† **859** HISPANIC RECOMBINANT 8 (REC 8) SYNDROME: SEGREGATION ANALYSIS. Ann C. M. Smith, Karen Spuhler, Eva Sujansky, Thomas Williams, Thomas McConnell, Arthur Robinson. The Children's Hospital, Univ. of Colorado School of Medicine, National Jewish Hospital and Research Center, Denver; Univ. of New Mexico, Albuquerque.

Pericentric inversions have been reported for almost all human chromosomes. While some (e.g., inv 9) are benign, others represent a significant reproductive risk for recombinant offspring. Reported risks range from 1-10% depending upon the chromosome involved and the mode of ascertainment. Thirty-one probands representing 24 Hispanic kindreds have been documented to have rec(8)dup q secondary to a parental inv(8)(p23q22). The clinical phenotype associated with rec(8)dup q includes a characteristic facies, congenital heart disease, and developmental and postnatal growth retardation (Am. J. Med. Genet., 10:229, 1981). Detailed pedigree and cytogenetic data collected from the 24 kindreds (223 individuals from 67 sibships) was analyzed by segregation analysis. The risk for an inv(8) carrier parent was 7% for rec(8) offspring and 60% for inv(8) carrier offspring. The risk for SAB/stillbirth (11%) was not significantly increased above the general population. Only one of the two possible recombinants was identified in these kindreds. All kindreds have, to date, been of Hispanic background with origins in southern Colorado and northern New Mexico. The Hispanic background, geographic localization, and common ancestry in three kindreds suggest a single origin of the Hispanic inv(8).

862 POST COUNSELING REPRODUCTION IN COUPLES WITH A CYSTIC FIBROSIS (CF) CHILD. Mark W. Steele, Joan B. Rodnan, Lynn Rosser, Marguerite Bryce. Univ. of Pittsburgh and Children's Hosp., Dept. of Pediatrics, Pittsburgh, PA

Reproduction in 44 couples with a CF child was compared to that of the general USA population. Based on race, maternal age, parental occupation & education, & sex-sibship order of the affected child (AC), each of 22 of the CF couples also was matched with 1 of 22 couples having either a Down Synd. (DS) or a neural tube defect (NT) child & 1 of 22 couples having a cerebral palsey (CP) child. By religion, ½ of CF parents and ¼ of DS/NT & CP parents were Roman Catholic. All couples were observed for at least 3 yrs. after dx of their affected child (AC) and recurrent risk (RR) counseling; ie, for: CF RR 25%, no prenatal dx (PD); DS/NT RR 2/6%, PD available; CP RR <0.5%, no PD. When the AC was the 1st born, almost 60% of all the CF couples initiated another pregnancy (similar to the behavior of the USA population) compared to ∿27% of DS/NT & CP couples (by χ^2, $p<0.001$). When the AC had a sib, further reproduction was curtailed in all 3 types of families compared to the USA population. These data, like others, suggest little correlation between reproductive behavior and RR or availability of PD; but do suggest an effect of influences revolving about parental perception of the AC within a context of their "ideal" for sibship size (the average being 2 in the USA). If the birth of a 1st born CF child seems not to deter reproduction, future development of prospective CF gene carrier testing with counseling of at risk couples is unlikely to do so either; except, perhaps, to encourage use of PD of CF in the fetus when a reliable technique for that purpose should also become available.

● **860** CYSTINOSIS: DEFECTIVE FIBROBLAST LYSOSOMAL CYSTINE TRANSPORT. M.L. Smith, A.A. Greene, and J.A. Schneider. University of California, San Diego, Department of Pediatrics, La Jolla, California.

Defective cystine efflux has been demonstrated in lysosomes from both peripheral leukocytes and cultured EBV-lymphocytes from cystinotic patients. However, this abnormality has been difficult to demonstrate in fibroblast lysosomes, raising the question of whether lysosomal transport is the only defect in cystinosis. We have substantiated that some previous methods of loading fibroblast lysosomes with cystine, using cystine dimethyl ester (CDME), lead to cystine accumulation in a non-lysosomal as well as the lysosomal compartment (E. Harms, personal communication). If fibroblasts are removed from their anchorage substrate and incubated with 0.25 mM CDME for 15 min., only lysosomes are loaded with cystine as shown on Percoll gradients. In cystine-loaded lysosomes from two normal control fibroblast cultures the 20 min. cystine efflux was 19% and 20% without ATP and 56% and 74% with 2 mM MgATP. Lysosomes from two cystinotic fibroblast cultures had no cystine efflux over 20 min. both with and without ATP. We have used the dye, dipropylthiodicarbocyanine iodide to measure lysosomal membrane potential. MgATP causes a positive shift in membrane potential, in both cystinotic and normal lysosomes. In both cases, inhibitor and anion effects are consistant with an electrogenic proton pump. All of these findings support the concept of an abnormal lysosomal cystine transport protein as the primary defect in this disease.

● **863** DETECTION OF GENE DELETIONS IN THE HUMAN TYPE II PROCOLLAGEN GENE IN 8 PATIENTS WITH ACHONDROPLASIA USING GENE DOSAGE ANALYSIS. C.M. Strom[1], C.E.L. Eng[1], T. Christides[1], C. Belles[1], and R. Pauli[2] (Spon. by Lawrence M. Gartner). [1]Univ. of Chicago, Pritzker School of Medicine, Dept. of Pediatrics, Chicago. [2]Univ. of Wisconsin, Dept. of Pediatrics and Medical Genetics, Madison.

Type II collagen is the major collagen of cartilage. This study analyzed type II procollagen gene dosage in 34 patients with achondroplasia. Human genomic DNA was digested with BamHI, EcoRI and HindIII, and Southern filters prepared. Experiments using simultaneous hybridizations with pgHCol(II)A (Strom and Upholt, Nuc. Acids Res. 12:1025) and pG44 (an X-linked probe) demonstrated that, when normalized for the amount of X-linked hybridization, the DNA from 8 patients with sporadic achondroplasia exhibited half the procollagen hybridization as compared to the DNA of normal controls. The DNA of 2 of the achondroplasts exhibited a deletion of at least 20 kb from the 3' end of the gene as demonstrated by analogous experiments using pgHCol(II)E and F. The DNA from the other 6 achondroplasts with deletions have only been analyzed using pgHCol(II)A. The observations were confirmed using densitometric analysis. Incubations with varying restriction enzyme concentrations revealed that incomplete digestion was not responsible for the observations. The ends of the deletions have yet to be found. A genetic defect involving the type II procollagen gene is implicated in the etiology of achondroplasia. (Supported by March of Dimes, AM-33910 and HD-04583).

● **861** THE MOST FREQUENT HUMAN AUTOSOMAL RECESSIVE DISEASE. P Speiser, B Dupont, P Rubinstein, A Piazza, A Kastelan, MI New, Cornell Univ Med Col, Memorial Sloan-Kettering Cancer Ctr, New York Blood Ctr, New York NY; Stanford Univ Med Ctr, Stanford CA; Tissue Typing Ctr, Zagreb, Yugoslavia

Nonclassical steroid 21-hydroxylase deficiency (nc21OHD) is an autosomal recessive disease which results in a phenotypically variable syndrome of postnatal hyperandrogenism. Its prevalence is unknown because basal serum 17-OHP levels are not sufficiently elevated for detection with the microfilter paper technique used in screening for classical 21-OHD. We therefore ascertained the frequency of the nc21OHD gene using ethnic group-specific HLA-B-nc21OHD associations in conjunction with ACTH testing in obligate heterozygote parents. Confirmation of this approach was obtained by the affected sib pair method of Thomson and Bodmer. The gene frequency for nc21OHD was highest in Ashkenazi Jews (19.1%) and was also high in Hispanics (13.6%), Yugoslavs (12.5%), and Italians (5.8%). In other Caucasians studied it was 0.1%. Disease frequencies were 1/27 for Ashkenazi Jews, 1/53 for Hispanics, 1/63 for Yugoslavs, 1/333 for Italians, and 1/1000 for other Caucasians. Carriers of the HLA-B14 marker had a 32-fold increased risk of nc21OHD compared to controls. Linkage disequilibrium betweeen HLA-B14 and nc21OHD was significant in Ashkenazi Jewish, Hispanic, and Italian patients, but not in Yugoslavs or other Caucasians. **Conclusion:** It appears that nc21OHD is the most frequent autosomal recessive genetic disorder in man.

864 EXPLORING THE MECHANISM OF NUCLEAR RNA TRANSPORT WITH MUTANT HUMAN tRNA GENES. Janet A. Tobian, Jose G. Castano and Michael A. Zasloff. (Spon. by James B. Sidbury). NIH, Human Genetics Branch, NICHD, Bethesda, MD.

Transfer RNA biosynthesis in eucaryotic cells involves processing of a primary transcript to mature tRNA by cleavage of sequences from the 5' and 3' ends. Following processing within the nucleus, mature tRNA is transported into the cytoplasm. We have recently demonstrated the existence of a tRNA transport system in Xenopus oocytes (Proc. Natl. Acad. Soc., 80, 6436-6440, (1983)). Studies of a cloned human variant tRNA $(met)_i$ gene, which contains a base substitution in the loop IV region, suggested that nucleolytic processing and transport might be functionally linked. The primary transcript of the variant gene is processed slowly and the mature variant tRNA species, although accurately processed, accumulates in the nucleus and is not transported. In order to define the nucleotides in a normal human tRNA $(met)_i$ gene involved in processing and transport, we have generated, by in vitro methods, mutants which contain one or a few C→T transitions in the tRNA coding sequence. The effect of these altered nucleotides on processing has been assessed in vivo by micro-injection of cloned mutant tRNA genes into the oocyte nucleus and in vitro by reaction of the mutant primary transcripts with the purified processing nucleases. The transport properties of the mutant tRNA genes have been analyzed following micro-dissection of nuclear and cytoplasmic components from oocytes injected with mutant tRNAs in the nucleus. Nucleotide changes independently affecting transport or processing have been observed showing the processes are not functionally linked.