Identification of Common Mutations Causing Wilson Disease in Egyptian Children

Thesis Submitted for partial fulfillment of MD degree in clinical and chemical pathology

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Abstract

Wilson Disease is caused by excessive accumulation of copper in hepatic and extrahepatic tissues due to defective excretion of copper into bile. As a result, copper accumulates particularly in the liver, kidney, brain, and cornea.

ATP7B gene, is responsible for transport of copper into bile from hepatocytes and its incorporation into apoceruloplasmin to form ceruloplasmin.

Wilson Disease is an autosomal recessive disorder, with high frequency in certain ethnic groups. Early diagnosis is mandatory to initiate early treatment so as to prevent morbidity and mortality.

Clinical features are highly variable, so the diagnosis may be easily missed. This is especially critical since the disease is readily treatable by chelating agents such as penicillamine or trientine, or by oral zinc that blocks copper absorption.

Key Words:

Elution buffer - base pair – Identification .

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And who sent me those who were of help “

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Marwa El-Sharkawy

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- **AASLD:** American Association for the Study of Liver Diseases.
- **ATOX1**: Antioxidant protein 1.
- **ATP7B:** Adenosine triphosphate.
- **BI-PASA**: Bidirectional PCR amplification of specific alleles.
- **Buffer AE:** Elution buffer.
- **Buffer AL:** Lysis buffer**.**
- **Buffer AW:** washing buffer.
- **Bp:** base pair.
- **CCS**: Copper chaperone of superoxide dismutase.
- **CMT1, CTR1**: Copper membrane transporter 1.
- **COX**: Cytochrome-c oxidase.
- **CVS:** Chorionic villous sampling.
- **ddNTPs**: dideoxynucleotide triphosphates
- **dNTPs:** deoxynucleotide triphosphates.
- **EDTA:** Ethylene diammine tetraacetate.
- **ICC**: Indian childhood cirrhosis .
- **ID:** Identification.
- **Kb:** Kilobase, Unit of length for DNA fragments equal to 1000 nucleotides**.**
- **KF rings:** Kayser-Fleischer rings .
- **KRT8**: Keratin8 gene.
- **KRT18** :Keratin18 gene**.**
- **NAFLD:** Nonalcoholic fatty liver disease.

- **PCR:** Polymerase chain reaction**.**
- **POP:** Performance Optimized Polymers.
- **ROS:** Reactive oxygen species.
- **Rpm:** Revolutions per minute**.**
- **SOD1:** Superoxide dismutase.
- **TGN:** Trans-Golgi network.
- **TE buffer:** Tris-HCl EDTA.
- **USA**: United States of America.
- **WD**: Wilson disease.

Introduction

Introduction

Copper is an essential metal that is an important cofactor for many proteins, the liver utilizes some copper for metabolic needs, synthesizes and secretes the copper-containing protein ceruloplasmin, and excretes excess copper into bile **(Roberts and Schilsky, 2008).**

Wilson disease (WD) is a recessive disorder of copper transport, in which the incorporation of copper into ceruloplasmin and excretion of copper through the bile are impaired. As a result, copper accumulates particularly in the liver, kidney, brain, and cornea. Clinical features are highly variable, so the diagnosis is easily missed. This is especially critical since the disease is readily treatable by chelating agents such as penicillamine or trientine, or by oral zinc that blocks copper absorption **(Cox and Roberts, 2006).**

The WD disease gene encodes ATP7B copper transporter, a gene of 21 exons, spanning approximately 100 kb. The 4.3 kb open reading frame encodes a 1,465 amino acid protein with features characteristic of the P-type ATPases **(Bull et al., 1993 and Tanzi et al., 1993).**

Mutational analyses of WD patients and their relatives have identified more than 518 distinctive variants as reported in the WD mutation database. Most of these variants are probable disease-causing variants (379 out of 518 variants). The remaining variants are reported as possible normal variants **(Kenney and Cox, 2007).**

It was hypothesized that the mutations tend to occur in a population-specific manner. Some mutations appear to be population specific, whereas others are common in many populations **(Wu et al., 2001)**.

The diagnosis of WD is based on the results of several clinical and biochemical tests, each has its limitations, and only the combination of clinical, biochemical and genetic tests provides a powerful and reliable tool for the diagnosis **(Merle et al.,2007).**

In Egypt, where viral hepatitis (HCV, HBV) is by far the major player in the field of liver disease, WD seems to be under diagnosed and clinical data on large cohorts are limited owing to its low frequency, being a rare disease. Increasing awareness about diagnosis and management based on detection of the mutational spectrum as a rapid screening approach may be applied for Egypt in the future **(El Karaksy et al., 2011)**

The aim of this study is to identify mutations in ATP7B gene in Egyptian diseased children by genetic study for early diagnosis and implementation of effective treatment before the onset of clinical symptoms. Also, help screening other family members to identify at risk individuals with presymptomatic gene mutation carriers.

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Review of Literature