THE DIAGNOSTIC ODISSEY OF A ML II PHENOCOPY: THE FIRST WESTERN PATIENT WITH LYSET DEFICIENCY

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INTRODUCTION

The *LYSET* gene (*TMEM251*; OMIM619332) discovered in 2021 codify the transmembrane protein 251 that regulates the lysosome biogenesis by activating the mannose-6-phosphate (M6P) pathway. Only two homozygous variants were associated to DMAN diasease in 6 reported cases - 5 Pakistani sibs and 1 Iranian girl. DMAN is an autosomal recessive ultrarare and severe progressive skeletal dysplasia with coarse facies, distended abdomen, short stature and severe physical disability, a phenocopy of mucolipidosis II/III (ML).

CASE

A female patient, daughter of consanguineous parents, born in 2008, had hand contractures and typical face since 5 months old. Clinically diagnosed at 2 years old with contractures and joint limitations in hands, feet and shoulder (Fig.1). Cardiac auscultation revealed systolic murmur and echocardiogram showing thickening of mitral and aortic valves, those with mild regurgitation, but without hemodynamic effect. It was also observed functional tricuspid regurgitation. Biochemical analizes were performed to investigate the clinical suspicion of ML and MPS. Eight different enzymatic activities presented increased levels in plasma and one normal level in leukocytes. Thin-layer chromatography, dosage of GAGs and OLS/SOLS chromatography in urine detected abnormal sialic acid profile and normal GAGs in urine. These results suggested the diagnosis of ML II. Sanger sequencing and aCGH were negative to variants in *GNPTAB* and *GNPTG*. Exome was performed and no suspicious variants were detected, however the *LYSET* gene had not yet been discovered (Fig.2).

Figure 1: Authorized images of the patient.

In an effort to reanalyze the patient with more advanced methods and knowledge, the whole genome was recently realyzed presenting the homozygous nonsense variant NM_001098621.4:c.112C>T (p.Gln38Ter) in *LYSET* gene. The variant is not predicted to undergo nonsense mRNA decay (NMD), however, the gene was recently discovered and its mechanism of pathogenicity is still being established.

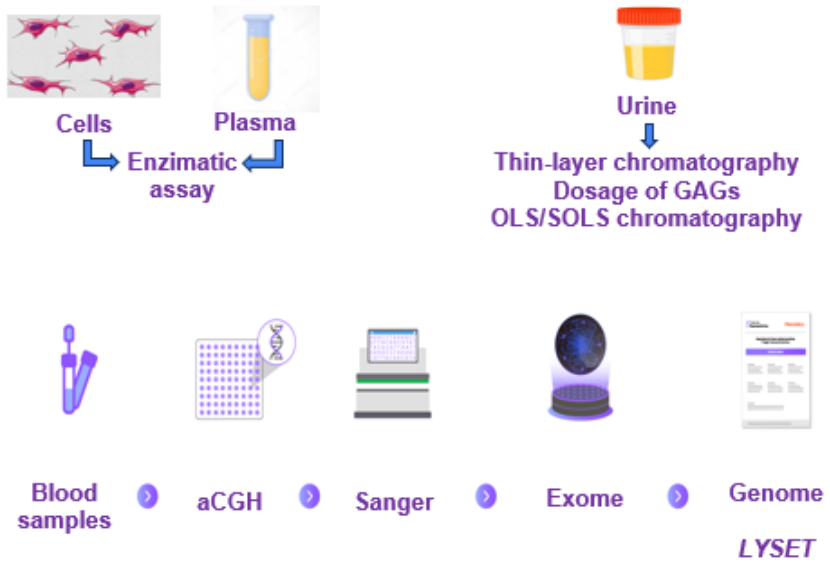

Figure 2: Analyses carried out in the diagnostic odyssey.

Table 1: Biochemical characterization of

Biochemical Investigation	Sample	Patient	Reference Values
Fibroblasts available for analysis	-	No	-
Age at biochemical Investigation (y.o.)	-	3	-
α–Iduronidase	Plasma	279	6,8-13,7
β– Glucuronidase	Plasma	832	30-300
α–Mannosidase	Plasma	2013	17-56
β– Hexosaminidases A	Plasma	2606 / 4599	550-1675
β– Hexosaminidases B	Plasma	457 / 15966	265-1219
Total β– Hexosaminidases	Plasma	3063 / 22675	1000-2857
% Hexosaminidases A/ Total β– Hexosaminidases	Plasma	85/30	45-79
Quitotriosidase	Plasma	78 / 159	8,8-132
α–Iduronidase	Leukocyte	44	32-56
Dosage of GAGs	Urine	88 (79-256)	< 9 years: 44-106 mg/L
Thin-layer Chromatography of GAGs	Urine	sialic acid / anomalous bands	Normal
Chromatography OLS/SOLS	Urine		

GAGs: Glycosaminoglycans; - : not available.

The discovery of the relationship of the *LYSET* gene with lysosomal biogenesis was determinant to the diagnostic conclusion (Fig.3). Cases of dysostosis multiplex can be extremely challenging due to the rarity of the disease and clinical similarity to ML. How *LYSET* causes disease is still being investigated. This is the first western report of a challenging case DMAN in an extensive diagnostic odyssey. *LYSET* gene must be considered in the differential diagnostic when M6P-labeled lysosomal enzymes are altered. It is extremely important that the biological functions of the *LYSET* gene be characterized, in addition to being now correlated with severe forms of an inheritable disease, it is also hypothesized that it may be related to highly pathogenic viruses that depend on lysosomal hydrolase activity for infection, and for cancer cell proliferation in nutrient-deprived environments.

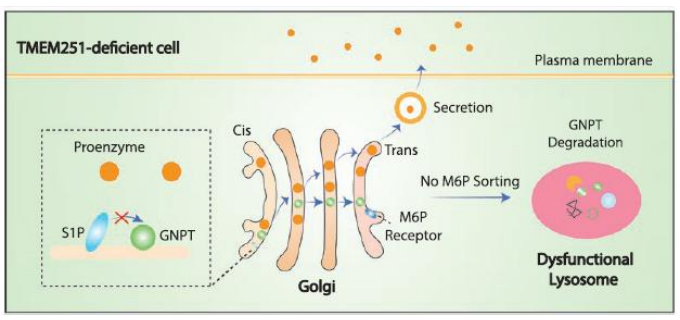

Figure 3: TMEM251 binds to GNPT and S1P, which retains GNPT in the Golgi and facilitates its cleavage by S1P. Then, GNPT transfers the lysosomal trafficking signal M6P onto the lysosomal proenzymes. Next, these proenzymes are recognized and sorted by MPRs and trafficked to lysosomes. When TMEM251 is deficient, GNPT is mis-trafficked to lysosomes and subsequent degradation, leading to loss of M6P modification and hypersecretion of lysosomal proenzymes (Qiao et al., 2023).

SUPPORT