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BIOTINIDASE DEFICIENCY: OUTCOMES OF 37 YEARS-EXPERIENCE OF NEWBORN SCREENING IN TURIN, ITALY

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***ABSTRACT* – Objective:** We describe our 37-year experience of newborn screening for biotinidase deficiency.

Patients and methods: From January 1987 to December 2023, we screened 1,289,389 newborns. Newborn screening was performed using a quantitative evaluation of biotinidase activity on dried blood spots (DBS) collected within the first 72 hours of life. In case of a positive result, the second-tier test is performed. If abnormal values are confirmed, newborns are referred to a quantitative measurement of serum biotinidase activity and a clinical and molecular evaluation.

Results: We screened 1,289,389 newborns and found 52 patients with profound or partial biotinidase deficiency (incidence 1:25,000). The D444H variant was detected in 58% of patients, mostly with partial biotinidase deficiency. The Q456H mutation was found in most patients (62%) with profound biotinidase deficiency. The complex allele A171T/D444H in *cis* configuration was observed in three patients (5.7%) affected from profound or partial biotinidase deficiency depending on the mutation presented in *trans* (in compound heterozygosity with the protective allele D444H in *trans*, in homozygosity and compound heterozygosity with the R211H mutation, respectively). All identified patients were treated and regularly followed up at our center. Biotin therapy was administered at 10 or 20 mg/day in patients with partial or profound biotinidase deficiency, respectively. On this treatment, all patients were asymptomatic on long-term follow-up. Moreover, no adverse effects were reported, even in patients treated for over 35 years.

Conclusions: This experience confirms the effectiveness of newborn screening and early treatment in biotinidase deficiency.

Keywords: Biotin, Biotin-responsive, Biotinidase, Biotinidase deficiency, Biotinidase deficiency incidence, BTD gene, Metabolic disease, Multiple carboxylase deficiency, MCD, Mutation, Newborn screening.

INTRODUCTION

Biotinidase deficiency (OMIM 253260) is an autosomal recessive inborn error of metabolism leading to biotin shortage[1]. This depletion results from the inability to recycle endogenous biotin and to utilize protein-bound biotin from the diet[2]. Biotin plays an essential role as a cofactor of four human carboxylases (i.e., pyruvate carboxylase, propionyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase) and its deficiency leads to multiple carboxylase deficiency, a complex and potentially life-threatening disorder[1,2]. The enzymatic defect impairs the metabolism of several amino

acids, glucose, and fatty-acid synthesis[2]. The natural clinical presentation of biotinidase deficiency is extremely variable. Lethargy, muscular hypotonia, grand mal myoclonic seizures, ataxia, developmental delay, sensorineural hearing loss, ophthalmological disorders (i.e., conjunctivitis, optic atrophy), skin rash and/or alopecia are the most common clinical findings in untreated or late-treated patients[3-5]. Biotinidase deficiency can be effectively treated by pharmacological doses of biotin, preventing or improving most signs and symptoms. However, optic atrophy, hearing loss, and cognitive disabilities can be irreversible and unresponsive to treatment[5]. After the discovery and the biochemical characterization of biotinidase deficiency in 1983, newborn screening has made it possible to identify patients with biotinidase deficiency at birth, allowing treatment anticipation and optimizing long-term clinical outcomes. At our department, in 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, biotinidase deficiency has been incorporated into the neonatal screening program since early 1987, and all detected patients have been followed at our clinic from the neonatal period onward. In this paper, we describe our 37-year experience in this field.

PATIENTS AND METHODS

We conducted a comprehensive review of newborn screening for biotinidase deficiency at our Department of Pediatrics, AOU Città della Salute e della Scienza (Turin, Italy), from January 1987 to December 2023, analyzing the corresponding long-term clinical outcomes. Before 2017, newborn screening for biotinidase deficiency was performed using a semiquantitative colorimetric assay. This method allowed us to distinguish normal samples (purple-colored) from biotinidase-deficient ones (straw-colored), determining the quantity of p-aminobenzoate (PAB) hydrolyzed from N-biotinyl-p-aminobenzoate. Since 2017, newborn screening for biotinidase deficiency has been performed using a quantitative evaluation of biotinidase activity on dried blood spots (DBS) collected within the first 72 hours of life. In case of a positive result, the second-tier test is performed. If abnormal values are confirmed, newborns are referred to a quantitative measurement of serum biotinidase activity and a clinical and molecular evaluation. The reference range of quantitative evaluation of biotinidase has been settled within 80–370 UI/dL, while biotinidase activity on serum ranges from 3.1 to 6.7 nM PAB/min/ml. Profound biotinidase deficiency is defined by enzyme activity levels less than 10% of the median serum enzyme activity, which corresponds to a value of biotinidase activity on DBS <30 UI/dl; partial biotinidase deficiency is defined as levels of enzyme activity ranging from 10 to 30% of the median serum enzyme activity, corresponding to a value of biotinidase on DBS ranging from 30 to 80 UI/dL. The molecular analysis is performed by conducting a full gene sequencing in all affected patients and a targeted mutation analysis in parents, following the acquisition of informed consent. In all genetically confirmed affected patients, treatment with biotin (10-15-20 mg/day) was started from the neonatal period onwards. Their progress has been continuously monitored at our department, ensuring ongoing observation and care to the present date. The scientific research was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013). The study was conducted without additional clinical, diagnostic, and therapeutic practices and all data were reported in anonymized form. All subjects provided written informed consent for inclusion and publication.

RESULTS

Among 1,289,389 newborns screened, 52 were found to have biotinidase deficiency, resulting in an incidence rate of 1:25,000. Notably, all identified patients were born to non-consanguineous Italian parents. A comprehensive overview of patients' clinical, biochemical, and molecular characteristics is provided in Table 1. A total of 13 patients were affected from profound biotinidase deficiency (incidence of 1:100,000). Eight (62%) of them harbored the missense mutation Q456H in compound heterozygosity. Thirty-nine patients were affected from a partial biotinidase deficiency (incidence of 1:33,000). Most of them exhibit the protective mutation D444H, the most common molecular finding affecting 30/39 (77%) of patients. Three patients exhibited the mutation D444H in *cis* with the A171T mutation, showing a different biochemical phenotype depending on the second allele in *trans*. Overall, 20 different mutations were found in all detected patients affected from biotinidase deficiency, and these pathologic variants were subsequently verified in their respective parents (D444H, Q456H, A171T, A171T/D444H, C33FS*36, C245Y, E218D, G34S, T532M, c. 595_596del, c. 594_506delCGT, C186Y, D348N, M399I, P497S, Q275H, R157H, R211H, R538C, V457L, Y949C).

Table 1. Clinical, biochemical, and molecular characteristics of 52 patients detected among 1,289,389 newborns screened for biotinidase deficiency.

Patient	Gender	Biochemical data				Genotype		Biotin treatment (mg/day)	Follow-up (years)	Clinical symptoms
		Biotinidase activity		Biotinidase on BDS**		Allele 1	Allele 2			
		Serum activity*	% of median serum activity	1° value at NBS	2° value at NBS					
1	Female	0.8	17	–	–	NA	NA	10	37	No symptoms
2	Female	0	0	–	–	A171T/D444H	R211H	20	33	No symptoms
3	Female	0	0	–	–	Q456H	C186Y	20	33	No symptoms
4	Female	0	0	–	–	Q456H	E218D	20	32	No symptoms
5	Female	1.4	30	–	–	NA	NA	20	30	No symptoms
6	Male	0.6	13	–	–	A171T/D444H	D444H	20	29	No symptoms
7	Male	0	0	–	–	Q456H	E218D	20	28	No symptoms
8	Female	0	0	–	–	Q456H	G34S	20	25	No symptoms
9	Male	0	0	–	–	Q456H	G34S	20	23	No symptoms
10	Male	0	0	–	–	A171T/D444H	A171T/D444H	20	15	No symptoms
11	Male	0	0	–	–	C245Y	Q456H	20	14	No symptoms
12	Male	0	0	–	–	M399I	Q456H	20	9	No symptoms
13	Male	0	0	–	–	C245Y	Q456H	20	9	No symptoms
14	Female	1	22	–	–	D444H	A171T	10	9	No symptoms
15	Male	1.2	24	–	–	NA	NA	10	9	No symptoms
16	Male	1.2	23	–	–	NA	NA	10	8	No symptoms
17	Male	1.3	26	40	90	D444H	Q456H	10	7	No symptoms
18	Female	1.2	25	35	67	D444H	Q456H	10	7	No symptoms
19	Male	–	–	69	62	D444H	Q456H	10	7	No symptoms
20	Male	–	–	57	65	D444H	Q456H	10	7	No symptoms
21	Female	–	–	63	43	D444H	T532M	10	7	No symptoms
22	Female	–	–	61	69	D444H	C33Ffs*36	10	7	No symptoms
23	Male	–	–	59	63	D444H	Q456H	10	6	No symptoms
24	Male	–	–	43	62	D444H	c. 594_506delCGT	10	6	No symptoms
25	Female	–	–	29	NA	NA	NA	20	6	No symptoms
26	Male	–	–	60	62	D444H	Q456H	10	6	No symptoms
27	Male	–	–	50	55,5	D444H	T532M	10	6	No symptoms

Continued

Table 1. Clinical, biochemical, and molecular characteristics of 52 patients detected among 1,289,389 newborns screened for biotinidase deficiency.

Patient	Gender	Biochemical data				Genotype		Biotin treatment (mg/day)	Follow-up (years)	Clinical symptoms
		Biotinidase activity		Biotinidase on BDS**		Allele 1	Allele 2			
		Serum activity*	% of median serum activity	1° value at NBS	2° value at NBS					
28	Male	–	–	53	65	D444H	R157H	10	6	No symptoms
29	Male	–	–	58	66	D444H	Q456H	10	6	No symptoms
30	Female	–	–	43	55	D444H	C33Ffs*36	10	6	No symptoms
31	Female	–	–	56	54	D444H	A171T	10	5	No symptoms
32	Male	–	–	72	77	D444H	Y949C	10	5	No symptoms
33	Male	–	–	52	84	D444H	A171T	10	4	No symptoms
34	Male	–	–	61	122	D444H	D444H	10	4	No symptoms
35	Male	–	–	51	49	D444H	D444H	10	4	No symptoms
36	Male	–	–	55	62	D444H	c. 595_596del	10	4	No symptoms
37	Male	–	–	71	70	D444H	C33Ffs*36	15	3	No symptoms
38	Male	–	–	46,8	77	D444H	Q456H	10	3	No symptoms
39	Female	–	–	65	66	D444H	C33Ffs*36	10	3	No symptoms
40	Male	–	–	32	36	Q275H	P497S	20	3	No symptoms
41	Female	–	–	<14	NA	V457L	R538C	20	3	No symptoms
42	Female	–	–	33	78	A171T	D444H	10	3	No symptoms
43	Male	–	–	48	60	A171T	D444H	15	3	No symptoms
44	Male	–	–	63	53	D444H	D348N	10	3	No symptoms
45	Female	–	–	59	58	D444H	Q456H	15	2	No symptoms
46	Female	–	–	56	63	D444H	Q456H	10	2	No symptoms
47	Female	–	–	53	55	D444H	Q456H	15	2	No symptoms
48	Male	–	–	63	80	NA	NA	10	0,5	No symptoms
49	Female	–	–	59	80	NA	NA	10	0,5	No symptoms
50	Female	–	–	50	45	NA	NA	10	0,5	No symptoms
51	Female	–	–	71	70	NA	NA	10	0,5	No symptoms
52	Male	–	–	37	34	NA	NA	10	0,3	No symptoms

NA: not available.

*Normal value = 3.1-6.7 nM PAB/min/ml.

**Normal value = 80-370 UI/dL.

Table 2 depicts the prevalence of any mutation and the corresponding biochemical ranges of biotinidase activity, which invariably depends on the pathologic variant presented in *trans*. All identified patients exhibited no signs and symptoms of disease during the neonatal period when treatment with biotin was started. The clinical follow-up extended from 0.3 to 37 years, with an average duration of 9.6 years. Compliance with biotin therapy remained complete even during long-term monitoring, and no adverse effects were reported. Regular metabolic, ophthalmologic, and audiological assessments revealed no signs or symptoms of biotinidase deficiency.

Table 2. Genetic prevalence and biochemical range in 42 patients with profound or partial biotinidase deficiency.

Mutation	Number of alleles mutated	Range serum activity, % of median serum activity (range)	Biotinidase on DBS (range)
D444H	32	0.6–1.3 (13–26)	35–122
Q456H	19	0–1.3 (0–26)	35–90
A171T	5	1 (22)	33–84
A171T/D444H	4	0–0.6 (0–13)	–
C33Ffs*36	4	–	43–71
C245Y	2	0 (0)	–
E218D	2	0 (0)	–
G34S	2	0 (0)	0
T532M	2	–	43–63
c. 595_596del	1	–	55–62
c. 594_506delCGT	1	–	43–62
C186Y	1	0 (0)	–
D348N	1	–	53–63
M399I	1	0 (0)	–
P497S	1	–	32–36
Q275H	1	–	32–36
R157H	1	–	53–65
R211H	1	0 (0)	–
R538C	1	–	<14
V457L	1	–	<14
Y949C	1	–	72–77

DISCUSSION

The first implementation of neonatal screening for biotinidase deficiency was in 1985 in Virginia. Shortly after this, in 1987, we introduced the same neonatal screening program in our department. Biotinidase deficiency meets the major criteria for inclusion in newborn screening programs[5], being 1) a potentially life-threatening disorder, if untreated; 2) a clinically and biochemically well-defined disease; 3) an early detectable disease by an inexpensive test; and 4) a treatable disease with safe and effective therapy[1].

The combined incidence of profound and partial biotinidase deficiency found at our center is markedly higher than the worldwide incidence of 1:40,000-1:60,000[6] due to our region's high incidence of partial deficiency. However, a variable incidence between countries was described[7].

Molecular analysis showed a prevalent mutation harbored by more than half of patients with profound biotinidase deficiency (Q456H in compound heterozygosity). This mutation was also described as the most common in profound biotinidase deficiency by other authors[8], in agreement with our experience. Frequently associated mutations in *trans* were the E218D, G34S, and C245Y. Furthermore, the double mutation A171/D444H in *cis* was the second most frequent finding in patients with profound biotinidase deficiency, being present in 15% of patients. This allelic complex was also present in one patient affected from partial biotinidase deficiency, resulting from the association of the protective mutation D444H in *trans*, according to previous observations[9].

From a clinical point of view, in our experience, all detected patients with biotinidase deficiency treated since the neonatal period were asymptomatic at either short- or long-term follow-up. Furthermore, no adverse effects were registered, and compliance with treatment was optimal even after a long follow-up period (over 30 years). On the other hand, patients affected from undiagnosed or late-diagnosed biotinidase deficiency may suffer from severe and/or irreversible neurological damage, potentially leading to death[10]. If newborn screening is not performed, clinical diagnosis is invariably complex, as biotinidase deficiency mimics various neurological conditions, including neuromyelitis optica, optic atrophy, and myelopathies with or without vision loss[5]. In our experience, treatment of patients with partial biotinidase deficiency with lower-dose biotin than with profound deficiency (10 mg/day *vs.* 20 mg/day) was clinically safe even in the long-term period (no symptoms referred to either biotinidase deficiency or chronic biotin administration).

However, doubts have recently arisen about whether biotin therapy should be started in patients with partial biotinidase deficiency[11]. Recent experiences have shown that untreated (or discontinuously treated) patients with partial biotinidase deficiency may develop severe disease symptoms[12]. A new report from Ontario revealed that interruption of biotin therapy for a few months up to 1.5 years in children with partial biotinidase deficiency was followed by symptoms, including memory loss, fatigue, rashes, abdominal pain, alopecia, developmental delay, hearing difficulties, gait instability, recurrent infections and speech difficulties[13]. On the other hand, occasional discontinuation of biotin for a few days to a couple of weeks generally does not result in the occurrence of any symptoms[14]. These findings suggest that life-long preventive biotin treatment is necessary not only in cases of profound deficiency but also in partial biotinidase. Our experience of over 37 years in newborn screening for biotinidase deficiency has confirmed that biotinidase deficiency exhibits all the major criteria for inclusion in newborn screening programs, allowing the identification of the disorder at birth and the full prevention of disease complications[15]. However, unlike in the USA, newborn screening for biotinidase deficiency is still inconsistent in Europe[16]. We hope our long-lasting, successful experience will promote the worldwide extension of this practice. For now, neurologists should be aware of biotinidase deficiency in the differential diagnosis of unexplained neurological disorders in children and adults.

CONCLUSIONS

This experience confirms the effectiveness of newborn screening proving that selective disease screening may allow appropriate biotin treatment with curative or ameliorative effects in most patients with late-diagnosed biotinidase deficiency.

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Authors' contributions:

All authors contributed equally to the study. Beatrice Bracci and Damiano Mala conceived and designed this study under the guide of Marco Spada. They also performed the statistical analysis and, drafted and edited the manuscript. Collection and interpretation of data were possible thanks to Enza Pavanello, Pina Sauro, Alessandro Mussa and Varvara Guaraldo, who performed and analyzed the laboratory and molecular assays.

Availability of data and material:

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest:
The authors declare that they have no conflict of interest to disclose.

Ethics Approval:
The scientific research was conducted in accordance with the Declaration of Helsinki of 1975 (as revised in 2013). The study was conducted without additional clinical, diagnostic, and therapeutic practices and all data were reported in anonymized form.

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