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Identification of a novel SBF2 missense mutation associated with a rare case of thrombocytopenia using whole-exome sequencing

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Abstract We describe in this report a case of a 6-years-old female who presented at the age of 1 month with a mucocutaneous bleeding and suspected thrombocytopenia. The patient's condition was refractory to the known idiopathic thrombocytopenic purpura treatments and congenital form of Thrombocytopenia was suspected following the delivery of a male sibling with the same phenotype. The patient also manifested fair colored hair and skin relative to her family however she did not have any detectable neurologic or other immunologic abnormalities. In order to further understand this condition, we have used whole-exome sequencing of the patient's DNA as well as her father's with the assumption that her condition is transmitted in an autosomal recessive manner. We have identified a missense change c.659C>G (p.Thr220Arg) in the SBF2 (also known as MTMR13) gene that causes a mutation in the DENN domain of the protein. This mutation was validated by traditional Sanger sequencing and analyzed in the remaining family members were it was found to segregate in the homozygous state in the affected siblings and in the heterozygous state in both parents. This novel mutation in the DENN domain of SBF2 maybe interfering with its putative association with the Rab family of small GTPases which are important mediators of vesicle transport and membrane trafficking. In conclusion, we have identified a novel mutation that is associated with severe thrombocytopenia. The fact that this mutation is found in SBF2 gene may indicate that the underlying cause of thrombocytopenia in our patient is either a new variant form of Griscelli syndrome (through the Rab GTPases action) or a variant Charcot–Marie–Tooth type 4 disease as SBF2 truncating mutations were previously identified in sufferers of this disease. This finding will help to accurately diagnose and classify similar cases of congenital thrombocytopenia and provide further proof to the power of whole-exome sequencing in personalizing patients management from the point of diagnosis to treatment and followup.

Keywords Thrombocytopenia · SBF2 · Whole-exome sequencing

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Introduction

Conditions affecting platelets count vary from vitamin B12 deficiency to malignancies and they are collectively known as Thrombocytopenia. [1] It is often a challenging task to determine the exact cause of low platelet count and attempting to do so will require the performance of several diagnostic laboratory investigations. However, the main

question affecting the treatment of a patient is whether the disease is acquired or congenital as the latter will not be responsive to drugs often used to tackle acquired thrombocytopenia. Low platelet count can be a phenotype associated with several overlapping syndromes. One such syndromes is the Griscelli syndrome (GS). Griscelli syndrome is often divided into three major types depending on the presence or absence of neurologic and/or immunologic aberrations and the underlying mutations causing it. However, all variants share the phenotype of decreased pigmentation and bleeding problems due to low platelets count [2, 3].

Here we report a case of a 6-years-old girl who presented at birth with low platelet count and with fair colored hair and skin relative to her parents. The patient did not exhibit any gross neurological abnormalities and no other abnormalities could be noted. Therefore, we carried out whole-exome sequencing of the patient's DNA as well as her father in order to identify the genetic mutation underlying her disease.

Materials and methods

Patients samples

Peripheral blood was obtained from the patient, her siblings and her parents following obtaining their informed consent and according to the institutional ethical procedures. Anonymized DNA samples from hematologically normal individuals served as a control for the frequency of any possible mutation. Genomic DNA was prepared using the Qiagen QIAamp DNA Blood Mini kit according to the manufacturer's recommendations.

Whole-exome sequencing

Three micrograms of genomic DNA obtained from peripheral blood from the patient as well as her father was sheared using the Covaris S2 system. Exome capture was performed using the SureSelect Whole-Exome Enrichment version 1 kit from Agilent. Fragment libraries were prepared from the captured exomes for sequencing on the SOLiD 3 Plus platform (AB). 48,616,830 50-bp reads were obtained from the patient's exome, 91.28 % of which were mapped to the UCSC hg18 reference sequence of the human genome using the BioScope 1.3 analysis pipeline. The father's DNA yield was higher as 88,781,345 reads were obtained, 81.85 % of which were mappable. Identification of single nucleotide polymorphisms was achieved by the "highest" settings in the diBayes software incorporated in the Bioscope 1.3 analysis pipeline. Short-listing of candidate SNPs was performed by filtering the detected SNPs to include only those with more than 10× coverage and not detectable in dbSNP or 1000 Genome databases. In addition, pathogenic variations were assumed to exist in the homozygous state only in the patient and heterozygous in the father. Damaging nonsynonymous variations were determined by the SIFT software (http://sift.jcvi.org) [4]. Finally, single nucleotide variations that are predicted to be damaging with high confidence by SIFT, MutationTaster (http://mutationtaster.org) [5] and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) [6] were selected for further analysis. Modelling the effect of the candidate mutations was performed using the I-TASSER server [7].

PCR amplification and Sanger sequencing

Candidate SBF2 mutation was confirmed using Sanger sequencing and custom oligonucleotide primers on the 3730xl DNA Analyzer. The PCR primers used to amplify the c.659C>G of SBF2 are forward (5′-CCATAACATTA TGACTTATCAAC-3′) and reverse (5′-GTATACTGTCT ATGATTGTTG). The PCR conditions used one denaturation cycle at 95 °C for 15 min followed by 35 cycles of denaturation at 95 °C for 30 s, and annealing at 52 °C for 30 s and extension at 72 °C for 30 s. The final extension step was done at 72 °C for 10 min. All PCR reactions were performed using the Qiagen HotStarTaq DNA Polymerase on the Eppendorf MasterCycler S thermal cycler. The PCR products were purified using ethanol precipitation prior to performing the cycle sequencing reaction using the ABI BigDye v3.1 and the ABI 3730xl capillary DNA analyzer.

Results

Case history

The case in question relates to a 6-year-old female who presented at the age of 1 month as a case of thrombocytopenia. She was the product of full-term pregnancy and caesarian section due to ruptured membrane and fetal distress. With the exception of the low platelet count, other hematological laboratory test results fell within normal ranges (Table 1). The proband is not displaying any sign of jaundice, lymphadenopathy, organomegaly or dysmorphic features. The proband was initially treated as case of idiopathic thrombocytopenic purpura as the initial absence of family history and the mild clinical course favored this diagnosis. However, the proband's failure to respond to any of the treatment modalities (such as steroids, intravenous immunoglobulin anti-D and rituximab) and the subsequent birth of an affected sibling shifted the focus towards a suspected case of congenital thrombocytopenia. Blood films showed vastly

Table 1 Complete blood count and differential test results of the proband performed over a period of 18 months with 5–6 months intervals

	Range	9/2012	4/2012	11/2011	4/2011
White blood cell count (WBC)	5.00–17.00 (K/μL)	2.88	2.30	3.57	4.20
Red blood cell count (RBC)	4.00–5.20 (M/μL)	4.92	4.64	5.14	4.25
Hemoglobin (Hb)	10.20–15.20 (g/dL)	13.70	12.70	14.20	12.80
Hematocrit (HCT)	34.00–48.00 (%)	39.10	37.70	40.30	35.70
Mean cell volume (MCV)	78.00–94.00 (fL)	79.50	81.30	78.40	84.10
Mean cell hemoglobin (MCH)	32.00–36.00 (pg)	27.80	27.40	27.60	30.00
Platelets	150.00–450.0 (K/μL)	21.00	16.00	23.00	3.00
Automated neutrophils	30.00–60.00(%)	28.10	17.00	28.60	28.10
Automated lymphocytes	10.00–15.00 (%)	55.90	64.80	56.30	59.00
Automated monocytes	2.00–11.00 (%)	11.50	13.50	9.20	11.60
Automated eosinophils	1.00–4.00 (%)	3.10	3.00	4.80	0.50
Automated basophils	0.00–2.00 (%)	1.40	1.70	1.10	0.80

reduced number of platelets and a relative lymphocytosis supporting the CBC results (Table 1). However, bone marrow aspirations showed no evidence of malignancy and normal megakaryocytes producing platelets were noted. Flow cytometry analysis and megakaryocytes immunophenotyping showed normal expression of all surface antigen receptors tested. Both mother and child had the same ABO and Rh blood groups, which excluded the neonatal alloimmune thrombocytopenia. No evidence of viral infection could be found as viral screening for HCV, HIV, CMV and EBV were negative. The proband could not be diagnosed with GS based on phenotypical observations alone as only mildly fair colored hair and skin could be observed.

Exome sequencing

We have identified 13,204 single nucleotide variations (SNVs) in 44,377,442 mappable reads from the exome of our proband using the high stringency setting of the diBayes algorithm incorporated in the Bioscope program which filters out SNVs with low quality values. The filtering strategy adopted is shown in Fig. 1. No informative small indels were identified in this study. Approximately 90 % of the SNVs were covered at least 10 times by mappable reads. Only 744 SNVs were novel, i.e. not reported in dbSNP 131 or by the 1000 Genomes project. As the condition is presumed to be transmitted in an autosomal recessive fashion, only homozygous variations identified in the patient were selected for further analysis. SIFT prediction was applied on the remaining SNVs which left 25 SNVs that are presumed to be damaging to the protein function. As the father is a presumed carrier as he displays no signs of the disease, damaging, heterozygous and novel SNVs present in the father's exome were identified. Only one SNV remained after applying the final filter in which the SNV has to be predicted to be damaging by 2 additional programs, Poly-Phen2 and Mutation Taster. This SNV is a missense change at c.659C>G (p.Thr220Arg) in the SBF2 gene that causes a mutation in the DENN domain of the protein.

The p.Thr220Arg mutation was validated by the Sanger sequencing method in the affected child as well as the other members of her family, Fig. 2. The proband harbored the p.Thr220Arg in a homozygous state as well as her other affected sibling. Their remaining sibling who does not exhibit the disease harbored only the wild-type sequence. Both the parents carried the p.Thr220Arg in the heterozygous state. The p.Thr220Arg mutation was not detected in 200 normal chromosomes confirming its status as a strong candidate disease-causing mutation. No cases of GS were available, however, the p.Thr220Arg mutation was not found in two cases of suspected Chediack–Higashi syndrome that has some overlapping phenotypic features [3]. ClustalW alignment of amino acid sequences of SBF2 from human to drosophila show that the threonine residue at position 220 aa is highly-conserved and its change to arginine is likely to affect the conformation and function of the DENN domain of SBF2 (Fig. 3).

Discussion

We have performed whole-exome sequencing on two members of a family of 5 that had a rare and an explainable form of hereditary thrombocytopenia that was refractive to known treatment modalities. The procedure adopted utilized a stringent bioinformatics analysis criteria that filtered the possible SNVs down to one candidate mutation, p.Thr220Arg in the SBF2 gene. The SBF2 (also known as MTMR13) has been linked with Charcot–Marie–Tooth 4 type B2 (CMT4B2) disease that cause aberrant demyelination of sensory and motor axons that is also transmitted in

an autosomal recessive manner [8, 9]. Interestingly, our patient exhibited none of the typical phenotypic features of CMT4B2 and her only ailment is manifested in the chronically low platelet count. The mutations linking SBF2 to CMT4B2 are nonsense or splice-site mutations affecting the N-terminus part of the protein [10–13]. This is in contrast to the missense mutation we identified affecting the DENN domain, supposedly interfering with SBF2 function with completely abrogating it, as it is the case in CMT4B2 disease. However, future manifestation of CMT4B2 in our patient cannot be completely excluded from occurring later in her life.

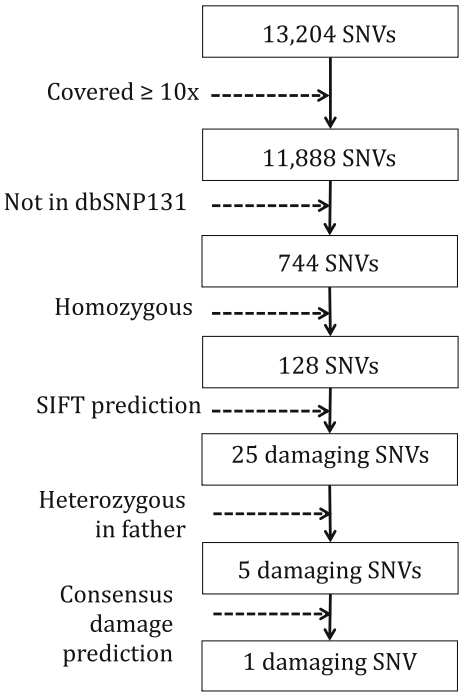

Fig. 1 Bioinformatic analysis pipeline used in this study. SNVs are single nucleotide variations

SBF2 is a pseudo-phosphatase that belongs to the myotubularin family of lipid phosphatases. In support of SBF2 expression in platelets and megakaryocytes, Gene Expression Omnibus (GEO) database search shows compelling evidence for the expression of SBF2 at the RNA level in both platelets (GDS3318) and megakaryocytes (GDS2513)[14, 15]. SBF2 is known to interact with MTMR2, an active lipid phosphatase thought to regulate endosomal function [16]. The DENN domain of SBF2 mediates its interaction with members of the RAB family of small GTPases [17]. One member of this family is Rab27a, the mutation in which is the underlying cause of the ashen mouse characterized by low platelet count, lightly-colored coat and is generally recognized as a model for the Griscelli syndrome, in which this gene is frequently mutated [18–22]. RAB28, another member of the RAB family of small GTPases, has been shown recently to interact with the DENN domain of SBF2 [23]. RAB28 is less characterized than RAB27A however it is thought to similarly function in vesicle transport and membrane trafficking [24]. SBF2 is a 1,849 aa protein (NP_112224) with a predicted molecular weight of 208 kD. Several attempts were made to purify the recombinant mutant SBF2 in order to study the possible effects of the substitution on SBF2 interactions mediated by the DENN domain, namely with the RAB GTPases. However, all attempts were unsuccessful and it is assumed the p.Thr220Arg substitution potentially has a role to play in the recombinant protein solubility.

Our bioinformatics analysis approach has been validated as the remaining four damaging SNVs are now reported in the dbSNP137 and the latest draft of 1000 Genomes project as single

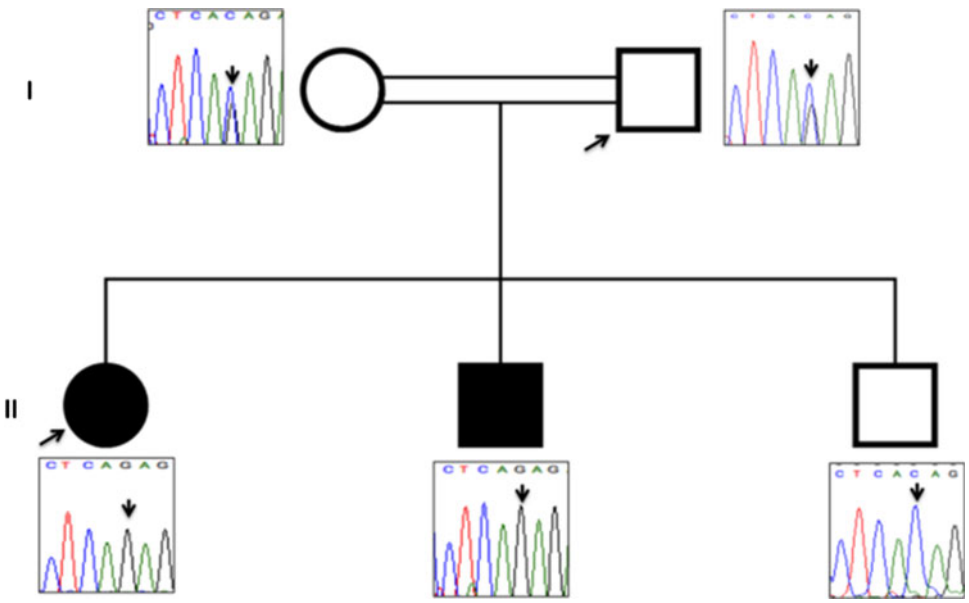

Fig. 2 Pedigree diagram of the family used in this study. Sequence chromatograms of the SBF2 c.659C>G mutation is also shown for every member with the *arrow* pointing at the affected nucleotide. *Black shaded areas* indicate affected individuals. *Diagonal arrows* indicate proband (*black circle*) and her father subjected to whole-exome sequencing

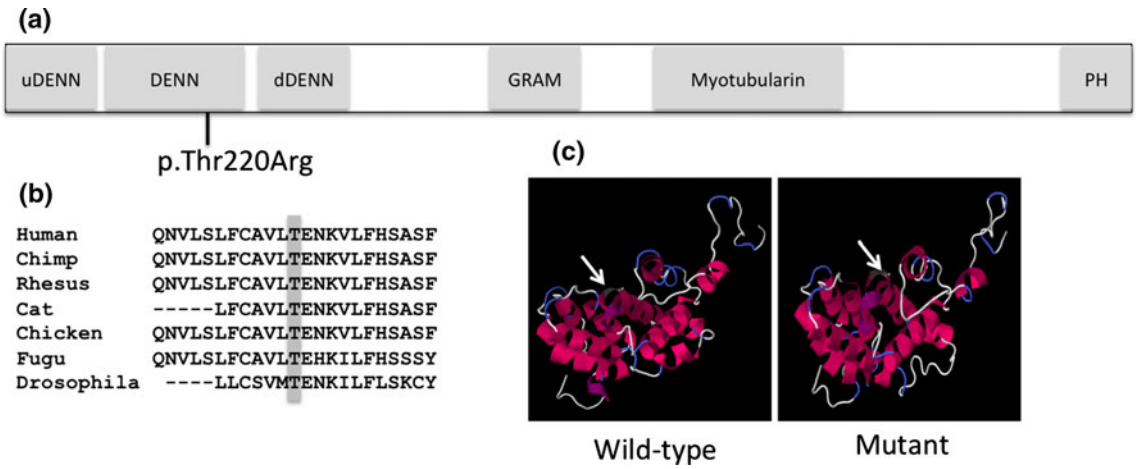

Fig. 3 The p.Thr220Arg mutation in the SBF2 gene. **a** Schematic diagram showing the SBF2 protein domains and the relative position of the p.Thr220Arg mutation. **b** The threonine residue at position 220 is highly conserved in evolution as shown by the ClustalW alignment of amino acid sequences of SBF2 from human to drosophila. **c** computer modeling of the effect of the p.Thr220Arg mutation on the DENN domain of SBF2. The *arrow* points to the affected residue. The modeling was performed by the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/

nucleotide polymorphisms. The genes affected are RELN (rs115913736), FERMT3 (rs149000560), NFS1 (rs138646150) and OR1L6 (rs117995259). SBF2 p.Thr220Arg remains a novel SNV not previously reported in the latest SNP database.

This study shows the usefulness of whole-exome sequencing in a clinical setting. In this study we report a patient who was presented with low platelet count, a condition that can occur in a multitude of symptoms. The SBF2 p.Thr220Arg mutation is highly likely to be the novel cause of the low platelet count and the relative fairness of hair and skin in our patient. SBF2 functional pathways and its involvement in the Rab family of small GTPases helps to potentially diagnose our patient as a variant GS or potentially CMT4B2 sufferer with thrombocytopenia being the main manifestation. Further work is required to elucidate the potential role of SBF2 in platelet function in order to fully understand the disease of our patient.

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