08.01
Functional analysis of the Huntington's disease gene promoter
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The basis for the highly specific neuronal vulnerability in Huntington's disease (HD) has not been determined. Recent studies have demonstrated that variation in HD protein expression occurs in the striatum, with affected regions showing increased HD immunoreactivity. Experiments in HD and SCA1 transgenic mice suggest a correlation between phenotypic severity and expression of the mutant transgene. To gain insights into control of HD expression, and to investigate the possibility of cell-cell differences in transcription, we have analysed the 5' upstream region of the HD gene in a neuronal (SK-N-SH) and a non-neuronal (JEG3) cell line. Reporter gene assays demonstrated the presence of a region which is critically responsible for HD transcription, comprising two copies of a transcription factor binding sequence in a tandem repeat, which act synergistically. Electrophoretic mobility shift assays and site-directed mutagenesis experiments suggest that Sp1 mediates transcription at these sites. This region of the HD gene is polymorphic and a single Sp1 site is associated with reduced levels of transcription. The biological bases for some of the differences in HD gene expression between neuronal and non-neuronal cell lines will also be presented.

08.02
The analysis of a ribozyme approach to target retained DMPK transcripts in myotonic dystrophy
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Myotonic dystrophy (DM) affects an estimated 1 in 8000, and is the commonest form of muscular dystrophy in adults. The mutation which underlies the disease is a (CTG) triplet repeat expansion in the 3'UTR of a protein kinase gene DMPK. The recent findings of nuclear retention of expansion derived transcripts, binding of a CUG-BP to tracts of CUGs, and a putative role for this protein as an alternative splicing factor, appear to indicate that the underlying mechanism in DM may be the consequence of a gain of function for RNA rather than a loss of DMPK protein. As a possible mechanism to correct this nuclear retention of DMPK transcripts we have developed several ribozymes targeted to different portions of DMPK transcripts. These ribozymes, based on both hammerhead and P ribozyme systems, have been introduced into COS-7 cells and fibroblasts from myotonic dystrophy patients. The efficacy of these ribozymes has been studied by RT-PCR analysis of target transcripts, and by analysis of the distribution of CUG-BP and DMPK protein using immuno-fluorescence. Preliminary results indicate that ribozymes could provide a mechanism for the removal of retained transcripts.

08.11
Functional analysis of DNA variants identified in the promoter/5'-UTR of the neurofibromatosis type 1 (NF1) gene.
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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder mapping to chromosome 17q11-2. A 950 bp fragment of the promoter region of the NF1 gene (-755 to +196 relative to the transcription start site) was screened using dye-labeled fingerprinting. Five base changes were found, at positions 412, -402, +16, +25 and +132. Incomplete linkage of some of these variants with the affected allele in family studies and the identification of three of the changes in control samples, made it unlikely that these changes caused the disorder. To determine if the changes had any effect on the normal expression of the NF1 gene, HeLa cells were transfected with luciferase reporter constructs containing sequence from -489 to +484 of the NF1 promoter/5'-UTR. The constructs containing changes at -412 and -402 gave luciferase levels not significantly different from the wild type construct. However, all three changes in the 5'-UTR gave a 60-70% increase in expression. The molecular mechanism underlying NF1 is thought to be haploinsufficiency. Therefore the increased expression reported here for the changes in the 5'-UTR may affect the clinical phenotype of the disease in the corresponding patients.

08.12
Different types of mutation in KVLQT1 underlie Romano-Ward and Jervell and Lange-Nielsen syndromes.
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The autosomal dominant Romano-Ward (RWS) and the autosomal recessive Jervell and Lange-Nielsen (JLNS) syndromes are allelic conditions caused by mutation in either of the genes KVLQT1 and Kc. Both syndromes are characterised by life-threatening cardiac arrhythmias and prolongation of the QT interval on electrocardiogram (ECG), which is a consequence of a delay in the normal cardiac repolarisation. The gene products of KVLQT1 and Kc associate to reconstitute a K+ channel with properties of Iks, the slow component of the delayed rectifier current in heart. We previously showed that mutation in KVLQT1 accounts for most cases of JLNS. Recent clinical data has indicated that parents of children with JLNS may have prolonged QT intervals and are at risk of sudden death. We now show that mutations in KVLQT1 which underlie JLNS differ from those that cause RWS. This may account for differences in symptoms in heterozygotes for the two conditions, explaining why carriers of missense mutations tend to suffer from RWS whereas nonsense mutations only rarely give rise to symptoms except in the homozygous state. In vitro expression studies of mutations should provide further data on the severity of individual mutations in JLNS allowing better clinical management.

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08.14
Functional analysis of mutations in the L1 gene that cause neurological disease in man
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Mutations in the L1 gene underlie the development of a clinically variable neurological disease and over 60 different mutations have been described. Many of these changes alter only a single amino acid and may highlight critically important residues for L1 ligand interaction or downstream activity. L1 has a multitude of binding partners at the neuronal surface including itself. Studies on mutations distributed across the entire extracellular surface of L1 will therefore provide valuable insight into binding requirements. We have engineered 14 different L1 ECD mutations into two different vectors for transient expression in mammalian cells. Expression of full length constructs indicates that with the exception of a single mutation (W9S) in the signal peptide all missense mutations allow L1 to reach the cell surface. Expression of the mutations in the context of the ECD only allows us to produce a soluble version of L1 that are used in homophilic binding assays. Results to date indicate that whilst some mutations severely reduce binding (e.g. R184Q) others do not (e.g. G121S), that immunoglobulin domain 2 is an essential component of the L1 homophilic binding site and that the patient pathology is due to different types of L1 malfunction.

08.17
Analysis of Pilomatrixomas in Myotonic Dystrophy.
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Myotonic dystrophy is a progressive neuromuscular disease caused by the expansion of a CTG repeat in the 3' untranslated region of a protein kinase gene (DMPK). The function of DMPK remains unclear although the identification of a subfamily of serine/threonine protein kinases that includes both DMPK and the WARTS gene in Drosophila melanogaster may provide some insight. WARTS is thought to have a tumour suppressor function, which may be representative of this subfamily of proteins, and a characteristic of DM is the occurrence of multiple benign tumours that arise from hair follicles. These pilomatrixomas are rare in the general population, hence the increased incidence associated with DM may imply a role for DMPK in the aetiology of these lesions. We have previously shown that expansion of the trinucleotide repeat results in nuclear retention of DMPK transcripts derived from the expanded allele. The DMPK protein level would be expected to decrease by 50% as a consequence. Pilomatrixomas may arise from cellular overproliferation if a second mutational event eliminates the remaining functional DMPK allele. We have used SSCP analysis on paraffin embedded pilomatrixoma tissue samples from DM patients as well as from several cases of multiple occurrence in the general population. Our results have included the identification of a new coding polymorphism within DMPK. The contribution of haploinsufficiency to the DM phenotype will be considered in view of the... [Abstract truncated at 1500 characters]