saline embryos. Interestingly, this pattern is reversed just after tracheo-oesophageal separation (E11.5) with Shh downregulated in the ventral trachea and strongly expressed in the dorsal oesophagus. In Adriamycin embryos, this precise dorsoventral pattern appears disturbed with a more uniform Shh expression in some E10.5 embryos and in the undivided foregut of the affected E11.5 embryos. Conclusions: The dorsoventral allocation of non-respiratory and respiratory fates within the foregut is undisturbed in mouse embryos destined to develop OA. The fault lies in the process of separation of foregut components. The spatial pattern of Shh expression before and after the separation makes it a good candidate for the initiation of this process. Adriamycin may interfere with the process by disturbing the dorsoventral Shh pattern in the foregut.

M124

Jagged and Notch expression in developing human biliary epithelium and reactivation in disease on abnormal ductular proliferative cells.

DM Flynn, AJ Strain, S Nijaar, MA Kilby, DA Kelly and HA Crosby.

Liver Research Laboratories and Birmingham Childrens Hospital, Birmingham UK

Introduction: Alagille syndrome is a paediatric cholestatic disorder associated with interlobular bile duct paucity. It is due to mutations in Jagged1, a ligand for the Notch signalling pathway. However the role of Notch signalling in the liver is as yet unknown.

Aim: to determine Jagged and Notch receptor expression in human ductal plate and paediatric normal liver and in paediatric cholestatic liver disease.

Methods: RT-PCR of whole liver tissue from fetal, normal and diseased (Alagille, bile atresia and alpha-1 antitrypsin deficiency) paediatric liver for Notch1-4 and Jagged1 and 2 and Delta1 and 4 were performed. Double immunofluorescent staining with cytokeratin19 was performed to determine localisation of ligands/receptors with respect to biliary cells and developing ductal plate.

Results: RT-PCR showed that mRNA for all 4 receptors was present in fetal liver (10-16 weeks gestation); and all normal and diseased paediatric liver samples. Ligand mRNA expression was limited to Jagged1. Confocal microscopy showed that only Notch3 was expressed in fetal liver, on mesenchymal cells adjacent to ductal plate. Notch 1,2 and 4 were expressed on normal biliary cells, with upregulation of Notch3 on stromal cells adjacent to ductular proliferative process in biliary atresia and A1AT. Interestingly Notch3 expression in Alagille was variable, with some samples showing marked Notch3 staining on abnormal biliary cells. Jagged1 was expressed on ductal plate cells, on the luminal aspect of normal biliary cells and markedly on ductular proliferative cells in all diseases examined.

Discussion: Expression of Notch ligand and receptors during biliary cell development and again in cholestatic liver disease, suggests that the Notch signalling pathway may be important both for normal bile duct development as well as reversion to an immature biliary phenotype in cholestasis. In Alagille the abnormalities in Jagged1 may lead to unusual Notch expression patterns seen. Elucidation of the precise role of Jagged and Notch in biliary cell development is important and may lead to increased understanding of pathogenesis of paediatric cholestatic disease.

M125

Hes6 regulates myogenic differentiation

Cossins, J*, Vernon, A E* , Zhang, Y*, Philpott A* and Jones P H*

The Notch signalling pathway regulates cell fate decisions during embryonic and adult life in all metazoan organisms. Notch mutants occur in leukaemia, in oncogenic viruses and are causative of cancer in animal models. The enhancer of split proteins (EoS) are Notch target genes in Drosophila. Using expressed sequence tag searches we identified a transcription factor homologous to EoS in mice and humans, Hes6. We found that Hes6 protein binds to the same DNA motif as EoS in vitro and represses transcription in reporter assays in vivo. Hes6 is expressed in murine embryonic muscle but not in adult muscle. We hypothesised that down regulation of Hes6 may be required for terminal muscle differentiation. When overexpressed in C2C12 mouse myoblast cells, Hes6 impairs normal differentiation, blocking induction of the cyclin dependent kinase inhibitor, p21(Cip1), and decreasing the number of cells withdrawn from the cell cycle as assessed by bromodeoxyuridine labelling.

Hes6 is well conserved in Xenopus, so we used this system to study the effect of Hes6 on myogenesis in vivo. In situ hybridisation in embryonic Xenopus reveals that Hes6 is co-expressed with the early muscle marker, MyoD. Overexpression of Hes6 in Xenopus embryos results in an expansion of the myotome, but despite this increase in myogenic cells, terminal muscle differentiation is suppressed, paralleling the phenotype seen in vitro. Analysis of Hes6 mutants indicates that whilst the DNA binding activity of Hes6 is not essential for its myogenic phenotype, protein-protein interactions mediated by the C terminus of the protein are required. Thus we demonstrate a novel role for Hes6 in multiple stages of muscle formation, and that down regulation of Hes6 expression is required for terminal differentiation of muscle. We are currently investigating expression of Hes6 in muscle tumours and characterising its interaction partners.

M126

nNOS in Developing Rat Spinal Nociceptive Responses.

C.E. Urch and A. H. Dickenson

Nitric oxide (NO) is a diffusible chemical messenger known to be involved in modulating the spinal neuronal response to noxious primary afferent stimuli via the activation of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is crucial for maturation and activity of developing neuronal circuits. Studies have indicated NO role in inflammatory and neuropathic pain models, with hyperalgesia attenuated by administration of nNOS antagonists. NO is not a simple adjunct to the NMDA excitatory pathway: produced by primary afferent terminals, postsynaptic and GABAergic neurons, it is diffusible acts in a retro- or antegrade manner or on bystander neurons and the descending inhibitory pathways. Different NMDA receptor antagonists have been shown to have differing efficacies depending on the postnatal age of the rat in parallel with the alterations in the subunit composition. In order to investigate the role and possible alterations in the nNOS pathways postnatally, different NOS inhibitors were applied in vivo at various postnatal ages (p14,21,28 and adult) and the effect on dorsal horn neuronal responses to noxious electrical stimuli were noted. This was correlated with expression of nNOS and the NR1 (NMDA subunit) in the spinal cord. The results revealed a dose dependent inhibition of the C fibre, post discharge and windup evoked response, with both antagonists 7 N I and L-NAME. There was no difference between any age groups. However the primary evoked response was significantly different between the p14 age group and the p21, p28