A rare *NAGLU* mutation in an Egyptian family with Sanfilippo B syndrome

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Background/aim
Sanfilippo syndrome is an autosomal recessive lysosomal storage disorder caused by the deficiency of α-*N*-acetylglucosaminidase (NAGLU). This leads to lysosomal accumulation of heparan and heparan sulfate. The aim of this study was to analyze the *NAGLU* gene in an Egyptian family with two family members manifesting a mild Sanfilippo syndrome type B phenotype which includes mild coarse facial features, hearing loss, clear corneas, and heavy eyebrows with synopherous, mild joint stiffness, mild dementia, and gastrointestinal symptoms.

Subjects and methods
A consanguineous Egyptian family with four siblings was studied. They were three girls and a boy aged 9, 7, 3, and 1 (6/12) years, respectively. Measurement of glycosaminoglycans, two-dimensional electrophoresis, and *N*-acetylglucosaminidase activity were performed to all four siblings. Mutation analysis of the *NAGLU* gene was performed using PCR followed by Sanger sequencing of the amplified fragments.

Results
Quantitation of glycosaminoglycans and electrophoresis were done and the diagnosis of Sanfilippo syndrome was confirmed by *N*-acetylglucosaminidase enzyme (NAGLU) deficiency. A missense mutation NM_000263.3:c.934 G>A; p.D312N was detected in exon 5 of the *NAGLU* gene in a homozygous pattern in the two affected sisters and in heterozygous form in the two carriers' sister and brother.

Conclusion
A rare missense mutation p.D312N was identified in an Egyptian family with Sanfilippo syndrome type B as homozygous in two affected sisters and heterozygous in the carriers' sister and brother. This rare mutation was previously reported only in three families from France, Iran, and Jordan.

Keywords:
D312N mutation, Mucopolysaccharidosis IIIB, NAGLU gene, Sanfilippo B syndrome

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Introduction

Sanfilippo syndrome type B, also known as Mucopolysaccharidosis type IIIB (MPS IIIB) (OMIM 252920), is an autosomal recessive metabolic disorder caused by mutations in the *NAGLU* gene. This leads to deficient activity of α-*N*-acetylglucosaminidase (Naglu, EC 3.2.1.50), and results in lysosomal accumulation of glycosoaminoglycans (GAGs), heparan and heparan sulfate (HS) (Neufeld and Muenzer, 2001). Sanfilippo B syndrome is caused by more than 170 different mutations in the *NAGLU* gene (Zhao *et al.*, 1996; Beesley *et al.*, 1998; Schmidtchen *et al.*, 1998; Zhao *et al.*, 1998; Bunge *et al.*, 1999; Weber *et al.*, 1999; Yogalingam and Hopwood, 2001; Emre *et al.*, 2002; Lee-Chen *et al.*, 2002; Beesley *et al.*, 2005; Chinen *et al.*, 2005; Champion *et al.*, 2010; Mohammed and Fateen, 2019). The gene for human α-*N*-acetylglucosaminidase (*NAGLU*) was identified in 1996; the 8.5 kb gene consists of six exons and is mapped to chromosome 17q21.1 (Zhao *et al.*, 1996). The NAGLU cDNA encodes a 743 amino acid protein that has six potential *N*-glycosylation sites (Weber *et al.*, 1996).

The clinical phenotype of Sanfilippo syndrome type B includes behavioral abnormalities, attention-deficit disorder, and aggressive behavior along with hyperactivity, delayed speech, and sleep disturbance. Other symptoms associated with the disease involve developmental delay, neurodegeneration, mild hepatosplenomegaly, joint stiffness, dysostosis, and progressive mental retardation (MR) leading to death in the second decade. The wide spectrum of clinical features of MPS IIIB patients is associated with molecular heterogeneity in the *NAGLU* gene.

In our previous study conducted by Mohammed and Fateen (2019), the genetic analysis of *NAGLU* gene in a cohort of Egyptian patients with Sanfilippo syndrome type B showed identification of 10 different mutations,

including three novel mutations (K255Rfs*18, E153Rfs*39, and Q350*) and seven previously reported ones (Y558*, L550P, R297*, R482W, G79C, Y140C, and W268R). This was the first study of *NAGLU* gene analysis in Egyptian patients with Sanfilippo B syndrome, which provided valuable information for genetic counseling and further clinical genetic studies of MPS IIIB syndrome.

Héron *et al*. (2011) reported a patient with Sanfilippo B syndrome in France with a compound heterozygous mutation of p.D312N and p.R565Q. This was the first report of the p.D312N mutation. Yassaee *et al*. (2017) reported an affected Iranian patient with Sanfilippo syndrome type B with a homozygous p.D312N mutation. In the same year, Froukh (2017) identified p.D312N mutation in a Jordanian consanguineous family with Sanfilippo syndrome type B in a homozygous pattern in the affected daughter and in heterozygous pattern in the carrier parents and their carrier siblings.

Participants and methods

This study was performed on an Egyptian consanguineous family with four siblings of three girls and one boy with ages 9, 7, 3, and 1 (6/12) years, respectively. Written informed consent was obtained from the parents of the children according to the guidelines of the Medical Research Ethics Committee of the National Research Centre before the study.

Phenotype

The older sister showed mild coarse facial features, hearing loss, clear corneas, and heavy eyebrows with synopherous, mild joint stiffness, mild dementia, gastrointestinal symptoms (diarrhea), and normal electroencephalogram (EEG) record. She sleeps well, is friendly, can talk to an extent, and is obedient. However, the younger sister has a more severe phenotype including neurological deterioration (severe MR), delayed speech, attention-deficit hyperactive disorder (ADHD), aggressive hyperactivity, sleep disturbances, gastrointestinal symptoms (diarrhea), severe dementia, hirsutism, mild autism, normal MRI of the brain, and mild hepatosplenomegaly.

Biochemical diagnosis

The MPS IIIB family was diagnosed by quantitative measurement of GAGs and two-dimensional electrophoresis of urinary GAGs (Whiteman and Henderson, 1977). The enzyme activity, α-*N*-acetylglucosaminidase (NAGLU) (MPS IIIB; EC 3.2.1.50) was assayed in the plasma using fluorogenic substrates (Marsh and Fensom, 1985).

Molecular analysis

Genomic DNA was extracted from peripheral blood lymphocytes of the four siblings, according to the standard salting-out protocol (Miller *et al*., 1988). The entire coding regions of the *NAGLU* gene were amplified using 10 overlapping primers previously described (Beesley *et al*., 1998). The coding regions as well as their exon/intron boundaries were amplified to identify potential splice site variation as well. The PCR reaction using 100 ng of genomic DNA, 25 pmol of each primer, 1× NH4 reaction buffer (Thermo Scientific, Waltham, Massachusetts, USA), 4% (v/v) DMSO (dimethyl sulfoxide), 1.0 mmol/l MgCl, 0.2 mmol/l dNTPs, and 0.5 µl (2.5 units) (Fermentas, EU, Canada) DNA polymerase (Thermo Scientific, Waltham, Massachusetts, USA) (Beesley *et al*., 1998) was set. Standard PCR cycling conditions were: initial denaturation at 96˚C for 10 min, 35 cycles of denaturation at 96˚C for 1 min, annealing at 60–64˚C for 1 min, extension at 72˚C for 1 min, and an additional extension at 72˚C for 10 min (Beesley *et al*., 1998). The PCR products were purified by a QIA quick PCR purification kit (Qiagen, Hilden, Germany) and directly sequenced in both directions using the Big Dye Termination kit (Applied Biosystems, Foster City, California, USA) and analyzed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. Mutations were named according to ACMG guidelines using NM_000263.3 as the reference sequence. The A of the first ATG was considered as nucleotide +1 (Richards *et al*., 2015).

Both parents are the obligate carriers, especially the detected mutation in homozygous pattern, so each parent will be a carrier of an allele of the detected mutation.

Mutational pathogenicity score analysis

The potential impact of missense mutations on protein function was assessed using three bioinformatics tools: polymorphism phenotyping (PolyPhen) (*http://coot.embl.de/PolyPhen/*) (Adzhubei *et al*., 2010) sorting intolerant from tolerant (SIFT) (*http://blocks.fhcrc.org/sift/SIFT.html*) (Kumar *et al*., 2009), and protein variation effect analyzer (PROVEAN) (*http://provean.jcvi.org/index.php*) (Choi and Chan, 2015).

Results

The Egyptian consanguineous family with Sanfilippo syndrome type B has two affected women, one

carrier woman and one carrier man as shown in the pedigree (Fig. 1).

Biochemical findings

The biochemical results have shown that the two affected sisters showed much higher GAG levels than the reference age range (Fateen *et al*., 2014). The electrophoretic separation of their urinary GAGs showed heparan and HS spots. Deficient NAGLU activity confirmed the diagnosis of MPN IIIB. Both carrier siblings showed normal electrophoretic pattern. The carrier sister showed moderately raised GAG level than the reference age range and the carrier brother showed a high normal GAG level for the reference age range. Both carriers showed different patterns of NAGLU enzyme activity. The carrier sister showed normal NAGLU activity while the carrier brother showed a reduced NAGLU activity (Table 1 and Fig. 2).

The enzyme assay is not accurate in detecting the carrier level, due to the wide range of overlap between the carrier and the noncarrier persons.

Molecular analysis

Sanger sequencing has shown missense mutation NM_000263.3:c. 934 G>A; (p.D312N) in the Egyptian consanguineous family in two patterns; homozygous pattern in the two affected daughters and heterozygous pattern in the two carriers' siblings: daughter and son (Fig. 3).

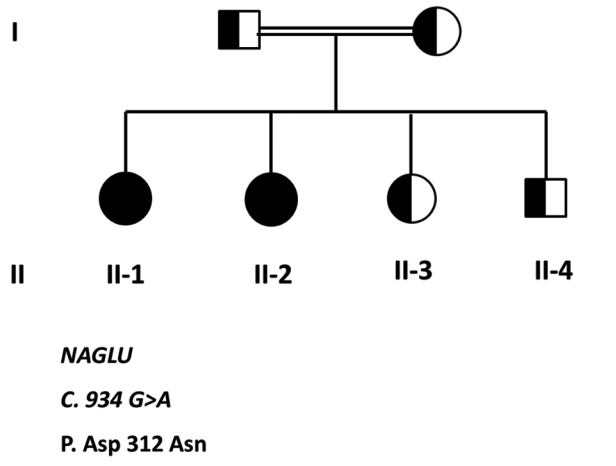
Mutations' pathogenicity score

The pathogenic potential prediction analysis using the three bioinformatics tools, Polyphen, SIFT, and PROVEAN has shown that the missense identified mutation c.934 G>A (p.D312N) in the *NAGLU* gene predicted that it has a pathogenic potential on the NAGLU protein function (Table 2).

Discussion

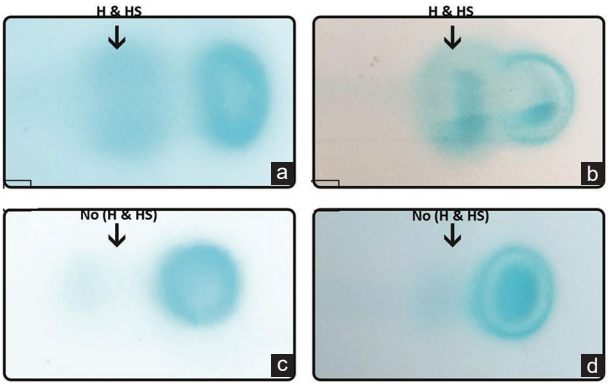
Sanfilippo syndrome (MPS type III) is an autosomal recessive lysosomal storage disease, characterized by progressive mental deterioration and behavioral problems with mild somatic disease. Sanfilippo syndrome caused by a deficiency in one of the four lysosomal enzymes involved in the degradation of GAGs (Neufeld and Muenzer, 2001). Based on the enzyme deficiency, four different subtypes, MPS IIIA, B, C, and D, are recognized (Neufeld and Muenzer, 2001). Sanfilippo syndrome type B is characterized by very low allele frequencies of different

Figure 1



Pedigree for Sanfilippo type B (Mucopolysaccharidosis IIIB) family. The pedigree represented two affected women (II-1 and II-2), a carrier woman (II-3) and a carrier man (II-4) of carrier parents. Black/white color indicates carrier cases, and black color indicates affected cases.

Figure 2



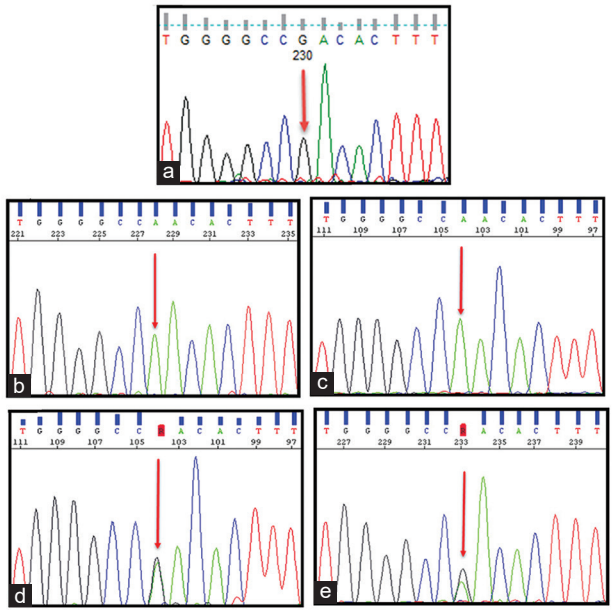
Two-dimensional electrophoretic separation of urinary glycosaminoglycans for all four siblings: (a) heparan (H) and heparan sulfate (HS) spots in the older affected sister; (b) heparan (H) and heparan sulfate (HS) spots in the younger affected sister; (c) normal pattern in the carrier sister (no H and HS spots), (d) normal pattern in the carrier brother (no H and HS spots).

Table 1 Age, sex, and biochemical findings of the four siblings

Children	Sex	Age at study (years)	Age at diagnosis (years)	NAGLU level (μmol/l/h) the reference range of NAGLU activity (10-45 μmol/l/h)	Electrophoretic pattern (heparan and heparan sulfate) (H and HS) spots	GAGs levels (mg/mmol creatinine) normal range in the curve according to the age
1	Female	11	9	0.1	(H and HS) spots	15.9
2	Female	9	7	0.2	(H and HS) spots	13.5
3	Female	5	3	13.7	Normal pattern	19.1
4	Male	2 (6/12)	1 (6/12)	3	Normal pattern	19.4

NAGLU, N-acetylglucosaminidase; GAG, glycosaminoglycan.

Figure 3



Sequencing electropherograms showing homozygous mutations in the *NAGLU* gene in the family: (a) the normal pattern, (b and c) homozygous c.934 G>A mutation in the two affected female siblings, (d) female sibling carrying the heterozygous mutation, and (e) male sibling carrying the heterozygous mutation. The arrow indicates the site of base substitution.

Table 2 D312N mutation pathogenicity score

Mutation	Polyphen	SIFT cutoff=0.05	PROVEAN cutoff=−2.5
D312N	Probably deleterious (damaging)	*P*<0.05 not tolerated (damaging)	*P*<−2.5 deleterious-4.68

PROVEAN, protein variation effect analyzer; SIFT, sorting intolerant from tolerant.

mutations and the majority of mutations is unique to individual families. The allelic heterogeneity is expected to contribute to a wide spectrum of clinical phenotypes in MPS IIIB patients (Weber *et al.*, 1999; Yogalingam and Hopwood, 2001).

In this study, an Egyptian family was diagnosed and confirmed by quantitative biochemical analysis of GAG levels, electrophoretic separation of urinary GAGs, and α-*N*-acetylglucosaminidase enzyme (NAGLU) activity. The affected sisters showed heparan and HS spots, much higher GAG levels than the matching age ranges and have NAGLU enzyme deficiency. Both carrier siblings showed normal electrophoretic pattern. The carrier sister showed moderately raised GAG levels than the reference age range and the carrier brother showed a high normal GAGs level of the reference age range. Both carriers showed different patterns of NAGLU enzyme activity, the carrier sister showed normal NAGLU activity and the carrier brother has a reduced NAGLU activity (Table 1). The two affected sisters presented with mild phenotypes for their ages (11 and 9) years. Although both affected sisters grew up in the same family and showed the same genotype, they showed different phenotypes. The mother according to an advice given by her relatives tied the younger sister from her hands to the chair to limit her movement and to try to restrict her uncontrolled activity. As a result, she threw herself from the balcony. Subsequently, she got a fracture in her backbone and pelvic bone fracture. We assume that these environmental conditions and the accident resulted in worsening of her aggressive behavior and cognitive ability than her older sister. The younger sister and brother (the carriers) showed normal behavior, and normal mental and physical development.

In this study, molecular analysis of the *NAGLU* gene to this family showed the missense mutation p.D312N that was detected in two states: homozygous pattern in the two affected sisters and heterozygous pattern in the two carriers' siblings: a sister and a brother. Sanfilippo syndrome type B is inherited in an autosomal recessive manner. Accordingly, both parents are obligate carriers with heterozygous mutation p.D312N. Although the carrier sister has normal NAGLU activity, she exhibited heterozygous mutation p.D312N the same genotype pattern as her carrier brother that has a reduced NAGLU enzyme activity. The biochemical analysis has a certain limitation for the carriers' diagnosis of Sanfilippo B syndrome; therefore, the molecular analysis of *NAGLU* gene is strongly required for the carriers' diagnosis within the affected families (Valstar *et al.*, 2008).

The mutation p.D312N is considered a rare mutation in the *NAGLU* gene. It is identified for the first time in Egypt in this MPS IIIB family, and was not detected in our previous study for the molecular analysis of *NAGLU* gene in a cohort of Egyptian patient with Sanfilippo syndrome type B (Mohammed and Fateen, 2019). The mutation p.D312N is associated with mild phenotype in this family.

Héron *et al.* (2011) conducted a parallel comparative study of the incidence and natural history of patients diagnosed with Sanfilippo syndrome in France (n = 26), UK (n = 28), and Greece (n = 25) from1990 to 2006. The mutation p.D312N (Heron *et al.*, 2011) was first reported as a compound heterozygous mutation with p.R565Q (Bunge *et al.*, 1999) in an affected patient in France. The patient was diagnosed before the age of 5 years with MPS IIIB. However, their study did not describe any phenotypes for the detected mutations. They focused on the Sanfilippo B patient phenotypes in general, including coarse features, hepatomegaly, language delay, abnormal behavior, and epilepsy (Heron *et al.*, 2011). We assumed this patient came from an Iranian origin since he carried one allelic p.R565Q mutation which was the first

mutation identified for Sanfilippo B syndrome in Iranian population (Najmabadi *et al*., 2011). The frequency of p.R565Q mutation is 3.4% of *NAGLU* mutations among different populations worldwide (Lee-Chen *et al*., 2002; Tang *et al*., 2013). Conversely, in our study, both affected sisters did not show epilepsy.

In a recent Iranian study conducted by Yassaee *et al*. (2017), the homozygous mutation p.D312N was identified by whole exome sequence in an affected MPS IIIB patient. The patient was a 10-year-old boy, born healthy to consanguineous parents with no history of metabolic or neurodegenerative disorder in the family. He was healthy until the age of 6 years. However, progressive neurodevelopmental deterioration and seizures were observed at the age of 7 years. At the age of 8 years, he lost speech and cognition. ADHD and MRI findings determined brain damage. The electrophoretic separation of urinary GAGs showed an inadequate band of HS. The concentration of GAGs was moderately outside the age-specific range (6.6, reference range: 1.9–4.3 mg/mmol creatinine). These results agreed with some of our observations for the younger affected sister including neurological deterioration, delayed speech, ADHD, aggressive hyperactivity, and sleep disturbances. However, there are some differences, the younger sister in our report has severe MR, gastrointestinal symptoms (diarrhea), severe dementia, hirsutism, mild autism, normal (MRI) of the brain, and mild hepatosplenomegaly. The Iranian patient showed slow deterioration in his phenotype compared with our younger sister that showed fast deterioration. In addition, our study has some differences in biochemical findings for both affected sisters that showed (H and HS) spots, have higher GAG levels than the reference age ranges, and the NAGLU enzyme activity deficiency (Table 1).

The study performed by Froukh (2017) for diagnosing the genetic causes in 10 consanguineous families with intellectually disabled children from Jordan using exome sequencing and homozygosity mapping technique, the homozygous mutation p.D312N was identified in a 7-year-old girl. The mutation appeared in heterozygous states in the parents and the other siblings. The affected sister presented with clinical phenotype of Sanfilippo syndrome type B including intellectual disability, MRMR, motor delay, microcephaly, slower mental development, hyperactivity, aggressive behavior, hearing loss, congenital hip dislocation, sleep disturbance, coarse face coarse hair, clear corneas, mild joint stiffness, and abnormal EEG record, and did not show neurologic deterioration or seizures (Froukh, 2017). These findings agreed with some of the findings in the affected sisters in our report like hearing loss. However, in general, they presented mild phenotypes with mild coarse facial features, mild joint stiffness, and mild dementia. Furthermore, the older sister showed normal EEG record, slept well, is friendly, can talk to an extent, and is obedient. Moreover, in our study, both affected sisters did not show delay motor activity, congenital hip dislocation, or microcephaly.

On the basis of pathogenicity score prediction analysis using the bioinformatics tools, Polyphen (Adzhubei *et al*., 2010), SIFT (Kumar *et al*., 2009), and PROVEAN (Choi and Chan, 2015), the missense mutation c. 934 G>A p. (Asp312Asn) in the *NAGLU* gene has the substitution of aspartic acid with asparagine at position 312 leading to deleterious effect of a pathogenic potential on NAGLU protein function.

Conclusion

We have identified the missense mutation p.D312N in the *NAGLU* gene in the MPS IIIB Egyptian family. This mutation is considered a rare mutation that was previously reported in only three countries worldwide: France, Iran, and Jordan. The mutation p.D312N is associated with mild phenotype in MPS IIIB Egyptian family. Molecular analysis is strongly recommended for carrier diagnosis of Sanfilippo B syndrome since the biochemical analysis has certain limitation due to the overlap between the NAGLU enzyme activity ranges of normal and heterozygous individuals.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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