7

A unique point mutation in the PMP22 gene is associated with Deafness, Charcot-Marie-Tooth and Anticipation. V.E. Kimonis[1], M.J. Kovach[1], J-P. Lin[2], S. Boyadjiev[3], K. Campbell [1], L. Mazzeo[1], K. Herman[1], W. Frank [4], B. Llewellyn[5], E.W. Jabs[3], and D. Gelber [1]. [1]Southern Illinois University School of Medicine, Springfield, IL., [2]Genetics Study Section, Skin Biology Laboratory, NIAMS, NIH, Bethesda, MD., [3]Center of Medical Genetics, Dept. of Pediatrics, Johns Hopkins Hospital, Baltimore, MD,[4]Illinois State Police Laboratory, Springfield, IL.

We have identified a large US family with 33 affected individuals with Charcot-Marie-Tooth (CMT) syndrome, sensorineural deafness and evidence of anticipation. Originally reportedby Kouseff et al (1982), this disorder has not been previously characterized at the molecular level. All affected individuals in this family demonstrate slow nerve conduction velocities resembling CMT1A, and electron microscopy of sural nerve reveals severe demyelination of axons. There is moderate to profound deafness by adulthood. Audiological studies suggest auditory neural dysfunction as well as a minor degree of peripheral cochlear impairment. Members of the younger generations exhibit earlier onset and progressively more severe manifestation of the disease ($p<0.05$); the proband and another male dying in infancy from complications of CMT. Individuals from this family do not have the common 1.5 Mb duplication of CMT type 1A. We have mapped the disease to a0.6 Mb interval on chromosome 17p11.2 - p12 (Z max 9.01 with D17S1357 at theta=0.03). This critical region lies within the CMT type 1A duplication region but excludes the DFNB3 recessive deafness locus. Sequence analysis of PMP22 detected a unique G to C transversion at position 248 in exon 4 predicted to result in a substitution ofproline for a conservedalanine at codon 67 in transmembrane domain 2. This mutation was only observed in affected individuals, and not in 50 unrelated controls. The observed anticipation could not be explained by expansions of the three known trinucleotide repeats. PMP22 glycoprotein probably functions as a gap protein and is highly expressed by Schwann cells in compact myelin of the peripheral nervous system. PMP22 is structurally similar to theconnexins and point mutations in the transmembrane region of the latter have been implicated insyndromic and non-syndromic deafness. Presently the only known gene expressed in the cochlear, that maps to position 17p11.2-p1214, this study illustrates the pleiotrophic nature of PMP22 and its role in hearing.

8

Prenatal diagnosis and carrier detection of albinism.
A. Rosenmann[1], E. Rosenmann[2], I. Bejarano-Achache[3], A. Blumenfeld[3]. Departments of Ophthalmology[1], Pathology[2], and Molecular Biology[3], Hadassah University Hospitals, Jerusalem, Israel.

Oculocutaneus Albinism Type I (OCA I) comprises several phenotypes of hypopigmentation and low vision, caused by lack of tyrosinase activity, which results from homozygosity or compound heterozygosity of the defective autosomal recessive tyrosinase gene (TYR). Because of the severe visual handicap, affected families seek genetic counseling and prenatal diagnosis. Prenatal diagnosis of OCA I can be achieved by light and electron microscopic examination of melanogenesis in fetal scalp biopsies or by molecular genetic tests. The identification of the two mutated copies of TYR is achieved either by direct screening for mutations or by a combination of mutation detection and haplotype analysis, following the identification of only one mutation.

We describe our experience in prenatal diagnosis of OCA I in families, with absolute and relative indications, using the histologic and molecular strategies. During the last ten years we performed 36 tests and diagnosed 7 albino and 29 normal fetuses. The diagnoses were confirmed in the newborns and in the aborted fetuses. 31 prenatal tests were done by fetal scalp biopsies using the histological approach. During the last 16 months we have performed 5 molecular genetic tests on DNA extracted from CVS or amniocytes, based on our population screen.

We screened by molecular analyses 115 unrelated Israeli albinos and their families. In certain ethnic groups we found at least one mutation in all, and two mutations in over 90% of albinos. In these groups we offer mutation detection to normally pigmented unrelated spouses of albinos and of diagnosed carriers pertaining to OCA I families. Based on our findings, prenatal molecular diagnosis of OCA I becomes possible in an increasing number of Israeli albino families.

9

Novel Protein Truncation Test to detect *COL2A1* Nonsense Mutations in Stickler syndrome. Ravi Savarirayan[1], Susanna Freddi[2], John F Bateman[2]
1.Victorian Clinical Genetics Service, 2.Department of Pediatrics, University of Melbourne; Royal Children's Hospital, Victoria, Australia, 3052

Stickler syndrome (hereditary arthro-ophthalmopathy) is a dominantly inherited disorder of connective tissue that affects the eyes, ears, joints and skeleton. It is the most common autosomal dominant connective tissue dysplasia, the commonest cause of inherited retinal detachment and an important cause of premature osteoarthritis. The diagnosis and classification of Stickler syndrome is made challenging by clinical variability and genetic (locus) heterogeneity. Half to two-thirds of affected families show linkage to the gene encoding type II procollagen, with all reported mutations resulting in a premature stop codon. Defects in type XI collagen are also associated with Stickler syndrome, with mutations in both the $\alpha1$(XI) and $\alpha2$(XI) chains having been characterized. We have developed a novel molecular test that will facilitate screening of families with Stickler syndrome due to COL2A1 nonsense mutations. This test employs an RNA-based approach, due to the large size of the gene with 52 exons, and messenger RNA extracted from transformed lymphoblasts is used as source material for the protein truncation test (PTT). Five overlapping primer sets were designed to cover the entire coding region of the COL2A1 gene. Resulting PCR products were then transcribed and translated *in vitro*, with the presence of a smaller protein product indicating a stop codon mutation in the corresponding PCR product. RNA-based approaches such as this can be compromised by low levels of mutant mRNA, due to the phenomenon of nonsense-mediated mRNA decay triggered by premature stop codons. To overcome this problem the protein synthesis inhibitor *cycloheximide* was added to cells prior to mRNA extraction and PTT. To develop this test we selected a candidate family with seven affected living members spanning two generations. Employment of our methodology led to the detection of a protein truncating mutation in all affected individuals and subsequent mutation analysis confirmed its predicted protein truncating nature. This screening test should become a powerful tool in the diagnosis of this group of Stickler patients, who are at risk of the disastrous ocular and other complications of this disorder such as premature osteoarthritis. It would enable these individuals to undergo regular medical surveillance with access to preventative or ameliorative treatment and accurate counseling.

10

Gestational age and risk for congenital defects: a population-based study. S.A. Rasmussen, C.A. Moore, L. Paulozzi, E. Rhodenhiser. Division of Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

The relationship between gestational age and congenital defects has not been fully studied. Previous studies have focused on birth weight, an unreliable indicator of gestational age in infants with congenital defects. We investigated the relationship between gestational age and congenital defects using data from the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based surveillance system that actively ascertains birth defects diagnosed in the first year of life among infants whose mothers reside in the metropolitan Atlanta area. For this study, only live-born singletons were included. We excluded premature infants (less than 37 weeks gestation) with isolated prematurity-related defects, such as undescended testes, inguinal hernia, patent ductus arteriosus, or persistent foramen ovale, from the analysis. Among 264,392 infants with known gestational age born between 1989 and 1995, 7852 (3.0%) had a congenital defect. Premature infants were more than two times as likely to have congenital defects than infants of term (37-41 weeks) gestation (risk ratio = 2.44, 95% C.I. 2.32-2.58). This relationship was evident for nearly all types of congenital defects. The frequency of congenital defects was highest among infants in the 29-32 week gestational age category (RR = 3.39). The risk ratios were 2.66, 2.21 and 0.55, in the 20-28, 33-36 and 42-45 week categories, respectively, using the 37-41 week category as referent. Among infants with birth defects, 22% were premature. Recognition of the correlation between prematurity and risk for congenital defects may guide clinical evaluation of these infants. This correlation may have several causes, including (1) congenital defects and prematurity may share some risk factors, and (2) some congenital defects may predispose the pregnancy to premature delivery. Further study of the association between prematurity and congenital defects may provide insight into the basic mechanisms of these relatively common problems.