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INVESTIGACION
SECRETARIA DE SALUD
INSTITUTO NACIONAL DE PEDIATRIA

CTNS gene study emphasizes diagnostic value of eye examination in cystinosis

TESIS

PARA OBTENER EL DIPLOMA DE ESPECIALISTA EN
GENETICA MEDICA

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MEXICO D.F. 2013

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To the Editor:

Cystinosis (MIM #219800) is an autosomal recessive and infrequent inborn metabolic disease, with an estimated incidence of 1 per 100-200,000 Caucasian live births. This condition is caused by a defect in cystine lysosomal transport, leading to intracellular accumulation of this amino acid in the kidneys, eyes, brain, thyroid, muscles, pancreas and other tissues. Classic nephropathic cystinosis (CNC) should be suspected in all children who show failure to thrive and signs of generalized proximal tubular dysfunction or renal Fanconi syndrome (RFS). Slit-lamp examinations reveal pathognomonic corneal and conjunctival deposits of typical cystine crystals (CC) in virtually all affected individuals after 12-16 months of age, whereas the absence of these crystals in children older than 2 years excludes this disease. A diagnosis of CNC can be confirmed by demonstrating elevated cystine content in isolated polymorphonuclear (PMN) leukocytes or pathogenic mutations in the *CTNS* gene. Cysteamine-based treatment early in life can prevent or delay end-stage renal disease.[1]

A diagnosis of CNC is difficult to confirm in children living in Mexico and most Latin American countries who present with RFS because cystine levels can be measured only at a few locations, such as those served by the Brazilian Multicenter Cystinosis Group.[2] In Mexico, the molecular diagnosis of CNC was first implemented in 2006, with the mutational spectrum assessed in 11 Mexican cystinotic families, including the description of four new mutations.[3] In the present study, approved by the Ethical and Research Committee, 15 non-related Latin American patients clinically suspected of having CNC,

but who did not undergo cystine PMN testing, were referred for molecular assessment of *CTNS* gene; most of these patients presented with complete or incomplete RFS, with or without ophthalmologic examination. None of them were under treatment with topical cysteamine eye drops and only two of these patients (CTNS-95 and CTNS-112) received oral cysteamine therapy previous of molecular analysis due to their phenotype (Table 1).

Molecular *CTNS* gene analysis confirmed the diagnosis of CNC in 6 of the 15 patients (40%), all from Argentina or Venezuela. Corneal CC was also observed in five of these patients, all of whom were diagnosed before 5 years of age, justifying the initiation of cysteamine treatment. Each of these five patients had two severe and previously reported *CTNS* gene mutations predicting the infantile or classic nephropathic phenotype.[2-4] The founder mutation c.?_(62_853)del or 57-kb deletion, which may have originated in Germany 1,300 years ago, is the most prevalent *CTNS* mutation in CNC patients of European heritage (40-65%),[4] and observed in homozygous state in 37.2% of patients in a Brazilian cohort.[2] Similary, in the present study this mutation was identified in four of the six *CTNS* alleles from the three Argentinean patients, findings consistent with the European genetic contribution to Latin American populations. Factors such as the very small sample of patients analyzed in this study and may be consanguinity (not referred in our patients), prevent the establishment of a Latin American *CTNS* mutational spectrum. However the presence of homozygous mutations in four of the six patients with confirmed CNC suggests a low *CTNS* allelic heterogeneity, at least in Argentinean and Venezuelan patients.

The remaining nine patients (60%) had a normal *CTNS* genotype; of these, four were not assessed ophthalmologically. Of the remaining five patients analyzed, three did not have corneal CC deposits, with crystals present only in two (Table 1). It is important to note, that the patient CTNS-112 despite having a normal result in the *CTNS* molecular study had a

high index of clinical suspicion, so the cystine PMN measurement was later performed in a laboratory in France, that confirmed the diagnosis of cystinosis (2.6 nmol half-cystine/mg cell protein, reference value: <0.1 nmol half-cystine/mg cell protein, Table 1). This patient and also the may be CTNS-95 patient without cystine measurement, illustrates the limitations described when a screening method is employed to identify the *CTNS* genotype,[1,3,4] since the single strand conformation analysis (SSCP) has a sensitivity ranging from 70-90% and some mutations may have been missed by the molecular diagnostic protocol used (e.g. heterozygous deletions for one or more exons or mutations in *CTNS* gene promoter). Moreover, if the molecular *CTNS* gene analysis is employed as a first diagnostic tool to initiate treatment, it might need around of three months to be completed at least in our laboratory. Although recently implementation of the full automated sequencing instead of the SSCP analysis, could lead to a more accurate and prompt diagnosis, a feature particularly helpful for those RFS patients under 12 months of age, when the corneal CC may go unnoticed.

It is important to note that eight of the nine patients with normal *CTNS* genotypes were older than 16 months, an age sufficient for proper diagnostic ophthalmologic evaluation. This is relevant because other algorithms, based on direct automated sequencing of the *CNTS* gene and its promoter along with transcript analysis, have a diagnostic accuracy rate of nearly 100% in patients with unequivocal corneal CC deposits and typical RFS, even in the absence of direct measurement of cystine.[6] Thus, while every child with RFS should be suspected of having cystinosis, the predominance of a normal *CTNS* genotype in 8 of 13 (61.5%) patients with RFS reinforces the need to immediately perform slit-lamp examinations in all patients with RFS over 1 year of age, prior to measuring cystine or the

molecular evaluation of the *CNTS* gene, both of which are more complex and/or time-consuming methods not readily available throughout Latin America.

Acknowledgments

We thank M. Díaz-Morales M. for technical assistance and F. Cavagnaro-Santa María, R. W. Márquez, A. Méndez-Parra, N. Guelbert, F. Gómez-Pizarro, A. Sebayo and F. Funes for kindly referring their patients.

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Table 1. Primary clinical data and *CTNS* genotype in studied Latin American patients.

Patient ID	Country	Age at referral	Renal abnormality / age at presentation	Corneal cystine crystals / age at eye evaluation	*CTNS* genotype*	CONCLUDING REMARKS
CTNS-35	Chile	?	?	?	Normal	Reconsider diagnosis and perform ophthalmologic examination
CTNS-36	Argentina	?	?	?	[c.?_(62_853)del]; [c.?_(62_853)del]**	CNC diagnosis confirmed
CTNS-37	Argentina	4 years	RFS / 8 months	Present / 18 months	[c.18_21delGACT]; [c.18_21delGACT]	CNC diagnosis confirmed
CTNS-69	Venezuela	4 years	Incomplete RFS *** (without glycosuria) / 6 months	Absent / ?	Normal	Reconsider diagnosis
CTNS-71	Venezuela	3 years	RFS / 7 months	Present / 24 months	[c.646dupA]; [p.Gln128*]	CNC diagnosis confirmed
CTNS-74	Venezuela	3 years	RFS / 6 months	Present / 24 months	[c.646dupA]; [c.646dupA]	CNC diagnosis confirmed
CTNS-76	Venezuela	3 years	Incomplete RFS (without glycosuria) / ?	Absent / ?	Normal	Reconsider diagnosis
CTNS-77	Chile	1 years 4 months	Incomplete RFS (without glycosuria) / ?	Absent / 15 months	Normal	Reconsider diagnosis
CTNS-82	Venezuela	1 year 6 months	RFS / 14 months	Present / 12 months	[c.40delC]; [p.Ser270del]	CNC diagnosis confirmed
CTNS-91	Colombia	22 years	RFS / 24 months. ESRD	?	Normal	Reconsider diagnosis and perform ophthalmologic examination
CTNS-95	Colombia	9 years	Incomplete RFS (without ricketts) / ?	Present / 6 years	Normal	Reconsider ophthalmologic reassessment and cystine measurement to confirm diagnosis
CTNS-100	Colombia	11 years	RFS / 24 months	?	Normal	Reconsider diagnosis and perform ophthalmologic examination
CTNS-112	Chile	3 years, 11 months	RFS / 12 months	Present / 20 months	Normal	Subsequently, cystine measurement confirmed the diagnosis of CNC.
CTNS-116	Venezuela	2 years	RFS/ 9 months	?	Normal	Reconsider diagnosis and perform ophthalmologic examination
CTNS-117	Argentina	5 years	RFS / 5 months	Present / 3 years	[c.?_(62_853)del]; [c.?_(62_853)del]	Diagnosis of CNC confirmed

Footnotes of Table 1.

[*] Characterized through identification of delta 57-kb deletion and mutational screening by SSCP as previously described.[3] Genotype annotation according to HGVS Nomenclature and transcript RefSeq ID NM_001031681.2 and the cystinosin isoform 1 precursor reference protein sequence NP_001026851.2.

[**] A 57-kb deletion encompassing the first nine exons and introns and part of exon 10 of the *CTNS* gene and the entire adjacent *SHPK* gene.

[***] Traditionally defined as the absence of one or more components typically excreted in urine of patients with RFS; e.g., without glycosuria, appreciable phosphaturia or rickets.[7]

?: Not referred. RFS: Renal Fanconi syndrome. CNC: Classic nephropathic cystinosis. ESRD: End-stage renal disease.