Molecular Genetics and Metabolism Reports 13 (2017) 80–82

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Neonatal screening for biotinidase deficiency: A 30-year single center experience

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A B S T R A C T

We reviewed the outcome of newborn screening for biotinidase deficiency performed at our department since 1987. Among 1,097,894 newborns screened, 461 were recalled, and 18 were identified as affected by complete or partial biotinidase deficiency (incidence 1:61,000, false positive rate 0.04%). The common missense mutation Q456H was found in 80% of patients with profound biotinidase deficiency. Of them, one patient harbored the novel mutation M399I in compound heterozygosity (M399I/Q456H). The complex allele A171T/D444H *in cis* was found in two patients with profound biotinidase deficiency (in homozygosity and in compound heterozygosity with the R211H mutation, respectively) and in one patient with partial biotinidase deficiency (in compound heterozygosity with the protective allele D444H *in trans*).

All detected patients were treated and followed up at our Center until present. Biotin therapy (10–20 mg/day) allowed the full prevention of clinical symptoms in all patients with no adverse effects. These excellent outcomes confirm that newborn screening for biotinidase deficiency is a very effective secondary prevention program.

1. Introduction

Biotinidase deficiency (OMIM 253260) is an autosomal recessive inborn error of metabolism leading to biotin shortage. As biotin is the essential cofactor of the four human carboxylases (namely propyonil-CoA carboxylase, methylcrotonyl-CoA carboxylase, acetyl-CoA carboxylase, and pyruvate carboxylase), its deficiency results in multiple carboxylase deficiency, a complex, life-threatening disorder impairing gluconeogenesis and organic acids metabolism. Clinically, untreated biotinidase deficiency can present with variable neurological and dermatological signs, including seizures, hypotonia, feeding difficulties, developmental delay, ophthalmologic problems, hearing loss, ataxia, alopecia, and skin rash [1]. The vast majority of these symptoms improve with the administration of pharmacological doses of biotin, although late treatment is not usually fully effective in reversing neurological, ophthalmologic, and audiological sequelae [2].

After the discovery and characterization of biotinidase deficiency in 1983 [3], a rapid colorimetric method for measuring biotinidase activity on dried blood spots was developed [4]. This addressed the first neonatal screening for biotinidase deficiency in 1985 [5], allowing for the possibility of treatment anticipation and optimization of clinical outcomes.

At our department, the Regional Reference Center for Newborn Screening of Piemonte and Valle d'Aosta and the Regional Reference Center for diagnosis and treatment of inborn errors of metabolism, neonatal screening for biotinidase deficiency was incepted since the early 1987 and all identified patients have been followed at our clinic from the neonatal period to adulthood. Here we report our 30-year experience in this issue.

2. Methods

We reviewed the outcome of newborn screening for biotinidase deficiency performed at our department from January 1987 to December 2016 and the correspondent long-term clinical outcome. Newborn screening was performed using the previously described colorimetric assay to determine biotinidase activity [4]. Briefly, enzyme activity was screened in newborns by determining the amount of *N*-biotinyl-*p*-aminobenzoate (PAB) hydrolyzed to *p*-aminobenzoate on dried blood spots collected in the third day of life. The first tier test was performed by a semiquantitative colorimetric assay, distinguishing normal (purple colored) from biotinidase deficient (straw-colored) samples. Newborns screened positive were recalled for re-determination of biotinidase activity on dried blood spot and, in case of confirmed

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http://dx.doi.org/10.1016/j.ymgmr.2017.08.005
Received 20 July 2017; Received in revised form 26 August 2017; Accepted 26 August 2017
Available online 20 September 2017

Table 1
Clinical, biochemical and molecular characteristics of 18 patients detected among 1,097,894 newborns screened for biotinidase deficiency.

Patient	Gender	Biotinidase activity		Genotype		Biotin treatment (mg/day)	Follow-up (years)	Clinical symptoms
		Serum activity*	% of median serum activity	Allele 1	Allele 2			
1	Female	0.8	17	NA	NA	10	30	No symptoms
2	Female	0	0	A171T/D444H	R211H	20	26	No symptoms
3	Female	0	0	Q456H	C186Y	20	26	No symptoms
4	Female	0	0	Q456H	E218D	20	25	No symptoms
5	Female	1.4	30	NA	NA	20	23	No symptoms
6	Male	0.6	13	A171T/D444H	D444H	20	22	No symptoms
7	Male	0	0	Q456H	E218D	20	21	No symptoms
8	Female	0	0	Q456H	G34S	20	18	No symptoms
9	Male	0	0	Q456H	G34S	20	16	No symptoms
10	Male	0	0	A171T/D444H	A171T/D444H	20	8	No symptoms
11	Male	0	0	C245Y	Q456H	20	7	No symptoms
12	Male	0	0	M399I**	Q456H	20	2	No symptoms
13	Male	0	0	C245Y	Q456H	20	2	No symptoms
14	Female	1.0	22	NA	NA	10	2	No symptoms
15	Male	1.2	24	NA	NA	10	2	No symptoms
16	Male	1.2	23	NA	NA	10	1	No symptoms
17	Female	1.2	25	NA	NA	10	1	No symptoms
18	Male	1.3	26	NA	NA	10	0.5	No symptoms

NA: not available.
* Normal value = 3.1–6.7 nM PAB/min/ml.
** Previously unreported mutation.

abnormal results, referred to clinical evaluation and quantitative measurement of serum biotinidase activity. Normal serum biotinidase activity, set on the basis of measurements in 120 healthy subjects, ranged from 3.1 to 6.7 nM PAB/min/ml. Profound and partial biotinidase deficiency were defined as < 10% and 10–30% of median serum enzyme activity, respectively. Serum biotinidase activity was also assessed in heterozygous parents of patients with genotyped biotinidase deficiency. Molecular analysis was performed by full gene sequencing in affected patients and by targeted mutation analysis in parents after informed consent. All detected patients were treated with biotin (10–20 mg/day) since the neonatal period and followed at our department up to now.

3. Results

Among 1,097,894 newborns screened, 461 were recalled, and 18 were identified as affected by biotinidase deficiency (incidence 1:61,000, false positive rate 0.04%, positive predictive value 3.9%, estimated cost per test 0.60 €). All detected patients were born from non-consanguineous Italian parents. Patients' clinical, biochemical, and molecular characteristics are summarized in Table 1. Ten patients were affected by profound biotinidase deficiency, with undetectable serum biotinidase activity. Of them, 8 (80%) harbored the missense mutation Q456H in compound heterozygosity. The novel mutation M399I was identified in one patients with profound biotinidase deficiency (Table 1, patient 12). The complex allele A171T/D444H *in cis* was the second most common molecular finding, either associated with profound and partial biotinidase deficiency depending on the second allele *in trans* (Table 1, patient 2, 6, and 10).

Eight patients were ascertained by 13–30% median serum biotinidase activity, consistent with partial biotinidase deficiency. Their serum biotinidase activity ranged from 0.6 to 1.4 nM PAB/min/ml. Genotype was available in one patients with the partial form, harboring the protective D444H mutation (Table 1, patient 6).

Overall, 9 different mutations were identified in patients with biotinidase deficiency and confirmed in parents (Q456H, A171T/D444H *in cis*, E218D, G34S, C245Y, R211H, C186Y, M399I, D444H). Their *in vivo* biochemical effect assessed in heterozygous parents of patients with biotinidase deficiency is reported in Table 2.

Table 2
In vivo serum biotinidase activity in 16 heterozygous parents of patients with profound or partial biotinidase deficiency.

Number of subjects	Mutation	Range serum activity (nM PAB/min/ml)	% of median serum activity
5	Q456H	2.3–2.5	50–54
4	A171T/D444H	2.1–3.9	45–85
1	E218D	2.4	52
1	G34S	2.1	46
1	C245Y	3.6	78
1	R211H	3.3	72
1	C186Y	3.6	78
1	M399I	3.5	76
1	D444H	3.7	80

All identified patients were asymptomatic at first clinical evaluation in the neonatal period, when treatment with biotin was started. Clinical follow-up lasted 13.6 ± 10.8 years. Compliance with biotin therapy was complete even on long-term follow-up. No adverse effects were reported. All patients underwent regular metabolic, ophthalmologic, and audiological evaluations revealing no signs or symptoms related to biotinidase deficiency.

4. Discussion

Shortly after the first pilot study of neonatal screening for biotinidase deficiency performed in Virginia in 1985, this condition was readily included in the screening program of our Region. Our department, indeed, has been traditionally dedicated to the implementation of innovative screening procedures [6–8]. Also a posteriori, biotinidase deficiency meets the major criteria for its inclusion in neonatal screening programs, being a severe disease if untreated, early detectable by rapid and economical methods, and effectively treated by a simple and inexpensive therapy. During the last 30 years, the screening for biotinidase deficiency of over one million newborns allowed the identification of 18 patients at the pre-symptomatic stage. The combined incidence profound and partial biotinidase deficiency in our Region overlapped that reported worldwide [9], being comparable to

that of many other disorders commonly included in neonatal screening programs [10,11]. A very low false positive rate characterized newborn screening for biotinidase deficiency at our clinic. In spite of the relative rarity of the disease, this parameter was even better than that advocated for expanded newborn screening programs by tandem mass spectrometry [12]. The long-lasting screening experience at our Center probably played a significant role on this achievement. On the other hand, the positive predictive value was low for this mass screening program; however, since this parameter is strictly dependent on the prevalence of the disease, this finding is not surprising. As for the estimated cost per test, newborn screening for biotinidase deficiency was cheaper than other single-disease screening programs performed at our Center, including galactosemia, cystic fibrosis, congenital adrenal hyperplasia, and hypothyroidism (around 2 € for each test).

From a molecular point of view, 80% of patients with profound biotinidase deficiency showed the same missense mutation Q456H in compound heterozygosity. This molecular finding was already identified as a common cause of biotinidase deficiency especially in patients with European ethnic backgrounds [13], in agreement of our findings. Three patients with different biochemical phenotypes shared the same complex allele A171T/D444H *in cis*. Consistently with previous observations, profound biotinidase deficiency resulted from homozygosity for this allele [14], as well as from its association with the R211H mutation *in trans*. On the other hand, the association of the allele A171T/D444H *in cis* and the protective mutation D444H *in trans*[15] resulted in partial biotinidase deficiency. *In vivo* biochemical data in heterozygous subjects are consistent with these findings.

From a clinical point of view, early biotin therapy allowed the full prevention of clinical symptoms in all detected patients with biotinidase deficiency. In particular, no adverse effects were registered and compliance to treatment was optimal even in patients on long-term follow-up (7 patients followed and treated for > 20 years). These excellent outcomes are in stark contrast with those of patients with late diagnosis of biotinidase deficiency, suffering from irreversible neurological damages if treated late and being even at risk of death if left untreated [16]. If newborn screening is not performed, indeed, the clinical diagnosis of biotinidase deficiency is invariably arduous, as biotinidase deficiency can mimic atopic dermatitis and a wide range of neurological conditions, including neuromyelitis optica, optic atrophy, and myelopathies [17–23]. Despite these clinical evidences and the cost-effectiveness of newborn screening for biotinidase deficiency [24], however, the application of this preventive procedure is still not uniform in Europe (differently from the U.S.).

We hope that our long-lasting successful experience could promote the universal extension of this practice. Neurologists, moreover, should be aware of the opportunity of including biotinidase activity as an adjunct to the diagnostic work-up of unexplained central neurological disorders in patients not screened for biotinidase deficiency in the newborn period.

References

[1] B. Wolf B, Disorders of biotin metabolism, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, 2001, pp. 3935–3962 (New York: McGraw-Hill).

[2] B. Wolf, R. Spencer, T. Gleason, Hearing loss is a common feature of symptomatic children with profound biotinidase deficiency, J. Pediatr. 140 (2002) 242–246.

[3] B. Wolf, R.E. Grier, W.D. Parker, S.I. Goodman, R.J. Allen, Deficient biotinidase activity in late-onset multiple carboxylase deficiency, N. Engl. J. Med. 308 (1983) 161.

[4] G.S. Heard, J.R. Secor McVoy, B. Wolf, A screening method for biotinidase deficiency in newborns, Clin. Chem. 30 (1984) 125–127.

[5] G.S. Heard, B. Wolf, L.G. Jefferson, K.A. Weissbecker, W.E. Nance, J.R. McVoy, A. Napolitano, P.L. Mitchell, F.W. Lambert, A.S. Linyear, Neonatal screening for biotinidase deficiency: results of a 1-year pilot study, J. Pediatr. 108 (1986) 40–46.

[6] M. Spada, S. Pagliardini, M. Yasuda, T. Tukel, G. Thiagarajan, H. Sakuraba, A. Ponzone, R.J. Desnick, High incidence of later-onset fabry disease revealed by newborn screening, Am. J. Hum. Genet. 79 (2006) 31–40.

[7] M. Spada, F. Porta, L. Vercelli, V. Pagliardini, L. Chiadò-Piat, P. Boffi, S. Pagliardini, G. Remiche, D. Ronchi, G. Comi, T. Mongini, Screening for later-onset Pompe's disease in patients with paucisymptomatic hyperCKemia, Mol. Genet. Metab. 109 (2013) 171–173.

[8] A. Ponzone, M. Spada, L. Roasio, F. Porta, A. Mussa, S. Ferraris, Impact of neonatal protein metabolism and nutrition on screening for phenylketonuria, J. Pediatr. Gastroenterol. Nutr. 46 (2008) 561–569.

[9] B. Wolf, Worldwide survey of neonatal screening for biotinidase deficiency, J. Inherit. Metab. Dis. 14 (1991) 923–927.

[10] B.L. Therrell, M.A. Lloyd-Puryear, K.M. Camp, M.Y. Mann, Inborn errors of metabolism identified via newborn screening: ten-year incidence data and costs of nutritional interventions for research agenda planning, Mol. Genet. Metab. 113 (2014) 14–26.

[11] F. Porta, S. Pagliardini, V. Pagliardini, A. Ponzone, M. Spada, Newborn screening for galactosemia: a 30-year single center experience, World J. Pediatr. 11 (2015) 160–164.

[12] P. Rinaldo, S. Zafari, S. Tortorelli, D. Matern, Making the case for objective performance metrics in newborn screening by tandem mass spectrometry, Ment. Retard. Dev. Disabil. Res. Rev. 12 (2006) 255–261.

[13] K.J. Norrgard, R.J. Pomponio, K.L. Swango, J. Hymes, T.R. Reynolds, G.A. Buck, B. Wolf, Mutation (Q456H) is the most common cause of profound biotinidase deficiency in children ascertained by newborn screening in the United States, Biochem. Mol. Med. 61 (1997) 22–27.

[14] K.J. Norrgard, R.J. Pomponio, K.L. Swango, J. Hymes, T. Reynolds, G.A. Buck, B. Wolf, Double mutation (A171T and D444H) is a common cause of profound biotinidase deficiency in children ascertained by newborn screening the the United States, Hum. Mutat. 11 (1998) 410.

[15] K.L. Swango, M. Demirkol, G. Hüner, E. Pronicka, J. Sykut-Cegielska, A. Schulze, E. Mayatepek, B. Wolf, Partial biotinidase deficiency is usually due to the D444H mutation in the biotinidase gene, Hum. Genet. 102 (5) (1998) 571.

[16] P. Ferreira, A. Chan, B. Wolf, Irreversibility of symptoms with biotin therapy in an adult with profound Biotinidase deficiency, JIMD Rep. (2017), http://dx.doi.org/10.1007/8904_2017_12.

[17] B. Wolf, Why screen newborns for profound and partial biotinidase deficiency? Mol. Genet. Metab. 114 (2015) 382–387.

[18] S. Yilmaz, M. Serin, E. Canda, C. Eraslan, H. Tekin, S.K. Ucar, S. Gokben, H. Tekgul, G. Serdaroglu, A treatable cause of myelopathy and vision loss mimicking neuromyelitis optica spectrum disorder: late-onset biotinidase deficiency, Metab. Brain Dis. 32 (2017) 675–678.

[19] B. Girard, C. Bonnemains, E. Schmitt, E. Raffo, C. Bilbault, Biotinidase deficiency mimicking neuromyelitis optica beginning at the age of 4: a treatable disease, Mult. Scler. 23 (2017) 119–122.

[20] B. Wolf, Biotinidase deficiency should be considered in individuals exhibiting myelopathy with or without and vision loss, Mol. Genet. Metab. 116 (2015) 113–118.

[21] A. Tourbah, Biotin and demyelinating diseases—a new connection? Mult. Scler. 21 (2015) 1608–1609.

[22] S. Chamney, V.G. Jothi, E. McLoone, Biotinidase deficiency, bilateral optic atrophy, and a visual field defect, Neuroophthalmology 37 (2013) 251–253.

[23] B. Wolf, Biotinidase deficiency masquerading as multiple sclerosis? Mult. Scler. (2017), http://dx.doi.org/10.1177/1352458517702551.

[24] L. Vallejo-Torres, I. Castilla, M.L. Couce, C. Pérez-Cerdá, E. Martín-Hernández, M. Pineda, J. Campistol, A. Arrospide, S. Morris, P. Serrano-Aguilar, Cost-effectiveness analysis of a national newborn screening program for biotinidase deficiency, Pediatrics 136 (2015) e424–432.