55 GENETIC ANALYSIS OF FACIAL CLEFTS.

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The study included 1499 families with at least one child affected with cleft lip± palate (CL±P) or cleft palate (CP). The families were ascertained in Southern Poland by multiple selection with probability $\hat{\pi}$ = 0.949 and $\hat{\pi}$ = 0.737, respectively. Complex segregation analysis was applied in an attempt to discriminate between the hypotheses of two alleles from single autosomal locus and that of multifactorial inheritance. The empiric recurrence risk of CL±P and CP equalled: 1.6 ± 0.66 and 0.93 ± 0.75, respectively. The heritability was: 54.8% (CL±P) and 52.8% (CP). The complex segregation analysis of CP did not distinguish between recessive model with incomplete penetrance (χ^2 = 18.27) and that of multifactorial inheritance (χ^2 = 20.38). For CL±P, the most plausible hypothesis (χ^2 = 16.86) appeared to be that of dominant inheritance with low penetrance (t = 0.277) and relatively high frequency of phenocopies (x = 0.683). The above models with the lowest χ^2 were used for computation of more precise recurrence risk figures for genetic counseling.

Presented results warrant further investigations on the genetic background of craniofacial anomalies. Future studies should concentrate on the improvement of the method of analysis and on the elimination of genetic heterogeneity of CL±P and CP malformations.

56 PREVALENCE AND MOLECULAR BASIS OF α-THALASSEMIA IN GREECE

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The prevalence and molecular basis of α-thalassemia in Greece was studied. To estimate the prevalence of α-thalassemia, 227 cord blood samples were examined by haematological and gene mapping techniques. Amongst the 227 newborns, 16(7.05%) α-thalassemia 2($-\alpha^{3.7}/\alpha\alpha$) and 2(0.88%) α-thalassemia 1 ($--^{Med}/\alpha\alpha$ and $-(\alpha)^{20.5}/\alpha\alpha$) heterozygotes were found. One individual was a double heterozygote for α-thalassemia 2 and a dysfunctional α-gene arrangement [$-\alpha^{3.7}/-(\alpha)^{?}$]. These results give an overall incidence for α-thalassemia in the Greek population of 8.4%. Gene mapping in 16 Greek individuals with HbH disease showed that eight had the genotype $--^{Med}/-\alpha^{3.7}$, four the genotype $-(\alpha)^{20.5}/-\alpha^{3.7}$ three the genotype $--^{Med}/\alpha\alpha^{T}$ and one the genotype $\alpha\alpha^{T}/\alpha\alpha^{T}$. These results demonstrate a wide diversity of molecular abnormalities of α-thalassemia in Greece which explains the observed heterogeneity of clinical phenotype.

57 A STRUCTURAL DEFECT OF TYPE III COLLAGEN CAUSING EHLERS-DANLOS SYNDROME TYPE IV

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The 22 year-old propositus had signs typical of Ehlers-Danlos syndrome type IV, i.e. thin and fragile skin, bleeding tendency from childhood, and recurrent haemato-pneumothorax, with normal joint mobility. His similarly affected father had died from internal hemorrhage at age 32 yrs. This syndrome has been attributed to deficient production of type III collagen (Pope et al., PNAS 72: 1314, 1975).

Cultured skin fibroblasts from the patient secreted reduced amounts of type III procollagen. After pepsin treatment, little normal type III collagen of 300 kDa was visualized after electrophoresis; instead, a more intense 230 kDa band was apparent, which after reduction gave rise to 75 kDa components en lieu of 100 kDa α1(III) chains. Two-dimensional CNBr mapping of the 230 kDa component revealed a type III collagen peptide pattern, but α1(III)CB3 and CB6 were missing. Thus, pepsin treatment had generated a disulfide-linked α1(III) homotrimer which was shortened by one fourth at the N-terminus of the molecule. Its thermal stability was decreased when compared to its normally structured counterparts.

Findings are best explained by a model in which a structural alteration in the CB6 region of the α1(III) chain impairs correct triple-helix formation in the N-terminal fourth of the collagen molecule. Molecules containing one, two or three mutant chains become unstable, and may be less efficiently secreted and processed.

We conclude that one mutant allele (dominant inheritance) of the α1(III) collagen gene codes for abnormal α1(III) chains which cause triple-helix disruption in the collagen molecules in which they are incorporated. The exact nature of the mutation at the gene level remains to be elucidated. These findings definitively establish the role of genetic defects of type III collagen in the pathogenesis of Ehlers-Danlos syndrome type IV.

58 BONE MARROW TRANSPLANTATION IN MURINE MODELS FOR LYSOSOMAL ENZYME DEFICIENCIES.

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Bone marrow transplantation (BMT) has been proposed as a therapy for lysosomal storage diseases. In the present study, the distribution of donor enzyme and the clinical effects after BMT were studied in two murine models for lysosomal enzyme deficiency. In the galactosylceramidase deficient Twitcher mice (model for Krabbe's disease), prolonged survival was seen after BMT (> 50 days; survival of untreated Twitchers: 30-40 d.). A benificial effect on the neurological symptoms was not observed. Six weeks after transplantation an increase in enzyme activity was measured in organs rich in bone marrow derived cells (spleen, liver and lung) but not in kidney and central nervous system (CNS). Similarly, in the ß-glucuronidase deficient C3H-mice increased enzyme activity was observed upon BMT in spleen plasma, leukocytes, lung and liver. Remarkebly, enzyme levels were also increased in kidney and peripheral nervous system, tissues which are not known to contain cells of hematopoietic origin. These data suggest that uptake in these tissues may depend on the type of enzyme involved. Uptake of donor-derived ß-glucuronidase was also found in isolated liver parenchymal cells. By subcellular fractionation, the increased enzyme activity was found to be present in the lysosomal fraction. Both in Twitcher and in C3H mice enzyme activity in the CNS was not increased after BMT, indicating that treatment of lysosomal storage diseases, which primarily affect the CNS requires a different approach.

59 LOW PLASMA MEMBRANE FLUIDITY IN JUVENILE NEURONAL CEROID LIPOFUSCINOSIS (NCL) LYMPHOCYTES.

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NCL is a group of genetic lipid storage disorders of unknown etiology which share the intracellular accumulation of a lipoperoxide containing material, possibly membrane breakdown products. The storage could be due to a yet undefined degrading enzyme deficiency or to an alteration of the lipid substrate. We investigated the plasma membranes of isolated intact lymphocytes from 7 patients with juvenile NCL and from 30 control children with steady-state fluorescence polarization. The membrane fluidity was determined by the anisotropy measured. Fluorescent probes used were diphenylhexatriene (DPH), a cationic analogue (TMA-DPH), and a set of n-(9-anthroyloxy) fatty acids (nAS; n = fatty acid carbon numbers 6,7,9,12, and 16, respectively). Membrane fluidity was significantly decreased in NCL vs. control lymphocytes when measured with the labels TMA-DPH ($P<.005$), 7AS ($p<.01$) and 9AS ($p<.01$). Fluidity was less significantly decreased when measured with the other probes. The results indicate a decreased fluidity of the outer membrane leaflet in NCL lymphocytes. Explanations of these changes include lower fatty acid unsaturation or raised levels of cholesterol, sphingomyelin, or protein. Further studies should focus on the composition of intact NCL membranes.

60 TYPE I HEREDITARY TYROSINEMIA: LACK OF IMMUNOLOGICALLY DETECTABLE FUMARYLACETOACETASE ENZYM PROTEIN IN TISSUES FROM PATIENTS.

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Type 1 hereditary tyrosinemia is an inborn error of tyrosine metabolism and presents itself shortly after birth as a severe liver and kidney disease. It is characterized by a profound deficiency of fumarylacetoacetase in liver, kidney and white blood cells. To investigate the nature of the enzym defect in more detail extracts of livers derived from patients were examined with immunochemical methods. Fumarylacetoacetase was purified (500x) from beef liver and antibodies against this protein were raised in rabbits. These antibodies cross react with the human liver enzyme. Analysis of liver extracts from type 1 hereditary tyrosinemia patients by immunodetection on blots showed the absence of cross reacting material in these livers. These findings could also be shown in extracts from extrahepatic tissues (kidney) and white blood cells (lymphocytes). Lack of cross reacting material could also be shown in cultured fibroblasts and amniotic fluid cells from patients. Thus the mutation causing type 1 hereditary tyrosinemia may lead to the synthesis of an aberrant enzym with a very short half-life, may affect the expression of the structural gene at the transcription/tranlational level or affects the proper expression of a regulatory gene. At present a human cDNA library is screened for probes in order to discriminate between these possibilities.