Hereditary xerocytosis - spectrum and clinical manifestations of variants in the PIEZO1 gene, including co-occurrence with a novel β-globin mutation

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A B S T R A C T

Hereditary xerocytosis (HX) is a rare, autosomal dominant congenital hemolytic anemia (CHA) characterized by erythrocyte dehydration with presentation of various degrees of hemolytic anemia. HX is often misdiagnosed as hereditary spherocytosis or other CHA. Here we report three cases of suspected HX and one case of HX associated with β-thalassemia.

Sanger method was used for sequencing cDNA of the PIEZO1 gene. Variants were evaluated for potential pathogenicity by MutationTaster, PROVEAN, PolyPhen-2 and M-CAP software, and by molecular modeling. Four different variants in the PIEZO1 gene were found, including three substitutions (p.D669H, p.D1566G, p.T1732 M) and one deletion (p.745delQ). In addition, in the patient with the p.T1732 M variant we detected a 12-nucleotide deletion in the β-globin gene leading to a deletion of amino acids 62AHGK65. The joint presence of mutations in two different genes connected with erythrocytes markedly aggravated the presentation of the disease. Bioinformatic analysis and molecular modeling strongly indicated likely deleterious effects of all four PIEZO1 variants, but co-segregation analysis showed that the p.D1566G substitution is in fact non-pathogenic.

Identification of causative mutations should improve the diagnosis and management of HX and provide a new insight into the molecular basis of this complex red blood cell abnormality.

1. Introduction

Hereditary xerocytosis (HX), also known as dehydrated hereditary stomatocytosis (DHS), is a rare red blood cell membrane disorder resulting in hemolytic anemia with diverse presentation and iron overload. Dominantly inherited missense mutations in the PIEZO1 gene encoding a large mechanosensitive ion channel, affecting mainly its highly conserved COOH-terminus, have been identified in HX patients [1]. They increase the cation permeability of the membrane, which leads to erythrocyte dehydration. Red blood cells (RBCs) from HX patients are characterized by a higher Na⁺ content and a decreased K⁺ content relative to healthy RBCs [2]. Besides the PIEZO1 mutations, also mutations in KCNN4 encoding the Gardos channel have been detected in some HX cases [3,4]. The clinical presentation of hereditary xerocytosis is markedly heterogeneous, ranging from normal hemoglobin values to severe anemia, with borderline macrocytosis, increased cell hemoglobin content (MCH) and increased mean corpuscular hemoglobin concentration (MCHC) [5]. Another type of RBC disorder with well-understood genetic and molecular etiology is β-thalassemia. It is a heterogeneous group of hemoglobin disorders due to a decreased or absent production of normal β-globin chains. A special form of β-thalassemia defects, called dominant β-thalassemia, is caused by point mutations or small insertions or deletions leading to the production of highly unstable β-globin, which results in the formation of an unstable tetrameric α2β2 protein. When such mutations generate a premature stop codon, the mutated mRNA may undergo degradation through a surveillance mechanism called nonsense-mediated mRNA decay (NMD). The NMD is not absolutely efficient and the mRNAs escaping degradation give rise to truncated β-globin chains, resulting in highly variable symptoms among carriers of the same variant. Heterozygotes for a dominant β-thalassemia mutation show typical anemia symptoms [6], whereas activation of NMD only leads to a mild anemia or is symptomless. Combined defects of the red cell membrane with other lesions affecting erythrocytes are very rare and difficult to diagnose properly. Only few HX cases associated with a thalassemia or RBC enzymopathy have been reported [7]. The aim of our study was to...
elucidate the molecular basis of hereditary xerocytosis in four Polish patients with suspected HX or an undiagnosed congenital hemolytic anemia. Four previously uncharacterized variants in the PIEZO1 gene were identified and, in one patient, a co-existing deletion in the β-globin gene. Homology modeling was performed in order to predict the effects of the identified variants on the structure of PIEZO1 and β-globin proteins and thereby their likely mechanism of pathogenicity. Family co-segregation studies were used to verify the predicted pathogenicity of two of the novel variants.

2. Materials and methods

2.1. Case presentation

2.1.1. Patient 1

A 16-year-old girl has been under the care of the outpatient department of a hematology division since birth due to hemolytic anemia of unknown etiology. She was hospitalized many times as she required packed red blood cells transfusions which occurred mainly during infections. Additionally, growth hormone deficiency, familial adenomatous polyposis (FAP) and Gilbert Syndrome were diagnosed. Mother and sister of the proband were asymptomatic. The father of the patient was unavailable for genetic studies. It is only known from an interview that he exhibited no symptoms of hemolytic anemia.

2.1.2. Patient 2

An 18-year-old girl with hemolytic anemia of unknown etiology has been observed since birth. She was hospitalized occasionally for packed red blood cells transfusions. Initially she was diagnosed with congenital dyserythropoietic anemia type II. A flow cytometry test with eosin-5′-maleimide (EMA) staining for red cell membrane disorders showed lowered fluorescence intensity compared with normal samples. At the age of 3, splenectomy was performed with no beneficial effect; no thrombotic event has been reported. Finally, other possible causes of anemia were excluded and diagnosis of HX was established. Mother, father and brother of the proband were asymptomatic.

2.1.3. Patient 3

A 59-year-old man complaining of weakness and fatigue since 2012. Lab studies revealed occasional mild anemia accompanied by elevated reticulocyte count and mild hyperbilirubinemia. In December 2017 he had an episode of severe abdominal pain located in the left upper quadrant of the abdomen. Gallstones were diagnosed and managed conservatively. Physical examination revealed no abnormalities. No family members were available for analysis. It is only known from an interview that the both parents exhibited no symptoms of hemolytic anemia.

2.1.4. Patient 4

A 3-year-old boy has been under the care of the outpatient department of a hematology clinic since birth due to hemolytic anemia of unknown etiology. Hereditary spherocytosis, enzymopathy of red cells and autoimmune hemolytic anemia were excluded. Since hemoglobin analysis showed elevated levels of hemoglobins F and A2, β-thalassemia was diagnosed. Red cell morphology demonstrated target cells in association with stomatocytes and microspherocytes (Fig. 1), and red cells displayed profoundly decreased osmotic fragility. He still requires regular and frequent packed red blood cells transfusions. Parents of the patient 4 refused to donate blood for the purpose of genetic analysis.

The hematological data of the probands are shown in Table 1. Informed consent has been obtained from the patients or their parents, as appropriate, and the study protocol approved by the Ethics Committee of the Medical University of Warsaw.

Fig. 1. Peripheral blood film (PBF) of patient 4 showing target cells (1), stomatocytes (2), microspherocytes (3) and leptocytes (4).
A homology model of β-globin lacking the 62AHKG65 fragment was obtained using the SWISS-MODEL server [12]. As a template the 3D structure of human hemoglobin was used (PDB code: 1DXT0) [17].

3. Results

All the probands exhibited variable degrees of anemia with the presence of stomatocytes in the blood smear, and reticulocytosis. Sequencing of the PIEZO1 cDNA showed four previously uncharacterized variants, one in each patient (Table 2, Supplemental Figure 1S).

They were confirmed by sequencing corresponding regions of their genomic DNA. In patient 1 the c.2005 G > C variant causes a substitution of aspartic acid with histidine at position 669 (p.D669H). In this patient symptoms of anemia/jaundice have been noted since birth and she was occasionally transfused. The most significant laboratory findings were: hemoglobin (Hb) concentration of 8.1 g/dL and a significantly decreased number of red blood cells with slightly elevated red blood cell distribution width (RDW). Her mother and sister have never been anemic, and DNA sequencing did not show the presence of the c.2005 G > C substitution. p.D669H was categorized as a disease-causing mutation by all the bioinformatics software used. Molecular modeling showed that the D669H replacement destabilizes the protein structure only slightly (~0.4 kcal/mol, see Fig. 2). It should be mentioned, however, that the loop separating TM14 and TM15 (residues 650–690) carries a stretch of negatively charged residues D651, D664, E665, D669, E673 and E679, all of which are generally conserved in the homologous sequences and therefore are expected to be functionally important. Thus, the D669H substitution, which markedly changes the charge distribution along this loop could affect intermolecular interactions of PIEZO1 with an unidentified partner.

Patient 2 carries the c.4697 A > G substitution changing aspartic acid to glycine at position 1566 (p.D1566G). This variant was also characterized variants, one in each patient (Table 2, Supplemental Figure 1S).

...down.

Fig. 2. Structure of T561-P960 fragment of Piezo1. The F649-S676 loop is stabilized by two salt bridges between D669 and E673, and K601. The D669H substitution present in Patient 1 destroys a network of interactions, bringing about destabilization of this loop. Residue 669 is depicted as a balls-and-sticks model; aspartate present in unmutated protein is shown. Arrow marks its ionic interaction with K601.

Bioinformatically classified as disease-causing, but less harmful than the variant in patient 1. Indeed, patient 2 showed a milder form of hemolytic anemia than patient 1. Since no reasonable template

Table 2

| Patient | Gene | Nucleotide variants (Variant dbSNP) | Amino acid mutations | Type | Prediction | MutationTaster | PROVEAN | PolyPhen2 | M-CAP |
|---------|------|-----------------------------------|----------------------|------|------------|----------------|---------|----------|-------|
| 1       | PIEZO1 | c.2005 G > C | p.D669H | missense | “disease causing” | – 5.354 | 1 | 0.649 |
| 2       | PIEZO1 | c.4697 A > G | p.D1566G | missense | “disease causing” | – 3.665 | 0.364 | 0.750 |
| 3       | PIEZO1 | c.2233,2235delACG | p.745delQ | deletion | “polymorphism” | 0.285 | – | – |
| 4       | PIEZO1 | c.5195C > T | p.T1732M | missense | “disease causing” | – 4.135 | 1 | 0.508 |
| 4       | β-globin | c.187,198del12 | p.62-65delAAGHK | deletion | “disease causing” | – 31.814 | – | – |

MutationTaster is a web-based application for evaluation of DNA sequence variants for their disease-causing potential. PROVEAN predicts whether an amino acid substitution or indel has an impact on the biological function of a protein; scores equal to or below – 2.5 are considered „deleterious”. PolyPhen2 predicts whether an amino acid substitution or indel has an impact on the biological function of a protein; scores range from 0 (tolerated) to 1 (deleterious). M-CAP is a pathogenicity classifier for rare missense variants in the human genome; scores above 0.025 are considered “deleterious”.

Table 1

| Patient | Age (years)/sex | RBC [10^6/μL] | Retic [%] | Ht [%] | Hb [g/dL] | MCV [fL] | RDW [%] | MCH [pg] | MCHC [g/dL] | Ferritin [μg/mL] | Total bilirubin [mg/dL] | LDH [U/L] | OF | EMA [%] | HbA₂ [%] | HbF [%] |
|---------|----------------|--------------|---------|-------|----------|---------|---------|---------|------------|----------------|-------------------|-----------|-----|---------|--------|--------|
| Normal value | – | 4.2–5.4 | 0.6–2.6 | 37–47 | 12–16 | 81–99 | 11–15 | 27–31 | 32–36 | 4.4–207.0 | 0.2–1.3 | *143–279 | *470–900 | > 86 | < 2.5 | < 1 |
| 1 | 16/F | 2.41 | 3.4 | 22.4 | 8.1 | 92.9 | 17.4 | 33.6 | 36.2 | 637.6 | 12.0 | 121* | dec | 115 | nd | nd |
| 2 | 18/F | 3.36 | 1.8 | 28.3 | 9.2 | 84.2 | 25.2 | 27.4 | 32.5 | 2560.8 | 1.0 | nd | dec | 79 | nd | nd |
| 3 | 59/M | 3.51 | 3.6 | nd | 12.9 | 100.6 | 15.3 | nd | nd | 829.5 | 1.7 | 359# | nd | nd | nd | nd |
| 4 | 3/M | 2.54 | 3.2 | 27.6 | 7.5 | 111 | 28.8 | 29.4 | 26.5 | 12.3 | 4.2 | 3400* | dec | 135 | 5.5 | 6.0 |

RBC - red blood cell, Retic - reticulocyte; Ht - hematocrit; Hb - hemoglobin; MCV - mean corpuscular volume; RDW - red blood cell distribution width; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration; LDH - lactate dehydrogenase; OF - osmotic fragility; EMA - the eosin-5′-maleimide binding test; HbA₂ - hemoglobin A₂; HbF - hemoglobin F; dec - decreased; nd - not detected.
structures were available for the Q1298-E1677 region of PIEZO1, predicted as an extramembrane sub-domain, the effect of the D1566G replacement could not be studied by homology modeling. It is expected that the introduction of a glycine residue should destabilize the structure. Notably, sequencing of family members has revealed that the variant occurs also in the asymptomatic brother and father of the patient, indicating that it should be regarded as benign, in contrast to the bioinformatic predictions.

In patient 3 the c.2233_2235 CAG deletion removes one glutamine residue from the stretch of glutamines at positions p.745–749. This deletion was categorized as non-disease-causing, in accordance with the mild symptoms of compensated anemia presented by this patient. In spite of these results, molecular modeling showed that the Q deletion would change the size and consequently the charge distribution on a putative helical region, which could affect intermolecular interactions (Supplemental Figure 2S). No family members of the patients were available for analysis.

Patient 4 carried the c.5195C > T variant which results in the substitution of threonine with methionine at position 1732 (p.T1732M). Patient 4 presents with macrocytosis, massively decreased MCH, and increased RDW. This variant was classified as disease-causing. Residue 1732 is located at the interface of transmembrane helices TM27 and TM28. The T1732M substitution was found to stabilize the protein structure markedly, by -2 kcal/mol. Such excessive stability could compromise the functioning of PIEZO1. On the other hand, its paralog Piezo2 in the mouse, pongo and human does contain a residue from the stretch of glutamines at positions p.745–749.

The T1732M variant (Supplemental Figure 3S). We performed in the cases of hereditary xerocytosis due to an increased risk of venous thrombosis [19]. The majority (83%) of HX cases are caused by nonsense mutations in the PIEZO1 gene, and rare cases related to the KCNN4 gene, which encodes the Gardos channel, have also been reported [20].

In the present analysis of four unrelated putative or diagnosed HX cases with highly different presentations we found four different variants in the PIEZO1 gene, including three substitutions and one deletion. The p.D669H variant of patient 1 affects the same position as the causative D669Y mutation described recently [20,21] in three unrelated families. The p.D1566G (patient 2) and p.T1732M (patient 4) variants, and the p.745delQ deletion (patient 3) are noted in dbSNP and scored of “unknown significance”.

The factors responsible for the strikingly different clinical manifestations of HX and its severity are still poorly understood. Some studies suggest that the clinical features of HX are, at least to some extent, related to the location of the causative mutation [20]. When non-critical parts of the PIEZO1 channel are affected, the resulting defect can vary greatly in strength. Such was the case for our patients 1 and 3 presenting with, respectively, typical strong and mild HX features. In both of them the mutations located to regions encoding peripheral helices of PIEZO1, exons 16 and 17, respectively [21].

Patient 4 was unusual as he carried two apparently unrelated mutations affecting the RBC: a missense mutation in the PIEZO1 gene and a 12-nucleotide in-frame deletion in the β-globin gene. Both these mutations were confirmed as pathogenic by different algorithms, and molecular modeling of the T1732M mutated PIEZO1 protein showed its markedly higher stability compared to the normal variant. At face value this is not a typical signature of pathogenic mutations. However, one should bear in mind that protein functioning requires a considerable degree of conformational flexibility, therefore an excessive structural stability can actually be detrimental. Notably, de novo-designed proteins often show substantially higher stability than their natural counterparts, which suggests that their conformations (and the underlying amino acid sequences) shaped by evolution are not the most stable structures possible but rather represent a trade-off optimum between high stability and the flexibility required for functioning. On the other hand, one should note that a paralog of Piezo1, Piezo2, does contain a methionine at position corresponding to residue 1732 of Piezo1 in the mouse, pongo and human, which does not compromise its activity. Taken together, these informations suggest that the T1732M substitution in PIEZO1 could affect the erythrocyte stability, albeit only functional studies of this PIEZO1 variant will prove that this is indeed so.

Dominantly inherited β-thalassemias are due to mutations that lead to production of a truncated or elongated and highly unstable β-globin chain. Mutations of this type have been found in many different ethnic groups, with very low frequency. Affected individuals have moderate to severe anemia, splenomegaly and the hematological features of β-thalassemia – elevated HbA2 and imbalanced globin chain synthesis [22]. Combined defects of red cell membrane and metabolism are very rare and difficult to diagnose properly, and carrihership for a metabolic defect can modify the clinical picture of the patient. In fact, reports on the
impact of co-occurring mutations responsible for various defects of the red blood cell are inconclusive. It has been noted that the concomitance of β-thalassemia and hereditary spherocytosis can in fact reduce the degree of hemolysis [23]. In contrast, Fermo et al. [7] state that the hemolytic effect of hereditary spherocytosis associated with pyruvate kinase deficiency and the β-thalassemia trait does not differ from the typical HX cases. Furthermore, the coexistence of mutations in the erythrocyte membrane protein 4.2 gene and α-thalassemia-causing deletions results in a decreased hemoglobin concentration, microcytosis, and an increased red cell distribution width value [24]. Similarly, hereditary xerocytosis due to a PIEZO1 mutation combined with the hemoglobin C (HbC) trait was associated with increased clinical severity [25]. Glogowska et al. [26] presented a patient with a PIEZO1 mutation co-inherited with heterozygous β-globin Cincinnati. A patient with an almost identical β-globin mutation (β-globin Geneva) exhibited only mild microcytic anemia [27], while in the former case the clinical severity was more profound and the patient had to be transfused regularly. In contrast, a deletion affecting codons 63–65 (c.189_195del TCATGGC) in a family from China leads to the β(0)-thal phenotype, but one should note that it causes a shift in the reading frame and a premature stop codon that causes nonsense-mediated decay of the mutant messenger RNA [28].

The severe clinical symptoms of patient 4 are unlikely to have been caused by the (previously not reported, therefore uncharacterized) β-globin defect alone. The deletion of the four amino acid residues 62–65 of β-chain results in a deformation of the heme pocket, particularly its distal side, indicating a consequent disorder in the oxygen-ion complex formation. The absence of the distal histidine important for the Fe-O coordination must itself have a similar effect. Whether the clinical picture of the patient is caused by a strong modifying effect of the otherwise non-detrimental mutation in PIEZO1, or is combined effect of two strongly deleterious mutations, cannot be decided at present.

To summarize, four previously clinically uncharacterized mutations in the PIEZO1 gene have been identified in as many patients with diverse forms of hemolytic anemia. For two of them the predicted clinical significance and molecular modeling consistently pointed to their pathogenicity, while in the other two cases the results were less clear-cut. Notably, family studies excluded a pathogenic effect of the otherwise non-detrimental mutation in PIEZO1, or is combined effect of two strongly deleterious mutations, cannot be decided at present.

Author contribution
KM, MG carried out experiments and wrote the manuscript, AAS, TU carried out experiments and wrote the manuscript, BB was responsible for the supervision of the study and manuscript revision.

Declaration of competing interest
None of the authors has any potential conflict of interest.

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Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcmd.2019.102378.

References
[1] R. Zarychanski, V.P. Schulz, B.L. Houston, Y. Maksimova, D.S. Houston, B. Smith, J. Rinehart, P.G. Gallagher, Mutations in the mechanotransduction protein PIEZO1 are associated with hereditary xerocytosis, Blood 120 (2012) 1908–1915, https://doi.org/10.1182/blood-2012-04-422533.
[2] J. Narla, N. Mohandas, Red cell membrane disorders, Int J Lab Hem 39 (2017) 47–52, https://doi.org/10.1111/ijlh.12657.
[3] X. Shang, X. Yu, Update on the genetics of thalassemia: what clinicians need to know, Best Pract Res Clin Obstet Gynaecol 39 (2017) 3–15, https://doi.org/10.1016/j.bpbgon.2016.10.012.
[4] E. Fermo, C. Vercellati, A.P. Marcello, A. Zaninoni, R. van Wijk, N. Mirra, C. Curcio, A. Corelezzi, A. Zanella, W. Barcellini, P. Bianchi, Hereditary xerocytosis due to mutations in PIEZO1 gene associated with heterozygous pyruvate kinase deficiency and beta-thalassemia trait in two unrelated families, Case Rep Hematol 2017 (2017) 2769570, https://doi.org/10.1155/2017/2769570.
[5] J.M. Schwarz, D.N. Cooper, M. Schuelke, D. Seelow, MutationTaster2: mutation prediction for the deep-sequencing age, Nat. Methods 11 (2014) 364–362, https://doi.org/10.1038/nmeth.2890.
[6] Y. Choi, G.E. Sims, S. Murphy, J.R. Miller, A.P. Chan, Predicting the functional effect of amino acid substitutions and indels, PLoS One 7 (2012) e46688, https://doi.org/10.1371/journal.pone.0046688.
[7] I.A. Adzhubei, S. Schmidt, L. Peddini, V.E. Ramensky, A. Gerasimova, P. Bork, A.S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods 7 (2010) 248–249, https://doi.org/10.1038/nmeth0410-248.
[8] K.A. Jagadeesh, A.M. Wenger, M.J. Berger, H. Guturu, P.D. Stenson, D.N. Cooper, J.A. Bernstein, G. Morin, J. Perrin, V. Proulle, J. Schymkowitz, J. Borg, R. Nys, F. Rousseau, L. Serrano, The FoldX web field, Proteins 47 (2002) 393–402, https://doi.org/10.1002/1097-0134(200202)47:4<393::AID-PRE-9>3.0.CO;2-H.
[9] V. Picard, C. Guitton, I. Thuret, C. Rose, L. Bendelac, K. Ghazal, P. Aguilar-Martinez, J. Schymkowitz, J. Borg, R. Nys, F. Rousseau, L. Serrano, The FoldX web field, Nucleic Acids Res. 33 (web server) (2005) W382–W388, https://doi.org/10.1093/nar/gki286.
[10] J. Rinehart, P.G. Gallagher, Mutations in the mechanotransduction protein PIEZO1-hereditary xerocytosis and Gardos-channelopathy: a retrospective series of 126 patients, Haematologica 104 (2019) 1554–1560, https://doi.org/10.1182/blood-2015-04-642496.
[11] E. Fermo, C. Vercellati, A.P. Marcello, A. Zaninoni, R. van Wijk, N. Mirra, C. Curcio, A. Corelezzi, A. Zanella, W. Barcellini, P. Bianchi, Hereditary xerocytosis due to mutations in PIEZO1 gene associated with heterozygous pyruvate kinase deficiency and beta-thalassemia trait in two unrelated families, Case Rep Hematol 2017 (2017) 2769570, https://doi.org/10.1155/2017/2769570.
[12] K. Arnold, L. Bordoli, J. Kopp, T. Schwede, The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling, Bioinformatics 22 (2006) 195–201, https://doi.org/10.1093/bioinformatics/btl770.
[13] E. Krieger, G. Koraimann, G. Vriend, Increasing the precision of comparative models with YASARA NOVA—a self parameterizing force field, Proteins 47 (2002) 393–402, https://doi.org/10.1002/1097-0134(200202)47:4<393::AID-PRE-9>3.0.CO;2-H.
[14] I. Andolfo, R. Russo, A. Gambale, A. Iolascon, Hereditary stomatocytosis: an un-
[23] E. Miraglia del Giudice, S. Perrotta, B. Nobili, L. Pinto, L. Cutillo, A. Iolascon, Coexistence of hereditary spherocytosis (HS) due to band 3 deficiency and beta-thalassaemia trait: partial correction of HS phenotype, Br. J. Haematol. 85 (1993) 553–557, https://doi.org/10.1111/j.1365-2141.1993.tb03347.x.

[24] M. Maciag, A. Adamowicz-Salach, A. Siciwka, J. Spychalska, B. Burzynska, The use of real-time PCR technique in the detection of novel protein 4.2 gene mutations that coexist with thalassaemia alpha in a single patient, Eur. J. Haematol. 83 (2009) 373–377, https://doi.org/10.1111/j.1600-0609.2009.01289.x.

[25] E. Yang, E.B. Voelkel, K. Lezon-Geyda, V.P. Schulz, P.G. Gallagher, Hemoglobin C trait accentuating erythrocyte dehydration in hereditary xerocytosis, Pediatr. Blood Cancer 64 (2017), https://doi.org/10.1002/pbc.26444.

[26] E. Glogowska, E.R. Schneider, Y. Maksimova, V.P. Schulz, K. Lezon-Geyda, J. Wu, K. Radhakrishnan, S.B. Keel, D. Mahoney, A.M. Freidmann, R.A. Altura, E.O. Gracheva, S.N. Bagriantsev, T.A. Kalifa, P.G. Gallagher, Novel mechanisms of PIEZO1 dysfunction in hereditary xerocytosis, Blood 130 (2017) 1845–1856, https://doi.org/10.1182/blood-2017-05-786004.

[27] G. Stamatoyannopoulos, B. Woodson, T. Papayannopoulou, D. Heywood, S. Kurachi, Inclusion-body beta-thalassemia trait. A form of beta thalassemia producing clinical manifestations in simple heterozygotes, N. Engl. J. Med. 290 (1974) 939–943, https://doi.org/10.1056/NEJM197404252901705.

[28] S. He, L. Lin, Y. Wei, B. Chen, S. Yi, Q. Chen, X. Qiu, H. Wei, G. Li, C. Zheng, Identification of a novel β-globin mutation (HBB: C.189_195delTCATGGC) in a Chinese family, Hemoglobin 40 (2016) 277–279, https://doi.org/10.1080/03630269.2016.1200073.