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CASE REPORT

The Identification of a Novel Thiopurine S-Methyltransferase Allele, *TPMT*45*, in Korean Patient with Crohn's Disease

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Abstract: Pediatric Crohn's disease (CD) carries a higher genetic susceptibility and an increased risk of a more aggressive disease course than adult CD. Treatment of CD is based on immunomodulatory drugs, such as thiopurines. The enzyme mainly involved in drug metabolism is thiopurine S-methyltransferase (TPMT). An increased concentration of drug metabolites can cause adverse drug effects, such as myelosuppression and hepatotoxicity; therefore, assessing the activity of TPMT is essential both before and during treatment. *TPMT* genotyping result is not affected by previous thiopurine dose and currently is the primary component of TPMT activity and disease monitoring. Until now, more than 40 allelic variants of the *TPMT* gene have been reported, with most of them having an uncertain or no enzyme function. In this article, we report the first case of a novel *TPMT* allele, *TPMT*45*, that was identified in a Korean girl with CD whose findings suggested decreased TPMT activity. This newly observed variant is caused by a single nucleotide polymorphism resulting in nonsense mutation (c.676C>T, p. R226*) and the partial loss of amino acids in the TPMT protein. Initially, the patient began azathioprine at a standard dosage (1.5 mg/kg/day), and her laboratory results, including red blood cell (RBC) TPMT activity (6-methylmercaptopurine 2.68 nmol/mL/h and 6-methylmercaptopurine riboside 4.82 nmol/mL/h) along with thiopurine metabolite levels (6-thioguanine nucleotides 479.3 pmol/8×10^8 RBC), suggested an enzyme deficiency. The thiopurine dose was reduced to half (0.7 mg/kg/day), and the follow-up metabolite results as well as the associated inflammatory markers were continuously within reference ranges. Along with an improvement in the patient's subjective reports and clinical symptoms, the patient demonstrated a good treatment response to the adjusted dose. The results of our report illustrate the importance of *TPMT* genotyping and pharmacogenetic-based thiopurine dose adjustment. Further research should focus on the functional characterization and impact on this novel allele's treatment effect.

Keywords: *TPMT*45*, Crohn's disease, thioupurine, single nucleotide polymorphism, TPMT activity, drug metabolism

Introduction

Crohn's disease (CD) is a chronic relapsing and remitting inflammatory bowel disease (IBD), and its incidence is increasing worldwide.[1,2] Approximately 20–25% of patients are pediatric, and children have greater genetic susceptibility and typically a more aggressive disease course than adult CD patients.[3,4] The treatment of pediatric CD includes immunomodulatory agents, such as thiopurines and methotrexate,[1,3] and disease monitoring markers include pediatric Crohn's disease activity index (PCDAI), C-reactive protein (CRP) and fecal calprotectin.[4,5]

Thiopurine drugs, such as azathioprine, 6-mercaptopurine, and thioguanine, are widely used to treat IBD, as well as leukemia, rheumatic diseases, and solid organ transplant rejection.[6,7] Thiopurines are S-methylated by the enzyme thiopurine S-methyltransferase (TPMT) and are thereby converted to metabolites, including thioguanine nucleotides (TGNs) and methylated mercaptopurine (MMPN).[7,8] TGNs are involved in the inhibition of de-novo purine synthesis in hematopoietic stem cells,[8–10] while MMPN is associated with hepatotoxicity.[9] In patients being treated with thiopurines, measuring these metabolites in washed red blood cells (RBCs) can be used for drug monitoring in association with TPMT activity.[11,12]

The activity of TPMT is largely affected by the *TPMT* gene polymorphism, which is inherited as a monogenic, autosomal codominant trait.[8,13,14] To date, more than 40 allelic variants of the *TPMT* gene have been reported to the *TPMT* nomenclature committee and assigned allele names (http://www.imh.liu.se/tpmtalleles).[10] Most of these alleles have a single nucleotide polymorphism that causes amino acid changes that reduce TPMT activity.[7] According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines, patients with *TPMT* variants demonstrate moderate to severe myelosuppression; therefore, 30–70% of the standard dose is recommended for heterozygous *TPMT*-deficient patients (ie, TPMT intermediate metabolizer phenotype), while 10% of the standard dose is typically prescribed for homozygous *TPMT* deficient patients (ie, TPMT poor metabolizer phenotype).[13] Previous studies have shown that three *TPMT* alleles (*TPMT*2, *TPMT*3A*, and *TPMT*3C*) contribute to approximately 95% of TPMT deficiencies.[8,10,15] Ethnic variability in the alleles exists, with *TPMT*3A* being most commonly deficient in Caucasians and Southwest Asians, whereas *TPMT*3C* is most likely to be deficient in Africans and Southeast Asians.[6,7,15] Among the Korean population, *TPMT*3C* and *TPMT*6* are the two most common variant alleles;[15–17] the allele frequencies of *TPMT*1* (wild-type), *3C*, and *6* are 98.1%, 1.4%, and 0.2%, respectively.[15]

Since pediatric CD involves a genetic susceptibility, pharmacogenetic tests carried out both before and during treatment can contribute to personalized therapy through dose adjustments and attempts to reduce adverse effects. In this article, we report the first case of a novel *TPMT* allele, *TPMT*45*, found in a Korean girl with CD whose findings suggested TPMT deficiency. This study was reviewed and approved by the Institutional Review Board of Samsung Medical Center in Seoul, Korea (approval number: 2012–04-106-020). Parental informed consent including the case details and images published was obtained since the patient was under the age of 19. The research was carried out in accordance with the World Medical Association Declaration of Helsinki.

Case Report

A 17-year-old girl was referred to our hospital in April 2020 with a 4-month history of abdominal pain, diarrhea, hematochezia, and weight loss >10% of her baseline body weight. One month ago, she was admitted to another hospital. At that time, laboratory tests showed a white blood cell count of 4.74 x10^9/L, an absolute neutrophil count of 3.81×10^9/L, an absolute lymphocyte count of 0.74×10^9/L, a hemoglobin of 90 g/L, a hematocrit of 25.4%, a platelet count of 397 x10^9/L, an erythrocyte sedimentation rate (ESR) of 77 mm/h, a CRP of 41.2 mg/L, and albumin level of 35 g/L; other laboratory tests were unremarkable. The stool occult blood test was positive and the fecal calprotectin level was >1000 μg/g. Ileocolonoscopy revealed multiple deep ulcers from the terminal ileum to the rectum. The upper gastrointestinal endoscopy findings were unremarkable. Magnetic resonance enterography revealed enhanced wall thickening in the entire colon and ileum without evidence of stricturing or penetrating complications. Pelvic magnetic resonance imaging showed perianal inflammation at the 5-o'clock direction. The patient was diagnosed with CD of an A2, L3, B1p, G0 phenotype according to the Paris classification.[18] The patient's PCDAI was 60, indicating severe CD, and the simple endoscopic score for CD was 32. Right after diagnosis, the patient was prescribed azathioprine (50 mg once daily, 1.4 mg/kg/day), mesalazine (1000 mg twice daily, 56.0 mg/kg/day), prednisolone (20 mg twice daily, 1.1 mg/kg/day), and exclusive enteral nutrition for CD treatment. After three weeks of azathioprine treatment, the patient was referred to our hospital.

According to our clinic's protocol, every patient with IBD is routinely performing *TPMT* genotyping. Briefly, *TPMT* genotyping was performed according to the following procedures. Peripheral blood from the patient was collected in a vacuum tube containing ethylenediaminetetraacetic acid as a preservative. Total genomic deoxyribonucleic acid (DNA) was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. All coding exons with their intron-exon boundaries

of the *TPMT* gene (NCBI reference sequence: NM_000367.2) were amplified by polymerase chain reaction (PCR). PCR was performed using a GeneAmp PCR system 9700 thermal cycler (Applied Biosystems, Foster City, California, USA). Cycle sequencing was carried out using BigDye Terminator Cycle Sequencing Ready Reaction Kits (Applied Biosystems) and an ABI 3130xl genetic analyzer (Applied Biosystems).

The sequencing results showed that the patient had a single nucleotide polymorphism (SNP) of 676C>T (Figure 1), resulting in a premature stop codon (Arg226*) in exon 10. In silico analysis using MutationTaster[19] (Genome Reference Consortium Human Build 37_ensembl 69) was performed and predicted this SNP as deleterious. This SNP fulfilled the inclusion criteria for the *TPMT* Nomenclature Committee for novel alleles[10] and was named *TPMT*45* (exon 10, c.676C>T, p.R226*rs761990479). Another variant was not observed, which confirmed the patient's *TPMT* genotype as *TPMT*1/*45*.

A thiopurine metabolite analysis was performed using an API4000 tandem mass spectrometer (Applied Biosystems, Foster City, California, USA) equipped with an Agilent Technologies Series 1200 high-performance liquid chromatography system (Agilent Technologies, Santa Clara, California, USA) and an electrospray ionization source that was operated in positive-ion detection mode.[20] Intra- and inter-assay coefficients of variation for the thiopurine metabolite measurements were <10%.[20] The lower limits of quantification were 0.1 mol/ L for 6-TGN and 0.5 mol/L for 6-MMPN. The concentration of the 6-TGN was 479.3 pmol/8×10^8 RBC, indicating a high risk of leukopenia, while the concentration of 6-MMPN was 609.5 pmol/8×10^8 RBC, which suggested a lower risk of hepatotoxicity (<5700 pmol/8×10^8 RBC).

To evaluate the RBC TPMT activity profile, mesalazine was withheld from the patient for 48 hours prior to blood sampling. The specimen obtained from the patient was sent to the Mayo Clinic Laboratories (Rochester, Minnesota, USA) for testing. Using three different substrates and a liquid chromatography-tandem mass spectrometry method, RBC TPMT activity was shown to be low in two of the three substrates: 2.68 of 6-MMPN (reference range: 3.00–6.66), 4.82 of 6-MMPN riboside (reference range: 5.04–9.57) and 4.19 of 6-methylthioguanine riboside (reference range: 2.70–5.84), with all units in nmol/mL/h.

Collectively, laboratory results of this patient suggest TPMT deficiency.[9] The dosage of azathioprine was reduced to 25 mg once daily (0.7 mg/kg/day), and prednisolone was tapered since the patient was a non-responder to steroids. She maintained exclusive enteral nutrition for eight weeks and was treated with infliximab. The patient's clinical symptoms improved, even with the azathioprine reduction and steroid tapering. The patient's PCDAI score decreased to zero, and all follow-up laboratory test results (including complete blood cell counts, ESR, CRP, and albumin) were all within normal limits. The concentration of 6-TGN decreased to 364.0 pmol/8×10^8 RBC, which was within the therapeutic range (235.0–450.0 pmol/ 8×10^8 RBC).

The patient is on maintenance therapy and regularly visits the outpatient department for a PCDAI check and routine laboratory follow-up. After three months on a decreased dose, the patient's PCDAI was still zero, and follow-up laboratory tests were all within normal limits, which indicated that there was no evidence of severe leukopenia or hepatotoxicity. Follow-up level of stool calprotectin was 5.5 μg/g, while the blood thiopurine metabolite levels of 6-TGN and 6-MMPN were 415.3 pmol/8×10^8 RBC and 159.3 pmol/8×10^8 RBC, respectively. The patient's treatment course along with her major laboratory findings are shown in Figure 2, and clinical laboratory results are listed in Table 1.

Discussion

This is the first report of a patient with *TPMT*45*, which is only the third nonsense mutation (c.676C>T; p.Arg226*) to be identified among *TPMT* alleles after *TPMT*3D* and *TPMT*37*.[10,21] Since nonsense mutations result in a premature stop codon, the synthesized messenger ribonucleic acid (mRNA) may undergo degradation to prevent production of a truncated protein.[22] It is possible that a decreased amount of protein production may contribute to thiopurine metabolism. The increased initial concentration of the thiopurine metabolite (6-TGN) in this patient
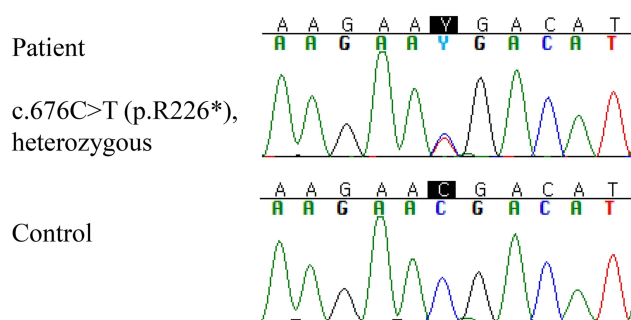
Figure 1 Sequence analysis result of the *TPMT* gene.
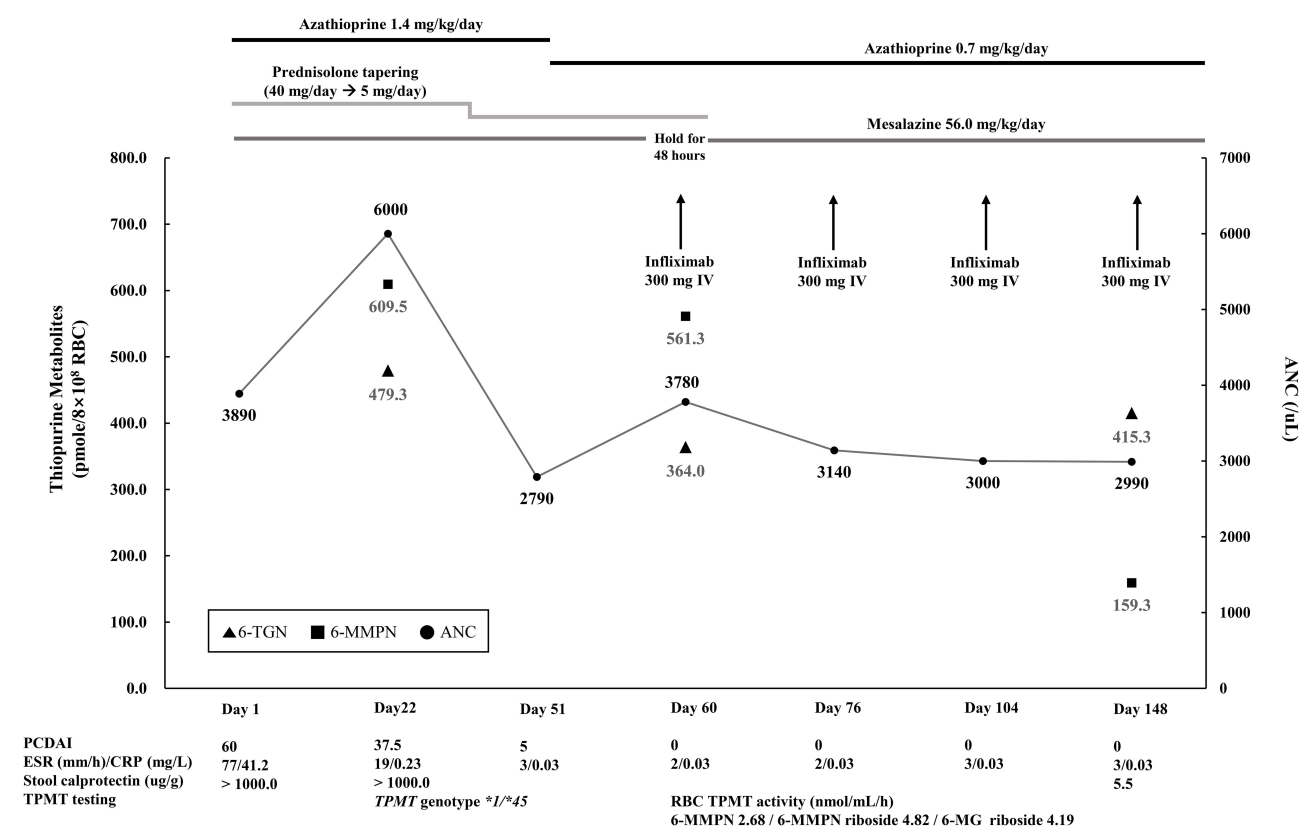
Figure 2 Treatment course of the patient.
Notes: Treatment Day 1 is corresponding to the first day of azathioprine use (date unscaled). PCDAI and other laboratory results normalized, following a reduction in azathioprine to half of the initial dose.
Abbreviations: PCDAI, Pediatric Crohn's Disease Activity Index; ANC, absolute neutrophil count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; 6-TGN, 6-thioguanine nucleotides; RBC, red blood cell; TPMT, thiopurine S-methyltransferase; 6-MMPN, 6-methylmercaptopurine; 6-MG, 6-methylguanine.

also supports this assumption, showing correlation with the RBC TPMT activity profile results.

Pediatric CD is more aggressive than adult CD, with a stronger impact of genetic determinants.[1,3] Many pediatric

CD patients begin thiopurine therapy at a standard dosage (eg, 1.0–2.0 mg/kg/day) and when available, testing of the TPMT activity (genotype or phenotype) is also suggested before initiating treatment.[3,23] In most clinical settings,

Table 1 Clinical Laboratory Results

Variable (Unit)	Reference Range	Azathioprine Day						
		1	22	51	60	76	104	148
WBC (/mm^3)	3800–11,000	4740	7210	5590	6370	5420	5940	5830
ANC (/mm^3)	1900–7400	3890	6000	2790	3780	3140	3000	2990
ALC (/mm^3)	1100–3900	740	1070	2390	2050	1800	2400	2450
Hb (g/dL)	11.2–14.8	9.0	9.3	11.2	11.2	11.1	12.0	11.8
Hct (%)	39.0–50.0	25.4	29.3	36.0	35.8	34.9	37.3	36.2
PLT (/mm^3)	150,000–400,000	397	250	236	224	278	210	230
ESR (mm/h)	1–20	77	19	3	2	2	3	3
CRP (mg/L)	<3.0	41.2	0.23	0.03	0.03	0.03	0.03	0.03
Albumin (g/dL)	3.2–4.8	3.5	4.1	4.2	4.4	4.5	4.4	4.7
Stool calprotectin (μg/g)	<50.0	>1000.0	>1000.0					5.5

Notes: Azathioprine day 1 indicates the first day of azathioprine use, and azathioprine was reduced to half of the initial dose in day 51.
Abbreviations: WBC, white blood cell; ANC, absolute neutrophil count; ALC, absolute lymphocyte count; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

including our patient's, thiopurine treatment had already been established and was ongoing prior to assessing the TPMT activity results. According to the 2018 updated CPIC guidelines, the recommended dose for heterozygous *TPMT* deficient patients should be 30–70% of the standard dose.[13] However, many of the *TPMT* alleles have an uncertain functional status, resulting in an indeterminate phenotype.[24] In the case of unknown phenotypes, dose adjustments should be made only after reaching a steady state based on disease-specific guidelines, with evaluation of RBC TPMT activity and close monitoring.[25] In pediatric CD patients whose enzyme activity is affected, a dose reduction by a half is recommended.[3] After adjusting our patient's azathioprine amount to 50% of the starting dose, PCDAI and laboratory results were normalized and persistently within the reference ranges, indicating a good treatment response.

Genetic evaluation in CD patients had different views among studies and guidelines. The European Crohn's and Colitis Organisation (ECCO) guidelines did not recommend routine genetic study for diagnosis and classification of CD.[26,27] In one study of IBD patients, performing *TPMT* genotyping before treatment was not beneficial in predicting adverse reactions to azathioprine.[28] On the other hand, updated ECCO guideline for pediatric CD suggested pre-treatment *TPMT* genotyping for optimized thiopurine treatment since up-front thiopurine dose reduction showed decreased risk of hematological adverse drug reactions.[29]

Previous studies have emphasized the importance of measuring biomarkers (such as TGNs) to predict a patient's response to thiopurines and to minimize any adverse reactions.[9,23,30] These tests are also helpful in assessing the TPMT phenotype; however, the concentrations of TGNs rely on prior thiopurine dosing. Measurement of the RBC TPMT activity also aids in evaluating patients' TPMT phenotype; however, it can be increased after thiopurine administration; therefore, cautious interpretation is required.[13] Since TPMT activity is measured using RBCs, other conditions that may affect the RBC turnover rate or any history of blood transfusions can interfere with the results.[13,23,31] In our patient's case, she had not received a transfusion but had begun taking azathioprine three weeks prior to our hospital visit.

Compared to biomarkers, *TPMT* genotype has the advantage of unchanging during treatment. In many medical centers, patients undergo *TPMT* testing before they start thiopurines. This method is faster, more cost-effective, and less labor-intensive than the phenotyping method.[7] Genotyping can be a representative method,

with previous study results have shown a high concordance of 94.5%[7] and 98.4%[32] between TPMT genotype and phenotype. Another meta-analysis revealed that *TPMT* genotype is the primary component of TPMT activity,[33] so it is important to perform genetic testing in routine practice to determine the best individual dose.

SNP occurred in our patient resulted in a nonsense mutation (c.676C>T; p.Arg226*). In a study that investigated the TPMT structure of murine, six amino acid residues were found to interact directly with 6-MPN, and they were conserved across mammalian species. Among them, Arg147 and Arg221 (Arg152 and Arg226 in human TPMT) were the only polar amino acids near 6-MPN that could be a candidate for substrate deprotonation and substitution of Arg147 influences the enzyme's kinetics more significantly than Arg221.[34] These findings could suggest that the position of our patient's SNP is involved in some part of the TPMT enzyme's reaction.

In our novel allele (*TPMT*45*), the nucleotide change occurred near the 3`-end of the DNA sequence. Despite the existence of premature stop codon, this variant may avoid mRNA degradation through an escape pathway,[35] which is variable among species.[36] Since this variant is predicted to generate a protein missing the last 20 amino acids near the C-terminus, it, therefore, produces a relatively less defected enzyme than the other alleles. This relatively less defected protein may explain the patient's initial thiopurine metabolite level (6-TGN) being close to the upper limit, and two substrates' RBC TPMT activity (6-MMPN and 6-MMPN riboside) being close to the lower limit of the reference values.

Nonetheless, most clinically relevant results are obtained from the TPMT enzyme activity measurements since they determine the actual metabolic status of patients.[7] Other blood inflammatory biomarkers also allow follow-up assessment, especially during maintenance therapy.[3] Combining the PCDAI score, CRP level, and fecal calprotectin drop has great utility in predicting disease activity before and after induction therapy.[4] Fecal calprotectin is particularly useful in pediatric CD due to its role as a non-invasive marker of mucosal recovery.[3]

Collectively, compared to the adult CD patients genetic evaluation as well as biomarker levels are important in the monitoring and risk assessment of pediatric CD patients.[3,29] In this case, a novel *TPMT* variant was identified, and this nonsense mutation is assumed to affect the activity of the TPMT enzyme. Our prediction was supported by the patient's clinical manifestations and

laboratory findings obtained after adjusting the dose of azathioprine.

Conclusion

To the best of our knowledge, this is the first report of the novel allele, *TPMT*45*. This nonsense mutation is related to the partial amino acid loss of the TPMT enzyme. The thiopurine metabolite concentrations and RBC TPMT activity results implied a deficiency of the enzyme in this patient. The patient's daily thiopurine amount was reduced to half of the initial daily drug dose, and currently showing a good response to this patient-specific treatment. The results of our report illustrate the importance of TPMT genotyping and pharmacogenetic-based thiopurine dose adjustment. Nevertheless, there has been no functional study, and clinical evidence including TPMT enzyme activity of TPMT*45 is insufficient as data has been obtained only from a single case. Further research should focus on the functional characterization and impact on its treatment effect.

Abbreviations

CD, Crohn's disease; IBD, inflammatory bowel disease; PCDAI, Pediatric Crohn's Disease Activity Index; CRP, C-reactive protein; TPMT, thiopurine S-methyltransferase; TGN, thioguanine nucleotide; MMPN, methylated mercaptopurine; RBC, red blood cell; CPIC, Clinical Pharmacogenetics Implementation Consortium; ESR, erythrocyte sedimentation rate; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; mRNA, messenger ribonucleic acid; ECCO, European Crohn's and Colitis Organisation.

Data Sharing Statement

The data are available upon reasonable request to the corresponding author, Soo-Youn Lee.

Ethics Statement

This study was reviewed and approved by the Institutional Review Board of Samsung Medical Center in Seoul, Korea (approval number 2012-04-106-020). The research was carried out in accordance with the World Medical Association Declaration of Helsinki, and the parents provided written informed consent since the patient was under age 19.

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