karyotype analysis.

X-inactivation studies performed on fibroblast cells of the proband revealed that she has an extremely skewed X-inactivation (100%) with preferencial use of the translocated X chromosome, as is usually observed in balanced X;autosome translocations (15).

FISH mapping of the chromosome Xp11.22 breakpoint. In order to map the translocation breakpoints, we initially used the karyotype information and the available physical maps to establish genomic contigs in the regions of interest. FISH experiments were performed with a set of YAC and BAC clones selected in the contigs and spanning the Xp11.2 and 20q11.2 genomic regions (data not shown). FISH studies allowed the identification of two BAC clones, RP11-957H19 and RP11-473J14, spanning, respectively, the translocation breakpoints of the 20q11.22 and Xp11.22 regions (Fig. 1).

BAC RP11-957H19 covers a segment of 181 kb on the 20q11.22 region and contains 3 known transcripts: a gene

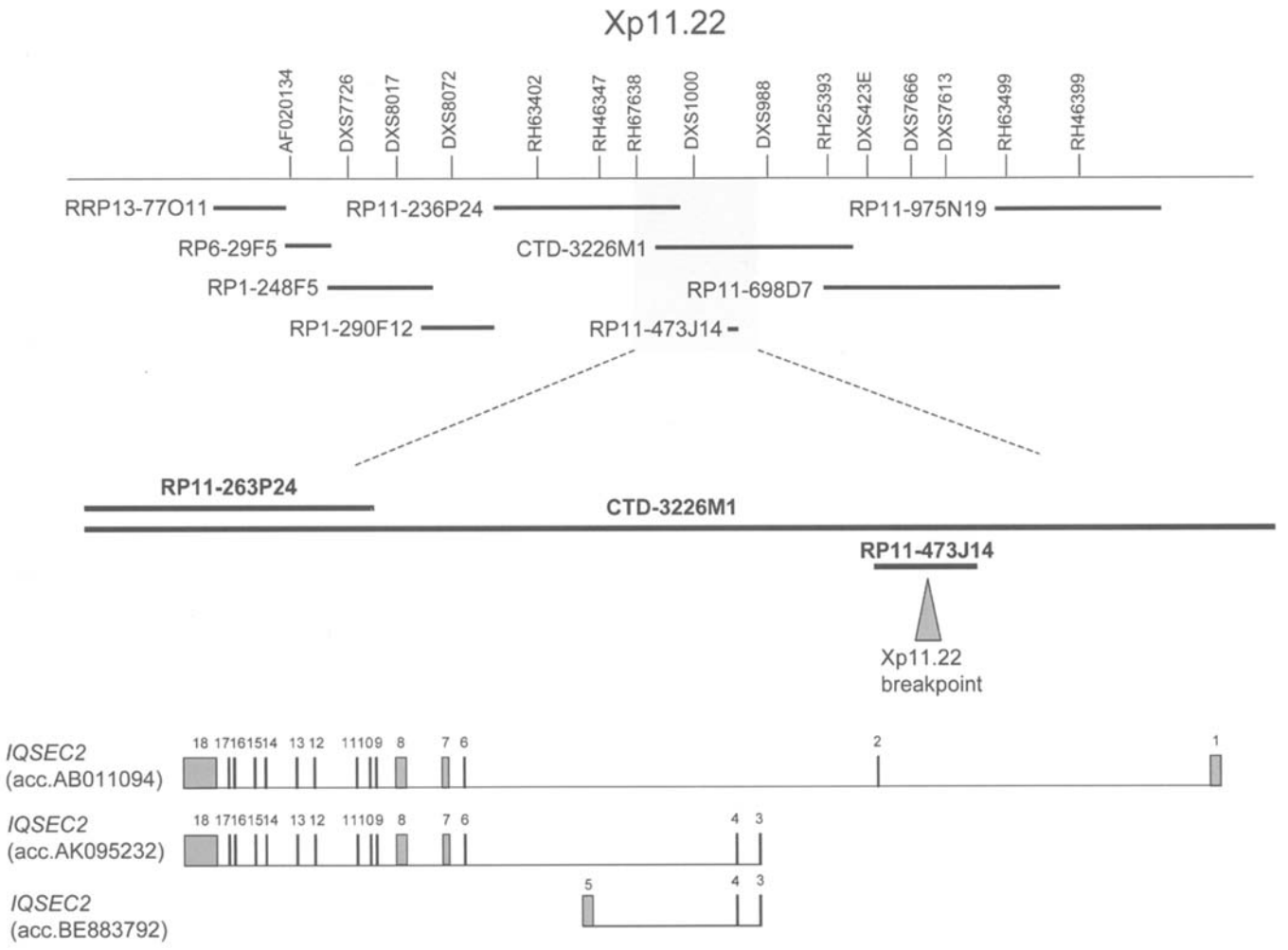

Figure 2. Schematic representation of the translocation breakpoint region in Xp11.22. At top, a physical map of the Xp11.22 locus is represented and the position of the DNA markers is shown. Overlapping genomic BACs spanning the region are indicated as plain lines. BAC clone RP11-473J14 is also shown. Below, the genomic structure of the *IQSEC2* gene (at least 88.5 kb) with its 18 coding exons and alternative transcripts is represented. The arrowhead indicates the X chromosome breakpoint, located in the first intron of *IQSEC2* (28.5 kb).

called *ASIP,* encoding a paracrine signaling molecule, the *AHCY* gene, coding for an S-adenosylhomocysteine hydrolase and the *ITCH* gene, encoding an E3 ubiquitin protein ligase.

BAC RP11-473J14 spans 11.2 kb in Xp11.22, inside the first intron (28.5 kb) of the *IQSEC2* transcript (acc. AB011094) that spans ≥88.5 kb (Fig. 2). The genomic clone RP11-473J14 has been entirely sequenced. Bioinformatic analysis of its genomic sequence revealed that *IQSEC2* is the only transcript contained within this clone. Among the genomic clones used in this study, RP11-473J14 in particular is specific for the longest isoform (see below), which is presumably interrupted by the translocation in this patient. The IQSEC2 protein is characterized by the presence of a domain known to function as a guanine nucleotide exchange factor, by an IQ calmodulin-binding region motif and by a pleckstrin homology (16,17).

Three different isoforms have been reported in both human and mouse for this transcript (AB011094, AK095232 and BE883792) (17) (Fig. 2). The three alternative splice forms belong to the UniGene cluster Hs.496138. The longer isoform (AB011094), which is presumably interrupted, is only represented by mRNA from the brain, although the possible involvement of all three isoforms cannot be ruled out. Further experiments are needed to clarify this issue. The third splice variant, acc. BE883792, represents a shorter isoform of the transcript and encodes a 183-aminoacid protein.

Expression studies of Iqsec2. In order to obtain information on the expression pattern of the *IQSEC2* gene, we performed expression analysis of the murine homolog *Iqsec2* by RNA *in situ* hybridization (ISH) on murine embryo tissue sections from E14.5 and E16.5, and adult brain. Experiments were performed using two different probes, a first RNA probe that hybridizes to exons 4-5 and corresponds to human BE883792 (murine cDNA clone IMAGE:803296) and a second probe that hybridizes to the 3'UTR and corresponds to the human isoforms AB011094 and AK095232 (murine cDNA clone IMAGE:6530479). ISH experiments performed on the serial sagittal sections of embryonic day 14.5 embryos show a clear signal restricted to the central and peripheral nervous systems, as shown in Fig. 3. The same results were obtained with the two different riboprobes described above.

Fig. 3 displays the results obtained with riboprobe transcribed from the cDNA clone IMAGE:803296 (data not shown for riboprobe transcribed from clone IMAGE:6530479). This experiment shows that, at the E14.5 and E16.5 cells transcribing this gene, numerous tissues are found, including

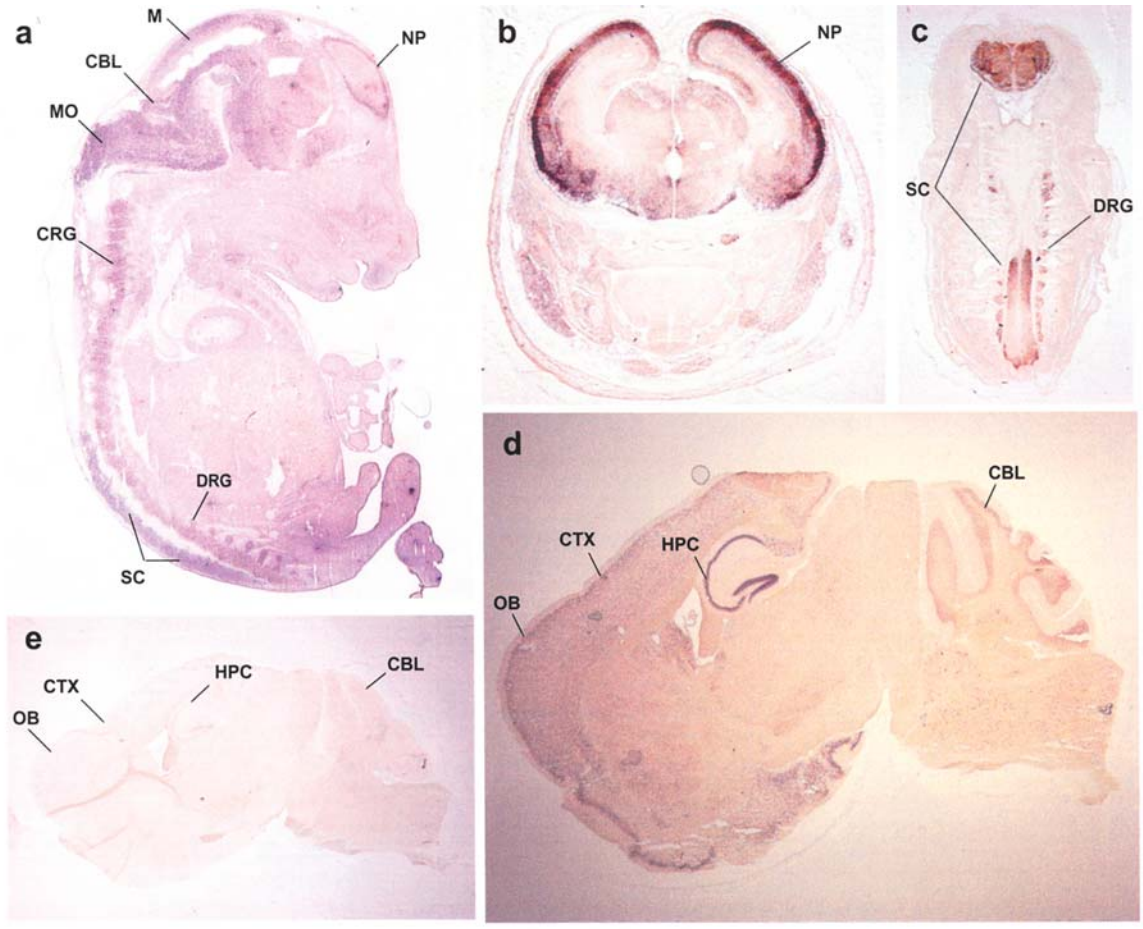

Figure 3. Expression analysis of *Iqsec2* mRNA during embryonic and postnatal development. *Iqsec2* (probe from clone IMAGE:803296) is highly expressed in the central and peripheral nervous systems. (a) RNA *in situ* hybridization on sagittal sections from E14.5. Signal corresponding to *IQSEC2* is evident in numerous tissues, including the neopallial cortex, the roof of the midbrain, cerebellum, medulla oblongata, spinal chord and dorsal root ganglia. (b and c) *IQSEC2* expression on coronal sections from E16.5. Of note is strong expression in the neopallial cortex (b) and both in the upper and lower region of the spinal cord and dorsal root ganglia (c). (d) Expression analysis on adult mouse brain sagittal sections. *Iqsec2* mRNA expression is found particularly in the hippocampus, the cerebral cortex, cerebellum and olfactory bulb. (e) RNA *in situ* hybridization on an adult mouse brain sagittal section using sense riboprobe as a negative control. Abbreviations: CBL, cerebellum; NP, neopallial cortex; CTX, cerebral cortex; MO, medulla oblongata; HPC, hippocampus; OB, olfactory bulb; SC, spinal chord; DRG, dorsal root ganglia; M, midbrain.

the neopallial cortex, midbrain, cerebellum, medulla oblongata, spinal cord and dorsal root ganglia (Fig. 3a-c). Significant and specific expression was also detected in the pituitary primordium and trigeminal ganglion (data not shown).

In situ hybridization analysis of postnatal mouse brain (week 8) revealed expression of the Iqsec2 gene in the hippocampal formation comprising hippocampus proper and dental gyrus, in the olfactory bulb, the cerebral cortex and the cerebellum (Fig. 3d).

Mutation analysis. In order to exclude the possible involvement of *ARX, MECP2* and *CDKL5*, mutation analysis for these transcripts was performed on patient AD as described (8,14) (Table I). This analysis revealed the absence of pathogenic mutations.

The observation that the *IQSEC2* gene is interrupted in AD and is highly expressed in the central and peripheral nervous systems makes it a good candidate for neurological disorders. To further assess the role of the *IQSEC2* gene in ISS and X-linked Rett-like phenotype with early-onset seizures, we screened a cohort of 40 unrelated female patients available at the RTT Italian biobank (www.biobank.unisi.it). These included 11 classic RTT patients, 20 atypical RTT patients (6 with early-onset seizures) and 9 RTT-like patients. All had already been screened for mutations in *MECP2* and *CDKL5*. Mutation analysis was performed on coding exons and splice junctions for all isoforms of the I*QSEC2* gene and on the genomic DNA of the probands. No causative mutations were identified.

Discussion

Although hypsarrhythmia, West Syndrome and ISS are not the same, they are used interchangeably because one almost always accompanies the other. The incidence of this type of epilepsy is about 1:2-4000 children. It usually occurs in the first months of age and is characterized by seizures, mental retardation and chaotic brain activity on the EEG (hypsarrhythmia), and is accompanied by developmental arrest or regression. In most cases, the etiology is unknown.

We report on a female patient (AD) with a balanced *de novo* X;autosome translocation [46,X,t(X;20)(p11.2;q11.2)] who showed developmental regression that started at the age of 6 months and the early onset of infantile spasms at

around 15 months of age, refractory to medical treatment. Chromosome breakpoints in the patient were defined and precisely assigned to the corresponding genomic regions of chromosome X and 20, and further research identified a transcript on the short arm of the X chromosome, interrupted by the translocation breakpoint. Only one of the three *IQSEC2* variants (acc. AB011094) seems to be truncated by the re-arrangement and, intriguingly, this variant is represented by a cDNA clone from brain. One hypothesis is that this isoform is preferentially expressed in the human brain and that its disruption is linked to the patient's phenotype, or that the impairment of all three variants is involved in the phenotype observed in patient AD. A functional copy of the gene is present in the patient, due to the reported *IQSEC2* escape of X inactivation in humans (17), and in order to assess whether one or more isoforms are missing, expression studies of the different splice variants should be conducted. We postulate that the severity of the phenotype could be caused by the absolute requirement of at least two functional copies of the gene within each cell in affected tissues, such as the central nervous system; the retained half dosage of the functional gene could be insufficient for the normal life of cells.

The clinical manifestations observed in our patient share similarities with two other X-linked conditions known to be associated with infantile spasms, ISS and Rett-like syndrome with early-onset seizures, caused by mutations in the *ARX* and *CDKL5* genes. To date, mutations in *ARX* and *CDKL5* account for a small percentage of cases. Several pieces of evidence suggest the existence of additional loci, which may be associated with this group of disorders (3).

As has been shown in other genetic diseases, the characterization of chromosomal rearrangements may be a powerful tool for the identification of genes responsible for genetic disorders. These considerations prompted us to hypothesize that *IQSEC2* could represent a possible candidate gene for the above-mentioned disorder.

IQSEC2 codes for a guanine nucleotide-exchange protein (GEP) and has two paralogs (*IQSEC1* and *IQSEC3*). These proteins activate ADP-ribosylation factors (ARFs), which are guanine nucleotide binding proteins belonging to the Ras superfamily of GTPases that modulate the formation of vesicles during intracellular protein trafficking, and are called ARF-GEPs (16). IQSEC2 and the other ARF-GEPs are involved in the activation of ARFs through the acceleration of the replacement of bound GDP with GTP. These proteins are characterized by the presence of a Sec7 domain, first found in yeast, which may be responsible for their guanine-nucleotide exchange activity. IQSEC2 also contains a pleckstrin homology (PH) domain commonly found in eucaryotic signalling proteins and required for phosphoinositide binding. Several genes involved in Ras-homologous GTPase, such as *OPHN1, ARHGEF6, GDI1* and *PAK3,* have been proven responsible for different forms of X-linked mental retardation (18). *ARHGEF6* in particular, which is similar to *IQSEC2*, codes for a protein with guanine-nucleotide exchange activity.

RNA *in situ* hybridization studies revealed that *Iqsec2* is highly expressed during development in the central and peripheral nervous systems. A comparison of the expression patterns of *Iqsec2* and *Cdkl5* in murine adult brain showed a significant overlap of the two signals, as they are both expressed in the basal ganglia, olfactory bulb, cerebral cortex, cerebellum and hippocampus (9). This indicates that *Cdkl5* and *Iqsec2* expression may coexist in the same cells, suggesting a possible functional link between the two proteins. This, however, remains to be proved.

These data lead us to hypothesise that the phenotype observed in AD could be due to the interruption of the *IQSEC2* transcript. It should be noted that a familial case, showing a balanced translocation t(X;22)(p11.22;p11) and displaying an RTT phenotype with early-onset seizures, has previously been described (19). Unfortunately, this patient has not been fully characterized and we do not know whether the breakpoint is identical to that of AD. Nevertheless, these data indicate the involvement of the Xp11.22 region at the location of *IQSEC2*, and suggest the possible involvement of the transcript in the pathogenesis of this neurological phenotype.

Mutation analysis performed on a representative subset of patients did not reveal mutations. However this, for several reasons, does not exclude the possible involvement of *IQSEC2*. Additional testing on a larger cohort of patients, in particular male patients with infantile spasms who are negative for *ARX* mutation analysis, is necessary before any conclusions can be drawn.

We cannot rule out the possibility that the phenotype observed in our patient is not caused by the interruption of *IQSEC2* but is due, rather, to the haploinsufficiency of one of the three genes located in the genomic clones spanning the breakpoint on chromosome 20. These genes include the *ASIP* gene, which encodes a molecule involved in melanin biogenesis, the *AHCY* gene, which codes for a S-adenosylhomocysteine hydrolase, and the *ITCH* gene, encoding an E3 ubiquitin protein ligase. Previous studies have shown that mutations in the ubiquitin protein ligase E3A (*UBE3A*) gene cause Angelman syndrome (20,21), which partially overlaps with the clinical manifestations observed in our patient. In addition, *Mecp2* deficiency has been shown to cause reduced expression of *Ube3a* in the brain of *Mecp2* knockout mice (22). Recently, Nascimento *et al* reported that a mutation of the *UBE2A* gene, which encodes a ubiquitin-conjugating enzyme (E2), is the cause of a novel X-linked mental retardation syndrome (23). Further studies are needed to understand the possible relationship between the *ITCH* gene and the phenotype observed in patient AD.

To understand the role of genes on chromosome 20, further studies, such as the characterization of the breakpoint from a molecular point of view or expression studies on mRNA to investigate the presence of double copies of each gene, will be needed. Moreover, we cannot rule out the possibility of a position effect causing an alteration of expression in genes in the vicinity of the breakpoint; an example is the *JARID1C* gene (acc. NM_004187), which lies only 7 kb from 3' of *IQSEC2*. Mutations in this transcript have been associated with X-linked mental retardation (24), so an alteration of *JARID1C* gene expression could be involved in patient phenotype. Lastly, the possibility of a fusion transcript and the consequent possible dominant effect should be considered for further studies.

In conclusion, we believe that the data reported here could make the *IQSEC2* transcript a candidate gene for X-linked neurological disorders, although further studies on different cohorts of patients will be needed to validate this hypothesis.

Acknowledgements

We thank the TIGEM Mutation Detection facility, and Michele Zappella for the clinical assessment of MECP2/CDKL5-negative patients. This work was supported by the Italian Telethon Foundation (B.F.) and by grant GTF03005.

References

1. Stromme P, Mangelsdorf ME, Shaw MA, *et al*: Mutations in the human ortholog of Aristaless cause X-linked mental retardation and epilepsy. Nat Genet 30: 441-445, 2002.
2. Gecz J, Cloosterman D and Partington M: ARX: a gene for all seasons. Curr Opin Genet Dev 16: 308-316, 2006.
3. Weaving LS, Ellaway CJ, Gecz J and Christodoulou J: Rett syndrome: clinical review and genetic update. J Med Genet 42: 1-7, 2005.
4. Amir RE, van den Veyver IB, Wan M, Tran CQ, Francke U and Zoghbi HY: Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23: 185-188, 1999.
5. Archer HL, Evans JC, Edwards S, *et al*: CDKL5 mutations cause infantile spasms, early onset seizures and severe mental retardation in female patients. J Med Genet 43: 729-734, 2006.
6. Evans JC, Archer HL, Colley JP, *et al*: Early onset seizures and Rett-like features associated with mutations in CDKL5. Eur J Hum Genet 13: 1113-1120, 2005.
7. Raymond FL: X linked mental retardation: a clinical guide. J Med Genet 43: 193-200, 2006.
8. Scala E, Ariani F, Mari F, *et al*: CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. J Med Genet 42: 103-107, 2005.
9. Weaving LS, Christodoulou J, Williamson SL, *et al*: Mutations of CDKL5 cause a severe neurodevelopmental disorder with infantile spasms and mental retardation. Am J Hum Genet 75: 1079-1093, 2004.
10. Osoegawa K, Mammoser AG, Wu C, Frengen E, Zeng C, Catanese JJ and De Jong PJ: A bacterial artificial chromosome library for sequencing the complete human genome. Genome Res 11: 483-496, 2001.
11. Morleo M, Pramparo T, Perone L, *et al*: Microphthalmia with linear skin defects (MLS) syndrome: clinical, cytogenetic, and molecular charac-terization of 11 cases. Am J Med Genet A 137: 190-198, 2005.
12. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM and Belmont JW: Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet 51: 1229-1239, 1992.
13. Rugarli EI, Lutz B, Kuratani SC, Wawersik S, Borsani G, Ballabio A and Eichele G: Expression pattern of the Kallmann syndrome gene in the olfactory system suggests a role in neuronal targeting. Nat Genet 4: 19-26, 1993.
14. De Bona C, Zappella M, Hayek G, Meloni I, Vitelli F, Bruttini M, Cusano R, Loffredo P, Longo I and Renieri A: Preserved speech variant is allelic of classic Rett syndrome. Eur J Hum Genet 8: 325-230, 2000.
15. Van den Vayvers IB: Skewed X inactivation in X-linked disorders. Semin Reprod Med 19: 183-191, 2001.
16. Someya A, Sata M, Takeda K, Pacheco-Rodriguez G, Ferrans VJ, Moss J and Vaughan M: ARF-GEP(100), a guanine nucleotide-exchange protein for ADP-ribosylation factor 6. Proc Natl Acad Sci USA 98: 2413-2418, 2001.
17. Tsuchiya KD, Greally JM, Yi Y, Noel KP, Truong JP and Disteche CM: Comparative sequence and x-inactivation analyses of a domain of escape in human xp11.2 and the conserved segment in mouse. Genome Res 14: 1275-1284, 2004.
18. Ropers HH: X-linked mental retardation: many genes for a complex disorder. Curr Opin Genet Dev 16: 260-269, 2006.
19. Journel H, Melki J, Turleau C, Munnich A and De Grouchy J: Rett phenotype with X/autosome translocation: possible mapping to the short arm of chromosome X. Am J Med Genet 35: 142-147, 1990.
20. Kishino T, Lalande M and Wagstaff J: UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15: 70-73, 1997.
21. Matsuura T, Sutcliffe JS, Fang P *et al*: *De novo* truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 15: 74-77, 1997.
22. Samaco RC, Hogart A and LaSalle JM: Epigenetic overlap in autism-spectrum neurodevelopmental disorders: MECP2 deficiency causes reduced expression of UBE3A and GABRB3. Hum Mol Genet 14: 483-492, 2005.
23. Nascimento RM, Otto PA, De Brouwer AP and Vianna-Morgante AM: UBE2A, which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome. Am J Hum Genet 79: 549-555, 2006.
24. Jensen LR, Amende M, Gurok U, *et al*: Mutations in the JARID1C gene, which is involved in transcriptional regulation and chromatin remodeling, cause X-linked mental retardation. Am J Hum Genet 76: 227-236, 2005.