IN VITRO ANALYSIS OF IDS GENE MUTATION (C.934G>A P.G312S): A COMPREHENSIVE STUDY ON TWO SIBLINGS

Mihriban Tijen Tanyalcin[1], Ibrahim Tanyalcin[2]
[1]Tanyalcin Medical Laboratory Selective Screening and Metabolism Unit; Izmir,Turkey www.tanyalcin.com / tanyalcinlab@gmail.com
[2]Self-Employment; Brussels, Belgium website: http://babylone.ulb.ac.be/mutaframe/ ; http://i-pv.org/; http://lexicon.ibrahimtanyalcin.com

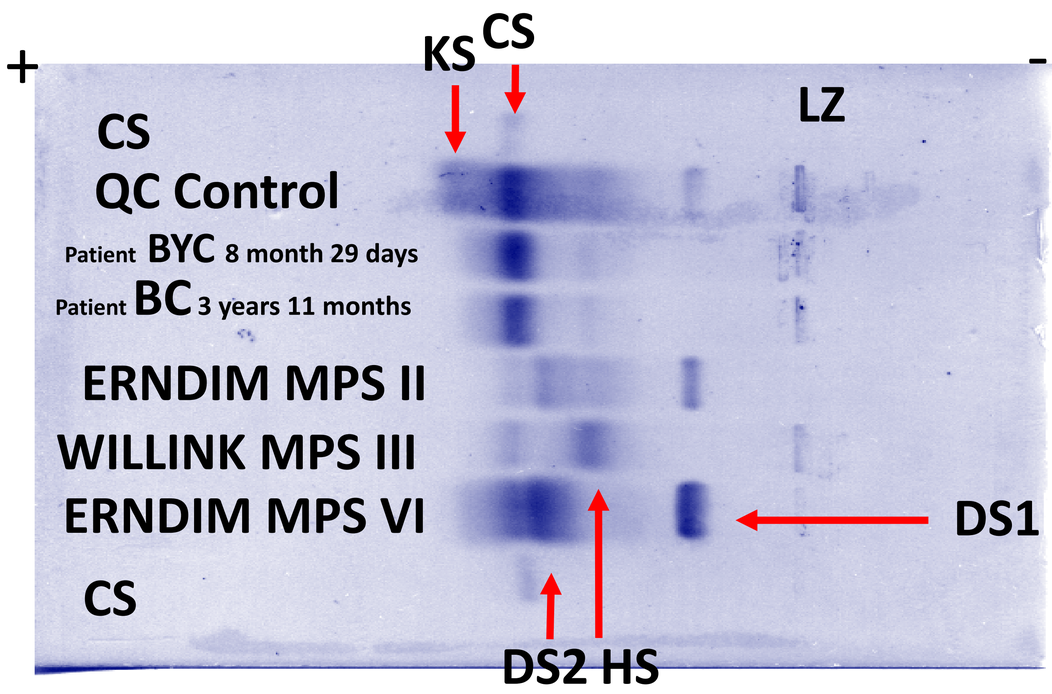
INTRODUCTION: In the complex and often mystifying world of genetic disorders, the intersection of clinical findings and genetic testing can sometimes present paradoxes that challenge our understanding and push the boundaries of medical science. This is particularly true in the context of our case: a mutation has been detected, signaling the presence of a genetic abnormality, yet, intriguingly, the results from the GAGE electrophoresis results are far from MPS II profile with clinically normal ; accompanying subnormal enzyme activity. The understanding of genetic mutations and their phenotypic implications is crucial in the realm of medical genetics.This case once again underscores the importance of simple yet manpower, time, skill, and effort demanding tests related to substrate accumulation in differential diagnosis and in making clear interpretations.

The investigation involved a multifaceted approach, including fluorometric analysis to measure iduronate-2-sulfatase activity in leukocytes and GAG electrophoresis to assess the presence of specific sulfates. Both patients exhibited an enzyme activity level of 1.3 nmol/h/mg-protein, marginally below the normal threshold (>1.5 nmol/h/mg-protein) and applied to our laboratory for GAG elctrophoresis

CASE PROFILES: AGES AND KEY DATA:

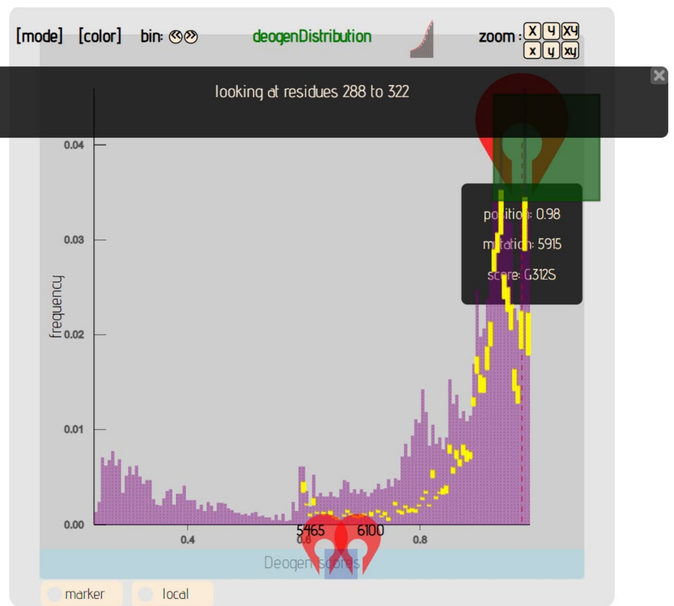
The investigation involved a multifaceted approach, including fluorometric analysis to measure iduronate-2-sulfatase activity in leukocytes and GAG electrophoresis to assess the presence of specific sulfates. Both patients are male siblings BYC ,8 months and BC 3 years old; as a result of detailed research due to a slight delay in speech in the older sibling, c.934G>A p.G312S mutation in the IDS gene has emerged from genetic screening without any other clinical findings.Both exhibited the same enzyme activity level of 1.3 nmol/h/mg-protein, marginally below the normal threshold (>1.5 nmol/h/mg-protein) and applied to our laboratory for GAG electrophoresis (GAGE). The samples from two brothers with codes 96888 BC and 96889 BYC have been studied on the cellulose acetate plate below, along with pathological patient samples.

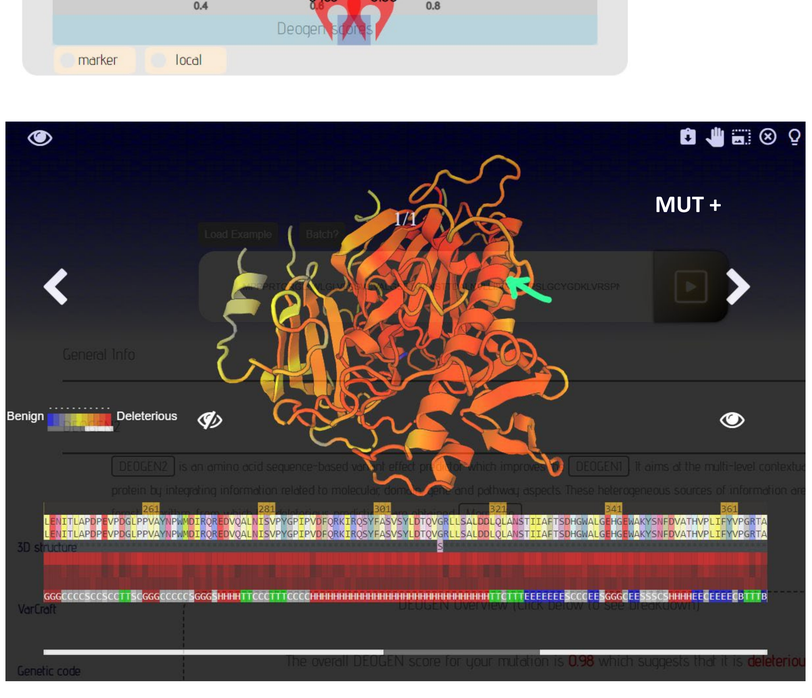
Total GAG excretions are normal in both patients and GAGE results indicated the absence of dermatan sulfate-1 (DS1) and dermatan sulfate-2 (DS2), keratan sulfate (KS) while heparan sulfate (HS) and chondroitin-sulfate (CS) excretion levels were within normal ranges: **NORMAL GAGE PROFILE**



RESULTS: Despite sharing the c.934G>A p.G312S mutation in the IDS gene, the in vitro analyses of the siblings revealed relatively normal metabolic functionality, with slight deviations in enzyme activity levels and normal GAGE profiles. This finding underscores the variability of phenotypic expression, even with the presence of known gene mutations. The same mutation is present in the patients' uncle, who is 40 years old and completely healthy and the enzyme activity in this individual is also marginally below the normal threshold. The family's residence is Akşehir, Konya, located in the Central Anatolia region.

The mutations were scrutinized using the bioinformatic tool, *Mutaframe (http://babylone.ulb.ac.be/mutaframe/).*





METHOD

Measurement of total GAG

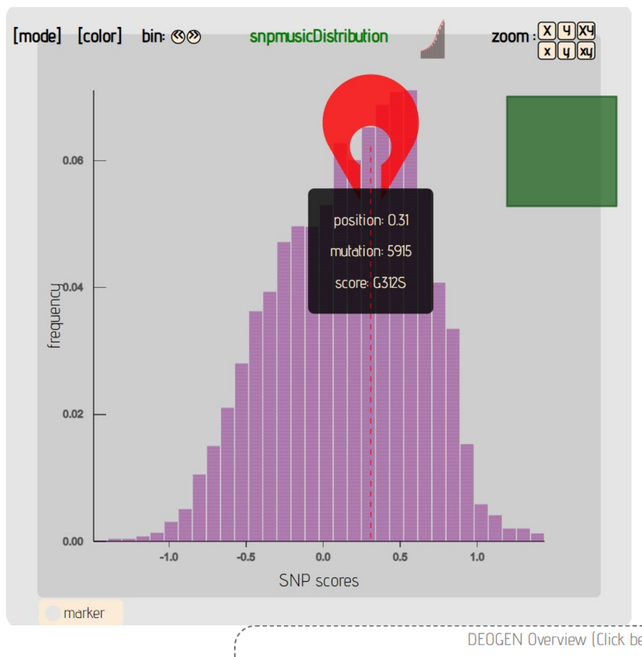
In this study, dimethylmethylene blue (DMB) dye method is used before GAG differentiation based on the method of (De Jong et al -1)

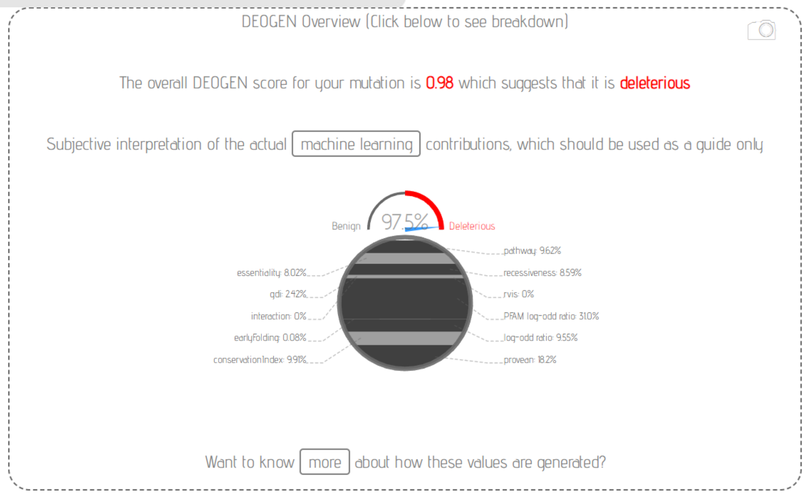
Measurements were carried out on microtiter plates at double wavelength 580/690 nm using regression equation polynomial second order.

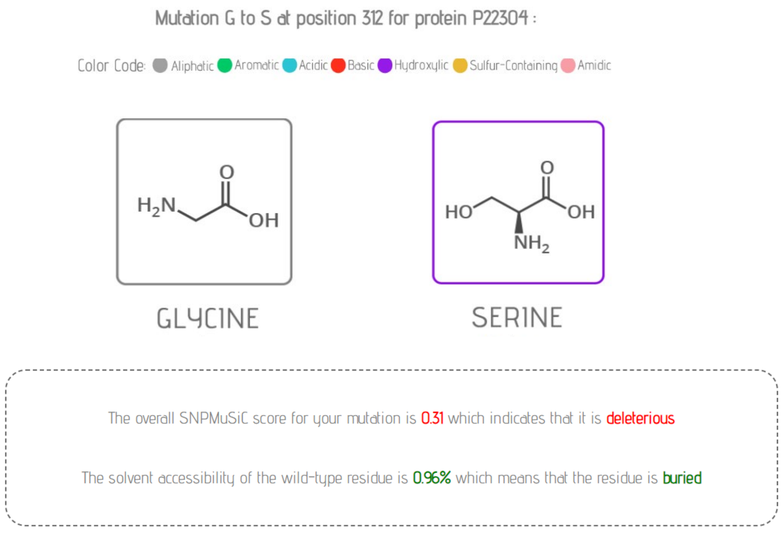
Isolation of GAGs and High Resolution Electrophoresis of isolated urinary GAGs are performed according to the paper. **http://journals.sagepub.com/doi/full/10.1177/2326409815613805**

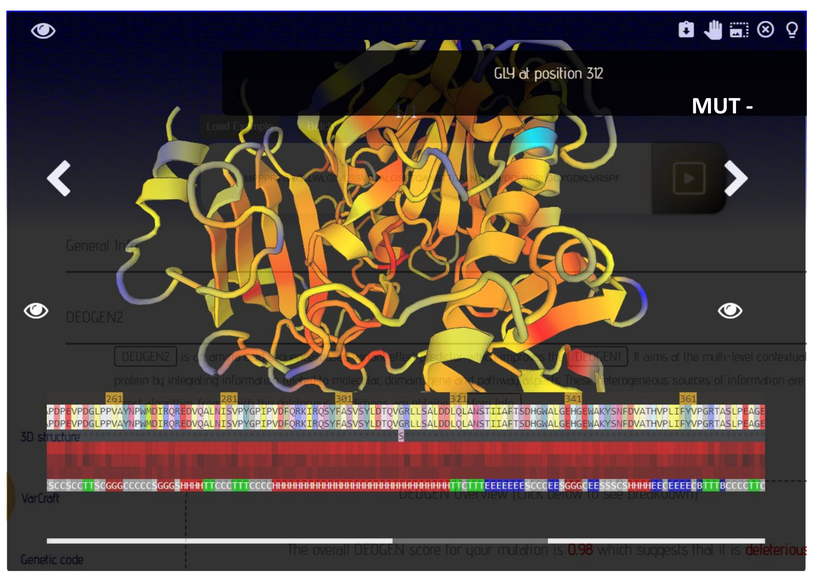
The IDSc.934G>A is documented in dbSNP as "rs903259179", the allele frequencies for general populations can be find at the linked below. In a dataset of the 2021PNAS paper for the genetic structure of the Turkish population from more than 3000 subjects) the A ("T" in genomic sequence) allele of IDS c.934 is very rare with only 1 allele was found in 5158. This variant is likely not a polymorphism in Turkish population.

https://www.ncbi.nlm.nih.gov/snp/rs903259179 The IDSc.934G>A is documented in HGMD with uncertain significance in ClinVar interpretation. This variant locates at the evolutionary conserved region, most of the predictions of the influences of this p.G312A variant by different methods showed it is likely damaging .The allele documented as DM in HGMD is only based on one Middle East patient with no clear clinical presentation and family history (Case 00080844 in the patient list data Table S3) published in Eur J Hum Genet 2017 . The allele may not be a disease causing variation or a quite mild mutation with borderline deficient enzyme activity. The Revised ClinVar classification as variation with uncertain significance may be more suitable description of this variation. c.934G>A p.G312S mutation in the IDS gene









CONCLUSION: This study emphasizes the importance of in vitro analysis as the gold standard for understanding the phenotypic implications of genetic mutations. While international mutation databases provide valuable genetic information, the actual expression of these mutations can vary significantly, as demonstrated by the cases of the two siblings. Our findings advocate for a comprehensive approach, integrating both genetic data and in vitro analysis, to accurately determine the phenotypic outcomes of genetic mutations, ensuring precise diagnosis and effective management of related disorders.

FOR DEMONSTRATION

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