WILSON DISEASE: PRACTICAL APPLICATIONS
OF MOLECULAR APPROACHES

GENETICS IN LIVER DISEASES

XIII FALK LIVER WEEK, FREIBERG, GERMANY
OCTOBER 8, 2006

DIANE WILSON COX
UNIVERSITY OF ALBERTA
WILSON DISEASE

- traditional features: clinical, biochemical
- genetic basis
- use of mutation (DNA) analysis in diagnosis
- importance of DNA diagnosis for sibs of patients
WILSON DISEASE VARIABILITY
- a diagnostic challenge

- autosomal recessive; 1 in 30,000
- hepatic: acute, chronic
- neurologic: tremor, rigidity
- hemolytic anemia
- other: psychiatric, kidney malfunction
- onset: 3 years to 55 (70?) years
TRANSPORT OF COPPER IN HUMANS

Oral Intake
(1.5-3 mg/24hr)

Intestinal Absorption

Menkes disease
(X Chromosome)

Plasma Albumin
(Rapid Clearance)

Other Tissues, Proteins:
Brain
Eye
Kidney
Enzymes

Cytochrome oxidase
dopa β mono oxygenase
lysyl oxidase
superoxide dismutase

Liver

MT apo-ceruloplasmin
(chromosome 3)

Wilson disease
(chromosome 13)

Fe (II)
ceruloplasmin
other proteins

Biliary Excretion
(1-3 mg/24hr)

D. W. Cox
U. Alberta
DIAGNOSTIC FEATURES

- Kayser-Fleischer rings
- decreased serum ceruloplasmin
- increased non-Cp serum copper
- increased urinary copper
- increased hepatic copper
Kayser-Fleischer Ring
<table>
<thead>
<tr>
<th></th>
<th>Hepatic</th>
<th></th>
<th>Neurologic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Confirmed</td>
<td>Query</td>
<td>Confirmed</td>
<td>Query</td>
</tr>
<tr>
<td>K-F Rings</td>
<td>89</td>
<td>50</td>
<td>81</td>
<td>69</td>
</tr>
<tr>
<td>Low Cp</td>
<td>90</td>
<td>65</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Urinary Cu &gt;1.6μM</td>
<td>100</td>
<td>90</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>
WILSON DISEASE 2006

- the gene
- new diagnostic aids
- presymptomatic diagnosis
CLONING OF THE GENE FOR WILSON DISEASE

A COPPER-TRANSPORTING MEMBRANE PROTEIN

1993


PREDICTED ATP7B PROTEIN

Copper binding domains

Phosphorylated D residue

ATP binding domain

Transduction

Conserved CPC motif

Channel

Modified from: Bull et. al, Nature Genet, 1993
WILSON DISEASE MUTATION DATABASE

Curators
Susan Kenney
Dr. Diane W. Cox

The Database has been prepared with the assistance of
Susan Kenney
Theresia Maier-Dobersberger
Siobhan Cashman

We acknowledge the support of the University Hospital Foundation.

The Wilson Disease Database and its contents are Copyright © 1999-2001 University of Alberta.

Download Database in Microsoft Excel Format - [114k] - Last Updated March 2006

Date submitted and name of submitter will be posted.
Download submission form, or contact Susan Kenney <kenney@ualberta.ca>
## MUTATIONS IN THE WILSON DISEASE GENE (ATP7B)

<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion</td>
<td>60</td>
</tr>
<tr>
<td>Insertion</td>
<td>21</td>
</tr>
<tr>
<td>Nonsense</td>
<td>20</td>
</tr>
<tr>
<td>Missense</td>
<td>168</td>
</tr>
<tr>
<td>Splice</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>291</strong></td>
</tr>
</tbody>
</table>

http://www.medicalgenetics.med.ualberta.ca/wilson/index.php
GENE DELETIONS

Large deletions: 15 - 20%

One deletion reported

Horn et al 2005

D. W. Cox
U. Alberta
### MUTATIONS IN WILSON AND MENKES DISEASE GENES

<table>
<thead>
<tr>
<th></th>
<th>Wilson (ATP7B)</th>
<th>Menkes (ATP7A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletion/insertion</td>
<td>27</td>
<td>39</td>
</tr>
<tr>
<td>Nonsense</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Missense</td>
<td>58</td>
<td>17</td>
</tr>
<tr>
<td>Splice site</td>
<td>7</td>
<td>23</td>
</tr>
</tbody>
</table>

Large Menkes deletions (15-20%) are excluded.

_Hsi & Cox Hum Gen. 2004 114:165_
HOMOZYGOUS MUTATIONS ATP7B FOR GENOTYPE/PHENOTYPE CORRELATIONS

- deletions, insertions (frame shift) and missense tend to have earlier onset (< 16 years) than missense mutations.
- different missense mutations are highly variable in phenotype (age and type of onset)
- variability in phenotype with same homoyzygous mutation
ASSESSMENT OF FUNCTION

of ATP7B MUTATIONS

- Disease-causing or normal?
- Type of defect?
  - toxicity  CHO
  - transport  yeast
  - trafficking  CHO
  - modelling
GROWTH CURVES: ATP7B IN YEAST

MUTANTS OF ATP7B

CRITICAL QUESTIONS

- IS THIS WILSON DISEASE?

- AFFECTED OR HETEROZYGOTE?
DIAGNOSIS BY HAPLOTYPE

BEFORE

NORMAL

AFFECTED

PRESYMPTOMATIC

AFTER

HETEROZYGOTE

HETEROZYGOTE

D. W. Cox

U. Alberta
OTHER COPPER DISEASES

?????????
BEDLINGTON TERRIERS
COPPER EXCRETION

Iron (II) → ATP7B → bile

CTR1 → copper(II) → ATOX1 → ATP7B

Cp → post Golgi → ATP7B
**MURR1 (COMMD1) GENE and POLYMORPHISMS**

**MURR1**

![Diagram of MURR1 gene with exons and polymorphisms](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age of onset</th>
<th>Polymorphism Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical WD (8)</td>
<td>9-26 yrs</td>
<td>c.471+63c&gt;g</td>
</tr>
<tr>
<td>Possible WD (16)</td>
<td>0.5-66 yrs</td>
<td>c.501T&gt;C</td>
</tr>
<tr>
<td>Not WD by Haplotype (3)</td>
<td>6-15 yrs</td>
<td>na</td>
</tr>
<tr>
<td>Unaffected (26)</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

SUMMARY

• DNA diagnosis is possible and useful

• DNA diagnosis is essential for sibs of known patients
WILSON DISEASE, COPPER TRANSPORT

Diane W. Cox

Gina Macintyre          Charles De Leeuw
Gloria Hsi              Jennifer Rauw
Veronica Coronado       Lisa Prat

Sue Kenney

Peter Bull               Steven Moore
Gail Billingsley         Manoj Nanji
Tsing Cheng              Gordon Thomas
Lara Cullen              Jingshi Wu
John Forbes              Cynthia Yu

Yeast collaborator: Moira Glerum

Clinical Collaborators  John Walshe, U.K.
                        Eve Roberts, HSC, Toronto
                        Marc Bilodeau, Montreal
                        Hisham Nazer, Riyadh, Saudia Arabia
                        Klaus Gutfreund and Wayne Martin, U. of Alberta
                        and many others.

*Canadian Genetic Diseases Network (NCE)
*Canadian Institutes for Health Research
*National Science and Engineering Council of Canada
Alberta Heritage Foundation for Medical Research
Canadian Liver Foundation
University of Alberta
Edmonton, Alberta, Canada

THANK YOU