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Different age at onset for psoriatic siblings discordant for the presence of Cw6 at the HLA-C locus *C. Enerbäck, T. Martinsson*, A. Inerot, J. Wahlström*, F. Enlund*, M. Yhr*, L. Samuelsson* and G. Swanbeck*. Department of Dermatology and *Clinical Genetics, Sahlgrenska University Hospital, Göteborg, Sweden

Psoriasis has a well-known HLA-association where HLA-Cw6 is considered to be the primary association based on serological typing. Recently there have been several publications showing linkage to the HLA-region. It is previously known that the frequency of Cw6 is higher among patients with an early onset, which we recently confirmed in a PCR-based study using sequence-specific primers in a large Swedish psoriasis population. In order to further clarify the importance of Cw6 for the age at onset of psoriasis, we now report a model where the variation of other genetic and environmental factors are reduced. In the PCR-SSP based typing of 104 complete families where psoriasis were present, we found 15 families with affected siblings discordant for the presence of Cw6 at the HLA-C locus. We tested the hypothesis that the Cw6 positive sibling would have an earlier age at onset than the Cw6 negative sibling using the non-parametric Wilcoxons signed rank test for paired observations. The difference in age at onset was significant with an average age at onset for the Cw6 positive sibling of 19 years as opposed to 27 of the Cw6 negative ones (p=0,025). This finding further establishes the role of an age-at-onset determining factor in the HLA region.

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Oxygen radical toxicity in mice deficient in adenine nucleotide translocator-1 (Ant1) and generation of a mouse line deficient in cellular glutathione peroxidase (cGPx). *L Esposito, KG Waymire, B Cottrell, GR MacGregor and DC Wallace.* Center for Molecular Medicine, Emory University, Atlanta, GA 30322.

Mitochondria from the skeletal muscle of mice deficient in the heart/muscle isoform of the adenine nucleotide translocator (Ant1) exhibited a severe defect in coupled respiration (Graham et. al. 1997). We hypothesized that the respiratory enzyme complexes in these animals would exist mostly in the reduced state and consequently generate increased levels of reactive oxygen species (ROS) including superoxide which would damage cellular components including DNA. Analysis of Ant-1 deficient mice for the levels of mitochondrial DNA (mtDNA) rearrangements by Long Extension (LX)-PCR have revealed that the hearts of 5-7 month old animals develop some mtDNA damage. Moreover, the mRNA and protein levels for the mitochondrial form of superoxide dismutase (MnSOD) as well as cellular glutathione peroxidase (cGPx) were upregulated in the hearts and skeletal muscle of these animals. To learn more about the importance of this compensatory induction of oxygen radical detoxification enzymes, we have created a null mutant for cGPx. We created our targeting vector by deleting both exons of the cGPx gene and replacing them with a neomycin resistance cassette whose expression is driven by the phosphoglycerate kinase promoter (PGKneo). Upon transfection of our vector into embryonic stem cells we isolated two properly targeted clones. One such clone yielded two male chimeric founders exhibiting germline transmission of the mutant allele. Homozygous mutant animals are being bred to assess the effect of cGPx deficiency on the integrity of the mitochondria and mtDNAs in animals with and without the Ant1 mutation.

Graham B.H., Waymire, K.G., Cottrell, B., Trounce, I.A., MacGregor, G.R., and Wallace, D.C. (1997) A mouse model for mitochondrial myopathy and cardiomyopathy resulting from a deficiency in the heart/skeletal muscle isoform of the adenine nucleotide translocator. Nature Genetics 16:226-234

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The Friedreich's ataxia mutation may originate from a premutation and shows size reduction when transmitted from parent to affected child. *S.M. Forrest[1], M. Delatycki[1], D. Paris[1], RJM Gardner[1], K Forshaw[1], G. Nicholson[2], N. Nassis[2], R. Williamson[1].* [1] The Murdoch Institute, Royal Children's Hospital, Parkville, Victoria,3052, Australia [2] University of Sydney, Department of Medicine, Molecular Medicine Laboratory, Concord Hospital, Sydney, New South Wales, Australia

Most trinucleotide repeat mutations cause dominant or X linked neurological disorders and show progressive increase in size from affected parent to affected child, the basis of phenotypic anticipation. Friedreich's ataxia (FA) is a recessive disorder in which gene dysfunction is due to an expansion of a GAA trinucleotide repeat in intron one which reduces mRNA and protein levels. We confirm that the severity of the disorder in our population depends upon the repeat length of the smaller allele, to a point where those with smaller alleles may be mis-diagnosed with other conditions such as spastic paraparesis. Two brothers were found to be heterozygous for the expansion and a point mutation (G to T) which leads to G130V. These patients have an atypical clinical phenotype. From studying 81 transmissions,we demonstrate that the repeat number of the expansion in FA usually decreases in size from parent to affected child, and that this affect is particularly marked in the paternal allele. No expanded alleles were found in the range between 22 and 332 trinucleotides with one important exception, a carrier with an intermediate repeat size of approximately 100. When this allele was transmitted to the affected child, the repeat increased in size either probably to 538 or possible to 1036. Analysis of a sperm sample from this carrier showed a major band for the expanded allele of 320 repeats. These data suggest that there may be a premutation for Friedreich's ataxia carriers, similar to that demonstrated for FraX-A and that expansion occurs in two stages, the first during meiosis followed by a second mitotic expansion.

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Gender-related association between the -93T→G/D9N haplotype of the lipoprotein lipase gene and elevated lipid levels in familial combined hyperlipidemia. *R. R. Frants[1], S. J. H. Bredie[2], H. Snieder[3], P. W. A. Reymer[4], P. N. M. Demacker[2], L. M. Havekes[5], D. I. Boomsma[3], A. F. H. Stalenhoef[2], J. J. P. Kastelein[4], M. J. V. Hoffer[1].* [1]MGC-Dept. of Human Genetics, Leiden University Medical Centre, Leiden, The Netherlands; [2]Dept. of Medicine, Division of General Internal Medicine, University Hospital Nijmegen, Nijmegen, The Netherlands; [3]Dept. of Psychophysiology, Free University, Amsterdam, The Netherlands; [4]Dept. of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; [5]TNO-PG, Gaubius Laboratorium, Leden, The Netherlands

Familial combined hyperlipidemia (FCHL) is a frequent cause of premature coronary artery disease. Affected family members are characterized by different combinations of elevated cholesterol and/or triglyceride levels. A reduction in lipoprotein lipase (LPL) activity has been observed in a subgroup of FCHL patients. Recently, we have demonstrated an increased frequency of mutations in the LPL gene in Dutch FCHL patients compared to normolipidemic controls. In the present study, we have applied a pedigree-based maximum log-likelihood method to study the effect of LPL mutations on the phenotypic expression of FCHL in families. In 40 FCHL probandi three different, previously reported, mutations in the LPL gene were identified resulting in amino acid changes, D9N, N291S, and S447X. The D9N mutation in exon 2 appeared to be in strong linkage disequilibrium with a T→G substitution at position -93 in the promoter region of the LPL gene. Using a pedigree-based maximum loglikelihood method, we demonstrated that the -93T→D9N haplotype is associated with significantly higher levels of LDL and VLDL cholesterol, and VLDL triglycerides. Interestingly, the effect was only observed in male carriers. In line with our previous observations these results further sustain that the LPL gene is a susceptibility gene for dyslipidemia which explains part of the variability in the phenotype observed among FCHL family members.

1801

Effect of CTG repeat expansion on chromatin structure and processing of DMPK mRNA in hybrid cell lines derived from myotonic dystrophy patients. *V.L. Funanage, R. Frisch, K.S. Singleton, P.Carango, P.A. Moses, and H.G. Marks.* duPont Hospital for Children, Wilmington, DE.

Myotonic dystrophy (DM), an autosomal dominant disease characterized by progressive muscle weakness and wasting, myotoniâ, cataracts, mental retardation, and cardiomyopathy, is associated with expansion of a polymorphic $(CTG)_n$ repeat in the 3′ untranslated region of the *DMPK* gene. The repeat expansion results in decreased levels of *DMPK* mRNA and protein, but the mechanism for this decreased expression is unknown. Otten and Tapscott (PNAS **92**: 5465, 1995) have recently demonstrated decreased chromatin sensitivity in the region of the repeat expansion in affected individuals and propose that this leads to decreased expression of the *DMPK* gene. These changes in chromatin structure could also interfere with expression of neighboring genes, *DMAHP* and *H59*. We have developed a PCR-based method to assay chromatin sensitivity of the region adjacent to the repeat expansion in somatic cell hybrids carrying either normal or affected *DMPK* alleles. Somatic cell hybrids carrying the expanded allele from either an adult-onset DM (133 CTG repeats) or a congenital DM (1700 CTG repeats) patient exhibited similar decreased sensitivities to *Pvu*II digestion in the region adjacent to the repeat expansion. Although *DMPK* mRNA expression was markedly reduced from $(CTG)_{133}$ or $(CTG)_{1700}$ alleles, a quantitative multiplex RT/PCR assay showed no inhibition of *DMAHP* or *H59* mRNA expression. Nested RT/PCR analysis of *DMPK* mRNA from somatic cell hybrids with the repeat expansions revealed that most of the *DMPK* transcripts expressed from the expanded alleles had spliced out exons 13 and 14, whereas full-length transcripts were expressed predominantly from the normal alleles. These results suggest that altered chromatin structure leads to a decrease in *DMPK* mRNA levels by affecting mRNA splicing at the 3′ end of the *DMPK* pre-mRNA transcript. Since the full-length transcript encodes a DMPK isoform that self-associates and shows peripheral association with membranes (Waring *et al.*, J. Biol. Chem. **271**:15187, 1996), our data suggest that a reduction in expression of the full-length transcript could cause the ion channel dysfunctions in DM.

1802

Frequency of intragenic haplotypes and association with (CAG)n repeat length at the *MJD1* locus in a large Azorean control group. *C. Gaspar[1,2], P. Maciel[1,2], L. Guimaraes[2], K. Arvidsson[1], S. Hayes[1], P. Coutinho[3], J. Sequeiros[2] and G.A. Rouleau[1].* [1]Centre for Research in Neuroscience, McGill University and The Montreal General Hospital Research Institute, Montreal, Canada; [2]Lab. Genetica Medica, ICBAS and UnIGENe, IBMC Universidade do Porto, Portugal; [3]Servico de Neurologia, Hospital Santo Antonio Porto, Portugal.

Machado-Joseph Disease (MJD) is an autosomal dominant spinocerebellar degeneration originally described in families of Portuguese-Azorean ancestry. We have previously shown evidence for the presence of linkage disequilibrium between the *MJD1* allele and several marker alleles ,suggesting either the existence of a founder mutation in the world MJD population or the presence of a predisposing haplotype. In the present study we analyzed a large group of control individuals in an attempt to identify an association between haplotype and (CAG)n length in normal chromosomes that might give an insight into the origin of the MJD mutation.

In this analysis we included a total of 225 unrelated individuals from the Azorean islands. All subjects were confirmed to be negative for the *MJD1* mutation. We typed all individuals for three intragenic single base-pair polymorphisms: $A^{669}TG/G^{669}TG$, $C^{987}GG/G^{987}GG$, TAA^{1118}/TAC^{1118}. In order to analyze the associations between intragenic polymorphisms and the size of the CAG repeats in normal chromosomes, we compared the frequencies of the alleles in three classes of normal chromosomes (according to size of (CAG)n), using Fisher's Exact test and chi-square.

In our control population we find evidence for an increased frequency of the haplotype formed by alleles ACA among the larger normal alleles, although the difference was not statistically significant. Furthermore, there is no exclusive association between larger normal alleles and a specific haplotype. These results seem to be incompatible with the suggestion that the expanded alleles in MJD might have originated from a pool of expression-prone normal alleles.