A COMPARISON OF WILSON’S DISEASE IN BRITAIN & INDIA
Bala P, Bavelek A, Curtis D, Pandit A, Quarrell O, Tanner S
Dept of Paediatrics, Centre for Human Genetics, Children’s Hospital, Sheffield, King Edward Memorial Hospital, Pune, India.

Wilson’s disease (WD) has a worldwide incidence of 1:3,500 to 1:100,000, variably presenting as either hepatic or neurological. We compared a cohort of WD patients from Britain and India by clinical presentation, linked markers and mutations. There were 25 Caucasian British families and 43 Indian families from the Pune region. DNA was obtained by consent from parents, affected children and siblings. Hepatic presentation of the index case occurred in 16 (64%) of the British families and 28 (65%) of the Indian families. Particular features of Indian hepatic cases were a young age of onset (below the age of 7 in at least 6 families), accompanying neurological symptoms (in 6 families) and Kaysler-Fleischer rings, features not seen in British cases. Haplotype analysis was performed using three linked microsatellite markers D13S314, D13S301 and D1S296 (1). Common mutation screening was performed using PCR and restriction enzyme digest (2).

In British families, haplotype analysis suggested most patients were compound heterozygotes. 20 Indian haplotypes were homozygous, suggesting identical mutations. There was no common British WD haplotype, but a common haplotype (3-5-4) was on 14% of Indian WD alleles. In British families, the common mutation H1069Q was on 5 alleles (10%) and G1269R on 3 alleles (6%). These mutations were not found in any Indian case. 1102T was homozygous in 2 Indian patients. In 27 Indian families, 45 siblings were investigated for carrier status using the linked markers. 15 were normal, 27 were carriers and 3 were found to have WD. In two cases the original diagnosis was reversed. In conclusion, WD should be suspected earlier than 7 years of age, especially in immigrant Asian families who may be consanguineous and have a much higher carrier rate of WD mutations. DNA analysis using linked markers can offer early diagnosis where there is already an affected WD child. WD haplotypes offer clues to identifying mutations. (1) Thomas et al (1995) Am J Hum Genet 56: 1315-1319. (2) Morris et al (1995) Gut 37: A20.

HYPERFERRITINAEMIA WITH CATARACTS: A NEW HEREDITARY CONDITION
J O Lindsay, A Mumford, M Hagan, U Hegde, R Hawkins, and J Arnold Dept Medicine, Ealing Hospital, Southall, UB1 3HW

Ferritin, the main intracellular iron storage protein, consists of two chains, a heavy (21KDa), and a light (19KDa). Ferritin synthesis is controlled at the level of mRNA translation by an iron responsive mechanism, and reflects the intracellular iron concentration. This control process depends on a highly conserved motif at the 5’ non coding region of the ferritin mRNA. The only familial condition associated with hyperferritinemia is genetic haemochromatosis.

We present the first kindred in the U.K. in which there is a familial association between early onset cataracts and hyperferritinemia in the absence of an acute phase response. The ferritin level in affected family members is independent of iron status. 13 members of a family were investigated after the detection of hyperferritinemia in the proband. 6 members from 3 generations had ferritin levels in excess of 800μg/dl, despite normal iron and transferrin saturations, and plasma viscosities. A liver biopsy from one patient demonstrated no excess stainable iron. Phlebotomy resulting in biochemical iron deficiency had no effect on ferritin levels. All 6 with raised ferritin levels had normal natural immediately after iron and transferrin saturations. The other family members had a normal ophthalmic examination. Direct cycle sequencing of PCR-amplified DNA from the 5’ end of the L-ferritin gene on chromosome 19 revealed a T to C mutation in the iron responsive element that was only present in affected family members. This represents the first described mutation in an Iron Responsive Element in the U.K. The description of this new autosomal dominant genetic condition has wide implications for the use of ferritin as a marker of iron overload in the screening and treatment of patients with haemochromatosis.
SHUNT INSUFFICIENCY AFTER TIPSS, BALLOON ANGIOPLASTY OR INSERTION OF PARALLEL SHUNT?

KJ Dabbs, AJ Stanley, DN Redhead*, R Jalan, PC Hayes. Dept. of Medicine and *Radiology, Royal Infirmary of Edinburgh

Shunt insufficiency after TIPSS occurs in about 42% of patients after one year. The purpose of this study was, to assess the best therapeutic approach, to treat this problem

Methods: We studied 160 consecutive patients, (mean age 55.9 years) undergoing TIPSS for variceal haemorrhage (n=135) or refractory ascites (n=25) in our unit. Shunt patency was assessed by direct portography at 3 and 6 months, then 6 monthly or whenever complications occurred. Balloon angioplasty (BA) was performed when the shunt was insufficient (PPG >12mmHg or 20% increase in PPG). Parallel shunt (PS) was inserted when the shunt was occluded, or required repeated dilatations with BA.

Results: Over a mean follow up of 17.1(±2.2) months, primary shunt patency was present in 66.2% of patients. Of the 54 patients who developed shunt insufficiency during follow up, 47 had BA and 7 had insertion of a PS without prior BA. Following B.A., 18 patients had an insufficient shunt at second portography and 9 patients had shunt insufficiency at third portography. A total of 13 patients required insertion of a PS, of whom 4 had shunt insufficiency at 6 months. Shunt insufficiency recurs in patients in whom the first episode of shunt dysfunction occurs early or is associated with total shunt occlusion.

Conclusions: Using a combination of BA and insertion of PS in selective patients, TIPSS patency could be maintained in over 90% of patients over a follow up period of 17.1 months. Earlier insertion of parallel shunts should be considered in patients presenting with severe shunt dysfunction. Further research should be directed, towards the development of covered stents

CEREBRAL HEPATITIS C: CURRENT OPINIONS AND PRACTICES OF BRITISH GASTROENTEROLOGISTS

N Balakumar, M Fowweather, P Clarke, E D Srivastava, M C Allison. Gastroenterology Unit Royal Gwent Hospital, Newport, South Wales.

Screening for the hepatitis C virus, and how far one pursues evaluation and treatment of those infected, are subjects of intense debate. We therefore sent a questionnaire to 342 physicians members of the BSG who replied (82%). Uncompleted forms were returned by 19 members not seeing patients in this regard. There were 263 completed forms, and 232 members (82%) had seen such referrals during 1995.

Results: Screening: Most members would offer screening to current intravenous drug users with transaminase elevations (78%), to those requesting tests for HIV (71%) and to spouses or partners of patients with chronic hepatitis C (66%). 50 believe consideration should be given to more widespread screening of high risk groups such as current or former IV drug users.

Contraversy: 31% recommand barrier methods to those infected, 49% for new relationships only, and 25% do not make recommendations.

Liver biopsy: Most physicians have a low threshold for biopsy. It is offered routinely by 35%, and by a further 28% for PCR-positive patients. 24 biopsy only those with raised transaminases.

Interferon: 56% said they offer interferon and 39% had treated at least one patient in their unit during 1995. Another 5% refer biopsy patients in for treatment and 25% sent theirs to tertiary referral centres. Eleven stated they wanted to treat locally but had no funding. Eight of these centres had been refused funding.

Conclusion: There is a need for consensus on screening, counselling and funding of therapy.

Meta-analysis of sclerotherapy and TIPS in the prevention of gastrointestinal rebleeding in patients with cirrhosis.

Patch D, Hamilton M, McCormick PA, Burroughs AKB
The Royal Free Hampstead NHS Trust, Pond St, London NW3

Introduction: TIPS is increasingly used in the treatment of portal hypertension. It is now being advocated for the secondary prevention of variceal rebleeding, though data from earlier surgical shunt trials had identified no survival benefit.

Aim: To evaluate sclerotherapy versus TIPS using meta-analysis in the prevention of variceal rebleeding.

Method: All published randomised controlled trials comparing TIPS with sclerotherapy in secondary bleed prevention in patients with cirrhosis were analysed. Mean follow up was 10 months. All but one are in abstract form. Data was obtained for survival, rebleeding and encephalopathy. Meta-analysis used both the Peto and Gart methods, with assessment of heterogeneity. End points were estimated according to the intention to treat principle.

Results: There were 8 trials with 515 patients. 255 received sclerotherapy, 260 received TIPS. One trial used banding ligation exclusively. Time to randomisation was heterogeneous. There was no significant difference between the groups in terms of death at one year. (Odds Ratio 1.39, 95%CI 0.9092,1.328). Modelling by random effect did not alter this. In terms of rebleeding, there was a significant reduction in the TIPS group. (OR 0.32, 95%CI 0.209, 0.49). The trade off was a significantly increased incidence of encephalopathy (OR 3.63, 95%CI 1.97, 5.61).

Conclusion: Confirming the results of previous surgical shunt trials, TIPS reduce the rebleeding rate when compared to sclerotherapy, but have no benefit in terms of survival, and are associated with a much higher incidence of encephalopathy. Because of this, it is not clear if TIPS is the treatment of choice. Further trials should include quality of life data and cost assessment, as well as comparing TIPS with drug therapy.

HOW TOXIC IS COPPER TO THE HEPATOCYTE?
N S Ashton, G S Evans and M S Tanner. University Department of Paediatrics, Stephenson Unit, Children's Hospital, Sheffield. S10 2TH

Liver damage in Wilson's disease and Indian Childhood Cirrhosis is associated with a massive accumulation of hepatic copper (Cu), but little is known about the cellular effects of increased liver Cu and its role in the aetiology of disease. In this study we have investigated responses of the hepatoma cell line, Hep G2 to Cu exposure.

Hep G2 cells were cultured in the presence of Cu (4-1000μM administered as CuSO4 to the growth medium) either acutely (4 days) or chronically (8 weeks). Cell numbers were assessed by the fluorochrome propidium iodide Cu concentrations above 32μM caused a significant reduction in cell growth (up to 80% reduction) within 2 days of exposure. In contrast, chronic exposure of Cu showed pronounced effects on growth and proliferation at concentrations less than 32μM. Colony forming efficiency (i.e. formation of colonies with greater than 40 cells) was impaired 2.5-fold when cells were chronically exposed to 16μM Cu but impaired 6-fold at 64μM Cu. FACs (fluorescent activated cell sorting) analysis revealed that with increasing Cu concentration there was a slowing down of Hep G2 passage through the cell cycle with an increase in their potential doubling time.

Short term effects on viability as measured by the uptake of the fluorochrome acridine orange showed no changes until 4 days of exposure and at concentrations above 32μM Cu. Viability was reduced to 35% of control at 64 μM and 10% of control above 125μM Cu. Stress responses, as measured by the lysosomal content of the cells, using acid phosphatase as a specific enzyme marker showed a 22% increase in activity at 16μM and 14% increase at 32μM Cu after 4 days of exposure.

In chronic, exposure of Hep G2 cells to Cu concentrations, which are comparable to those found in the serum (13-30μM), cause measurable signs of stress and inhibition of cell growth and proliferation. Such effects may undermine the ability of the hepatoctye to cope with damage from other xenobiotics so resulting in the rapid progression of these disease states. Cu concentrations four times higher than this are directly cytotoxic.
AGE RELATED DEVELOPMENT OF MUCIN GLYCOPROTEINS IN THE COLONIC MUCUS GEL LAYER OF CHILDREN

A. Aslam, R. D. Spicer, *A. P. Corfield, (introduced by B. Warren)
Department of Paediatric Surgery, Bristol Children's Hospital, Bristol, U.K. and *University Department of Medicine Labs., Bristol, U.K.

Mucin glycoproteins are the major component of the colonic mucus gel layer and interact with pathogens performing a protective function. We studied developmental changes in mucin glycoproteins in normal children from birth to the age of 15 years. We used colonic mucosal organ culture with radioactive mucin precursors [35S] sulphate and [3H]-glucosamine in dual isotope labeling experiments. After 24 hours culture the secreted and cellular mucus fractions were collected. Mucins were purified by gel filtration and the ratio of incorporation ([35S]/[3H]) was measured. The turnover of radioactive precursors was quantified by relating it to the DNA content of the mucosa ([35S] or [3H] per μg DNA). The total mucin in each sample was assessed by reactivity with Wheat germ agglutinin and the turnover of total mucin was quantified. Mucins were tested against 5 anti-mucin antibodies and 2 lectins. Three age groups were used guided by usual weaning and dietary changes, and had mucosal biopsies taken for culture. They were 0-3 months (9 subjects), 3 months-3 years (20), and 3-15 years (12).

The ratio of incorporation showed a statistically significant trend of increase with age in both secreted and cellular fractions. The turnover of both [35S] and [3H] and total mucin was significantly higher in the youngest age group in both fractions. This was followed by a sharp drop in turnover in the 3 month-3 year group followed by a gradual rise to a level about half that of early infancy in older children. The antibody 91SH (which detects human sulphated mucin) showed a significant trend towards increasing sulphation in the older age groups and this correlates with the increasing [35S] ratio, especially as the reactivity with the antibody PRJAS (which detects di- and tri-O-acetylated sialic acids) did not change. The other antibodies also showed no significant differences. This is the first time developmentally regulated changes in the colonic mucus glycoproteins have been identified and characterized.

LIVER REJECTION PROMOTES THE GROWTH OF DISTANT TUMOUR IN THE RAT.

M. Caplin1, T. Morris,2, RE Founder2, AP Dhillon2, SA Watson2, Departments of Academic Medicine1 and Histopathology2, Royal Free Hospital School of Medicine, London. Cancer Studies Unit1, University of Nottingham, Nottingham.

Introduction: Liver resection results in the release of a number of trophic agents which contribute to liver regeneration. It has previously been shown in an animal model that liver resection promotes the growth of intrahepatic metastases. Aim: To determine whether liver resection promotes the growth of distant tumour. Method: 20 BDIX rats (10 female and 10 male) were each given an injection of 1 × 106 cells of a colon cancer cell line DHDK12 into the muscle layer of the abdominal wall. After 1 week 10 rats (5 female = group I; and 5 male = group II) underwent 70% liver resection. As a control 10 rats (5 female = group III; and 5 male group = IV) underwent sham operation with laparotomy only. At 3 weeks post surgery all rats were terminated and growth of abdominal wall tumour was assessed. Results: There was a significant increase in the tumour growth between female rats group I vs. group III with tumour weight 0.354mg (SD 0.07) vs. 0.24mg (SD0.07) a difference of 47.5% p<0.05 and tumour cross-sectional area 87.7mm2 vs. 74.0mm2 a difference of 18.5% p<0.03. There was a smaller difference in tumour growth in males group II vs. group IV with tumour weight 0.41mg(SD0.09) vs. 0.39mg(SD0.09) p<NS and tumour cross-sectional area 104.7mm2(SD43.5) and 77.3mm2(SD14.6) p<NS. Overall in rats undergoing liver resection vs. sham operation there was an increase in tumour weight 0.38(SD0.08) vs. 0.32(SD0.11) a difference of 21% p=0.06 and a significant increase in tumour cross sectional area 121.7mm2 vs. 80.65mm2 a difference of 51% p<0.05. Conclusion: There is an increase in the growth of distant tumour following liver resection in the tumour growth compared to sham operated rats was more significant in the female rats. Those growth factors involved in liver regeneration may well promote the growth of more distant tumour. This study additionally raises the question of female sex hormones upregulating the effect of these growth factors.

Neoplasia, cell biology, immunology

A PROSPECTIVE RANDOMISED TRIAL OF CIMETIDINE THERAPY IN GASTRIC CANCER: AN INTERIM REPORT.


Cimetidine has been reported to improve the survival of patients with gastric cancer at all stages of disease. The aim of the fourth British stomach cancer Group trial was to assess the survival benefit of adjuvant cimetidine in gastric cancer.

The study is a randomised double-blind trial comparing cimetidine at 2 dose levels, 800 or 400mg twice daily with matching placebos. Eligible patients had biopsy proved gastric adenocarcinoma, with any stage of disease, were considered fit to enter the trial (life expectancy > 3 months) and were able to give informed consent. The study recruited 442 patients between February 1990 and March 1993 from 59 consultants in 39 hospitals throughout the UK. Analysis has been undertaken on an intention-to-treat basis. There is now 12 months follow up on all patients with a median follow up of 41 months.

The treatment allocation was balanced for sex, age, and stage. The median age of the patient in the study population is 68 years (range 23 - 88 years). The male to female ratio was 1.25. The majority of patients had stage III (31%) or stage IV (46%) disease, with 63 (15%) stage II and 36 patients (8%) stage I.

The trial results demonstrate no survival benefit for cimetidine when comparing the survival for all patients receiving cimetidine against placebo (z² = 1.77, p=0.18). When adjustment is made for dose (400 vs 800mg), stage (II, III, IV), age (<68, 68+) and sex, there is no survival benefit for the use of cimetidine. The median survival is 11 months for the cimetidine group and 12 months for the placebo group. The median survival within the cimetidine group is 11 months (95% CI 6 - 16 months) for 800mg BD and 12 months (95% CI 8 - 14) for 400mg BD.

The results of this trial do not support the use of cimetidine as an adjuvant therapy in gastric cancer. The need to continue trials of therapy in gastric cancer remains.