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Case Report

Phenylketonuria and Hirschsprung Disease—A Report of an Unusual Neonatal Presentation

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Abstract: We describe a term born boy of non-consanguineous Swiss parents with tetrahydrobiopterine (BH_4)-responsive Phenylketonuria (PKU) and Hirschsprung disease with unusual neonatal presentation. The child presented with floppiness, irritability, recurrent bilious vomiting and failure to pass meconium until 32 hours after birth, resulting in the clinical suspicion of an intoxication-type metabolic disease such as maple syrup urine disease (MSUD). Although the slightly elevated branched-chain amino acids in newborn screening on the fourth day of life initially supported the clinical suspicion of MSUD, the elevated Phenylalanine (Phe) of 650 µmol/L, low Tyrosine (Tyr) of 30 µmol/L, and a Phe/Tyr ratio of 22, led to the diagnosis of PKU. BH_4-testing resulted in a significant decrease of Phe from 1011 to 437 µmol/L within 24 h. Urinary pterins and dihydropteridine reductase (DHPR) activity were normal, supporting the diagnosis of BH_4-responsive PKU. Dietary restriction of Phe was initiated immediately, but oral feeding turned out to be difficult because of gastrointestinal symptoms. Intestinal motility disorder was suspected due to distended abdomen, obstructive symptoms and radiological findings with dilated intestinal loops and lack of intestinal gas in the anorectal region. Hirschsprung disease was confirmed by rectal suction biopsies and treated by a laparoscopically-assisted transanal pull-through (de la Torre) procedure. The boy is additionally compound heterozygous for two mutations in the phenylalanine hydroxylase (*PAH*) gene, which confirmed BH_4-responsive PKU. It is the first case to be described in the literature of the comorbidity of PKU and Hirschsprung disease.

Keywords: Phenylketonuria; maple syrup urine disease; Hirschsprung disease; intestinal motility disorder

1. Introduction

Newborn screening (NBS) for phenylketonuria (PKU) was initiated in Switzerland in 1965, using the classical "Guthrie-test" [1]. Since 2005, NBS for PKU using tandem mass spectrometry is performed at the University Children's Hospital Zurich. Phenylalanine (Phe) and the phenylalanine/tyrosine (Phe/Tyr) ratio are sensitive and specific tests to detect PKU [2–4]. The main clinical features of untreated PKU are intellectual disability and seizures, which become evident during the first months of life [5]. Maple syrup urine disease (MSUD) is an acute intoxication-type metabolic disease, that often presents with recurrent vomiting, coma and sepsis-like presentation during first days of life [6,7].

Thus, clinical presentations of PKU and MSUD are very different. Hirschsprung disease (HD) is a rare congenital birth defect of the enteric nervous system characterized by the absence of neuronal ganglia in the most distal segment of the intestine. The aganglionosis leads to an intestinal motility disorder with associated symptoms such as absence of meconium pass, abdominal distention and emesis. It can occur as an isolated disease or as part of a multisystem disorder [8]. We are presenting an infant, with both PKU and HD in whom the symptoms of HD and slight elevations of branched-chain amino acids in newborn screening initially led to the suspicion of MSUD.

2. Case Report

A first born male child of non-consanguineous Swiss parents was referred to the neonatology department due to recurrent bilious vomiting and absent meconium pass at 32 h after birth at term. Clinical examination showed pale skin, delayed time to recapillarisation and a distended abdomen, resulting in the clinical suspicion of intestinal atresia or motility disorder or intoxication-type metabolic disease, e.g., MSUD. X-ray and abdominal ultrasound demonstrated dilated intestinal loops and lack of intestinal gas in the anorectal region. Laboratory tests revealed normal blood count, creatinine, c-reactive protein (CRP), blood gas analysis and electrolytes. Ketonuria or hyperammonaemia were not observed.

Dry blood spot sampling for newborn screening (NBS) performed on day 4 during parenteral feeding revealed an elevated phenylalanine level of 650 µmol/L. Elevated plasma phenylalanine (768 µmol/L) and low plasma tyrosine (31 µmol/L) in quantitative amino acid analysis supported the tentative diagnosis of phenylketonuria but the additional slightly elevated branched-chain amino acids (leucine/isoleucine 306 µmol/L; valine 299 µmol/L) at first supported the clinical suspicion of MSUD. However, alloisoleucine was not detectable with the second-tier test, therefore MSUD could be excluded [9].

Administration of BH_4 showed a significant decrease of phenylalanine level (744 to 169 µmol/L) 24 h after administration of 20 mg/kg BH_4 on day 12 of life. DHPR activity as well as neopterin and biopterin concentrations in blood and urine were normal. Therefore, mild phenylketonuria (ORPHA79253) caused by phenylalanine hydroxylase deficiency was presumed. Genetic testing of the *PAH* gene revealed compound heterozygosity for two mutations in the *PAH* gene: c.612T>G, p.(Tyr204*) and c.1241A>G, p.(Tyr414Cys).

Mutation analysis of the parents confirmed that these two mutations are in *trans*. This genotype is associated with BH_4-responsive PKU (www.biopku.org).

Treatment with diet was implemented subsequent to BH_4 testing and as expected, the child required only mild Phe restriction. Over the next days, however, oral feeding turned out to be difficult and further gastroenterological workup indicated an intestinal motility disorder which caused recurrent vomiting and failure to thrive which triggered an increase of Phe levels. Rectal suction biopsies showed increased acetylcholinesterase staining and an absence of neuronal ganglia in all rectal biopsies, histomorphological typically for Hirschsprung disease. At 6 weeks of age, the patient underwent surgery. A laparoscopic mapping of biopsies revealed an affected zone up to the transverse colon. The aganglionotic segment was resected transanally by a pull-through procedure. Postoperatively, he recovered fully, but at the age of 3 months an intrasphincteric botulinum toxin injection was done due to recurrent obstructive symptoms. Impaired intestinal motility resulted in difficulties of feeding. Therefore, a percutaneous endoscopic gastrostomy (PEG) was inserted leading to an improvement of the feeding situation. By following a specific diet, phenylalanine values were kept stable within the targeted range (Table 1). In the follow-up visits, the patient presented in good health with full recovery from the gastrointestinal symptoms and normal neurological development.

Table 1. Concentration of phenylalanine, tyrosine, leucine/isoleucine, and valine during the first 51 days of life (DOL); measured from dried blood spots (DBS) by tandem MS with the standard newborn screening (NBS) method; concentrations in µmol/L.

DOL	Phe	Tyr	Leu/Ile	Val	Remarks
4	650	30	306	299	
7	442	45	91	87	
11	557	21	62	65	
12	744	45	127	120	before BH_4
12	722	58	143	116	4 h after BH_4
12	582	86	198	140	8 h after BH_4
12	403	80	162	119	12 h after BH_4
13	169	<10	61	44	24 h after BH_4
14	117	15	172	163	48 h after BH_4
15	28	41	227	178	
16	5	31	188	200	
17	18	42	223	220	
18	5	41	242	216	
19	3	49	232	194	
20	36	37	209	193	
21	12	19	203	159	
22	23	27	169	135	
23	95	71	193	160	
24	35	75	165	156	
25	29	23	123	129	
26	20	21	118	97	
27	68	19	119	94	
31	30	31	80	79	
34	22	39	165	151	
37	35	73	205	176	
40	45	100	220	187	
46	54	23	99	90	
47	77	13	68	70	
48	53	96	334	282	
49	138	22	133	141	
49	147	106	136	157	
50	236	48	131	129	
51	209	32	98	89	

3. Discussion

Our case of a mild BH_4-responsive PKU, detected by newborn screening, showed an initially untypical clinical presentation with gastrointestinal symptoms reminiscent of an intoxication-type metabolic disease such as MSUD. Slightly elevated branched-chain amino acids supported the initial clinical suspicion of MSUD. However, the distended abdomen, emesis and delayed passage of meconium were also highly indicative of Hirschsprung disease, a congenital disorder of the enteric nervous system. Therefore, the patient was evaluated by an interdisciplinary team of paediatric surgeons, neonatologists and paediatric endocrinologists. PKU was confirmed by persistently elevated phenylalanine blood concentrations. However, the elevated phenylalanine and branched-chain amino acids in the initial NBS specimen could have also been a result of the parenteral nutrition. Rectal suction biopsies showed aganglionosis up to the transverse colon. The concurrence of PKU and Hirschsprung disease has never been described in literature before and made the diagnosis of PKU initially difficult.

A multicentre cohort study described 21 co-existent conditions in a cohort of 30 PKU patients with six cases involving the gastrointestinal tract. The co-existent gastrointestinal disorders were Crohn disease, ulcerative colitis ($n = 2$), eosinophilic colitis, cystic fibrosis and oesophageal stenosis. A possible genetic background linking PKU to these disorders has not yet been elucidated [10]. None of these conditions, however, involved the enteric nervous system. Hirschsprung disease occurs as an isolated

phenotype in 70% of cases, but can also be associated with a variety of congenital abnormalities and chromosomal syndromes [11]. It is characterized by a variable pattern of inheritance which has not yet been fully explored. Genes with a crucial role in the pathogenesis of HD include *RET* [12], Endothelin B receptor [13] and *SOX* [14]. Comorbidities such as Waardenburg syndrome, MEN2, Mowat–Wilson Syndrome, Down Syndrome and other chromosomal anomalies have been reported [15]. Concerning other metabolic disorders, HD in associations with Smith–Lemli–Opitz [11] and Bardet–Biedl syndrome has been described [16]; an association with PKU has never been reported. As the pathogenesis and genetics of Hirschsprung disease are still to be discovered, reporting of associated comorbidities is of great value and importance.

In our case, the concurrence of PKU and Hirschsprung disease has not only made the diagnosis challenging, but also influenced the management. Dietary treatment was more difficult to manage due to the intestinal motility disorder and obstructive symptoms. It is essential to integrate a multidisciplinary team to provide the best care and treatment options for patients with such comorbidity constellations.

4. Conclusions

We present a case of BH_4-responsive PKU where Hirschsprung disease and not an intoxication-type metabolic disease caused the recurrent vomiting and feeding disorder. So, comorbidity can complicate the interpretation of newborn screening results.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Guthrie, R.; Susi, A. A simple phenylalanine method for detecting PKU in large populations of newborn infants. *Pediatrics* **1963**, *32*, 338–343. [PubMed]
2. Schulze, A.; Kohlmueller, D.; Mayatepek, E. Sensitivity of electrospray-tandem massspectrometry using the phenylalanine/tyrosine-ratio for differential diagnosis ofhyperphenylalaninemia in neonates. *Clin. Chim. Acta* **1999**, *283*, 15–20. [CrossRef]
3. Schulze, A.; Lindner, M.; Kohlmuller, D.; Olgemoller, K.; Mayatepek, E.; Hoffmann, G.F. Expanded newborn screening for inborn errors of metabolism by electrospray ionization-tandem mass spectrometry: Results, outcome, and implications. *Pediatrics* **2003**, *111*, 1399–1406. [CrossRef] [PubMed]
4. Wilcken, B.; Wiley, V.; Hammond, J.; Carpenter, K. Screening newborns for inborn errors of metabolism by tandem mass spectrometry. *N. Engl. J. Med.* **2003**, *348*, 2304–2312. [CrossRef] [PubMed]
5. Blau, N.; van Spronsen, F.J.; Levy, H.L. Phenylketonuria. *Lancet* **2010**, *376*, 1417–1427. [CrossRef]
6. Strauss, K.A.; Puffenberger, E.G.; Morton, D.H. Maple Syrup Urine Disease. In *GeneReviews*; Pagon, R.A., Adam, M.P., Ardinger, H.H., Wallace, S.E., Amemiya, A., Bean, L.J.H., Bird, T.D., Fong, C.T., Mefford, H.C., Smith, R.J.H., et al., Eds.; University of Washington: Seattle, WA, USA, 2013.
7. Abiri, M.; Talebi, S.; Uitto, J.; Youssefian, L.; Vahidnezhad, H.; Shirzad, T.; Salehpour, S.; Zeinali, S. Co-existence of phenylketonuria either with maple syrup urine disease or Sandhoff disease in two patients from Iran.: Emphasizing the role of consanguinity. *J. Pediatr. Endocrinol. Metab.* **2016**, *29*, 1215–1219. [CrossRef] [PubMed]
8. Parisi, M.A. Hirschsprung Disease Overview. In *GeneReviews*; Pagon, R.A., Adam, M.P., Ardinger, H.H., Wallace, S.E., Amemiya, A., Bean, L.J.H., Bird, T.D., Fong, C.T., Mefford, H.C., Smith, R.J.H., et al., Eds.; University of Washington: Seattle, WA, USA, 2015.

9. Fingerhut, R.; Röschinger, W.; Heck, M. A rapid and sensitive UPLC-MS/MS-method for the separation and quantification of branched-chain amino acids from dried blood samples of patients with maple syrup urine disease (MSUD). *Int. J. Neonatal Screen* **2016**, *2*, 2. [CrossRef]
10. MacDonald, A.; Ahring, K.; Almeida, M.F.; Belanger-Quintana, A.; Blau, N.; Burlina, A.; Cleary, M.; Coskum, T.; Dokoupil, K.; Evans, S.; et al. The challenges of managing coexistent disorders with phenylketonuria: 30 cases. *Mol. Genet. Metab.* **2015**, *116*, 242–251. [CrossRef] [PubMed]
11. Moore, S.W. Chromosomal and related Mendelian syndromes associated with Hirschsprung's disease. *Pediatr. Surg. Int.* **2012**, *28*, 1045–1058. [CrossRef] [PubMed]
12. Lyonnet, S.; Bolino, A.; Pelet, A.; Abel, L.; Nihoul-Fékété, C.; Briard, M.L.; Mok-Siu, V.; Kaariainen, H.; Martucciello, G.; Lerone, M.; et al. A gene for Hirschsprung disease maps to the proximal long arm of chromosome 10. *Nat. Genet.* **1993**, *4*, 346–350. [CrossRef] [PubMed]
13. Puffenberger, E.G.; Hosoda, K.; Washington, S.S.; Nakao, K.; deWit, D.; Yanagisawa, M.; Chakravart, A. A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* **1994**, *79*, 1257–1266. [CrossRef]
14. Kelsh, R.N. Sorting out Sox10 functions in neural crest development. *Bioessays* **2006**, *28*, 788–798. [CrossRef] [PubMed]
15. Patterson, K.; Toomey, K.E.; Chandra, R.S. Hirschsprung disease in a 46,XY phenotypic infant girl with Smith-Lemli-Opitz syndrome. *J. Pediatr* **1983**, *103*, 425–427. [CrossRef]
16. Islek, I.; Küçüködük, S.; Erkan, D.; Bernay, F.; Kalayci, A.G.; Görk, S.; Kandemir, B.; Gürses, N. Bardet-Biedl syndrome: Delayed diagnosis in a child with Hirschsprung disease. *Clin. Dysmorphol.* **1996**, *5*, 271–273. [CrossRef] [PubMed]