A Hunter Patient with a Severe Phenotype Reveals Two Large Deletions and Two Duplications Extending 1.2 Mb Distally to IDS Locus

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Received: 10 December 2013 / Revised: 16 April 2014 / Accepted: 22 April 2014 / Published online: 25 July 2014
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Abstract Mucopolysaccharidosis type II (Hunter syndrome, MPS II) is an X-linked lysosomal storage disorder caused by the deficit of iduronate 2-sulfatase (IDS), an enzyme involved in the glycosaminoglycans (GAGs) degradation. We here report the case of a 9-year-old boy who was diagnosed with an extremely severe form of MPS II at 10 months of age. Sequencing of the *IDS* gene revealed the deletion of exons 1–7, extending distally and removing the entire pseudogene *IDSPI*. The difficulty to define the boundaries of the deletion and the particular severity of the patient phenotype suggested to verify the presence of pathological copy number variations (CNVs) in the genome, by the array CGH (aCGH) technology. The examination revealed the presence of two deletions alternate with two duplications, overall affecting a region of about 1.2 Mb distally to *IDS* gene. This is the first complex rearrangement involving *IDS* and extending to a large region located distally to it described in a severe Hunter patient, as evidenced by the CNVs databases interrogated. The analysis of the genes involved in the rearrangement and of the disorders correlated with them did not help to clarify the phenotype observed in our patient, except for the deletion of the *IDS*

gene, which explains *per se* the Hunter phenotype. However, this cannot exclude a potential “contiguous gene syndrome” as well as the future rising of additional pathological symptoms associated with the other extra genes involved in the identified rearrangement.

Introduction

Mucopolysaccharidosis type II (MPS II or Hunter syndrome; MIM #309900) is an X-linked multisystemic progressive metabolic disorder caused by the deficit of activity of the iduronate 2-sulfatase (IDS) enzyme, which leads to the lysosomal accumulation of the glycosaminoglycans (GAG) heparan- and dermatan-sulfate. Albeit presenting as a continuum of pathological phenotypes, the disease mainly recognizes an attenuated and a severe form (Muenzer et al. 2009).

No strict genotype–phenotype correlation can be so far defined for the disease, nevertheless large deletions or insertions or important genomic rearrangements are always associated with severe phenotypes (Martin et al. 2008). Implications of large regions proximal or distal to the *IDS* gene have been previously described in some Hunter cases (Brusius-Facchin et al. 2012; Burruss et al. 2012; Probst et al. 2007; Honda et al. 2007; Dahl et al. 1995; Dahl et al. 1995; Beck et al. 1992; Clarke et al. 1992; Birot et al. 1996; Timms et al. 1997), mostly conferring to the MPS II phenotype other signs and symptoms associated with the extra genes implied in the rearrangement, such as *FMRI* and *MTMI* correlated with the Fragile X-syndrome (MIM#300624) and the X-linked myotubular myopathy (MIM#310400), respectively.

Communicated by: Gregory M. Pastores, MD

Competing interests: None declared

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We here report the case of a 9-year-old boy who received an early biochemical and clinical diagnosis of MPS II. The difficulty of precisely defining the molecular variation affecting the *IDS* gene using PCR mapping suggested to extend the analysis to the whole X chromosome through the array CGH (aCGH) technology.

Materials and Methods

For mutation analysis genomic DNA was extracted from peripheral blood leukocytes using the commercial kit QIAmp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Informed consent was obtained from the parents. *IDS* exons and their flanking regions were PCR-amplified; specific sets of primers were used for selective amplification of *IDS* exon 2 and 3 versus *IDSP1* homologous exons (Villani et al. 1997). PCR products were purified by using Microcon YM 100 (Millipore) columns and sequenced with the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). Sequence variations were confirmed by sequencing in both directions duplicate PCR products.

The presence of *IDSP1* gene was verified by applying the two PCR amplifications suggested by Lualdi (Lualdi et al. 2005) for the detection of the recombinants.

Array CGH analysis was carried out using a 244 K Agilent chip with a mean resolution of 30 kb (Agilent Technologies, Santa Clara, USA); the array was analyzed through an Agilent scanner (G2505C) and Feature Extraction software V.10.1.1.1. A graphical overview of the results was obtained using DNA Analytics software V.4.0.76. DNA sequence information refers to the public UCSC database [Human Genome Browser, February 2009, assembly hg19 (NCBI Build 37.5)].

For the interpretation of aCGH results, we followed the three-step workflow approach suggested by Poot (Poot and Hochstenbach 2010). To verify whether the same CNVs, or one partly overlapping with those under examination, had been previously described, we exploited the following publicly available databases: ISCA (The International Standards for Cytogenomic Arrays) database (<https://www.iscaconsortium.org/>), DECIPHER (Firth et al. 2009) (<http://decipher.sanger.ac.uk>), and the Database of Genomic Variants (Iafate et al. 2004) (<http://www.ncbi.nlm.nih.gov/dbvar/>). For the undescribed CNVs, the genes mapping in the four genomic regions of the rearrangement were identified through the UCSC Genome Browser on Human Feb. 2009 (NCBI 37.5/hg19) assembly. The content of protein-coding genes was analyzed using OMIM, UniProtKB, and GeneCards for protein function and tissue expression; also the genes located in regions flanking the

variation underwent the same analysis. The inheritance of the CNV was checked by evaluating the aCGH results of the patient's mother and sister.

Results

Clinical Description

Our 9-year-old patient was the second-born child of a non-consanguineous Italian couple of Caucasian ethnicity with no previous family history of neurological and/or metabolic disorders; however, the father is affected by hyperaldosteronism and chronically treated for hypertension. At the time of the patient's birth, the mother was 37 years old and the father was 41 and they had a healthy 11-year-old daughter. The patient was born at 36 weeks gestational age by cesarean section after a pregnancy complicated by threats of abortion. At birth, the patient weighed 2.5 kg (5th percentile) and he was 46 cm in length (5th percentile) with a head circumference of 33 cm (5–10th percentile). At two months of life, he presented with inguinal hernia which was surgically reduced. During the first year of life the child showed normal psychomotor development. Nevertheless, at 9 months of age he came to our attention for macrocephaly, mild facial dysmorphism, slight rigidity of the upper track, and claw hands. Suspecting a metabolic storage disease, biochemical and molecular genetics analyses were performed. Dosage of urinary GAG revealed a high urinary GAG/creatinine ratio equal to 914.41 mg/g (normal range values for the age: 30–300). IDS activity in cultured skin fibroblasts was 0.5 nmol/mg/h (n.v. 69.2), whereas other enzymes were normal, hence excluding multiple sulfatase deficiency and leading to the diagnosis of MPS II.

The first brain MRI examination performed at 11 months evidenced white matter lesions, ventricular expansion, and perivascular alterations.

At the age of 18 months the patient started enzyme replacement therapy (ERT) with idursulfase (Elaprase®) at the standard dosage of 0.5 mg/kg body weight per week. ERT was observed to be very well tolerated and safe, with no severe adverse events. Urinary GAG analysis performed just before the start of ERT detected a value of 493.19 GAG/g creatinine (normal range values for the age: 12–95); during ERT follow-up GAG levels reduced but never reached normal values, setting to an average level of about 300 mg GAG/g creatinine (normal range values for the age: 12–68) (data not shown). The search of anti-IDS, neutralizing antibodies performed during the follow-up of the patient resulted positive with a percentage of inhibition of 100% after 5 years of treatment.

Over the years the patient developed a progressive severe cognitive degeneration with a notable impairment of verbal communication. At present, cognitive functions are not testable; the adaptive behavior, evaluated by the Vineland Scale, showed in all domains an age equivalent to less than 1.5 years. At about 3 years of age, the patient started to receive occupational and speech therapies, in addition to similar services at school. At 4.5 years, the patient showed signs and symptoms of hypertensive hydrocephalus which was relieved through surgical derivation with a ventriculoperitoneal shunting. The frequent upper-respiratory tract infections, since the first months, were treated with antibiotic prophylaxis therapy. At 3.8 years he underwent adenotonsillectomy. The repeated ear infections lead to hearing problems with bilateral hypoacusia; at 4 years, he started to use hearing aids. A mild-moderate mitral insufficiency, which stabilized during the years, developed around 2 years of age. At 5 years he developed arterial hypertension and he started antihypertensive pharmacological therapy with good response and tolerance. The hepato-splenomegaly was present at the beginning of ERT but it slowly normalized 3.5 years post-therapy. He presented bone dysostosis with mild joint stiffness; up to today the patient did not require any surgical interventions or walking aids, but he uses wheelchair for long distances.

Array CGH Analysis

IDS mutational analysis allowed the sequencing of only exons 8 and 9, which did not carry any variations, and revealed the presence of an intragenic deletion. The absence of amplification of any fragments belonging to *IDSP1* pseudogene allowed to hypothesize that the deletion extended distally to this region. Several attempts to catch the telomeric boundary of the deletion through mapping PCR-methods, designing primers annealing to the region distal to *IDSP1*, failed. Thus, an in-depth analysis exploiting the aCGH technology was conducted to determine the extension of the deletion. This analysis revealed that the deletion extended for a total of 54.7 kb (148,568,786–148,623,440 bp). In addition, the aCGH analysis pointed out, in a telomeric position to the mentioned deletion, an 86.4 kb duplication (148,648,557–148,734,969 bp), a 198.7 kb deletion (148,830,445–149,029,121 bp), and a 644.6 kb duplication (149,105,821–149,750,457 bp) separated from one another by 3 regions spanning 25.1, 95.5, 76.7 kb, respectively (Fig. 1). The same rearrangement was detected in the patient's mother, but not in the patient's sister.

Discussion

Hunter syndrome is in most cases associated with point or small alterations of the *IDS* gene; only a little percentage of the patients present with intragenic or complete deletions of the gene or with complex rearrangements, including recombinational events between *IDS* and its pseudogene *IDSP1*. A suspect of "atypical rearrangement" should arise from the absence of PCR amplification of part of the exons (preferably the distal or the telomeric ones) or of the entire *IDS* gene and should lead to a deeper analysis of the nearby genes, included the *IDSP1* gene that should be investigated to verify the presence of recombinational events with the *IDS* gene. In case of negative results, the aCGH analysis should be afterwards recommended.

In the present paper we report the first case of a Hunter patient showing a complex rearrangement consisting of two deletions and two duplications extending far distally towards the telomere, involving on the whole a region of about 1.2 Mb of the X chromosome. To our knowledge, the entire complex rearrangement had never been reported so far in any of the publicly available databases.

A search for the CNVs partly or entirely overlapping with the single deletions in the ISCA database gave the following results: five variants previously described overlapping with the first deletion (four of them correlating with a pathogenic phenotype and one with an uncertain phenotype) and one pathogenic variant with the second deletion. The same search performed for the duplications gave an output of five cases overlapping with the first duplication (one pathogenic, three uncertain and one likely benign) and 11 cases with the second duplication of which four pathogenic, six uncertain, and one likely to be benign. Although none of the single CNVs analyzed gives an unambiguous phenotype as a result, on the whole the first deletion and the second duplication seem to have the most deleterious effect. This might be due to the presence in this region of three MIM-associated genes, *IDS*, *MAMLD1*, and *MTM1*, the only genes mapping in the rearranged region with which a specific pathology has been correlated (Table 1).

A search in the DECIPHER database, submitting the four CNVs separately evidenced a series of cases carrying deletions partly overlapping with the four variants detected by us. However, only one of them (case 256308) presented with a large deletion overlapping the entire chromosomal region involved in the rearrangement here described. The reported phenotype presented some symptoms in common with the subject described by us (delayed speech and language development, intellectual disability) along with other symptoms (soft skin, joint laxity and 2–3 toe syndactyly).

Fig. 1 (a) Array-CGH graphical output. Mapping of the rearrangement in position Xq28 and its magnification showing the details of the two deletions and of the two duplications; (b) UCSC Genome Browser graph of the protein-coding genes mapping in the X

chromosome region (148,500,000–150,000,000 bp) including the described rearrangement (red box: deleted segment; blue box: duplicated segment)

Table 1 Summary of the genes mapping in the deleted/duplicated X chromosome regions in the rearrangement analyzed

Chromosomal region involved	Loci involved	Chromosomal position (first-last bp)	Status	Gene product	Function of the gene product	Observations
148,568,786–148,623,440	<i>IDS</i>	148,560,295–148,586,884	Del*	Iduronate 2-sulfatase	Required for the lysosomal degradation of heparan sulfate and dermatan sulfate	Mutations in this gene are associated with mucopolysaccharidosis type II also known as Hunter syndrome
	<i>IDSP1</i>	148,606,539–148,607,956	Del	Iduronate 2-sulfatase pseudogene 1	Unknown	–
	<i>LOC100131434</i>	148,609,130–148,621,312	Del	Uncharacterized LOC100131434	Unknown	–
	<i>CXorf40A</i>	148,622,519–148,632,086	Del*	Chromosome X open reading frame 40A	May have an important role of cell protection in inflammation reaction	Associated to intrahepatic cholangiocarcinoma, and cholangiocarcinoma
148,648,557–148,734,969	<i>MAGEA9B</i>	148,663,309–148,669,116	Dup	Melanoma antigen family A, 9 -like	Not known, though may play a role in embryonal development and tumor progression	Associated with terminal osseous dysplasia, and enophthalmos
	<i>HSEY2</i>	148,674,172–148,676,974	Dup	Heat shock transcription factor family, X linked 2	Transcription factor	Associated with oligospermia, and male infertility
	<i>TMEM185A</i>	148,678,216–148,713,487	Dup	Transmembrane protein 185A	Transmembrane protein	This gene is best known for localizing to the CpG island of the fragile site FRAXF
						Associated with FRAXF syndrome and atypical autism
						Associated with melanoma
148,830,445–149,029,121	<i>LOC100420321</i>	148,730,846–148,731,851	Dup	Melanoma antigen family A, 11 pseudogene	Unknown	Associated with melanoma
	<i>LOC100420322</i>	148,839,670–148,840,322	Del	Melanoma antigen family A, 10, pseudogene	Unknown	Associated with melanoma
	<i>LOC100420334</i>	148,842,431–148,842,959	Del	Melanoma antigen family A, 11, pseudogene	Unknown	Associated with melanoma
	<i>TMEM185API</i>	148,850,544–148,854,484	Del	Transmembrane protein 185A pseudogene	Unknown	–
	<i>HSEY1</i>	148,855,726–148,858,517	Del	Heat shock transcription factor family, X linked 1	Transcription factor	Associated with oligospermia, and male infertility
	<i>MAGEA9</i>	148,863,600–148,869,399	Del	Melanoma antigen family A, 9	Not known, though may play a role in embryonal development and tumor progression	Associated with terminal osseous dysplasia, and enophthalmos

(continued)

Table 1 (continued)

Chromosomal region involved	Loci involved	Chromosomal position (first-last bp)	Status	Gene product	Function of the gene product	Observations
149,105,821–149,750,457	<i>MAGEA7P</i>	148,890,201–149,890,478	Del	Melanoma antigen family A, 7, pseudogene	Unknown	Associated with melanoma
	<i>DUTP4</i>	148,898,834–148,899,310	Del	Deoxyuridine triphosphatase pseudogene 4	Unknown	–
	<i>MAGEA8</i>	149,009,941–149,014,609	Del	Melanoma antigen family A, 8	Not known, though may play a role in embryonal development and tumor transformation or aspects of tumor progression	Associated with terminal osseous dysplasia, and enophthalmos
	<i>CXorf40B</i>	149,100,415–149,106,716	Dup*	Chromosome X open reading frame 40B	Unknown	Associated with include gastric cancer
	<i>LOC100272228</i>	149,106,766–149,185,018	Dup	Uncharacterized LOC100272228	Unknown	–
	<i>LOC643015</i>	149,282,608–149,284,952	Dup	Nucleolar protein 11 pseudogene	Unknown	–
	<i>MIR2114</i>	149,396,239–149,396,318	Dup	MicroRNA 2114	Post-transcriptional regulation of gene expression	Associated with include ovarian cancer
	<i>XRCC6P2</i>	149,399,291–149,438,002	Dup	X-ray repair complementing defective repair in Chinese hamster cells 6 pseudogene 2	Unknown	–
	<i>MAMLD1</i>	149,531,551–149,682,448	Dup	Mastermind-like domain containing 1	Transactivates the HES3 promoter independently of NOTCH proteins. HES3 is a non-canonical NOTCH target gene which lacks binding sites for RBPJ	Mutations in this gene are the cause of X-linked hypospadias type 2
	<i>MTM1</i>	149,737,047–149,841,616	Dup*	Myotubularin 1	Lipid phosphatase which dephosphorylates phosphatidylinositol 3-monophosphate (PI3P) and phosphatidylinositol 3,5-bisphosphate (PI(3,5)P2). Negatively regulates EGFR degradation through regulation of EGFR trafficking from the late endosome to the lysosome.	Mutations in this gene have been identified as being responsible for X-linked myotubular myopathy

For each gene the name, the position (based on NCBI 37.5/hg19 assembly), the deleted or duplicated status (Del = deleted gene; Dup = duplicated gene; * = partially deleted or partially duplicated gene) as well as the function of the gene product and the related observations are reported

Table 1 reports the genes mapping in each deletion and duplication detected in our case.

The first deletion (spanning region: 148,376,691–148,431,346 bp), partially matching with that previously defined by PCR mapping, leads to the removal of part of the *IDS* gene, of the entire pseudogene *IDSP1* and of a further locus whose function is still unknown (*LOC100131434*). The locus *CXorf40A* (chromosome X open reading frame 40A) is only partly affected by the deletion; such locus encodes for the endothelial-overexpressed lipopolysaccharide-associated factor 1 which might have an important role on cell protection in inflammation reaction.

The second deletion (148,638,257–148,789,779 bp) causes the elimination of five pseudogenes and three genes (*HSFX1*, *MAGEA9*, *MAGEA8*). *HSFX1* gene encodes for the heat shock transcription factor family X linked 1, whose function has not yet been cleared. The genes *MAGEA9* and *MAGEA8* are members of the *MAGEA* gene family, clustered at Xq28, coding for proteins with 50–80% sequence identity to each other, implicated in some inherited diseases. *MAGEA9* is one of the most frequently expressed cancer-testis (CT) genes in bladder tumors (Bergeron et al. 2009).

Among the genes mapping in the duplicated regions, we found another gene of the *MAGEA* cluster (*MAGEA9B*), *MAMLD1* and *MTM1*, along with other genes whose functions are still undefined. *MAMLD1* encodes a mastermind-like domain containing a protein that may function as a transcriptional co-activator. Mutations in this gene are the cause of the X-linked hypospadias type 2 (MIM#300758). *MTM1* gene encodes for the myotubularin protein, a phosphatase specifically acting on two types of phosphoinositides. Mutations in this gene are associated with the X-linked myotubular myopathy (Oliveira et al. 2013). In the case here described, *MTM1* gene encompasses the duplication boundary, thus being involved for 13% of its length.

Patients bearing large deletions involving the *IDS* gene have been previously described. Apart from the female cases, few reports described deletions encompassing the region proximal to the *IDS* locus, removing the fragile site mental retardation 1 (*FMRI*) and fragile site mental retardation 2 (*AFF2*) genes (Brusius-Facchin et al. 2012; Burruss et al. 2012; Probst et al. 2007; Clarke et al. 1992; Birot et al. 1996; Clarke et al. 1990), or extending both proximally and distally towards the telomeric sides of *IDS* (Brusius-Facchin et al. 2012; Honda et al. 2007; Birot et al. 1996; Timms et al. 1997). The phenotype observed in these cases spans from a severe to an early-onset severe form of Hunter disease presenting additional symptoms associated with the extra genes involved in the deletion, such as mental retardation associated with *FMRI* and *AFF2* genes

and muscular hypotonia correlated with the *MTM1* locus. Among the deletions extending only distally to the *IDS* locus reported in the literature (Beck et al. 1992; Timms et al. 1997), recently Brusius-Facchin (Brusius-Facchin et al. 2012) characterized a molecular rearrangement consisting of a 3.9 Mb deletion removing *FMRI*, *AFF2* and *IDS* and a 3.1 Mb duplication encompassing *MAMLD1* and *MTM1* in a patient with a peculiar Hunter phenotype accompanied with severe intellectual disability and hypotonia.

Similarly to some of the cases reported in the literature, one of the peculiarities of the patient here presented is the extreme severity of the pathology, which led to a very precocious clinical diagnosis. During his life he developed most of the symptoms clinically associated with Hunter disease, including hydrocephalus and arterial hypertension.

Another feature is the mild response to enzyme replacement therapy observed in this subject. Urinary GAGs showed a fluctuating pattern during the follow-up but did not normalize, evidencing, after 6 years of treatment, a reduction of 24% with respect to pre-therapy value (data not reported). The detection of anti-*IDS* neutralizing antibodies, detected after 4 years of ERT, might in part explain the GAG pattern registered during the follow-up. On the opposite, hepato- and splenomegaly improved, although slowly, and they were no longer detectable after 4 years of treatment.

Although therapeutic inclusion criteria or efficacy of ERT are beyond the aim of our work, given that important genomic rearrangements have always been associated with severe forms of Hunter syndrome, we believe that these cases should be included in the discussion related to a possible discontinuation of the therapy for the severe forms of the disease, as suggested by Muenzer (Muenzer et al. 2012).

Although gene deletions are in most cases linked to the severe form of the pathology associated with each gene, as for total or partial duplications the prediction of the resulting phenotype is not so straightforward. Pathogenic gene duplications often involve dosage-sensitive genes or genes coding for proteins which tend to aggregate (Conrad and Antonarakis 2007). Moreover a partial gene duplication might cause in turn gene disruption leading to the production of a nonfunctional protein according to which region of the gene is involved. Although the second duplication detected in our patient involves part of *MTM1* gene, it does not seem to affect the phenotype observed, as the child does not present with hypotonia, the typical symptom associated with the X-linked myotubular myopathy.

Although carrier of the same genetic alteration, the mother, clinically examined, did not present any signs or symptoms associated with the deleted or duplicated genes involved in the described rearrangement.

Conclusion

In conclusion, even though the patient here described carries a complex composite rearrangement, extending 1.2 Mb from the *IDS* locus, only the first deletion appears to be the main genetic determinant of the disease phenotype evidenced in the subject so far. The extension of the rearrangement to several extra genes does not seem to affect the peculiar clinical Hunter phenotype as no additional symptoms associated with these genes have been evidenced up to now, through patient's regular monthly check-ups. This might be partly determined by the fact that while pathological phenotypes have been so far associated with mutations or deletions of the two principal disease-related genes here involved, *MAMLD1* and *MTM1*, no potential effects of their total or partial duplication have been so far described. Unfortunately, this does not allow to exclude the presence of a "contiguous gene syndrome" as well as the potential rising of future additional pathological symptoms associated with the other genes involved.

Acknowledgments Part of the data in this manuscript were obtained from the ISCA Consortium database (www.iscaconsortium.org), which generates this information using NCBI's database of genomic structural variation (dbVar, www.ncbi.nlm.nih.gov/dbvar/), study nstd37. Samples and associated phenotype data were provided by ISCA Consortium member laboratories.

This study makes use of data generated by the DECIPHER Consortium. A full list of centers who contributed to the generation of the data is available from <http://decipher.sanger.ac.uk> and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust.

One Sentence Take-Home Message

First case described of a Hunter patient with two deletions alternate with two duplications, involving exons 1–7 of the *IDS* gene and extending 1.2 Mb distally to it.

Details of the contributions of individual authors

AZ: conception and design of the study, performing of *IDS* molecular analysis, analysis and interpretation of molecular data, writing and critical revision of the manuscript. RT: conception and design of the study, clinical data collection, writing and critical revision of the manuscript. AR: clinical data collection and analysis, critical revision of the manuscript. CR: aCGH analysis, interpretation of aCGH data, critical revision of the manuscript. NG: urinary GAG analyses, critical revision of the manuscript. MCa: critical revision of the manuscript. MCl: critical revision of the manuscript. MS: conception and design of the study, critical revision of the manuscript, final approval of the manuscript.

Name of one author who serves as guarantor

Maurizio Scarpa.

Details of funding

No funding was raised to conduct this study.

Details of ethics approval

Ethics approval was not required to perform the described studies.

A patient consent statement

Informed consent for genetic analyses in the patient and relatives was obtained from the subject involved in the study, parents or tutors.

Conflict of interest

Maurizio Scarpa has received research grants and honoraria and travel support for speaking engagements from Actelion, Shire HGT, Genzyme Corporation, and BioMarin.

Alessandra Zanetti, Rosella Tomanin, Angelica Rampazzo, Chiara Rigon, Nicoletta Gasparotto, Matteo Cassina, and Maurizio Clementi declare no conflicts of interest.

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