Mutation Analysis of the Iduronate-2-Sulfatase Gene among Egyptian Patients with Hunter Syndrome

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Original Article

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ABSTRACT

Background: Mucopolysaccharidosis type II (Hunter syndrome) an X-linked disorder mostly due to mutations in the gene responsible for production of the enzyme iduronate-2-sulfatase (IDS). It belongs to subgroup of diseases called mucopolysaccharidoses caused by accumulation of lysosomal material. It is a rare disease worldwide, having an incidence of 0.3–0.7 per 100,000 live births. Its primary cause is due to mutation affecting the IDS gene causing in accordance deficiency in the lysosomal enzyme activity. The IDS enzyme catalyzes the catabolism of glycosaminoglycans (GAGs), resulting in the accumulation of dermatan and heparan sulphates in different body tissues mainly in connective tissue, spleen, liver, and brain, with elimination of large amounts of them in the urine. The clinical spectrum can be divided into mild, intermediate, and severe variants. The patients clinically are characterized by coarsening of their facial structures, joint and bone deformities, short stature, changes in different body systems including cardiac, respiratory, ocular and olfactory systems and in severe cases troubled motor function, advanced learning complications and behavioral abnormalities can occur. To date, 792 variants have been reported by the HGMD associated with MPS type II have been identified.

The study was conducted on fifteen Egyptian male patients from unrelated families with Hunter syndrome to study the molecular basis of Hunter disease among Egyptian patients through determination of iduronate 2- sulfatase gene mutations. Patients had hepatosplenomegaly, ENT problems mostly due to enlarged adenoids, central nervous system (CNS) abnormality and delayed IQ. Five novel and four previously reported mutations were detected in nine of our patients. Our study being the first Egyptian study on Hunter syndrome has shown as other studies that most mutations of MPS II appear to be unique and there is a great variety between the different populations in the presentation of the disease with different mutations

Key Words: Glycosaminoglycans, hunter syndrome, IDS gene, lysosomal, mucopolysaccharidosis.

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INTRODUCTION

There are seven distinct clinical types and numerous subtypes of mucopolysaccharidoses (MPS) agreeing to the National Institutes of Health (NIH). Although each MPS is different, most people with MPS experience a period of normal development at the beginning of their life followed by a decline in physical and/or mental function **(Huang *et al.*, 2025**).

MPS is an inherited metabolic disorder caused by absence or severe deficiency of one of the lysosomal enzymes that there main function is degradation of glycosaminoglycans (GAGs). All MPSs show an autosomal-recessive inheritance pattern except for Hunter syndrome which is an X-linked (**Brusius-Facchin *et al.*, 2014**).

IDS enzyme appear to be responsible for breaking down of mucopolysaccharides (also known as glycosaminoglycans). These mucopolysaccharides are components of various tissues. The breaking down of mucopolysaccharides usually occurs in the lysosomes of the cells, where cells lysosomes' containing proteins and

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enzymes that aid in the different chemical reactions mainly digestion of various substances (**Beck *et al.*, 2007**). The enzyme is responsible for the catabolism of two subtypes of GAGs; the dermatan and heparan sulfates (DS and HS) in various tissues of the body (**Muenzer *et al.*,2024**).

The principal defect is caused by mutation that occur in iduronate sulfatase (IDS) gene which result in a decrease in the lysosomal enzyme activity (**Scarpa *et al.*, 2011**). The enzyme iduronate sulfatase catalyzes one of the steps in the degradation of the GAGs, leading to accumulation of both the heparan and dermatan sulphates in the various body tissues and organs mainly in connective tissue, liver, spleen, and brain, with elimination of huge quantities of them in the urine (**Muenzer *et al.*, 2012**).

The clinical spectrum of MPS type II patients varies significantly including mild, intermediate, and severe variants according to age of onset, severity of illness, and the rate of progression of the symptoms. The mild form mostly presents without cognitive association while the severe form shows an early onset, mental retardation, rapid progression and even death in the first or second decade of life (**Wei *et al.,* 2011; Zhang *et al.,* 2011**). The patients mostly present by coarsening of facial structures, several bone and joint deformities, short figure, different affections in different systems including the respiratory, the cardiac, ocular, olfactory systems, and in more severe patterns affection of the CNS by a defect in the motor function, progressive educational difficulties and social disturbance (**Scarpa *et al.*, 2011**).

The disease reported incidence varies in different geographical areas and even from one country to another, ranging from about 0.38 per 100,000 live newborns in Brazil to 1.09 per 100,000 live newborns in Portugal, being universally much lower in European countries compared with those of East Asian ones (**Zanetti and Tomanin., 2024**).

IDS human gene is found on Xq28 chromosome, spanning about 24 kb and consisting of 9 coding exons. A full-length complementary DNA (cDNA) clone of the gene exhibited an open reading frame about 1650 bp predicting a protein encoding 550 amino acids. There is an IDS pseudogene (IDSP) was identifiedof about 20 kb distal to the telomeric side from the functional IDS gene, sequences of the pseudogene are greatly homologous to exons 2 and 3 and introns 2, 3 and 7 of the IDS gene. By the year 2023, about 792 variants have been reported by the HGMD and were submitted to clinvar most of which are missense variants (**Zanetti *et al.*, 2024**).

Suspicion of Hunter syndrome, can be done using analysis of the urinary GAGs. Detecting of excessive excretion of dermatan and heparan sulfate. Yet this is not exclusively diagnostic for MPS type II as urinary GAGs can also increase in different types of MPSs. Detection of the IDS enzyme activity is also an essential tool in the diagnosis of Hunter syndrome. Absent or low level of the enzyme in a male patient is considered diagnostic, but absolute enzyme activity cannot predict the severity of the phenotype. IDS genetic testing seems to be the chosen way to predict the correlation between both the genotype and phenotype if present and is the only dependable means for identification of female carriers of the disease which is a critical element in the decisions taken for family plan counselling issues and also in prenatal testing (**Chiong *et al.*, 2017**).

AIM OF THE STUDY

Thus the aim of our study was to study molecular basis of Hunter disease among Egyptian patients through determination of iduronate 2- sulfatase gene mutations and correlating the different mutations with the severity of the syndrome.

MATERIAL AND METHODS

Fifteen Egyptian male patients from fifteen unrelated families with Hunter syndrome were included. Cases were referred from the Medical Genetics and Biochemical Genetics Departments, National Research Centre. Written informed consents were taken from the patients' guardians. Ethical approval was obtained according to Medical Research Ethics Committee at the National Research Centre (NRC).

All cases undergone clinical examination completely with distinct emphasis on any bone defects including the anthropometric measurements. Eye assessment, ultrasound for the abdomen, echo and skeletal examination, MRI brain, and IQ evaluation were also done.

Total GAGs in all urine samples were quantitatively determined by **De Dong *et al.* (1989)** method. Patients with high concentrations were subjected to GAGs extraction from the urine samples by using method of Whiteman (1973). IDS enzyme concentration was implemented fluorometrically using the 4- methylumbelliferyl-a-iduronate 2-sulfate as a substrate (**Shawky *et al.*, 2008; Gabrielli *et al.*, 2010**) the Biochemical Genetics Department, NRC undergone all the enzyme assay detection.

Sequencing analysis of the 9 exons of IDS gene (RefSeq) was performed for all patients at the Medical Molecular Genetics Department, NRC.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes of all patients and controls using the salting-out procedure as described by **Miller *et al.* (1988)**. The

concentration and purity of DNA was then determined by measuring the absorbance using The NanoDrop 2000c UV-Vis Spectrophotometer between 260 and 280 nm.

PCR and sequencing

PCR products were concentrated and purified from excess primers using QIA quick PCR purification (QIAGEN, Germany) kit and then utilized as templates for direct sequencing with the same PCR primers using ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Sequencing chromatograms were aligned with reference sequences (NG_008667.1) by Finch TV software.

Bioinformatic analysis for Pathogenicity of novel mutations

Pathogenicity of the novel mutations was detected by bioinformatic predictions of the defects in the protein variant using the bioinformatic tools; MutationTaster. The Mutation Taster is a free, web-based application used for rapid assessment of the disease-causing probablity of DNA sequence modifications. It incorporates information from various biomedical databases and uses recognized analysis tools. Analyses comprise splice-site changes, loss of protein features, changes affecting the amount of mRNA, frame shifts and others. Outcomes are assessed by a naive Bayes classifier 2, predicting disease potential.

RESULTS

The study included fifteen Egyptian male patients from unrelated families. The patients' ages ranged from 1 to 17 years (median 3.5) at the time of referral and +ve consanguinity of 66.6%.

Clinical results

Dysmorphic facies, frontal bossing, low anterior hair line and short neck were major phenotypic presentation in all of the patients. 6 patients (40%) had cardiac problems mainly thickened mitral valve, infiltrative cardiac disease, mild mitral stenosis, pericardial effusion, systolic murmur at apex, mitral valve prolapse, diastolic dysfunction, diffuse mitral and tricuspid valves and cardiomyopathy. 5 patients (33.3%) showed combined inguinal and umbilical hernias. 11 patients (73.3%) had hepatosplenomegaly (HSM). 7 patients (46.8%) had hearing difficulty with different degrees up to hearing loss, this is mainly due to enlarged adenoid. 3 patients (20%) had CNS abnormality and delayed IQ. 7 patients (46.8%) had limb abnormalities ranging from bowing of ulna and elbow, clinodactyly, nodular swellings on arms, shoulder, back and legs, knock knees, overriding toes, bradydactyly and others.

Biochemical results

Urinary GAGs were high for age in all cases also enzyme activity for IDS revealed null activity in all of our cases. Also urinary GAGs were separated using Two-dimensional electrophoresis revealing 2 big spots of the dermatan and heparan sulfate which are characteristic for MPS II patients (Figure 1).

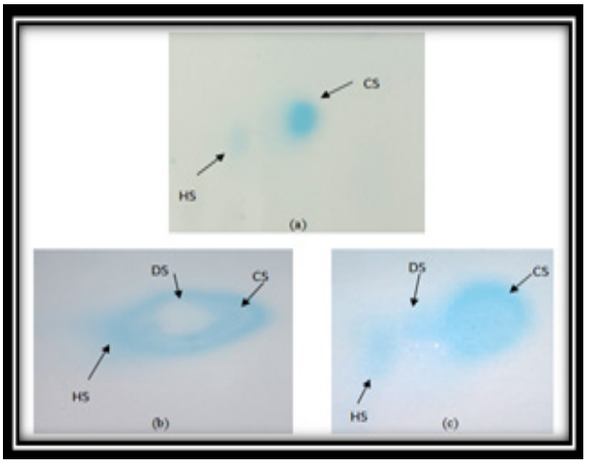

Figure 1: Pattern of urinary GAGs separation using two-dimensional electrophoresis: (a) normal control showing chondroitin and heparan sulphate spots, (b and c) two patients with MPS type II showing the characteristic dermatan and heparan sulphate spots together with the chondroitin spot.

Molecular Results

PCR of IDS gene showed successful amplification of the 9 exons. Amplicon length for the nine exons is 676 bp for exon 1, 561 bp for exon 2, 250 bp for exon 3, 508 bp for exon 4, 552 bp for exon 5, 514 bp for exon 6, 627 bp for exon 7, 623 bp for exon 8 and 610 bp for exon 9 as shown in (Figure 2).

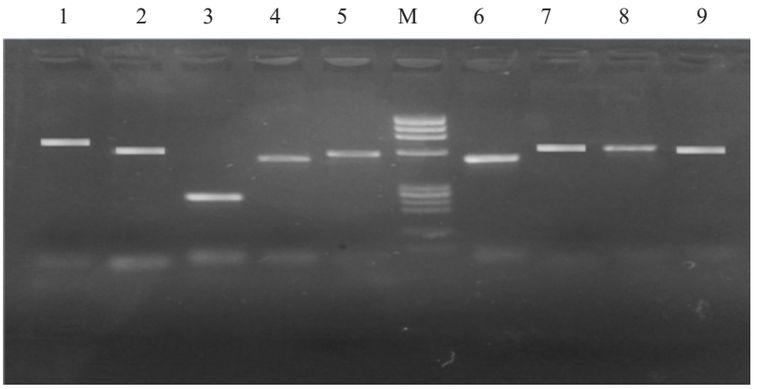

Figure 2: 2% ethidium bromide-stained agarose gel illustrating PCR products of amplification of the 9 exons of the IDS gene. Lane 1: exon 1 (676 bp), lane 2: exon 2 (561 bp), lane 3: exon 3 (250bp), lane 4: exon 4 (508bp), lane 5: exon 5 (552bp), M: Molecular weight marker (PhiX174 DNA/HaeIII digest), lane 6: exon 6 (514bp), lane 7: exon 7 (627 bp), lane 8: exon 8 (623bp), lane 9: exon 9 (610bp).

Successfully amplified PCR products were directly sequenced to detect mutations in the IDS gene. Sequencing of patients revealed pathogenic mutations in seven patients including three novel mutations and four previously reported ones. On the other hand, no other mutations were found in the rest of the patients (8-15). (Table 1) summarizes the detected mutations. Moreover, a recurrent polymorphism (c.438C/T) was detected in exon 4 in four of our patients.

Table 1: Mutations identified in IDS gene in the studied patients.

Patient	Mutation	Exon	Reference
1	c.1327C>T (p.Arg 443X)	9	(***Vafiadaki et al., 1998***) (***Zhang et al., 2011***) (***Amartino et al., 2014***)
2	c.411C>T (P.H138Y)	3	(***Brusius-Facchin et al., 2014***)
3	c.353C>T (p.T118I)	3	(***Guglielmo et al., 2000***)
4	c.217_219delCTC (p.L73del)	2	(***Kato et al., 2005***)
5	c.625_627delTTG (p. L208del)*	5	This study
6	c.302G>C (p.R101P) *	3	This study
7	c.1161_1162delinsA (p. S388Hfs*3)*	8	This study

*: Novel mutation

Patient 1 showed a reported in exon 9 c.1327C>T (p.Arg 443X), patient 2 showed a reported c.411C>T (P.H138Y) mutation. Patient 3 showed a reported mutation, c.353C>T (p.T118I), patient 4 showed a reported mutation in exon 2 c.217_219delCTC (p. L73del). Patient 5 showed c.625_627delTTG (p. L208del) a novel mutation (Figure 3), patient 6 showed one novel mutation c.302G>C (p.R101P) in exon 3 (Figure 4), patient 7 showed another novel one in exon 8 c.1161_1162delinsA (p. S388Hfs*3) (Figure 5).

Pathogenicity of the 3 novel mutations was detected by bioinformatics predictions of the defects in the protein variant using the following bioinformatics tools: MutationTaster. The results of the bioinformatics predictions ranged from 0.99-1.

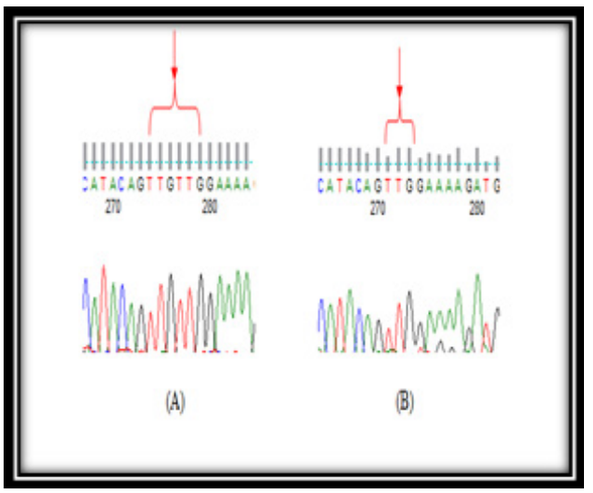

Figure 3: Sequence chromatogram of exon 5 of IDS gene: direct sequencing of the patient 5 revealed c.625_627delTTG (p. L208del) mutation. Site of deletion is denoted with the red arrow (B) compared to wild type shown in (A).

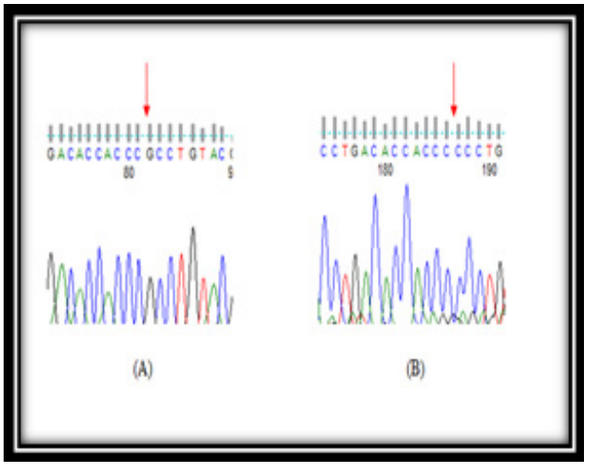

Figure 4: Sequence chromatogram of exon 3 of IDS gene: direct sequencing of the patient 6 PCR product revealed p.R101P mutation. Site of substitution is denoted with the red arrow (B) compared to wild type shown in (A).

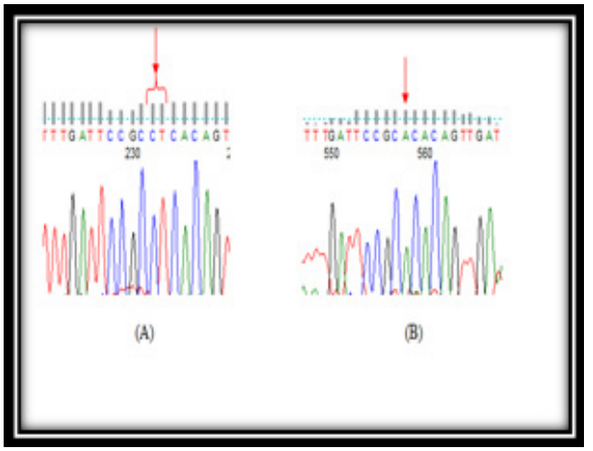

Figure 5: Sequence chromatogram of exon 8 of IDS gene: direct sequencing of the patient no.7 PCR product revealed c.1161_1162delinsA (p. S388Hfs*3) mutation. Site of insertion is denoted with the red arrow (B) compared to wild type shown in (A).

DISCUSSION

MPS type II inheritance is in an X-linked pattern that is due to gene mutations that controls IDS enzyme production. It belongs to subgroup of syndromes termed the mucopolysaccharidoses presenting by lysosomal accumulation in the different tissues (**Kosuga *et al.*, 2016**). It is a rare global disease, having 0.3–0.7 per 100,000 live births incidence (**Chiong *et al.*, 2017**).

The clinical spectrum of MPS type II varies significantly including mild, intermediate, and severe variants according to onset age of the disease, disease severity, progression rate. (**Wei *et al.*, 2011; Zhang *et al.*, 2011**). Clinically the patients can manifest by facial coarsening, abnormalities in both bones and joints, short stature, changes in different body systems, meanwhile in the more severe forms it present by motor function irregularities, learning problems and behavioral deviations (**Scarpa *et al.*, 2011**).

The human IDS gene can be mapped on Xq28 chromosome consisting of 9 exons encoding for a 550 amino acids. Identification of an IDS pseudogene (IDSP) about 20 kb distal to the telomeric sideof the functional IDS gene, sequences of the pseudogene are greatly homologous to exons 2 and 3 and introns 2, 3 and 7 of the IDS gene is an important finding.

Various genetic alterations have been reported in MPS type II: missense and nonsense mutations, splice mutations, small insertions and deletions, partial gene deletions, and deletions or rearrangements of the whole IDS gene (**Guo *et al.*, 2007**), with exonic point mutations comprising half the mutations, followed sequentially by small deletions, altered splicing, gross deletions, small insertions, complex rearrangements, small indels, and gross insertions/ duplications (**Zhang *et al.*, 2011**).

In Egypt, no cohort study has been done until now to be able to have accurate prevalence or incidence for MPS II. However, according to a study done by the Biochemical Genetics Department, National Research Centre, Hunter Syndrome comprised 16.5% of the total 278 MPS diagnosed cases among the studied group (**Fateen *et al.*, 2013**). Our study is the first molecular study done on Egyptian MPS II patients.

The study included fifteen Egyptian male patients from fifteen unrelated families with Hunter syndrome. The patients mostly presented with dysmorphic facies, frontal bossing, low anterior hair line and short neck.

The clinical assessment was followed by biochemical investigations in the form of quantitation of urinary GAGs which was found high for age in all patients. This was followed by bidirectional electrophoretic separation of the urinary GAGs which showed the dermatan and heparan sulfate spots which are characteristic for MPS II patients.

The IDS enzyme activity was measured for the patients and was found to be of null activity in most of the cases.

Molecular assessment was carried out by sequencing of successfully amplified fragments corresponding to the 9 coding exons of IDS gene for our 15 studied patients. Multiple sequence alignments were done to detect present variants. In silico functional analysis was carried out to know the pathogenic degree of the newly found variants by the usage of Mutation Taster and yet this need further investigation using other tools.

Sequencing of patients revealed pathogenic mutations in seven patients including three novel mutations and four previously reported ones. On the other hand, no other mutations were found in the rest of the patients (8-15). A reported polymorphism (c.438C/T) at exon 4 was found in four of the patients.

The c.1327C>T (p.Arg 443x) nonsense mutation, being the most reported mutation in the literature, presented with a severe phenotype and had a family history of his uncle having the disease. It was found in different ethnic groups and different studies being previously reported by **Vafiadaki *et al,* (1998)** in 2 cases one from UK and a female Hunter patient from Asia both with intermediate course harboring identical phenotypes to those already reported. It also occurred with an intermediate presentation in a study done at Minnesota University by **Jonsson *et al,* (1995)**. In another old study done by **Rathmann *et al,* (1996)** it was detected with an intermediate phenotype. It occurred twice in a study done by **Zhang *et al,* (2011)** on the Chinese population showing a mild form of the disease. A study done in 2013 from different countries of Latin America revealed the mutation in 5 patients from Brazil and one from Bolivia with different forms being severe and attenuated (**Brusius-Facchin *et al.,* 2014**). Also, in a study done on Argentinean population by **Amartino *et al,* (2014)** revealed that the mutation was linked to the severe phenotype of disease.

The c.411C>T (P.H138Y) missense mutation found in patient 2, who presented with an intermediate course, was also reported before in a study done on Latin American patients in 2014 with a position specific independent (PSIC) counts score 2.667 using the Polyphen program with great confidence assumed to affect either the protein function or structure (**Brusius-Facchin *et al.*, 2014**).

The c.353C>T (p.T118I) missense mutation found in patient 3 who presented with a severe course and had another affected dead brother. It was also found by **Balzano *et al,* (1997)** and **Villani *et al*, (1997)** in a study done on 40 Italian patients with MPS Type II presented with a mild form being detected in exon 3 supporting the observation that it is one of the most frequent regions harboring mutations in the IDS gene as reported before (**Li *et al.*, 1999**).

The c.217_219delCTC (p.L73del) deletion mutation was previously detected in a study done on 18 Japanese patients with MPS type II done by **Kato *et al,* (2005)** stated that the L73del mutation comprises the loss of only one amino acid of the beta sheets, which occurs on the IDS protein peripheral part thus may lead to preserving of the protein structure and activity. Also, this mutation was found in a patient with an attenuated phenotype. It was found in patient 4 who presented also with an attenuated form with mild HSM, hearing defect, an average IQ and umbilical hernia.

The c.625_627delTTG (p. L208del) is a novel mutation with a deleted codon found in the fifth patient at exon 5 with deletion of leucine amino acid at position 208. This patient presented with a severe form of coarse facies, high forehead, thickened lips, cloudy corneas, limited movement, clinodactyly, broad knees and ankles, short stature, HSM, pericardial effusion, infiltrative cardiac disease, thickened mitral valve, low IQ and high GAGs (35.5). The MutationTaster showed that this variant is deleterious and causing disease with a Probability: 0.99.

Another novel missense mutation was detected in patient 6 at exon 3 c.302G>C (p.R101P), where arginine was converted to proline at position 101(CGC>CCC). This patient showed a moderate phenotype with coarse facies, frontal bossing, depressed nose, overriding toes, HSM, umbilical hernia and moderately high GAGs, using MutationTaster this variant appeared to be deleterious and causing disease with a Probability: 0.99.

Patient seven sequencing showed the only insertion mutation at exon 8 c.1161_1162delinsA (p. S388Hfs*3) with insertion of adenine and deletion of 2 base pairs, thymine and cytosine, this is another novel mutation. This patient showed a severe presentation with coarse facies, frontal bossing, depressed nose, dolicocephaly, low anterior hair line, depressed nose, thickened lower lips, umbilical hernia, organomegaly and mental retardation. He also had high GAGs of 62.3. His mother was not available. MutationTaster for this variant appeared to be deleterious and causing disease with a Probability: 0.99.

The rest of the patients revealed no mutation by sequencing all the 9 exons and this was also reported in the literature, where in some studies no mutation was found and were only clinically and biochemically detected (**Gucev *et al,* 2011**). Also, in another study done by **Kim *et al,* (2003)** failed to detect any mutation in 2 patients. Also in eight patients, no mutation was detected throughout the entire coding region as detected by **Vafiadaki *et al,* (1998)** in a study done on 57 unrelated patients with MPS II from the UK. This might be due to the presence of pseudogene complexed in a recombination occurrence with IDS gene in about 13% of patients with Hunter syndrome containing sequences that are related to exons 2 and 3 as well as introns 2, 3, and 7 of the IDS gene, also this might be due

gene rearrangements thus further analysis might be needed using microarray. Unrevealed mutations may be intronic or present in the promoter region which arise the need for epigenetics studies.

The c.438C/T polymorphism at exon 4, where ACC was converted to ACT at codon 146, was detected in patients 1, 11, 13 and 15. Also it was reported in a patient in a previous study done in the United States by **Li *et al,* (1999)**. Another study revealed its presence in four Argentinean patients (**Amartino *et al.*, 2014**). Although this polymorphism was reported not to be pathogenic yet in patient 13 it was present without a detected mutation but the patient showed to be a typical MPS II patient clinically and was biochemically diagnosed.

Our study being the first Egyptian study on Hunter syndrome has shown as other studies that most mutations of MPS II appear to be unique and there is a great variety between the different populations in the presentation of the disease with different mutations. Yet about 57.1 % of mutations are clustered in exons 3 and 5 which can be considered hot spots exons for mutations in our population. This is in accordance with previous reports as detected by **Rathmann *et al,* (1996)**. These results are evidence of mutational heterogeneity for MPS II.

Genotype and phenotype correlation of MPS type II is challenging as it only occurs for a small subgroup of mutations, being mostly influenced by different factors that modulate the residual IDS activity. Thus, identification of MPS type II mutation among the population and genetic counseling for families are crucial goals in most studies.

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COMPETING INTERESTS

There were not any competing interests accomplishing the study.

DETAILS OF ETHICAL APPROVAL

The Medical Research Ethical Committee at the NRC approved the study protocol, and written informed consents were taken from all individuals involved in the study or their guardians.

CONTRIBUTIONS

- Prof. Dr. MSK: Responsible for manuscript supervision and reviewing the article.
- Dr. NMI responsible for revising the manuscript and results.
- Prof. MLE: Responsible for the molecular studies and reviewing the article.
- Prof. MSA: Responsible for clinical examination and evaluation of the patients.
- Prof. EF & Ass. Prof. MMI: Responsible for biochemical enzyme activity assays and reviewing the article.
- Ass. Prof. HHA: Responsible for revising the manuscript and results.
- Ass. Researcher NMS: Responsible for the molecular studies and reporting the work described in the article.

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