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LETTER TO THE EDITOR

Clinical, biomarker and genetic spectrum of Niemann-Pick type C in Egypt: The detection of nine novel *NPC1* mutations

To the Editor

Niemann-Pick type C (NPC) is a rare autosomal recessive lysosomal storage disorder caused by deleterious mutations in either *NPC1* (95%) or *NPC2* (4%-5%) genes. NPC1 (a large late endosomal/lysosomal transmembrane protein) and NPC2 (a soluble lysosomal protein) work collaboratively regulating the trafficking of intracellular cholesterol and thus lipids homeostasis. Their dysfunction leads to the lysosomal accumulation of unesterified cholesterol, sphingolipids and other lipids. Neuropathological sequelae include progressive neuronal degeneration, neuroaxonal dystrophy and demyelination.[1]

The availability of the substrate reducing iminosugar miglustat, which can stabilize the neurological symptoms in NPC patients,[1,2] places great emphasis on early diagnosis of NPC. Furthermore, new potential therapeutic agents, such as 2-hydroxylpropyl-β-cyclodextrin and arimoclomol are currently in clinical trials. In this study, we summarize the clinical and genetic characteristics of the first Egyptian NPC cohort. We also confirm the validity of a new biomarker, lysosphingomyelin-509 (Lyso-SM-509), measured in dried blood spots, as a diagnostic tool that can substantially improve the time needed for diagnosis of NPC.

Patients were recruited at the neurometabolic clinic at Cairo University Children's Hospital from January 2011 to June 2017. The ethical committee at Cairo University Children's Hospital approved the study, and informed consents were obtained from parents or legal guardians of all NPC patients. Twenty-three patients from 20 unrelated Egyptian families were confirmed genetically as NPC after excluding other sphingolipidoses, such as Gaucher and Niemann-Pick A/B through specific enzymatic assays. Disease onset was neonatal in eight patients (<3 months), early infantile in six (3 months-2 years), late infantile in three (2-6 years) and juvenile in six patients (6-15 years). Nineteen patients were offspring of consanguineous marriages (82.6%), while positive family history was reported in 13 families (65%) (Table 1).

Visceral manifestations in the form of cholestasis, organomegaly, ascites, vomiting and coagulopathy were dominant in the neonatal group, while neurological manifestations, such as psychomotor retardation/regression, abnormal muscle tone, epilepsy, ataxia, extrapyramidal symptoms, ocular abnormalities, and neuropsychiatric manifestations, were more dominant in the juvenile and infantile groups.

Through targeted DNA sequencing of *NPC1* and *NPC2* in 20 patients and whole exome sequencing followed by Sanger sequencing in three patients (19, 20, 21), pathogenic mutations were detected in all 46 alleles (Table 1). Twenty-one different pathogenic mutations were detected in *NPC1*, while none were detected in *NPC2*. Nine novel *NPC1* mutations were discovered. These include three missense (c.1588C>T;p.His530Tyr, c.1881C>A;p.Ser627Arg and c.1892T>C;p.Met631Thr), two small deletions (c.425_426delAA;p.Lys142Argfs*27 and c.2046_2048delGAC; p.Leu682_Thr683delinsPhe), two indels (c.3032_3038delins10bp; p.Cys1011* and c.2045_2048delinsA;p.Leu682_Thr683delinsTyr), one small insertion (c.2779dupG;p.Ala927Glyfs*38) and one large insertion (exon10-11dup). In silico analysis predicted all reported novel mutations as disease causing. Homozygous mutations were detected in the majority of Egyptian NPC patients (n = 20, 87%), further demonstrating the harmful impact of consanguineous marriages on autosomal recessive disorders. No founder *NPC1* mutations were detected in our cohort, as only one mutation was detected in two unrelated families (patients 4 and 13).

Determination of lyso-SM-509 in dried blood spots was performed by multiple-reaction-monitoring mass spectrometry.[3] Lyso-SM-509 levels were elevated in the blood of all tested patients (3.1 ± 1.67 ng/mL, Ref. range < 0.6 ng/mL), except for a single patient in the juvenile onset group (patient 18) who was borderline normal. He was confirmed genetically at 15 years and started miglustat therapy (200 mg, 3 times/day) a few months before biomarker analysis. A possible explanation is the negative correlation of lyso-SM-509 values with age[4], which is also evident in our adolescent patients. This may necessitate the determination of lower cutoff values for higher age groups. The Gaucher disease biomarker (Lyso-Gb1) was measured in four NPC patients and was borderline elevated (6.75 ± 1.9 ng/mL, Ref. range < 4.8 ng/mL). Chitotriosidase enzyme was evaluated in another six patients and was moderately elevated (173 ± 59 nmoL/mL/hour, Ref. range < 55 nmoL/mL/hour) (Table 1).

Here, we present for the first time the mutational spectrum of a large cohort of Egyptian NPC patients. We further confirmed the diagnostic utility of the new biomarker lyso-SM-509 in dried blood spots for the diagnosis of NPC.

TABLE 1 Clinical, biomarker, and mutational spectrum of Egyptian Niemann-Pick type C patients

		Clinical data									Biomarkers			Genetic mutations		
Clinical subtype	Patient no	Gender	Age at first presentation	Age at neurological onset	Age at diagnosis	Positive consanguinity	Family history	Visceral	Neurological	Neuropsychiatric	Lyso-SM-509	Lyso-Gb1	Chitotriosidase	Mutant alleles	Protein effects	References
Neonatal	1	F	1 mo	8 mo	2 mo	+	+	+	+	–	2.0	N/A	N/A	c.3380dupT (Hom)	p.Met1127Ilefs*131	Elmonem et al, (2016)[5]
	2	M	10 d	4 mo	36 mo	+	+	+	+	–	4.3	N/A	N/A	c.425_426delAA (Hom)	p.Lys142Argfs*27	*This study*
	3	F	12 d	–	6 mo	+	+	+	–	–	5.4	N/A	N/A	c.3041+5G>A (Hom)	Alternative splicing	Reunert et al, (2016)[6]
	4	M	40 d	6 mo	48 mo	+	–	+	+	–	2.8	N/A	N/A	c.2872C>T (Hom)	p.Arg958*	Sun et al, (2001)[7]
	5	M	5 d	4 mo	8 mo	+	+	+	+	–	4.5	N/A	202	c.2245+1G>A (Hom)	Alternative splicing	Héron et al, (2012)[8]
	6[a]	M	2 mo	9 mo	4 mo	+	+	+	+	–	3.3	N/A	N/A	c.2972_2973delAG (Hom)	p.Gln991Argfs*15	Schicks et al, (2013)[9]
	7[b]	M	1 mo	4 mo	7 mo	+	+	+	+	–	2.4	5.6	166	Duplication/multiple copies of exons 10 and 11	–	*This study*
	8	M	1 mo	–	36 mo	+	+	+	–	–	2.6	7.7	159	c.1588C>T (Het), c.2045_2048delinsA (Het)	p.His530Tyr, p.Leu682_Thr683delinsTyr	*This study, This study*
Early infantile	9[a]	F	10 mo	10 mo	18 mo	+	+	+	+	–	2.1	N/A	N/A	c.2972_2973delAG (Hom)	p.Gln991Argfs*15	Schicks et al, (2013)[9]
	10	F	8 mo	8 mo	37 mo	+	–	+	+	+	8.0	N/A	272	c.3032_3038delins10bp (Hom)	p.Cys1011*	*This study*
	11	F	18 mo	18 mo	26 mo	+	–	+	+	+	3.1	N/A	99	c.2779dupG (Hom)	p.Ala927Glyfs*38	*This study*
	12	F	15 mo	15 mo	36 mo	+	–	+	+	–	2.2	N/A	N/A	c.3591+1G>A (Hom)	Alternative splicing	Ribeiro et al, (2001)[10]
	13	M	18 mo	18 mo	36 mo	–	+	+	+	–	2.8	9.1	N/A	c.2872C>T (Hom)	p.Arg958*	Sun et al, (2001)[7]
	14	M	11 mo	11 mo	30 mo	+	–	+	+	–	3.0	5.6	N/A	c.451_452delAG (Hom)	Ser151Phefs*18	Sun et al, (2001)[7]
Late infantile	15	F	28 mo	28 mo	75 mo	–	+	–	+	+	3.3	N/A	N/A	c.1881C>A (Het), c.3182T>C (Het)	p.Ser627Arg, p.Ile1061Thr	*This study*, Millat et al, (1999)[11]
	16	F	42 mo	42 mo	84 mo	+	+	–	+	–	4.6	N/A	N/A	c.3557G>A (Hom)	p.Arg1186His	Carstea et al, (1997)[12]
	17	M	26 mo	26 mo	28 mo	–	+	–	+	–	2.2	N/A	N/A	c.1553G>A (Hom)	p.Arg518Gln	Zhang et al, (2014)[13]
Juvenile	18	M	8 y	8 y	15 y	+	+	–	+	+	0.6	N/A	N/A	c.1588C>T (Hom)	p.His530Tyr	*This study*
	19[a]	F	11 y	11 y	16 y	+	+	–	+	+	N/A	N/A	N/A	c.1892T>C (Hom)	p.Met631Thr	*This study*
	20[a]	F	11 y	11 y	14 y	+	+	+	+	+	N/A	N/A	N/A	c.1892T>C (Hom)	p.Met631Thr	*This study*
	21[a]	M	10 y	10 y	12 y	+	+	–	+	+	N/A	N/A	N/A	c.1892T>C (Hom)	p.Met631Thr	*This study*
	22	M	7 y	7 y	15 y	–	–	+	+	+	1.1	N/A	140	c.2046_2048 delGAC (Het), c.3467A>G (Het)	p.Leu682_Thr683delinsPhe, p.Asn1156Ser	*This study*, Carstea et al, (1997)[12]
	23	F	10 y	10 y	13 y	+	–	–	+	+	1.3	N/A	N/A	c.496C>T (Hom)	p.Pro166Ser	Park et al, (2003)[14]

Abbreviations: F, female; Het, heterozygous; Hom, homozygous; M, male; N/A, not available.
Patients 9 and 20 died during the course of the study.
[a] Patients 6 and 9 are siblings and so are patients 19, 20 and 21.
[b] Patient 7: Through multiplex ligation-dependent probe amplification (MLPA), a duplicated or multiple copies encompassing exons 10 and 11 were detected.

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