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1040

Molecular and clinical aspects of G6PD Aures (nt 143 T—>C) in an Arabian newborn . G. A. Niazi , Arabian Gulf university, College of Medicine, Manama, Bahrain.

G6PD deficiency,an X linked enzyme abnormality is highly prevalent in the Middle Eastern countries with an incidence varying between 5- 50 %. Inspite of the fact that this enzymopathy is frequently encountered, there is hardly any work on the molecular characterization and as such no new variant of G6PD has been identified from this region. DNA analyses were carried out recently on twenty randomly selected children (1- 6 yr.) with severe G6PD deficiency. Seven (3M; 4 F) out of 20 children were found to have nt 143 T—> C substitution, similar to one described in G6PD Aures; a variant recently reported from Algeria. The Aures mutation T—> C at nt 143 was detected by sequencing exon 3. It was confirmed by restriction digest analysis using a sense primer5' TGTCCCCAGCC TT 3' and an antisense primer 3' TGTCCGGACCAGGACGGGAC 5' to amplify a portion of exon 3. Digestion with Sau3A1 resulted in fragments of 68 bp and 77 bp in the normal and the 145 bp fragment was not cleaved in the mutant as this substitution of T—> C destroys the normal restriction site. 12 out of the remaining 13 children had G6PD Med (563 T) mutation whereas the mutation in the last child remained unidentified .G6PD Aures although has been reported earlier from an north African but it is a first example from Middle East which we feel is worth reporting as this variant is not only associated with favism but is also a cause for hyperbilirubinemia and neonatal jaundice in the newborns ,a clinical finding which has not been documented so far.

1041

Chinese achondroplasia is also defined by recurrent G380R Mutations of FGFR3. D.-M. Niu[1], C.-H. Chen[3,6], N.-H. Wang[3], L.-S. Chin[3], K.-J. Hsiao[2,4] [1]Depts. of Pediatrics, [2]Med. Res. and [3]Orthopedics, Veterans Gen. Hosp.-Taipei; [4]Institute of Genetics, [5]School of Medicine, National Yang-Ming University; [6]Cheng Hsin Rehabilitation and Medical Center, Taipei, Taiwan.

Achondroplasia is the most common form of human dwarfism and is transmitted as an autosomal dominant trait with complete penetrance. The homozygous of achondroplasia is incompatible with life. 80% to 90% of the achondroplasia are sporadic cases. Recently, Rousseau et. al. (Nature, 371:252-254, 1994) and Shiang et. al. (Cell, 78:335-342, 1994) have identified mutation (G380R) in fibroblast growth factor receptor 3 (FGFR3) causing achondroplasia. Further study showed that almost all the achondroplasia patients were caused by this unique mutation. To investigate if the homogeneity of mutations of achondroplasia is also present in Chinese patients, Chinese achondroplasia patients were studied for the presence of G380R mutation in FGFR3.

Nine unrelated families of achondroplasia were studied, including eight families with one sporadic affected member each, and one family with two affected members which are compatible with dominant inheritance trait. For detection of G380R mutation, genomic DNAs were extracted and FGFR3 PCR reactions were carried out using the conditions and primers described by Shiang et. al.. PCR products were digested by restriction enzymes Sfc1 and Msp1 separately. All the ten Chinese achondroplasia patients were found to have G to A transition at nt 1138 of FGFR3 cDNA which causing G380R mutation. Our results add further evidence to support that G380R is the unique mutation of FGFR3 causing achondroplasia, irrespective of ethnic background, which accounts for the common pathogenesis and common phenotype of achondroplasia across different ethnic groups.

1042

Cutaneous manifestations in a neonate with cobalamin C methylmalonic acidemia. S. Packman,[1] R. Howard,[1] I. Frieden,[1] D. Crawford,[1] D. Rosenblatt,[2] L. Sweetman,[3] C. Ohnstad,[1] K. Hart,[1] and M. Berrios.[1] University of California, San Francisco,[1] McGill University, Montreal, Canada,[2] and Childrens Hospital of Los Angeles.[3]

Eruptive dermatitis resembling the skin lesions of acrodermatitis enteropathica has been described in a number of aminoacidopathies and organic acidemias. In the multiple carboxylase deficiencies, the dermatitis is a manifestation of the untreated disease, while in methylmalonic and propionic acidemias, and maple syrup urine disease, the skin lesions have been ascribed to nutritional deficiency in the face of therapeutic protein or branched chain amino acid restrictions. We report a full term neonate who presented at birth with profound hypotonia, homocysteinemia and methylmalonic and methylcitric aciduria. There was no metabolic acidosis or hyperammonemia. Serum zinc concentration and biotinidase enzyme activity were normal. She rapidly developed cheilitis and a widespread erythematous rash, with erosions and desquamation. Skin histology showed epidermal pallor, psoriasiform hyperplasia and confluent parakeratosis. Upon treatment with isoleucine and valine restriction and daily intramuscular hydroxocobalamin, the baby's neurologic status improved and the skin rash resolved. Cobalamin C disease was subsequently diagnosed by complementation analysis and measurements of AdoCbl and MeCbl synthesis in cultured skin fibroblasts. We conclude that erosive dermatitis may be a *presenting* sign in cobalamin C type of methylmalonic acidemia, in the absence of long-standing nutritional restrictions or deficiency. Further, cobalamin C disease should be specifically added to a growing list of inherited and acquired metabolic disorders associated with eruptions clinically and histopathologically resembling acrodermatitis enteropathica.

1043

Induction of Mn-superoxide dismutase and production of superoxide radicals in mitochondria from patients with Complex I Deficiency. S. Pitkanan and B. H. Robinson, Depts. of Pediatrics and Biochemistry, University of Toronto, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

Mitochondria from cultured skin fibroblasts were isolated for patients and controls with complex I deficiency of the mitochondrial respiratory chain. Categories of patients included those with fatal infantile lactic acidosis (FILA), cardiomyopathy and cataracts (CC), tubulopathy and hepatopathy (TH), Leigh's disease (LD), cataracts only (CO) and controls (C). Production of superoxide radicals were measured using the luminometric probe lucigenin on addition of NADH. Superoxide production rates were highest with CO and decreased in the order CO >>TH>LD>C>FILA=CC. Quantity of Mn-superoxide dismutase, however, was highest in CC and FILA and lowest in CO only. Plots of MnSOD activity versus superoxide production showed an inverse relationship for most conditions with complex I deficiency. We hypothesise that oxygen radical production is increased when complex I activity is compromised. However, the observed superoxide production rates are modulated by the variant induction of MnSOD which decreases the rate, sometimes below that seen in control fibroblast mitochondria. Whether this variant induction relates to different sites of production or different reactive oxygen species is under investigation.

1044

Aminoglycoside toxicity: the isolation of a yeast gene involved in aminoglycoside resistance. T. R. Prezant, W. E. Chaltraw, Jr., N. Fischel-Ghodsian. Ahmanson Department of Pediatrics and Medical Genetics Birth Defects Center, Cedars-Sinai Research Institute, Los Angeles, CA.

Ototoxicity is the major irreversible toxicity of aminoglycosides, and can occur in a dose-dependent or an idiosyncratic fashion. We have identified one major group of mutations that cause a genetic predisposition to aminoglycoside ototoxicity (*Nature Genetics* 4:289-294,1993; *Am J Otolaryngol* 14:399-403,1993; *Pharmacogenetics*, in press). The susceptibility mechanism is related to the bactericidal function of aminoglycosides: interference with proofreading during bacterial protein synthesis. The mutations we described can cause the mitochondrial rRNA to be more similar to the bacterial rRNA at a site where aminoglycosides bind to it.

We are now searching for additional genes and their products that are related to aminoglycoside uptake, intracellular transport and metabolism, in order to identify additional candidates for both genetic susceptibility mutations and potential intervention and prevention of the ototoxicity. A yeast model system is used to functionally identify genes whose products interact with aminoglycosides, to elucidate the pathway of aminoglycoside action. Other researchers had demonstrated aminoglycoside resistance mutations in the mitochondrial small rRNA (*J Biol Chem* 257:5921-5928,1982) and in the plasmid membrane H^+-ATPase (*J Biol Chem* 264:21857-21864,1989), indicating a role for these gene products in mitochondrial protein proofreading and in drug uptake, respectively. We report the isolation of a novel gene which confers neomycin resistance to yeast transformants. The gene sequence has been established, and has partial homology to transport ATPases, but is distinct from the H^+-ATPase. This gene might also be involved in antibiotic transport, and biochemical characterization of transformants containing this clone are in progress. (Work supported by NIH grant R01 DC 02273)

1045

Iduronate-2-Sulfatase Deficiency in a Dog: Canine Hunter Syndrome. D.J. Prieur[1], M.J. Wilkerson[1], D.C. Lewis[1], N.G. Kennaway[2], J.R. Toone[3], D.A. Applegarth[3], H. Vallance[3], S.L. Marks[1], and R.K. Wood[1]. [1]Washington State University, Pullman, WA, [2]Oregon Health Sciences University, Portland, OR, and [3]University of British Columbia and Children's Hospital, Vancouver, B.C., Canada.

A five-year old male Labrador Retriever presented with progressive incoordination, visual impairment, and exercise intolerance. He had coarse facial features, unilateral corneal dystrophy, macrodactylia, and radiographic evidence of generalized osteopenia. Cultured dermal fibroblasts had lower iduronate-2-sulfatase (IDS) levels compared to normal dogs (affected: 2.0 vs. controls: (n=2) 13.5 and 17.0 units (% conversion of substrate)/hr/mg protein). Other lysosomal enzymes were within normal levels. By electrophoresis, urine of the affected dog contained a moderate band of heparan sulfate and a heavy band of dermatan sulfate (no bands visible in control dog urine). Southern blot analysis probed with full length human IDS cDNA did not disclose any deletions or genomic rearrangements. Light microscopy disclosed intracytoplasmic vacuolation in cells of multiple tissues, most prevalent in renal tubular epithelium, hepatocytes, bladder epithelium, adrenal gland, pancreatic acinar cells, myocardium, histiocytes of spleen and lymph nodes, and smooth muscle cells of the splenic trabeculae and intestine. Ultrastructurally, these vacuoles consisted of lamellar to floccular material characteristic of lysosomal storage material. Periodic acid-Schiff positive intracytoplasmic material was identified in multiple neurons in the medulla, cerebellum, and spinal cord gray matter horns. Scattered neuropile vacuolation and distal axonal swelling, most prominent in the cerebral grey matter and pontine and thalamic nuclei was also observed. The cerebellum had a marked paucity of Purkinje cells and thinning of neurons in the granular cell layer suggesting an acquired cerebellar abiotrophy. This represents the first report of mucopolysaccharidosis type II or Hunter syndrome in a dog and would appear to correlate best with the intermediate form of the human disease.

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