Correspondence

Mutation analysis and prenatal diagnosis of a family with Griscelli syndrome type 2: two novel mutations in the *RAB27A* gene

Griscelli syndrome type 2 (GS2; OMIM#607624) is a rare autosomal recessive disorder characterized by hypomelanosis with immunologic abnormalities and haemophagocytic lymphohistocytosis.[1] Neurological manifestations were reported in 67% of GS2 patients.[2] It is caused by mutations in the *RAB27A* gene.[3] The *RAB27A* gene encodes Rab27a, a member of the small GTPase superfamily, involved in vesicular fusion and trafficking.[3] Mutations in the *MYO5A*, *RAB27A*, or *MLPH* genes cause GS1, GS2 or GS3, respectively. It has been demonstrated that the tripartite protein complex (Rab27a/melanophilin/myosin Va) in melanocytes is needed for capturing mature melanosomes for transferring to keratinocytes.[4]

In this study, we report a Thai boy who was admitted to King Chulalongkorn Memorial Hospital for the first time at two years of age because of recurrent fever. He was the first child born to non-consanguineous parents. Hepatosplenomegaly was first noted at the age of 9 months. On physical examination, he was found to have silvery-gray hair and eyebrows, pale conjunctivae and hepatosplenomegaly with the liver edge palpable 7 cm below the right costal margin (span: 12 cm) and the spleen palpable 5 cm below the left costal margin. Lab investigation showed large clumps of pigment in the hair shafts seen under the light microscope (Fig. A), pancytopenia (haemoglobin B: 9.4 g/dL; white blood cell count: 4450 mm^3; platelet count: 20 000 mm^3), normal to slightly high immunoglobulin levels [IgG: 637.4 mg/dL (380-950 mg/dL); IgM: 143.6 mg/dL (28-112 mg/dL); IgA: 108.7 mg/dL (18-110 mg/dL); total IgE: 79.7 IU/mL (0-60 IU/mL)]. Bone marrow aspiration revealed hypercellular marrow, increased normally matured megakaryocytes, and increased histiocytes. Flow cytometry showed total T cells (CD3) of 3386 cells/mm^3 (72%; CD4 18% and CD8 42%), B cells (CD19) 15% and natural killer cells (CD16 & 56) 8%. Chest X-ray showed diffuse granular infiltration. At the age of three years, he developed ataxia and refused to walk. Magnetic resonance imaging of the brain showed almost symmetrical white matter lesions involving bilateral cerebellar hemispheres and parietooccipital regions.

After informed consent, genomic DNA and total RNA was isolated from peripheral leukocytes. Direct sequencing of polymerase chain reaction (PCR)-amplified complementary DNA representing the entire coding regions of *RAB27A* was performed. It revealed that the patient was compound heterozygous for two novel mutations, c.109A>T (p.K37X) and c.318T>G (p.S106R) (Fig. B). Genomic DNA was amplified and sequenced, confirming the existence of both mutations.

His conditions worsened and bone marrow transplantation using an unrelated donor was performed at the age of four years. He passed away a few weeks after the transplantation due to sepsis.

Identification of the disease-causing mutation has enabled more accurate genetic counseling. Five years after the proband passed away, the parents decided to have another child and requested a prenatal diagnosis. At 16 weeks of gestation, genomic DNA was isolated from amniocytes. PCR-restricted fragment length polymorphisms primers were used to specifically amplify exons 2 and 4 of the *RAB27A* gene. The PCR products were examined for the p.K37X and p.S106R mutations by restriction enzyme digestion using DdeI and BanI, respectively. The result revealed that the fetus carried the p.K37X, but not the p.S106R (Fig. C), suggesting that the fetus would be unaffected with GS2. The mother continued her pregnancy and gave birth to a healthy child.

We reported the first patient with GS2 in Thailand. To our knowledge, in Asia, only Turkish, Iranian, Jordanian, Indian and Pakistani descents were reported for GS2 mutations.[5-7] Our patient had silvery-gray hair and eyebrows with immunological defects which could help distinguish GS2 from other types. The hair shaft revealed the large irregular clumping of pigment which distributed around medulla when compared to normal hair. There was no difference between the characteristic of our Thai patient's hair shaft and other Asian's hair shaft.[8] The onset of the accelerated phase of our proband was within the previously observed range which was from the infancy to teens.[2] Serum triglyceride level of our patient was within a normal range while most reported patients had a high level of triglycerides.[9] At least 16 patients with GS2 were reported to undergo hematopoietic stem cell transplantation and all of them survived.[2,4,5,7,10]

The p.K37X is expected to be degraded by nonsense-mediated mRNA decay and the p.S106R changes a neutral polar serine to a basic polar arginine. ClustalX 1.83 software showed that serine at the 106 position of

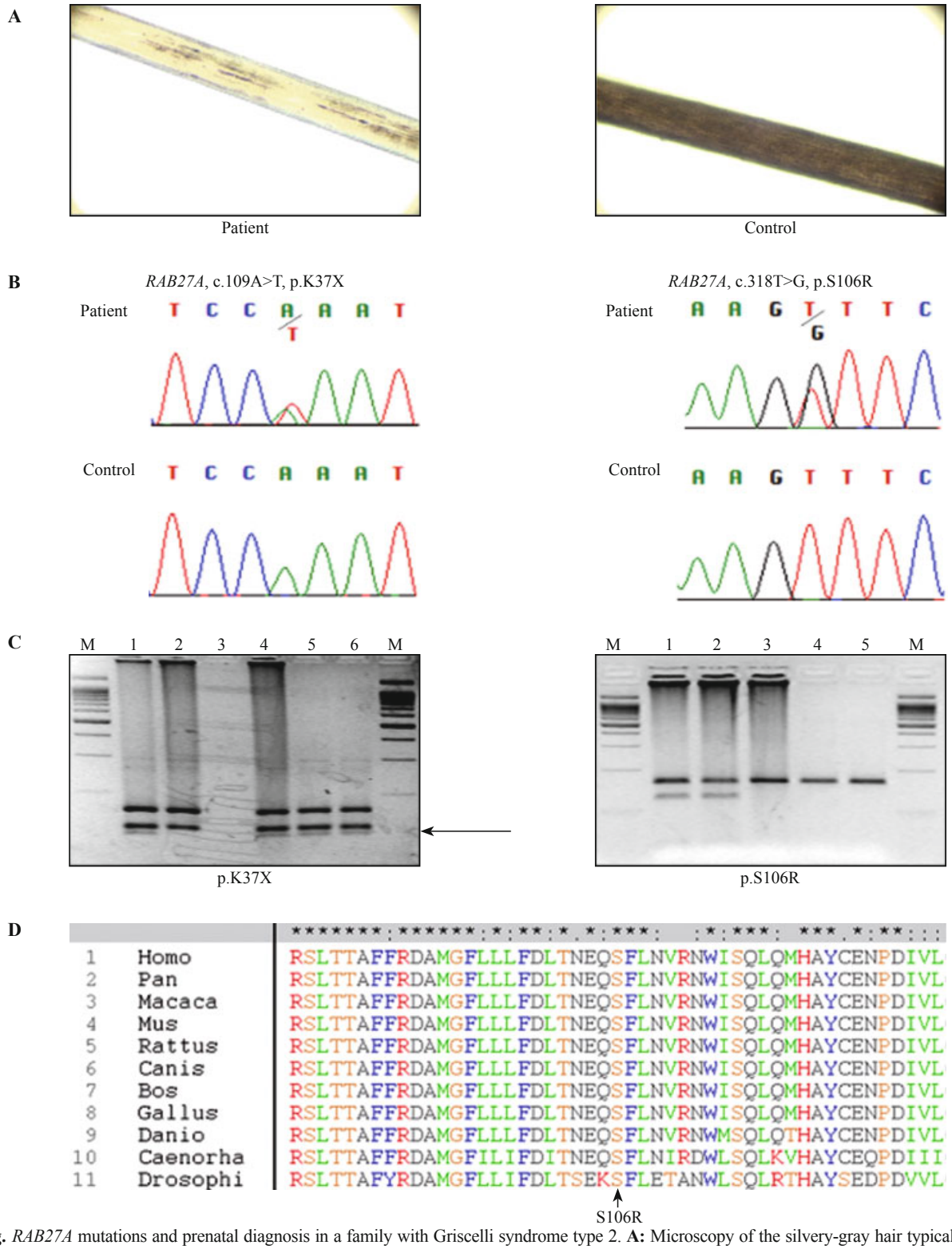

Fig. *RAB27A* mutations and prenatal diagnosis in a family with Griscelli syndrome type 2. **A:** Microscopy of the silvery-gray hair typical of Griscelli syndrome type 2 showing light hair color and large clumps of pigment; **B:** Electropherograms of the affected brother and a control in the upper and lower panels, respectively. The patient was compound heterozygous for c.109A>T (p.K37X) and c.318T>G (p.S106R); **C:** Prenatal diagnosis using polymerase chain reaction-restricted fragment length polymorphisms of *RAB27A*. The left panel is for p.K37X, M: 100 bp marker; lane 1: the proband, as a positive control; lane 2: the proband's father who did not carry the p.K37X; lane 3: a negative (no DNA) control; lane 4: the proband's mother who was a carrier for the heterozygous p.K37X; lanes 5 and 6: the fetal amniocytes, performed in duplicate. The light band (an arrow) in lanes 1, 4, 5 and 6 indicated that the proband (lane 1) and fetus (lanes 5 and 6) carried the p.K37X from the mother (lane 4). The right panel is for p.S106R, M: 100 bp marker; lane 1: the proband, as a positive control; lane 2: the proband's father who was a carrier for the heterozygous p.S106R; lane 3: the proband's mother who did not carry the p.S106R; lanes 4 and 5: the fetal amniocytes, performed in duplicate; **D:** Multiple sequence alignment of *RAB27A* in different species. The serine at 106 amino acid residue is 100% conserved across all species with their available sequences.

RAB27A was evolutionary conserved among all species available in the database (Fig. D). Polymorphism phenotyping-2 (http://genetics.bwh.harvard.edu/pph2/) and sorting intolerant from tolerant (http://sift.jcvi.org/) predicted that it was "probably damaging" and "deleterious", respectively. In addition, it was absent in 250 unrelated Thai controls.

In conclusion, we found two novel mutations in the *RAB27A* gene in a Thai boy with GS2. The identified mutations enabled us to perform a prenatal diagnosis for the subsequent pregnancy. The fetus was found to carry only one mutation. We reported the first case of a successful prenatal diagnosis for GS2.

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Crohn's disease with anorectal stenosis successfully treated with vedolizumab

Anorectal stenosis (ARS) is a rare complication of Crohn's disease (CD) and a risk factor for both fecal diversion (FD) and proctectomy,[1,2] especially in patients who failed anti-tumor necrosis factor-α (anti-TNF) therapy.[3,4] Herein, we presented a case of a 17-year-old female diagnosed with CD at age 7, with inflammation located in the small bowel and colon (Paris classification: L3L4b). Due to corticosteroid dependency, infliximab with concomitant azathioprine was introduced at age 9 and discontinued two years after, because of the appearance of a 3 cm tumor-mass, which resembled a lymphoma, in the oral cavity. The histopathologic examination confirmed orofacial granulomatosis. The therapy with anti-TNF agent (adalimumab) was continued. While treated with adalimumab and azathioprine, she presented with symptoms of ileus at the age of 14. The cause of the obstruction was ARS (4 cm in length), impassable by the coloscope, which was resolved by endoscopic balloon dilatation (EBD). The dose of adalimumab was increased to 80 mg per week and azathioprine was continued. Despite this therapy, she needed EBD every 3-5 months because of repeated obstructive ARS. She suffered from extreme fatigue and was not able to attend school. Pediatric Crohn's disease activity index (PCDAI) was 45. At that time, surgical therapy with FD was planned. Colonoscopy revealed severe proctitis with ARS and a simple endoscopic score for CD (SES-CD) of 11 in the rectum (Fig. A). However, a trial with vedolizumab (VDZ) was started prior to surgical therapy at age 16. The response to VDZ was assessed at week 10; she was in clinical remission (PCDAI=5), feeling energetic and again able to attend school. Colonoscopy revealed a dramatic endoscopic improvement (Fig. B) with SES-CD of 3.

The management of CD with ARS is challenging. Our patient had failed anti-TNF therapy, therefore a trial involving the biologic agent VDZ with a different mechanism of action was a logical treatment option. VDZ is a selective monoclonal antibody against