

ATP7B Mutation Analysis: Wilson Disease, A Difficult to Diagnose Case

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ABSTRACT

Wilson's Disease (WD) is a common metabolic disorder predominantly involving liver, brain, and eyes. Pancreatic, renal, psychiatric, and cardiac involvement have also been described. No single investigation can be considered diagnostic of WD; therefore, diagnosis is based upon a series of tests best interpreted using Wilson disease diagnostic index (WDDI). We present a difficult-to-diagnose, 9-year girl of consanguineous parents, with chronic liver disease and portal hypertension. Initial workup was equivocal with significantly low serum ceruloplasmin, normal urinary copper excretion and absent Kaiyser-Fleischer (KF) rings. Diagnosis was established by ATP7B mutation analysis. The patient was found homozygous for c.3955C>T (p.Arg1319Ter) in exon 19, a rare mutation described in literature, which results in premature truncation of peptide chain.

Key Words: ATP7B, Wilson disease, Copper, Mutations, Hepatolenticular degeneration.

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INTRODUCTION

Wilson's Disease (WD) is among the commonest genetic metabolic disorders of the liver. The gene responsible is ATP7B on chromosome 13.¹ The gene product is an enzyme-essential in incorporation of copper into ceruloplasmin within the hepatocyte membrane and excretion in bile and outside the body through feces. ATP7B gene mutation results in buildup of copper in the body.² The accumulated copper is deposited in the liver, cornea, brain and pancreas, resulting in protean manifestations ranging from liver disease, central nervous system (CNS) disease, psychiatric manifestations, hemolysis, pancreatitis and cardiac manifestations. Most common presentation is with hepatic and neurological involvement.³

The diagnosis of WD is straight forward, based on serum ceruloplasmin, urinary copper excretion, hepatic copper content, and eye examination for Kaiyser-Fleischer (KF) rings in most cases.⁴ Homozygous mutation of ATP7B gene is considered diagnostic for WD. More than 500 disease causing mutations have been described in the literature. In doubtful cases, where biochemical and histopathological features are equivocal, mutation analysis can be extremely useful investigation for accurate diagnosis of WD.

We present a case of WD with equivocal findings on routine tests in which the diagnosis was confirmed by mutation analysis.

CASE REPORT

A 9-year female presented to the Paediatrics Department of Holy Family Hospital, Rawalpindi, with history of abdominal distension and jaundice for 3 months and altered conscious level for 2 days. She did not have fever, joint pains or skin rash. Her parents were first cousins and she had two elder female siblings, one younger female sibling and one younger male sibling. Rest of the siblings were alive and healthy. Child was in grade 2 hepatic encephalopathy at presentation, jaundiced having hepatosplenomegaly and ascites. Patient had normal blood counts. Serum bilirubin was 5.9 mg/dL (direct 2.6 mg/dL). alanine aminotransferase (ALT) was 32 iu/mL, aspartate aminotransferase (AST) 21 iu/mL, gamma-glutamyl transpeptidase (γ-GT) 64 iu/mL, serum albumin 2.5 mg/dL and normal coagulation profile (INR 1.1). Serum ceruloplasmin was 7.7 mg/dL (range: 20-35 mg/dL), which was in favour of WD but urinary copper with penicillamine challenge was 140 ug/24 hours, which was only mildly raised (normal ug<50 ug/24 hours) and KF rings were absent. Workup for autoimmune hepatitis was unremarkable.

WD genetic mutation analysis was done at the Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan. Sanger sequencing of ATP7B gene identified a homozygous nonsense mutation c.3955C>T (p.Arg1319Ter) in exon 19 (Figure 1).

Patient improved with penicillamine, zinc and supportive care and liver functions normalised over a period of 8 months.

DISCUSSION

WD is among the commonest causes of childhood liver diseases; and even fewer treatable causes. Diagnosis of WD is usually straight forward, on the basis of serum ceruloplasmin levels, 24-hour urinary copper excretion estimation and the presence of KF

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rings on slit-lamp examination. Liver biopsy and calculation of copper content of dry weight of liver is not widely available. However, the diagnosis of WD is complicated because of subtle and diverse clinical features, and lack of accurate biochemical markers.⁵ Ceruloplasmin is an acute phase reactant and may be falsely negative in acute inflammation as well as in 15-36% of patients with WD and false positive in heterozygous individuals and aceruloplasminemia. KF rings are usually absent under 10 years of age. Copper estimation of dry weight of liver is an invasive test and not freely available. Urinary copper excretion is variable among individual patients.

 **Figure 1: Region of exon 19 of ATP7B gene from control individual (A). Region of exon 19 in proband (B). Arrows indicate the position of mutation.**

Under these circumstances, mutation analysis becomes an extremely useful adjunct to the diagnostic testing. However, the large number of known mutations (>500) and a very large gene complicate the situation.

In a recent meta-analysis, p.His1069Gln was most often encountered mutation in Europe, while p.Arg788Leu was common in Far East. P.Gln1399Arg was commonest in Saudi Arabia, p.His1069Gln was dominant in USA, and c.3402delC was predominant in Brazil.⁶ In another review, common mutations identified in North American, Russian and Swedish populations were single nucleotide substitution, c.3207C>A (His1069Gln) involving exon 14. Other slightly less common mutations were 3402delC and c.3809A>G.(Asn1270Ser).⁷

The mutation identified in our index case, i.e. c.3955C>T (p.Arg1319Ter), was a single nucleotide substitution resulting in premature truncation of the peptide chain at amino acid 1319. The mutation has been reported as pathogenic and is considered as a rare mutation with only 10 patients registered in The University of Alberta Wilson's Disease mutation registry. The reported cases are mainly from England and European countries with one each from China and Egypt. No case has been reported from the Indo-Pak subcontinent yet.⁸⁻¹⁰

It is inferred that mutation analysis is a useful adjunct to the diagnosis of WD because of wide clinical spectrum, subtle features and lack of accurate biochemical diagnostic tests. ATP7B gene is a very large gene, therefore, identification of common mutations in

Pakistani population will greatly help in genetic diagnosis of WD.

PATIENTS' CONSENT:

Informed Consent was taken from the father of the patient.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MAH: Conceptualization, data collection, write-up.

BZ: Genetic testing, literature review.

RMA, MAL: Critical review, proof reading.

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