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Case report

A new sporadic case of early-onset Loeys-Dietz syndrome due to the recurrent mutation p.R528C in the *TGFBR2* gene substantiates interindividual clinical variability

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Abstract. We report on a 2-year-old Polish girl with typical manifestations of Loeys-Dietz syndrome (LDS), a rare genetic condition belonging to the group of Marfan-related disorders. The characteristic LDS symptoms observed in the girl included craniofacial dysmorphism (craniosynostosis, cleft palate, hypertelorism), arachnodactyly, camptodactyly, scoliosis, joint laxity, talipes equinovarus, translucent and hyperelastic skin, and umbilical hernia. Mild dilatation of the ascending aorta and tortuous course of the left internal carotid artery were recognized during her second year of life. Molecular genetic testing revealed a heterozygous missense mutation (c.1582C>T, p.R528C) in the transforming growth factor beta receptor II gene (*TGFBR2*). This mutation has been previously associated with LDS in 5 unrelated cases, and was never reported in patients with other Marfan-related disorders. Comparison of the phenotypes of our patient and these 5 individuals with c.1582C>T showed that only the hallmark triad of the syndrome – consisting of hypertelorism, aortic root dilatation/aneurysm, and cleft palate or bifid uvula – was present in all 6 cases. Interestingly, none of the 5 individuals who underwent psychological evaluation showed developmental delay. The pattern of all other LDS features showed interindividual variability. Our data support the recently reported observation that symptoms of LDS can develop at a very young age, making early diagnosis and management essential for these patients. This is the first report on a Polish infant with typical LDS symptoms caused by a *TGFBR2* mutation.

Keywords: Loeys-Dietz syndrome, marfanoid habitus, Marfan-related disorder, *TGFBR1*, *TGFBR2*, missense mutation, arterial tortuosity.

Introduction

Loeys-Dietz syndrome (LDS) is a rare, recently characterized autosomal-dominant genetic condition with marfanoid habitus, belonging to the Marfan-related disorders, a subset of connective tissue diseases with skeletal, ocular, and cardiovascular involvement. The initial report on LDS appeared in 2005 (Loeys et al. 2005). The authors characterized 10 families with the syndrome and found heterozygous mutations in the genes encoding transforming growth factor beta receptor I (*TGFBR1*) or II (*TGFBR2*). A year later, Loeys et al. (2006) described 30 additional individuals

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with the diagnosis of typical LDS. Nearly all patients presented with a triad of symptoms, comprising arterial tortuosity and aneurysms, hypertelorism, and bifid uvula or cleft palate, and all harboured a *TGFBR1* or a *TGFBR2* heterozygous mutation. In addition, in the same work, Loeys et al. (2006) studied a cohort of 40 patients with a vascular Ehlers-Danlos syndrome-like (EDS IV) phenotype without characteristic collagen type III (*COL3A1*) anomalies or craniofacial dysmorphism typical of LDS, and identified heterozygous mutations either in *TGFBR1* or *TGFBR2* in 12 probands. LDS type I was proposed to stand for the syndrome with typical facial dysmorphic features, and LDS type II to describe the syndrome without craniofacial affectation. Both entities are characterized by aggressive arterial aneurysms (Loeys et al. 2006). Occasional findings in LDS type I encompass craniosynostosis, congenital heart defect, structural brain abnormality, hydrocephaly, and mental retardation/developmental delay (Loeys et al. 2005, 2006; Stheneur et al. 2008). In order to distinguish between the genetic background of LDS type I and type II, additional nomenclature subdivides cases into subtype A, resulting from *TGFBR1* mutations, and subtype B, caused by *TGFBR2* mutations (Loeys et al. 2006). Currently, with a growing number of LDS patients, a detailed clinical phenotype of the syndrome is beginning to emerge.

In this report, we describe a Polish girl with typical LDS type I caused by a recurrent *TGFBR2* mutation (p.R528C). We refer her clinical picture to the phenotype of 5 other LDS cases resulting from the same mutation, and to the total LDS type I population reported to date in the literature.

Case report

The proband, a 2-year-old girl of Polish descent, was born by elective Caesarean section after uneventful pregnancy (G2P2) at 39 weeks of gestation to nonconsanguineous and healthy parents: 30-year-old mother and 33-year-old father. At birth, her weight was 2800 g (10th–25th percentile), length 51 cm (75th–90th percentile), head circumference 34 cm (50th percentile), and Apgar score was 10 at 1, 3, and 5 minutes. Physical examination after birth showed craniofacial dysmorphism with prominent forehead, cleft soft and hard palate, and long slender fingers. An ultrasound scan of the brain at 3 months revealed external hydrocephaly and a subependymal cyst (8 mm in diameter) of the right lateral ventricle. On repeated ultrasound brain controls, mild dilatation of the lateral ventricles, with gradually decreasing external hydrocephaly were noted. Hearing tests performed at the age of 8 months showed initially right-sided and subsequently bilateral conductive hypoacusis. The girl was referred to our genetics clinic for diagnosis and first investigated at the age of 9 months. Upon examination, she presented with craniofacial dysmorphic features comprising scaphocephalic shape of the head with frontal bossing due to craniosynostosis (premature closure of sagittal suture), proptosis, hypertelorism, mild epicanthal folds, blue sclerae, short nose with flat nasal bridge, micrognathia, cleft hard and soft palate, and thin upper lip (Figures 1a, b, c, d). Her length and head circumference was then 65 cm (<3rd percentile) and 45 cm (50th–75th percentile), respectively. Enlarged, prominent anterior fontanelle and widening of subcutaneous veins on the skull were conspicuous (Figure 1c). Furthermore, arachnodactyly of the hands and feet, camptodactyly of the 5th fingers (Figure 1e), joint laxity, hyperelastic translucent skin, and umbilical hernia were noted (Figure 1a). Psychomotor development was apparently normal. Cardiac sonography at 16 months revealed a mild dilatation of the ascending aorta and a trace patent fora-

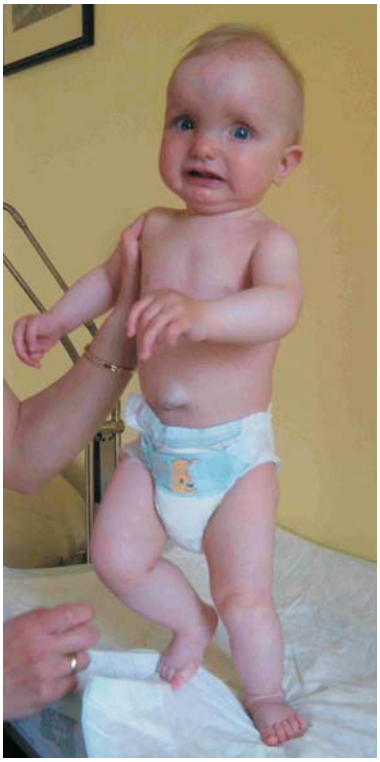

Figure 1A. A whole view of the proband, showing prominent umbilical hernia (age 9 months)

Figure 1B. Facial dysmorphic features of the proband (hypertelorism, prominent forehead, short nose with flat nasal bridge, thin upper lip, and discrete epicanthal folds)

Figure 1C. Lateral view of the proband (abnormal skull shape with frontal bossing, micrognathia, widened veins, and translucent skin on the head)

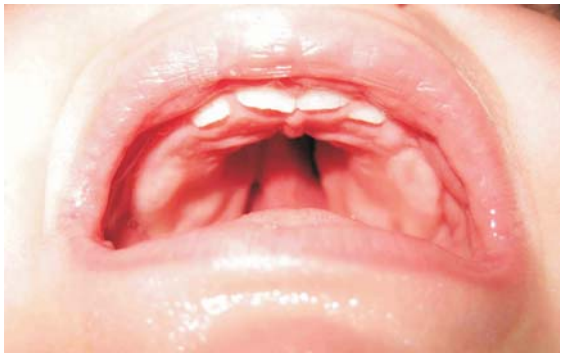

Figure 1D. Cleft soft and hard palate seen in the proband

men ovale (PFO) without haemodynamically significant interatrial shunting. The girl was reinvestigated in the genetics clinic at 19 months of age. Dysmorphic features seen during her first evaluation were still present. Her growth was slightly retarded, with body weight of 9.8 kg (< 3rd percentile), height of 77.5 cm (3rd–10th percentile), and head circumference of 48.5 cm

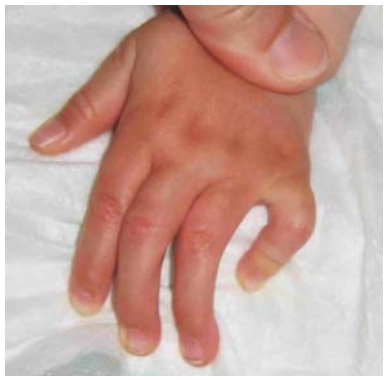

Figure 1E. Arachnodactyly of the hand and camptodactyly of the 5th finger noted in the proband

(50th–75th percentile). According to anamnesis, developmental milestones were achieved on time, with independent sitting at 7, walking at 12, and expressive speech (several first words) at 12 months. MRI of the brain showed no structural malformation, but magnetic resonance angiography of the brain vessels at 23 months of age disclosed abnormal pars cavernosa of the left internal carotid artery, described as most probable tortuous course of the artery, or less likely arterial aneurysm. Chromosomal analysis on peripheral blood lymphocytes with resolution of 550 bands per haploid genome revealed a normal female karyotype (46,XX). Except mild hypertelorism in the father, both parents had no signs of craniofacial dysmorphism. Family history was noncontributory.

To confirm our clinical diagnosis of LDS, we performed molecular genetic testing of the *TGFBR1* and *TGFBR2* genes (informed consent of the parents was obtained prior to genetic testing). All exons and flanking intronic regions of both genes were amplified and sequenced according to Matyas et al. (2006). In our proband, we detected a heterozygous substitution of C>T at position 1582 (c.1582C>T) in exon 7 (Figure 2), resulting in a missense mutation at codon 528 in the C-terminal fragment of the TGFBR2 protein (p.R528C). The mutation affects a highly conserved amino acid residue (arginine) and was predicted to damage protein function (cf. Matyas et al. 2006). However, the possibility that c.1582C>T has an effect on mRNA expression or splicing cannot be excluded. Additionally, 2 known *TGFBR2* polymorphisms, one homozygous for IVS2+7A>G (rs1155705) and the other heterozygous for IVS3-4T>A (rs11466512), were identified. Deletions/duplications of all *TGFBR1* and *TGFBR2* exons were excluded by multiplex ligation-dependent probe amplification (MLPA) using the SALSA kits P065 and P148, as described

elsewhere (Matyas et al. 2007). The mutation c.1582C>T was not detected in the leukocyte DNAs of the unaffected parents, but (germline) mosaicism cannot be excluded. Haplotype analysis using 8 short tandem repeat markers on 6 different chromosomes confirmed paternity and thus *de novo* occurrence of c.1582C>T (p.R528C).

prerequisite for adequate management of LDS patients. Thorough repeated clinical assessment of the cardiovascular system (echocardiography, angiography, MRI angiography), along with aggressive surgical repair of aneurysms, should be undertaken to prevent future vascular events (Yetman et al. 2007).

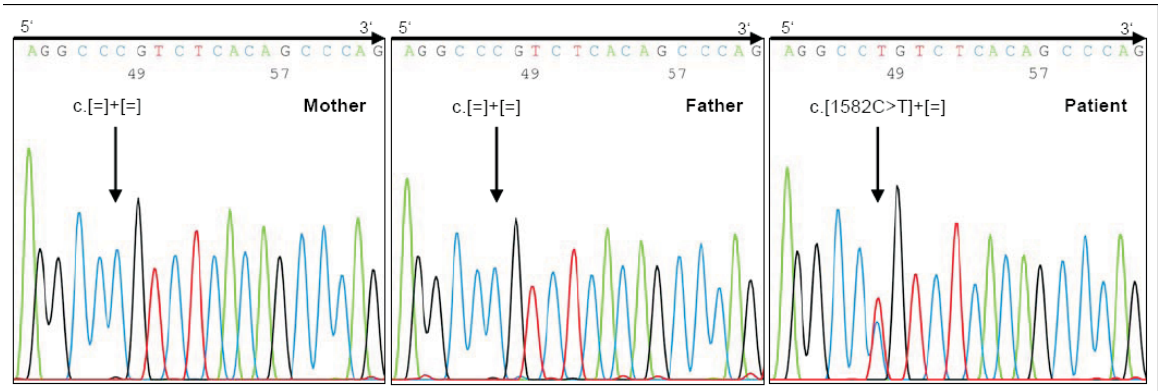

Figure 2. Partial forward sequence of the *TGFBR2* gene (exon 7) flanking the mutation c.1582C>T (p.R528C) in our patient and her parents. Arrows indicate the site of mutation

Discussion

So far, a series of about 80 LDS patients have been described in the literature, with a majority of adult individuals (e.g. Loeys et al. 2005; 2006; Sakai et al. 2006; Singh et al. 2006; Matyas et al. 2006; Akutsu et al. 2007; Togashi et al. 2007; LeMaire et al. 2007; Aalberts et al. 2008; Stheneur et al. 2008; Frederic et al. 2008). The clinical phenotype of LDS overlaps to some extent with Marfan syndrome (MFS), including the propensity for aortic aneurysm and dissection, and thus the condition may pose diagnostic difficulties and still be lumped together with MFS (Ades et al. 2006). In addition to the phenotypic overlap, MFS and LDS share partially common genetic background. Besides the *FBN1* mutations responsible for more than 90% of the cases, MFS with or without fulfilment of Ghent criteria, can be caused by mutations in the TGF receptor genes (Mizuguchi et al. 2004; Disabella et al. 2006; Matyas et al. 2006; Singh et al. 2006; Stheneur et al. 2008). LDS is caused solely by *TGFBR1* or *TGFBR2* mutations, and as a result it seems to have a distinct natural history. With respect to MFS, the syndrome conveys a higher risk of arterial dissection, without considerable dilatation of the affected vessel, and is characterized by marked arterial tortuosity or aneurysms throughout the arterial tree. Furthermore, the mean age of a deadly vascular episode without treatment is estimated at 26 years, which is at least a few years earlier than in MFS. Therefore, it is of great relevance to make a distinction between these 2 conditions. Early diagnosis is the

Here, we present a female infant with typical manifestation of LDS type I, initially seen and evaluated by us at the age of 9 months. Mutational analysis of the *TGFBR1* and *TGFBR2* genes revealed a *de novo TGFBR2* missense mutation (c.1582C>T, p.R528C). This mutation has been previously described in 5 unrelated LDS cases (Loeys et al. 2005; 2006; LeMaire et al. 2007; Stheneur et al. 2008). Interestingly, to our knowledge, the mutation has never been identified in the individuals with MFS or other MFS-related disorders (Shprintzen-Goldberg syndrome, thoracic aortic aneurysm and dissection), suggesting its specificity to LDS. Comparison of the phenotypes of all 6 individuals with c.1582C>T (p.R528C) showed that only the hallmark triad of the syndrome – consisting of hypertelorism, aortic root dilatation/aneurysm, and cleft palate or bifid uvula – was present in all the patients. The above features occur with the highest frequencies in overall LDS type I population, regardless of the mutation. Interestingly, none of the 5 affected individuals who underwent psychological evaluation presented with psychomotor retardation. The overall incidence of developmental delay in a subset of 67 LDS type I patients was estimated to be about 10% (Table 1). The lack of this feature in the patients with p.R528C *TGFBR2* mutation most probably results from the small number of individuals in our study. On the other hand, it is also possible that the mutation has little impact on neurocognitive development. The pattern of other LDS type I characteristics caused by the p.R528C *TGFBR2*

Table 1. Overview of clinical features observed in patients with the *TGFBR2* mutation p.R528C in relation to the overall LDS type I (LDS1) population. Presence or absence of a feature is indicated by + or –, respectively. Bold numbers highlight the features demonstrated in all or none of the analysed patients.

Clinical feature	Loeys et al. (2005)	Loeys et al. (2006) – case 1	Loeys et al. (2006) – case 2*	LeMaire et al. (2007)	Stheneur et al. (2008)	Our case	Total of p.R528C *TGFBR2*	Total of up to 77 LDS1 patients[#]
Craniofacial:								
Hypertelorism	+	+	+	+	+	+	**6/6**	48/74 (65%)
Cleft palate/abnormal uvula	+/+	+/+	–/+	–/+	–/+	+/–	**6/6**	57/74 (77%)
Malar hypoplasia	+	+	–	+	+	–	4/6	38/64 (59%)
Retrognathia	–	+	–	+	–	+	3/6	29/62 (47%)
Craniosynostosis	–	–	–	–	–	+	1/6	22/73 (30%)
Proptosis	–	–	–	–	–	+	1/6	?
Blue sclerae	–	+	–	–	–	+	2/6	23/64 (36%)
Cardiovascular:								
Aortic root dilatation/aneurysm	+	+	+	+	+	+	**6/6**	73/77 (95%)
Arterial tortuosity	+	+	+	+	–	+	5/6	26/38 (68%)
Aneurysm of other vessels	PAA	?	DAA	multiple	?	?	3/6–6/6	29/60 (48%)
Congenital heart defect	–	–	PDA	–	–	PFO	2/6	?
Mitral valve prolapse	+	–	–	–	+	–	2/6	6/28 (21%)
Musculoskeletal:								
Dolichostenomelia	–	–	–	–	+	–	1/6	19/66 (29%)
Arachnodactyly	+	+	+	–	+	+	5/6	37/66 (56%)
Camptodactyly	+	–	–	–	–	+	2/6	23/65 (35%)
Talipes equinovarus	–	+	+	–	–	–	2/6	23/65 (35%)
Joint laxity	–	+	+	+	+	+	5/6	44/66 (67%)
Pectus deformity	–	+	+	+	+	–	2/6	42/66 (64%)
Scoliosis	–	–	+	+	+	–	3/6	35/66 (53%)
Umbilical/inguinal hernia	?	–	+	+	–	+	3/5–4/6	5/17 (29%)
Skin:								
Velvety texture	–	+	?	+	–	+	3/5-4/6	14/52 (27%)
Translucency	–	+	+	+	–	+	4/6	18/52 (35%)
Hyperelastic skin	?	?	+	–	–	+	2/4–4/6	?
Nervous system:								
Developmental delay	–	**	–	–	–	–	**0/5**–1/6	7/67 (10%)
Hydrocephalus	–	?	?	–	–	+	1/4–3/6	6/59 (10%)

According to literature: Loeys et al. (2005, 2006); Sakai et al. (2006); Singh et al. (2006); Matyas et al. (2006); Akutsu et al. (2007); Togashi et al. (2007); LeMaire et al. (2007); Aalberts et al. (2008); Stheneur et al. (2008); * patient died at the age of 16 years, ** patient evaluated at the age of 6 months; ? = no data available; PAA = pulmonary artery aneurysm; DAA = descending aorta aneurysm; PDA = patent ductus arteriosus; PFO = persistent foramen ovale.

mutation showed more interindividual variability (Table 1).

Although our patient was very young, she presented with mild aortic root dilatation, while magnetic angiography of the brain vessels disclosed a corkscrew course (or aneurysm) of the left internal carotid artery. The reported case confirms that symptoms of LDS begin to develop at a young age and thus an early recognition that allows appropriate management and treatment of these patients is needed (Loeys et al. 2005; Stheneur et al. 2008). Genetic testing of *TGFBR1* and *TGFBR2*, especially in cases with mild or inconspicuous facial dysmorphism, should be regarded as the method of choice to verify tentative diagnosis of LDS.

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