Case report

A rare combination of Hereditary folate malabsorption (SLC46A1 gene variant) and beta-thalassemia trait

Dolat Singh Shekhawat[1], Siyaram Didel[1*], Abhishek Purohit[2], Kuldeep Singh[1]

[1]*Medical Genetics, Department of Pediatrics, All India Institute of Medical Sciences, Jodhpur, India*
[2]*Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur, India*

Article Info

[*]***Corresponding Author:***
Siyaram Didel, Associate Professor (Incharge PHO Div.)
Department of Pediatrics, All India Institute of Medical Sciences, Jodhpur, India
E-mail: drdsram2001@gmail.com

Keywords

Hereditary folate malabsorption disease, SLC46A1 gene, HBB, beta-thalassemia

Abstract

Background: Hereditary folate malabsorption is an autosomal recessive disorder caused by a pathogenic variant in SLC46A1, affecting proton-coupled folate transporter (PCFT) function. Infants with hereditary folate malabsorption often develop megaloblastic anemia and, without treatment, may experience serious neurodegenerative complications. Thalassemia is also an autosomal recessive genetic disorder. Major or compound heterozygous thalassemia is associated with severe complications and may require regular blood transfusions.

Case: A couple is seeking guidance on the recurrence risk of the condition that led to the loss of their two previous children. The second child's medical history and laboratory findings indicate a low vitamin B12 level 137.5 pg/mL, "reference range 211-911 pg/mL", elevated homocysteine 34.98 µmol/L, "reference range 3.7 -13.9 µmol/L", and ferritin levels at 1129 ng/mL "reference range 18.2 -341.2 ng/mL". Hematological results show a hemoglobin level of 6.4 g/dL, a total reticulocyte count of 3.39%, MCV of 82.7 fL, MCH of 26.9 pg, RDW of 19.0%, neutrophils at 27%, and lymphocytes at 42%. Hb-HPLC analysis revealed an HbA2 level of 4.6%. Whole-exome sequencing identified a homozygous pathogenic variant in the SLC46A1 gene (c.1127G>A), associated with hereditary folate malabsorption and a heterozygous pathogenic variant in the HBB gene (c.92+5G>C), linked to β-thalassemia. The first child's medical history also suggests low vitamin B12 levels and elevated homocysteine and ferritin levels. Hb-HPLC showed normal results, and genetic testing was not undertaken.

Conclusion: The homozygous SLC46A1 (c.1127G>A) variant is lethal, whereas a heterozygous state with SLC46A1 (c.1127G>A) and HBB (c.92+5G>C) may not be associated with complications like transfusion-dependent thalassemia.

Introduction

Hereditary folate malabsorption (HFM), or congenital folate malabsorption (OMIM#229050), is a rare autosomal recessive condition that can affect multiple organ systems and is potentially treatable. It results from homozygous or compound heterozygous variants in the SLC46A1 gene, which encodes the proton-coupled folate transporter (PCFT). Loss of function in this transporter impairs intestinal folate absorption and transport into the central nervous system. Importantly, HFM is a potentially treatable condition, making early diagnosis critical to prevent serious complications and optimize clinical outcomes [1]. This transporter is essential for folate absorption in the intestine and its transport across the choroid plexus. PCFT dysfunction causes folate deficiency in both serum and cerebrospinal fluid (CSF), manifesting as hematologic, immunologic, gastrointestinal, neurological symptoms and mitochondrial disease [2,3]. Intracranial calcifications are often observed in neuroimaging [4]. The diagnosis is established through various methods, including evidence of impaired absorption of an oral folate challenge[5], persistently low CSF folate levels despite corrected serum folate, or molecular genetic testing revealing pathogenic variants in SLC46A1. This report highlights the clinical presentation, biochemical profile, and genetic findings of a patient diagnosed with homozygous HFM and beta-thalassemia trait, representing the first documented case of its kind.

Case detail

A non-consanguineous couple visited the Hematology and Medical Genetics clinic seeking guidance on the recurrence risk of the condition that led to the loss of their two previous children. They expressed a desire to understand the underlying cause and explore preventive measures for future pregnancies. A detailed three-generation pedigree was constructed (Figure 1), and a comprehensive medical history was obtained.

The first child, a male, had a history of severe anemia, hepatosplenomegaly, and vitamin B12 deficiency (levels: 188 pg/mL, reference range 211-911 pg/mL) with hypersegmented neutrophils, elevated lactate dehydrogenase (LDH), and a fatal outcome at 2.5 years of age. Laboratory findings included ferritin levels of 900 ng/mL "reference range 18.2 -341.2 ng/mL", hemoglobin 6.2 g/dL, total reticulocyte count 2.39%, mean corpuscular volume (MCV) 72.7 fL, mean corpuscular hemoglobin (MCH) 25.9 pg, and red cell distribution width (RDW) 19.0%. Differential counts showed neutrophils at 22% (reference range 55–70%) and lymphocytes at 70% (reference range 20–40%). The Hb-HPLC profile was normal (Table 1). No genetic test was performed. These findings were suggestive of a complex underlying hematological and metabolic disorder requiring further investigation.

The second child, a male, was admitted to the Pediatric Intensive Care Unit (PICU) with severe anemia, congestive cardiac failure (CCF), mild tachypnea, severe acute respiratory distress syndrome (ARDS), and other complications. These included hyperglycemia (steroid-induced), hepatomegaly with the liver palpable 2 cm below the costal margin along the midclavicular line, splenomegaly with the spleen palpable 1 cm below the costal margin, thing, lusterless, and sparse hair. Despite these systemic manifestations, anthropometric measurements were normal. Laboratory findings indicated a low vitamin B12 level (137.5 µmol/L, reference range 211-911 pg/mL), elevated homocysteine (34.98 µmol/L, "reference range 3.7 -13.9 µmol/L"), and ferritin levels at 1129 ng/mL (reference range 18.2 -341.2 ng/mL). Hematological results showed hemoglobin at 6.4 g/dL, total reticulocyte count at 3.39%, MCV at 82.7 fL, MCH at 26.9 pg, RDW at 19.0%, neutrophils at 27% (reference 55–70%), and lymphocytes at 42% (reference 20–40%). Hb-HPLC showed HbA2 at 4.6%, with a maternal level of 4.2%, and normal findings for the father (Table 1). The neonatal, anthropometric, and clinical history, as documented in the medical records, is summarized in Table 2.

Suspecting an inborn error of metabolism (IEM) due to the clinical presentation and sibling history, further metabolic investigations, including tandem mass spectrometry (TMS), gas chromatography-mass spectrometry (GC-MS), amino acid, and acylcarnitine profiles, were performed but yielded normal results. After that, the family was advised to conduct further evaluation and clinical management based on the findings of the genetic report. Whole-exome sequencing (WES) identified a homozygous pathogenic variant in the SLC46A1 gene (c.1127G>A), a single heterozygous 5' splice variant in Intron 1 of the (c.92+5G>C), and a single heterozygous variant of uncertain significance in the KRT83 gene (c.950G>A). Unfortunately, the child succumbed on the 12th day of PICU admission due to multiple complications, including CCF, severe anemia, vitamin B12 deficiency, severe ARDS, stage 3 acute kidney injury (AKI), acute liver failure, and catecholamine-refractory septic shock.

The clinical and laboratory findings, along with the genetic data, strongly suggested that the deaths of both affected children were due to the same underlying condition. Genetic counseling was provided to the family, and WES was recommended for the parents to understand the recurrence risk better and to guide management in future pregnancies.

WES of the mother revealed a heterozygous pathogenic variant in the SLC46A1 gene (c.1127G>A), associated with HFM, and a heterozygous pathogenic variant in the HBB gene (c.92+5G>C), linked to beta-thalassemia. Additionally, a heterozygous missense variant of uncertain significance (VUS) in the KRT83 gene (c.950G>A) was identified, though its clinical relevance is unclear. WES of the father identified a heterozygous pathogenic variant in the SLC46A1 gene (c.1127G>A), as summarized in Table 3.

The second child harbored a homozygous SLC46A1 variant (c.1127G>A) inherited from both parents. Additionally, a heterozygous missense HBB variant (c.92+5G>C) was inherited from the mother.

The risks associated with genetic variations in future pregnancies were thoroughly explained. Each pregnancy carries a 25% risk of homozygous SLC46A1 gene variants. Additionally, there is a 25% chance of the fetus being heterozygous for SLC46A1 and heterozygous HBB gene variants.

Figure 1: Pedigree of the family.

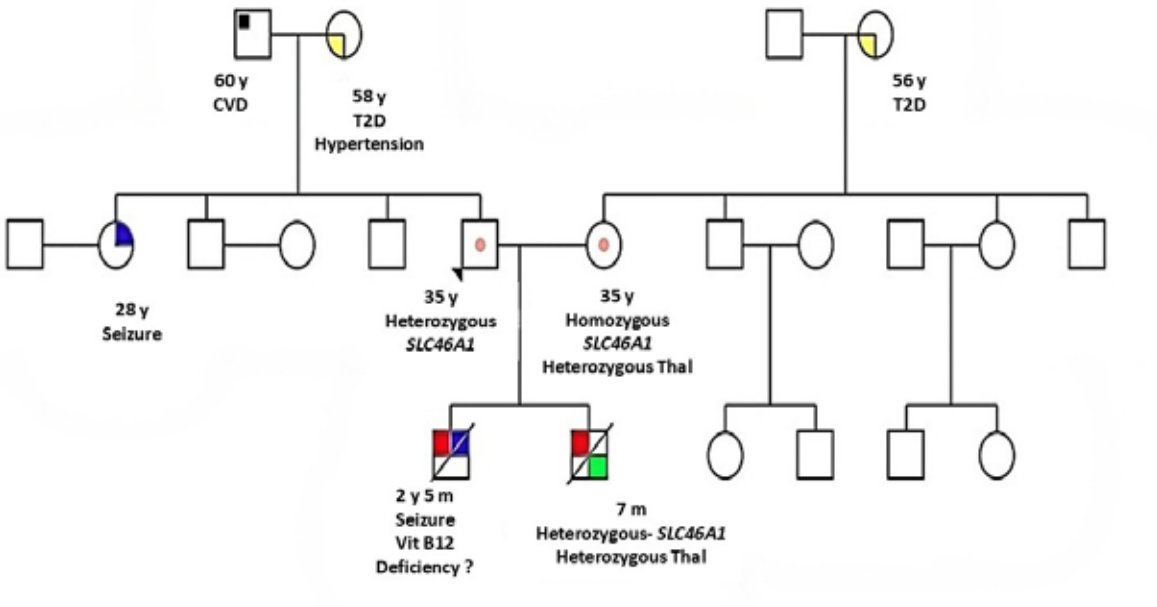

Table 1: Hb-HPLC profile of the couple and previous children.

Parameter	Second child (Ref.)	First child (Ref.)	Father (Ref.)*	Mother
HGB (g/dL)	6.4 (17-20)	6.2 (10.2-12.7)	14.3 (11-16)	10.7
RBC (109/L)	3.3 (3.5-7.5)	2.39 (4.1-6.7)	5.71 (3.5-5.5)	6
MCV (fL)	82.0 (95-125)	72.70 (80-96)	98.6 (82-100)	76.3
MCH (pg)	26.9 (30-42)	25.90 (27-31)	25.0 (27-34)	17.8
RDW-CV (%)	25.4 (11-16)	19.0 (10-15)	19.9 (11-16)	19
HPLC				
HbA (%)	88.8	92.1	92	91.4
HbA2 (%)	4	3.1	2.9	4.7
HbF (%)	1.6	0.9	0.9	0.7
P3 (%)	5.6	3.9	4.2	3.2

P3 – unknown peak at retention time 1.52 minute, Ref. -Reference range, (Ref.)* - reference range common for mother and father

Table 2: Development and clinical detail of sibling (as per available medical history).

Parameter	First child	Second child
Age of child at presentation	7 months	6 month
Birth history and anthropometry parameters at birth	Term, normal vaginal delivery; Birth Weight – 3.5 Kg	Term, normal vaginal delivery; Birth Weight – 3.2 Kg
Developmental history	Development as per the age	Development as per the age (sitting with own support, monosyllables, unidextrous reach, followed light and sound)
Anthropometry at presentation	Length – 68 cm (50th centile); Weight – 7.3 (3rd centile); Occipitofrontal Circumference – 44 cm (50th centile)	Length – 66 cm (25th centile); Weight – 6.7 (3rd centile); Occipitofrontal Circumference – 43 cm (40th centile)

Chief complaint at first presentation	Cough, low-grade fever, issues with feeding, significant pallor	Cough for one month (dry, non-spasmodic), low-grade fever for 2 days, difficulty in breathing, significant pallor
History of blood transfusion and treatment	Lowest Hb – 5.1 gm/dl; First transfusion at 6 months of age; Received – Vitamin B12, antibiotic, folate supplements	Lowest Hb – 4.1 gm/dl; First transfusion at 5 months of age; Received – Vitamin B12, folate supplements, antibiotics, immunoglobulin and antiviral
Final diagnosis made	As per available historical records – Pancytopenia under evaluation with B12 deficiency (166.9 µmol/L) with PBF s/o micro-ovalocytes, hypersegmented neutrophils and high LDH	Congestive cardiac failure, severe anemia, severe vitamin B12 deficiency (137.5 µmol/L) with hyperhomocysteinemia (34.98 µmol/L), severe ARDS (H1N1 Pneumonia), stage 3 acute kidney injury, acute liver failure, and catecholamine-refractory septic shock, multiple organ dysfunction, thalassemia minor
Cause of death	Death at the age of 2.5 years; 1a -??? Congestive cardiac failure; 1b - Severe anemia with ?? Systemic viral illness leading to decompensation	Death at the age of 8 months; 1a - Pulmonary Hemorrhage; 1b - Catecholamine refractory septic shock with respiratory distress syndrome; 1c - Severe H1N1 Pneumonia

Table 3: Findings of whole-exome sequencing in the father, mother, and child.

Subject	Gene	Zygosity	Genotype	Reference Sequence
Father	SLC46A1 Exon 3	Heterozygous	c.1127G>A, p.Arg376Gln	GRCh38
Mother	SLC46A1 Exon 3	Heterozygous	c.1127G>A, p.Arg376Gln	
	HBB Intron 1	Heterozygous	c.92+5G>C	
Second child	SLC46A1 Exon 3	Homozygous	c.1127G>A, p.Arg376Gln	
	HBB Intron 1	Heterozygous	c.92+5G>C	
First child	-	-	-	

The WES was performed using NovaSeq 6000 Illumina sequencing platform.

Discussion

A heterozygous missense variant in exon 3 of the SLC46A1 gene (chr17:g.28402276C>T) that results in the amino acid substitution of Glutamine for Arginine at codon 376 (p.Arg376Gln). The observed variant has previously been reported in patients affected with HNM and functional studies provide strong evidence that the variant has a damaging effect on the gene or gene product [6]. Clinical phenotype characterized by gastrointestinal, hematologic, immunologic, and neurological complications due to folate deficiency from the neonatal period onward. It is primarily caused by defective oral folate absorption and reduced CSF folate levels [2], highlighting the need to assess serum and CSF folate, including 5-methyltetrahydrofolate. Huddar et al. also reported a similar case of multisystem involvement with a severe phenotype and died undiagnosed without adequate health care at the age of 3 months [4]. In the present case, the second child died at 7 months of age, with the initial presentation occurring at 6 months. The suspected cause of death was multisystem involvement, possibly triggered by H1N1 pneumonia. The first child first presented with symptoms at 6 months of age and was diagnosed with vitamin B12 deficiency, for which treatment was initiated. However, the child passed away at 2.5 years of age despite ongoing management.

HFM is a treatable cause of neurological deterioration in children and should be considered in those presenting with concomitant megaloblastic anemia [7]. Diagnosis is confirmed by significantly low baseline serum folate levels (<0.1 ng/mL; normal range: 5–15 ng/mL) with minimal or no response to an oral 5-formyl-tetrahydrofolate load [8]. Studies suggest that

intramuscular folinic acid effectively restores CSF folate levels, whereas oral supplementation remains ineffective [5]. In the present case, folate level measurements were unavailable in the medical history of the deceased siblings. This case highlights the potentially fatal consequences when timely and appropriate treatment is not provided.

The reported genotype-phenotype associations of homozygous or compound heterozygous mutations in SLC46A1, resulting in loss of function of PCFT, suggest intracranial calcification [4], immunologic dysfunction, neurologic manifestations, and hematologic issues such as megaloblastic anemia and pancytopenia [9]. In the present case, the medical history of the second child indicates immunological dysfunction (H1N1 pneumonia), as well as hematological and gastrointestinal dysfunction. However, the medical history did not show episodes of seizures or other movement disorders in either sibling.

HFM results from homozygous or compound heterozygous variants in the SLC46A1 gene, with no reported cases involving heterozygous variants in both SLC46A1 and HBB. In this case, genetic analysis of the proband identified a homozygous pathogenic SLC46A1 variant (c.1127G>A) and a heterozygous HBB 5' splice site variant (c.92+5G>C). The clinical phenotype observed in deceased siblings may be attributed to the homozygous SLC46A1 variant or a possible compound heterozygous interaction with HBB.

Thalassemia major and intermedia, may experience folate deficiency due to increased folate utilization by the bone marrow during red blood cell production and ineffective erythropoiesis [10]. However, a recent case-control study suggests that individuals with β-thalassemia trait are not significantly more likely to exhibit folate deficiency compared to healthy controls [11]. In the present case, WES of the parents revealed that the mother carried heterozygous pathogenic variants SLC46A1 (c.1127G>A) and HBB (c.92+5G>C). However, genotype-phenotype correlation in the mother indicated an asymptomatic carrier. The presence of affected children in this context is particularly intriguing, especially considering that pathogenic variants in the SLC46A1 gene, encoding the proton-coupled folate transporter (PCFT), are typically associated with impaired folate absorption and related clinical manifestations.

Folate deficiency is central to HFM, resulting from impaired intestinal absorption and defective transport across the choroid plexus due to mutations in the SLC46A1 gene. In contrast, vitamin B12 deficiency is not intrinsically associated with HFM, as its absorption occurs via an independent pathway involving intrinsic factor and the terminal ileum [2]. However, serum vitamin B12 levels in reported HFM cases have shown considerable variability. For instance, Kumar M et al. [12] and Ahmad I et al. [7]documented elevated vitamin B12 levels as high as 2000 pg/mL, whereas Tan J et al.[13] and Sakurai Y et al.[14] reported markedly low levels of 105.48 pg/mL and 42 pg/mL, respectively. A case series by Manea E et al. [15] described both low and high vitamin B12 levels across different individuals, suggesting that vitamin B12 status in HFM patients may be influenced by factors such as nutritional intake, coexisting deficiencies, or supplementation history. In the present case, the patient exhibited a reduced vitamin B12 level (137.5 pg/mL).

Genetic counseling plays a crucial role in preventing genetic disorders [16]. HFM follows an autosomal recessive inheritance pattern, where each sibling of affected individuals has a 25% chance of being affected, a 50% chance of being a carrier, and a 25% chance of inheriting neither pathogenic variant if both parents are heterozygous for SLC46A1 [8]. Identifying both pathogenic variants within a family enables carrier screening for at-risk relatives and allows for prenatal and preimplantation genetic testing in high-risk pregnancies. In this case, the father was a carrier of SLC46A1 (c.1127G>A), while the mother was heterozygous for SLC46A1 (c.1127G>A) and HBB (c.92+5G>C). Both parents were healthy, with no history of illness. The deceased child was homozygous for SLC46A1 (c.1127G>A) and a carrier of HBB (c.92+5G>C). The likely cause of death was FHM, homozygous SLC46A1 (c.1127G>A) variant.

Conclusion

HFM is a treatable genetic disorder where early diagnosis is crucial. The homozygous SLC46A1 (c.1127G>A) variant is lethal, whereas a heterozygous state with SLC46A1 (c.1127G>A) and HBB (c.92+5G>C) is not associated with complications. Identifying both pathogenic variants in a family facilitates carrier screening for at-risk relatives and enables prenatal and preimplantation genetic testing in high-risk pregnancies.

Data Availability Statement

The data supporting this study's findings are available in this manuscript.

Ethical Approval and Consent

Consent was taken from both parents for this case report. All authors listed gave consent for the publication of this paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

All authors contributed equally to the writing, development, and finalization of the case report.

Funding

None.

References

1. Zhan H-Q, Najmi M, Lin K, Aluri S, Fiser A, Goldman ID, et al. A proton-coupled folate transporter mutation

causing hereditary folate malabsorption locks the protein in an inward-open conformation. J Biol Chem 2020;295:15650–15661. https://doi.org/10.1074/jbc.RA120.014757.
2. Zhao R, Aluri S, Goldman ID. The proton-coupled folate transporter (PCFT-SLC46A1) and the syndrome of systemic and cerebral folate deficiency of infancy: Hereditary folate malabsorption. Molecular Aspects of Medicine 2017;53:57–72. https://doi.org/10.1016/j.mam.2016.09.002.
3. Serrano M, Pérez-Dueñas B, Montoya J, Ormazabal A, Artuch R. Genetic causes of cerebral folate deficiency: clinical, biochemical and therapeutic aspects. Drug Discovery Today 2012;17:1299–1306. https://doi.org/10.1016/j.drudis.2012.07.008.
4. Huddar A, Chiplunkar S, Nagappa M, Govindaraj P, Sinha S, Taly AB, et al. Child Neurology: Hereditary Folate Malabsorption. Neurology 2021;97:40–43. https://doi.org/10.1212/WNL.0000000000012083.
5. Lubout CMA, Goorden SMI, van den Hurk K, Jaeger B, Jager NGL, van Koningsbruggen S, et al. Successful Treatment of Hereditary Folate Malabsorption With Intramuscular Folinic Acid. Pediatr Neurol 2020;102:62–66. https://doi.org/10.1016/j.pediatrneurol.2019.06.009.
6. Mahadeo K, Diop-Bove N, Shin D, Unal ES, Teo J, Zhao R, et al. Properties of the Arg376 residue of the proton-coupled folate transporter (PCFT-SLC46A1) and a glutamine mutant causing hereditary folate malabsorption. Am J Physiol Cell Physiol 2010;299:C1153-1161. https://doi.org/10.1152/ajpcell.00113.2010.
7. Ahmad I, Mukhtar G, Iqbal J, Ali SW. Hereditary folate malabsorption with extensive intracranial calcification. Indian Pediatr 2015;52:67–68. https://doi.org/10.1007/s13312-015-0571-8.
8. Goldman I, Diop-Bove N, Kronn D, Mahadeo M K, Min Hee S, Sandoval C. Hereditary Folate Malabsorption. vol. 2008–2025. GeneReviews® [Internet]. Seattle (WA). University of Washington, Seattle; 1993-2023; n.d.
9. Goldman ID. Hereditary Folate Malabsorption. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews®, Seattle (WA): University of Washington, Seattle; 1993.
10. Rivella S. Iron metabolism under conditions of ineffective erythropoiesis in β-thalassemia. Blood 2019;133:51–58. https://doi.org/10.1182/blood-2018-07-815928.
11. Thilakarathne S, Jayaweera UP, Herath TU, Silva R, Premawardhena A. Serum folate and dietary folate intake in beta thalassaemia trait: a case-control study from Sri Lanka. BMJ Open 2025;15:e086825. https://doi.org/10.1136/bmjopen-2024-086825.
12. Kumar M, Yoganathan S, Todari S, Suresh P, Chandran M, Danda S, et al. Hereditary Folate Malabsorption: A Rare Treatable Disorder with Hematological and Neurological Manifestations. Ann Indian Acad Neurol 2022;25:1238–1241. https://doi.org/10.4103/aian.aian_1118_21.
13. Tan J, Li X, Guo Y, Xie L, Wang J, Ma J, et al. Hereditary folate malabsorption with a novel mutation on SLC46A1: A case report. Medicine (Baltimore) 2017;96:e8712. https://doi.org/10.1097/MD.0000000000008712.
14. Sakurai Y, Toriumi N, Sarashina T, Ishioka T, Nagata M, Kobayashi H, et al. An infantile case of hereditary folate malabsorption with sudden development of pulmonary hemorrhage: a case report. J Med Case Rep 2022;16:268. https://doi.org/10.1186/s13256-022-03448-x.
15. Manea E, Gissen P, Pope S, Heales SJ, Batzios S. Role of Intramuscular Levofolinate Administration in the Treatment of Hereditary Folate Malabsorption: Report of Three Cases. JIMD Rep 2018;39:7–12. https://doi.org/10.1007/8904_2017_39.
16. Resta R, Biesecker BB, Bennett RL, Blum S, Estabrooks Hahn S, Strecker MN, et al. A New Definition of Genetic Counseling: National Society of Genetic Counselors' Task Force Report. Journal of Genetic Counseling 2006;15:77–83. https://doi.org/10.1007/s10897-005-9014-3.