Severe BSEP deficiency
Mutations, Immunohistochemistry and Malignancy

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Bile salt export pump (BSEP)

- ABC transporter
- Hepatocyte canalicular membrane
- Bile salt export pump of the liver
- Determinant of bile flow
BSEP deficiency

- Failure of bile salt transport
- Continuum of disease severity
- Progressive familial intrahepatic cholestasis
- Benign recurrent intrahepatic cholestasis
  - Hormonal
  - Viral
  - Drug induced
Severe BSEP deficiency

- **Presents** - jaundice, pruritus, malabsorption, failure to thrive
- **Progresses** - cholestasis, cirrhosis, liver failure
- Normal $\gamma$GT and cholesterol
  - $\uparrow$ Serum bile acids
  - $\downarrow$ Biliary bile acids
- Hepatic phenotype
  - 1/3 gallstones
Aims

• Determine the mutation profile of severe BSEP deficiency

• Correlate genotype with residual BSEP detection

• Determine if genotype affects malignancy risk
Patients

- Presentation suggestive of BSEP deficiency
- One individual, in each family, had persistent disease
- Excluded if cholestasis resolved completely
Mutation analysis

- **ABCB11** gene on chromosome 2q24-31
- 27 coding exons

**Analysed**
- Microsatellite marker typing
- Restriction enzyme digestion
- SSCP analysis
- Sequencing
- cDNA analysis
Mutation analysis

• 109 families
  - 82 different mutations
  - 52 novel

• Mutations
  - 9 nonsense - 15%
  - 10 small insertions/deletions - 14%
  - 15 splice-site - 25%
  - 3 whole-gene deletions - 3%
  - 45 missense - 79%
Canaliculus

Walker A and B motifs

ABC signatures

Nonsense

Deletion / Insertion
Canaliculus

Walker A and B motifs
ABC Signature
Missense
Mutation analysis

• 109 families in total
• 10 single mutation
  - 3 insufficient DNA
  - 7 deletions/rearrangements?
• 99 families with 2 mutations
• 36% homozygous
  - 23 consanguinity
  - 9 two copies E297G or D482G
• 64% compound heterozygotes
Founder/recurrent mutations

- 32% in ≥2 families
- 16% in ≥3 families

- Population specific
  - E1302X Greek
  - c.2012-8T>G UK
  - T127HfsX6 Saudi Arabia

- 58% of Europeans have E297G and/or D482G
  - E297G Northern Europe
  - D482G Central/Eastern Europe
Recurrent mutations

• CpG mutation hotspots
  - 33% of missense/nonsense mutations
  - 19% multiple families

• Can occur in all populations
  - R575X

• 49% at least one novel mutation
Genotype / phenotype correlations

- Mutation profile determined
- Genotypes sorted by likely severity
- Correlated with
  - Clinical outcome
  - Immunohistochemistry
BSEP Immunohistochemistry

• 2 Protein truncating mutations - no BSEP

• 45% of patients with E297G and/or D482G have some detectable BSEP

• E297G (29)
  - Absent 16, Abnormal 12, Normal 1

• D482G (14)
  - Absent 8, Abnormal 3, Normal 3

• 10 additional missense mutations some BSEP
BSEP
normal liver
Patient absent BSEP

MRP2
same patient
Patchy BSEP in patient

MRP2 in same patient
Patient, normal staining
Immunohistochemistry

- 88 patients
  - Absent 72%
  - Abnormal 22%
  - Normal 7%

- 93% patients have abnormal/absent BSEP

- Useful marker in severe disease

- Identify candidates for therapy?

- Detection threshold unknown
Malignancy risk

- HCC/CC rare paediatric tumours

- Increased risk
  - 10 HCC <5yrs
  - 2 CC

- Does genotype affect malignancy risk?
Genotype-outcome

• 128 patients

• Group 1 (21)
  - 2 protein truncating mutations
  - 8 HCC / CC

• Group 2 (107)
  - ≥1 missense /unknown mutation
  - 11 HCC / CC
Genotype-outcome

- 15% HCC / CC
- 74% before 5yrs
- 2 protein truncating mutations greatest risk
- 38% Group 1 vs 10% Group 2
- Relative risk 3.7 (CL=1.7-8.1 p=0.003)
Genotype-outcome

- 13 group 1 patients no malignancy

- However
  - 10 OLT/died
  - Only 3 retain native livers

- Real incidence higher?
Genotype-outcome

- 11 group 2 patients developed malignancy
- 9 missense mutations identified
  - 6 changes investigated
  - 5 result in splicing defects/no protein
- Some have 2 protein truncating mutations?
Conclusions

- >100 $ABCB11$ mutations now identified
- Abnormal or absent BSEP is a good marker for severe BSEP deficiency
- BSEP deficiency confers risk of HCC / CC
- 2 protein truncating mutations greatest risk
- Monitor all patients with native liver
Acknowledgements

- K Emerick, H Melin-Aldana, P Whittington - Children’s Memorial Hospital, Chicago, USA
- Y Meier, B Stieger - University Hospital, Zürich, Switzerland
- B Shneider - Mount Sinai School of Medicine, New York, USA
- R Kotalová - Charles University, Prague, Czech Republic
- M Jirsa - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
- P McClean - St James’s University Hospital, Leeds, UK
- H Verkade - University Hospital Groningen, The Netherlands
- É Sokal - Université Catholique de Louvain, Brussels, Belgium
- P Bilezikçi, F Özçay - Baskent University Ankara Hospital, Turkey
- N Papadogiannakis, B Fischler, B A Németh - Karolinska University Hospital, Sweden
- J Cielecka-Kuszyk, J Jankowska, J Pawłowska - Children’s Memorial Health Institute, Warsaw, Poland
- F Al-Hussaini, A Bassas, S Wali - Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia
- A Scheimann, M Finegold - Texas Children’s Hospital, Houston, USA