Muccopolysaccharidosis Type II, Two Novel Mutations

Nouran Sedky[1], Ekram M. Fateen[3], Mona S. Khalil[2], Nahed M Ibrahim[2], Mona M. Ibrahim[3], Mona S. Aglan[4], Heba A. Hassan[1] and Mona L. Essawi[1]

Original Article

[1]Department of Medical Molecular Genetics, [3]Department of Biochemical Genetics, [4]Department of Clinical Genetics, Division of Human Genetics and Genome Research, National Research Centre, Cairo, Egypt

[2]Department of Clinical and Chemical Chemistry, Faculty of medicine, Ksr Eleny Cairo University, Cairo, Egypt

ABSTRACT

Background: Mucopolysaccharidosis type II (Hunter syndrome; MPS II) is an X-linked lysosomal disorder caused by mutations detected in the gene that controls production of the enzyme iduronate-2-sulfatase (IDS). MPS II is considered to be a rare disease worldwide, with an incidence of 0.3–0.7 per 100,000 among live births. The primary defect of the disease is caused by a mutation on the IDS gene which disrupts the activity of the IDS lysosomal enzyme. The enzyme IDS catalyzes one of the steps in the catabolism of the glycosaminoglycans (GAGs), leading to accumulation of both heparan and dermatan sulphate in different tissues and organs of the body with excretion of large amounts of them in the urine. The clinical spectrum of MPS II includes mild, intermediate, and severe variants affecting mainly the connective tissues, skeletal system, brain, liver and spleen. To date, 792 variants have been reported by the HGMD associated with MPS type II have been identified.

This study was done for two Egyptian male patients with MPS type 2 which revealed 2 novel mutations in the two different patients with successful segregation of their mothers aligning with X-linked recessive mode of inheritance. We recommend a genotype phenotype correlation using larger cohorts of patients in the future studies.

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

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Corresponding Author: Nouran Sedky, Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, National Research Centre, Dokki12311, Cairo, Egypt. **Tel.:** 01221174972,
E-mail: nouran_sedky@hotmail.com

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INTRODUCTION

According to the National Institutes of Health (NIH), there are seven distinct clinical types and numerous subtypes of mucopolysaccharidoses (MPS). 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. Meanwhile some MPS types may be fatal in the first few months of life (**Huang *et al.*, 2025**).

MPSs are a group of inherited metabolic disorders caused by absence or severe deficiency of one of the lysosomal hydrolases that is responsible for the degradation of glycosaminoglycans (GAGs). All MPSs show autosomal-recessive inheritance; meanwhile Hunter syndrome is X-linked (**Brusius-Facchin *et al.*, 2014**).

Mucopolysaccharides (also known as glycosaminoglycans) are components of various body tissues. The breaking down of these mucopolysaccharides usually takes place in the lysosomes of the cells, these lysosomes contain enzymes that are main controller in different chemical reactions mainly digestion of various substances (**Beck *et al.*, 2007**). IDS enzyme is involved in the catabolism of two subtypes of GAGs; dermatan and heparan sulfates (DS and HS) in various tissues (**Muenzer *et al.*,2024**).

The primary defect of the disease occur due to a mutation in the iduronate sulfatase (IDS) gene (NM_001166550.4) leading to a deficit in the main action of the IDS enzyme (**Scarpa *et al.*, 2011**). Deficient IDS activity leads to multisystemic accumulation of its corresponding substrates, HS and DS in different tissues and organs mainly in connective tissue, brain, liver and spleen, with elimination of large amounts of these GAGs in the urine (**Muenzer *et al.*, 2012**).

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This in turn leads to a wide range of disease manifestations and reduced life expectancy. Hunter disease is inherited as an X-linked trait; therefore, it affects only male subjects. Yet, a few female patients' cases have been reported in literature, mostly due to an unbalanced inactivation of the X chromosome during the lyonization process in the female carriers (**Jurecka *et al.*, 2012**).

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

The clinical spectrum of MPS type II varies expressively including mild, intermediate, and severe forms according to different criteria comprising, age of onset, severity of the disease, and also of progression. The symptoms start at 1–1.5 years of age, especially in the severe forms. However, children are not commonly diagnosed precociously, due to the overlapping signs and symptoms with other pediatric neurological disorders, misleading the correct diagnosis. About two-thirds of the patients had a distinctive trait of heavy neurological impairment, mainly exhibited as significant developmental delay (**Zanetti and Tomanin., 2024**).

Mild forms usually presents without cognitive involvement while the severe forms shows an early onset, mental retardation, rapid progression of the disease and even death commonly in the first or the second decades of life (**Wei *et al.*, 2011; Zhang *et al.*, 2011**). The patients mostly are characterized by coarsening of facial features, bone and joint aberrations, short stature, alterations in heart, respiratory system, hearing and vision, and in more aggressive forms by distressed motor function, advanced learning problems and social irregularities (**Scarpa *et al.*, 2011**).

The human IDS gene is positioned on chromosome Xq28. It spans nearly 24 kb and consists of 9 coding exons. A full-length complementary DNA (cDNA) clone of the gene exhibited an open reading frame of 1650 base pair (bp) span translated into a protein encoding 550 amino acids. An IDS pseudogene (IDSP) has been identified 20 kb distal to the telomeric side of the functional gene, pseudogene's sequences are vastly homologous to exons 2 and 3 and introns 2, 3 and 7 of IDS gene. By the year 2022,2023 about 792 variants have been reported up to date by the HGMD and finally they were submitted to clinvar showing that most of which are missense variants [Human Gene Mutation Database (HGMD); http://www.hgmd.org/] (**Zanetti *et al.*, 2024**).

Urinary GAGs analysis can be a tool accustomed to rule out or suspect Hunter syndrome .In hunter syndrome there is excessive excretion of dermatan and heparan sulfate are detected in urine. Yet, this is not diagnostic to Hunter syndrome as urinary GAGs can be also prominent in other types of MPSs. Detection of the IDS enzyme activity is also a crucial tool in the identification of Hunter syndrome. Absent or low level of the enzyme in a male patient is considered diagnostic, but absolute enzyme activity cannot expect the severity of the phenotype simply. Genetic analysis of the IDS gene is the solitary approach to detect the correlation between the genotype and phenotype of the disease and also is the only reliable tool to detect the female carriers of the syndrome which is a critical aspect in family planning judgments and prenatal testing for the disease (**Chiong *et al.*, 2017**).

MATERIAL AND METHODS

Two Egyptian male patients with Hunter syndrome as well as mothers of patients were included and each patient's mutation was compared with his mother. Detection of the female carrier at risk in these families whenever available was done. Cases were referred from the Biochemical Genetics Department, National Research Centre (NRC). Written informed consents were taken from the patients' parents. Ethical approval was obtained according to Medical Research Ethics Committee at the NRC.

2.1 Clinical Evaluation:

Complete clinical examination with distinctive highlighting on bone abnormalities including anthropometric measurements. Eye assessment, abdominal ultrasound, echocardiography, skeletal examination, Head-cervical Magnetic Reasonance Imaging (MRI), developmental evaluation and Intelligence quotient (IQ) evaluation were undergone.

2.2 Biochemical analysis:

Total GAGs were quantitatively determined in all urine samples and evaluated by **De Dong *et al.*** (1989) technique. IDS enzyme, fluorometrically detected in all patients using the 4- methylumbelliferyl-a-iduronate 2-sulfate as a substrate (**Shawky *et al.*, 2008; Gabrielli *et al.*, 2010**)

2.3 Molecular Analysis:

Genomic DNA was extracted from peripheral blood leukocytes of the patient using salting-out technique as designated 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.

Polymerase chain reaction (PCR) was carried out for the 9 coding exons of IDS gene

Concentrated and purified PCR products from excess primers using the QIA quick PCR purification kit (QIAGEN,

Germany) and then employed as templates for the direct sequencing using the same PCR primers by means of ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Finch TV software was used to align reference sequences (NG_008667.1) of the Sequencing chromatograms of the patients.

Pathogenicity of the novel mutations was detected by Mutation Taster. It incorporates the different information from altered biomedical databases and utilizes recognized analysis tools. Analyses include splice-site changes, frame shifts, variations affecting the amount of mRNA, loss of protein features and others. A naive Bayes classifier 2, predicting the disease probability was used to evaluate the examination results.

RESULTS

3.1. Clinical results:

The patients showed a delay in development of all milestones, dysmorphic facies, Frontal bossing and low anterior hair line. Eyes showed thick eye brows, long eye lashes, Synophorus eyelids and puffiness of eyelids. Depressed frontonasal root with wide nose base and wide nostrils. Thick everted lower lip, large ear with hearing difficulty and a short neck. Upper and lower limbs showed bowing of ulna and elbow, clinodactyly, nodular swellings on arms, shoulder, back and legs with knock knees, right knee swelling and overriding toes.

3.2 Biochemical results

Urinary GAGs were high for age in both cases also enzyme activity for IDS revealed null activity as the of the urinary GAGs separation done using a Two-dimensional electrophoretic separation revealed big dermatan and heparan sulfate spots which are characteristic for MPS II patients (Figure 1).

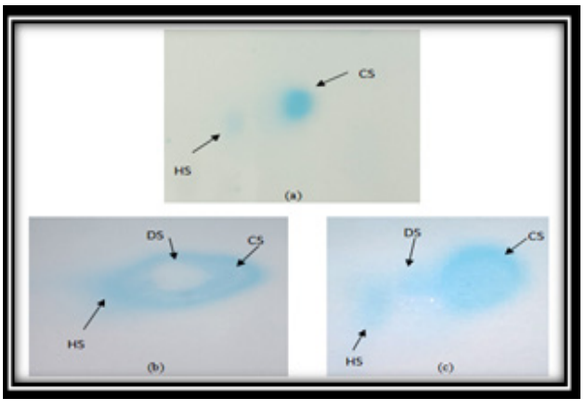

Figure 1: Pattern of separation of urinary GAGs using a two-dimensional electrophoresis: (a) normal control showing chondroitin and heparan sulphate spots, (b) patient 1 with MPS type II showing the characteristic dermatan and heparan sulphate spots together with the chondroitin spot, (c) patient 2 with MPS type II showing the characteristic dermatan and heparan sulphate spots together with the chondroitin spot. CS: chondroitin sulphate, HS: heparan sulphate, DS: dermatan sulphate.

3.3 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).

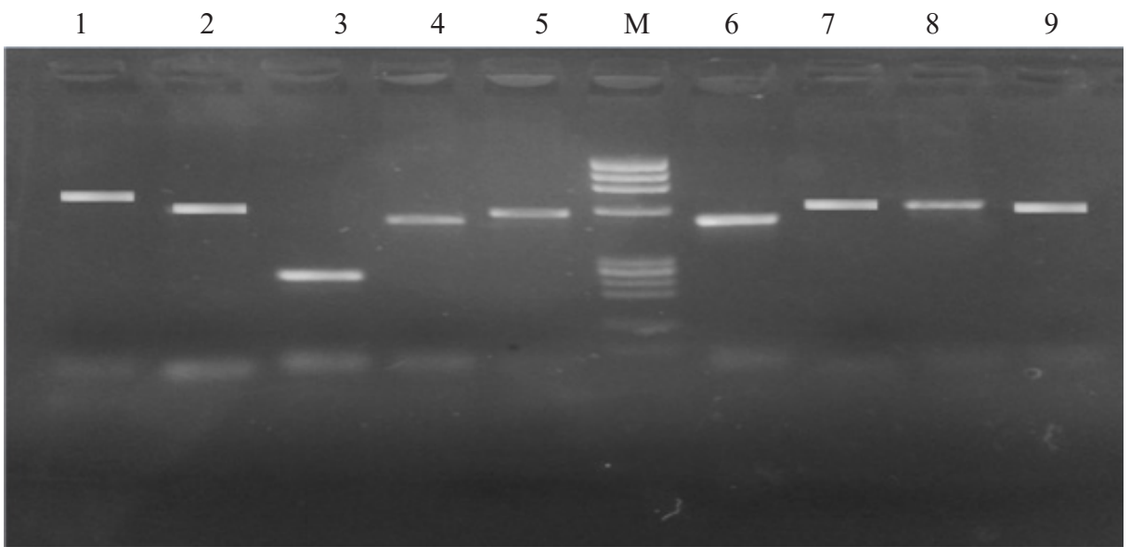

Figure 2: 2% ethidium bromide-stained agarose gel illustrating PCR amplified products of the 9 exons of the IDS gene. Lane 1: exon 1 (676 bp), lane 2: exon 2 (561 bp), lane 3: exon 3 (250bp), lane 4: exon 4 (508bp), lane 5: exon 5 (552bp), M: Molecular weight marker (PhiX174 DNA/HaeIII digest), lane 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 the patient 1 revealed pathogenic novel c.682C>T (p.P228S) (CCA→TCA) mutation in exon 5 (Figure 3), both his mother and brother (showed the same mutation) were also sequenced (Figure 4).

Patient 2 showed a novel mutation including deletion of one base c.70delC (p.L24Sfs*14), segregation of his mother and sister was done (Figure 5). This patient also showed 438C>T (silent mutation) which was previously reported.

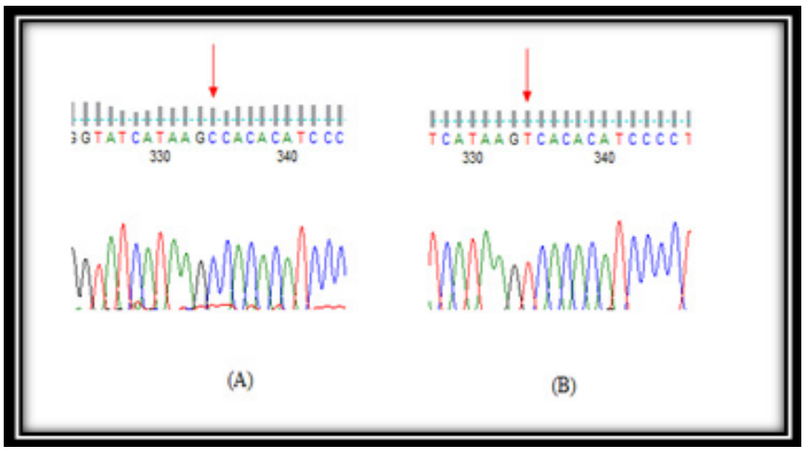

Figure 3: Sequence chromatogram of exon 5 of IDS gene: direct sequencing of the patient revealed p.P228S mutation. Site of substitution is denoted with the red arrow (B) compared to wild type shown in (A).

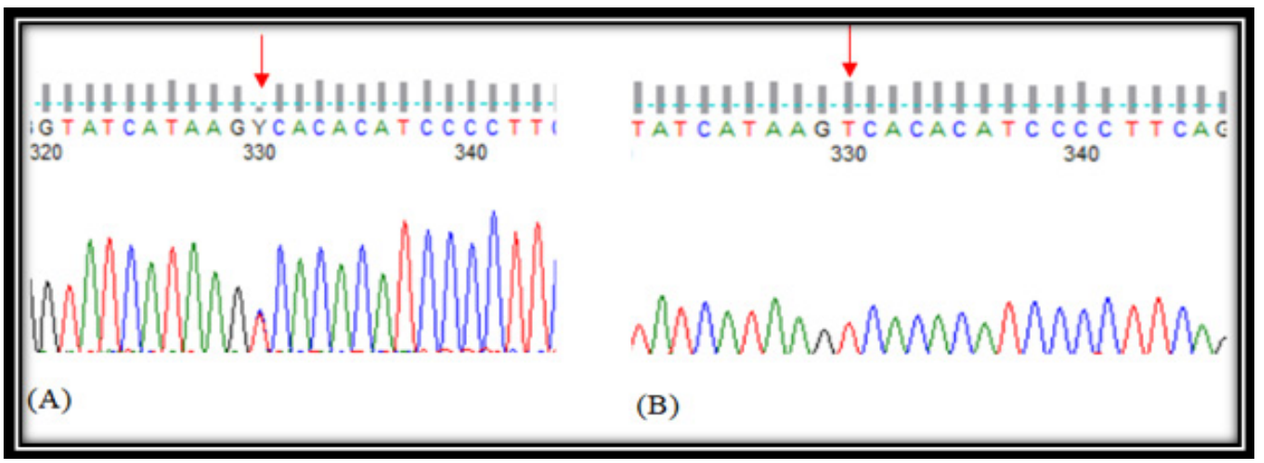

Figure 4: Sequence chromatogram of exon 5 of IDS gene: direct sequencing of the patient's mother shown in (A) and brother shown in (B).

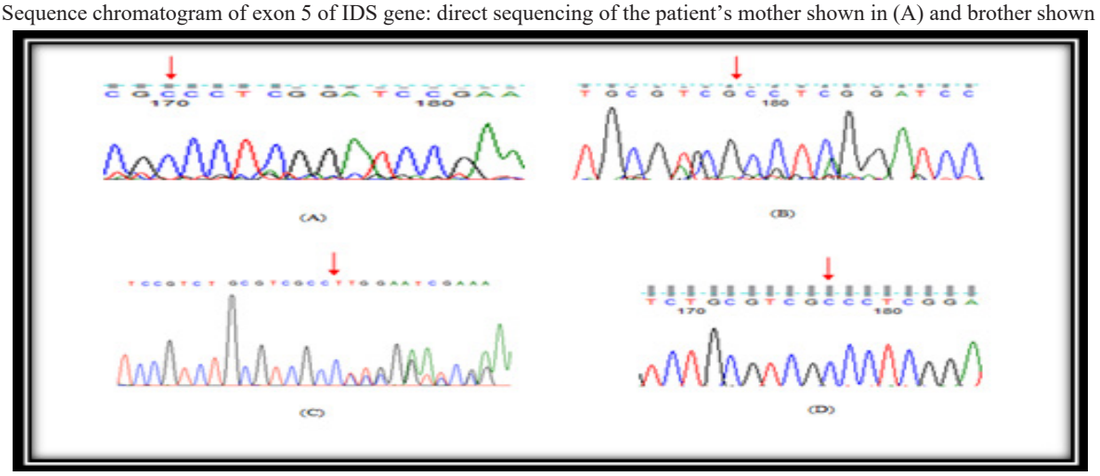

Figure 5: Sequence chromatogram of exon 1 of IDS gene: direct sequencing of patient1 revealed deletion of one base pair (C) at position 70 bp showing c.70delC. Site of deletion is denoted with the red arrow (B), wild type is shown in (A), the patient's mother is shown in (C) and the normal sister (D).

Pathogenicity of the novel mutation was detected by bioinformatics predictions of the defects in the protein variant using the following bioinformatics tool: MutationTaster. The results of the bioinformatics predictions ranged from 0.99-1 indicating a high probability of being disease causing.

DISCUSSION

MPS type II being an X-linked multisystem syndrome triggered by mutational changes in the gene controlling the production of the IDS enzyme which fit in to subgroup of syndromes called mucopolysaccharidoses caused by accumulation of lysosomal substance (**Kosuga *et al.*, 2016**). Worldwide it is a rare disorder, with a frequency of 0.3–0.7 per 100,000 live births (**Chiong *et al.*, 2017**).

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 including half the mutations, followed consecutively by small deletions, gross deletions, small insertions, altered splicing, complex rearrangements, gross insertions/duplications and altered splicing (**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**).

The patients presented with delay in development of all milestones, dysmorphic facies, Frontal bossing and low anterior hair line. Upper and lower limbs various deformities, eyes showed thick eye brows. Mental retardation was present in both patients.

The clinical assessment was followed by biochemical investigations in the form of quantitation of urinary GAGs which was found high for age. 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 and was found to be of null activity in the 2 patients.

Molecular assessment was carried out by sequencing of successfully amplified fragments corresponding to 9 coding exons of IDS gene. 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 through the available online bioinformatics program.

The novel missense mutation c.682C>T (p.P228S) which was detected in the first patient at exon 5, where proline was converted to serine at position 228, cytosine being changed to thiamine at position 682 cDNA (CCA>TCA). This patient and his brother suffer MPS II and he presented with a delay in all his milestones and a typical feature of the disease, with coarse facies and depressed nasal bridge and low anterior hair line, moderate HSM and mental retardation. Also, a past mitral valve replacement and other cardiac problems with hearing difficulty knock knees and overriding toes. His GAGs were very high (102.6 µg). The mother's family also had a history of an affected case before with MPS type II with no further data. Mutation Taster bioinformatics tool revealed that this variant is deleterious and disease causing with a Probability: 0.99.

The second patient revealed the c.70delC (p.L24Sfs*14) novel mutation. The one base deletion at position 70 of the cDNA resulted in a change in the reading frame. The Leucine amino acid at codon 27 was changed with serine and 14 codons downstream, the translation was stopped. It was detected in exon 1and the entire protein was destroyed. The patient showed a delay in all milestones, dysmorphic facies, HSM, difficulty in speech, hearing impairment, and mental retardation, his GAGs were high for age (59.5 µg) presenting with a moderate phenotype.

Segregation of the mother and sister revealed a carrier mother and a normal sister. Mutation Taster showed that this variant is deleterious and disease causing with a Probability: 1.

In conclusion, this study expands the molecular spectrum of MPS type II by detection of 2 novel mutations in two different patients with successful segregation of their mothers aligning with X-linked recessive mode of inheritance. We recommend establishing a genotype phenotype correlation using larger cohorts of patients in future studies.

DETAILS OF FUNDING

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COMPETING INTERESTS

There were not any competing interests accomplishing the study.

DETAILS OF ETHICAL APPROVAL

The study protocol was accepted and approved by The Medical Research Ethical Committee at the NRC and also

there were written informed consents that were taken from all individuals involved in this study or their guardians were obtained.

CONTRIBUTIONS

- Prof. Dr.Mona Salem Khalil: was in charge for thesis supervision and also reviewing the article.
- Dr. Nahed Mohamed Ibrahim responsible for revising the thesis and results.
- Prof. Mona L. Essawi: Responsible for the molecular studies and appraising the article.
- Prof. Mona S. Aglan: Responsible for the clinical evaluation of the patients and reviewing of the data.
- Prof. Ekram Fateen & Ass. Prof. Mona M. Ibraim: Responsible for biochemical enzyme activity assays and revising of the article.
- Ass. Prof. Heba Hasssan Amin: Responsible for revising the thesis and the molecular results.
- Ass. Researcher Nouran Mohamed Mohamed Sedky: Responsible for the molecular studies, recording of the results and the reporting the work described in the article.

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