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Niemann-Pick Disease Type C: Phenotypic variability often leads to delay in diagnosis. *C. Prasad*[1], *C. Pushpanathan*[2], *R. Morris*[3], *A. J. Davis*[3] *and F. E. Dougherty*[4]. Division of Genetics[1], Pathology[2] and Pediatrics[3], Janeway Child Health Center, St. John's, Newfoundland, Canada, and the Division of Genetics and Metabolism[4] Children's Hospital, Boston, MA.

Niemann-Pick Disease Type C (NP-C) a lipidosis, is caused by a unique biochemical defect in cholesterol esterification. The protean manifestations of this condition often cause diagnostic confusion in the early stages. We present 3 cases highlighting such phenotypic variations, and distinctive pathological findings of this disorder.

Case 1: A 2 year and 9 month old boy presented with **neonatal hepatitis, hepatosplenomegaly** and **developmental delay**. Initial investigations failed to establish a cause. A repeat study of the bone marrow showed foamy histiocytes, providing a diagnostic clue.

Case 2: A 14 year old boy presented with **chronic megaloblastic anemia, hepatosplenomegaly** and **short stature**. There were no neurological symptoms. Electron microscopic examination of muscle tissue showed complex lipid storage and cholesterol crystals in cytosol.

Case 3: A female infant born at 38 weeks gestation developed **neonatal hepatitis**. At 4 months of age she developed **respiratory failure** requiring aggressive ventilation until 2 year and 3 months. She had **developmental delay, generalized hypotonia** and **weakness**. A muscle, skin and nerve biopsy showed lamellar inclusions suggestive of NP-C.

In each of the three cases the definitive diagnosis was established by demonstration of impaired cholesterol esterification in skin fibroblasts. In conclusion, these cases illustrate the diverse, but common presentations of a rare disorder. Pulmonary manifestations (as in case 3) are rarely described in classical NP-C, but have been reported in NP-C 2. Unless specifically sought for, a delay in diagnosis is not uncommon. Skin biopsy is an effective screening tool, while demonstration of defective cholesterol esterification remains the gold standard for diagnosis. With limited treatment options, establishing an early diagnosis is invaluable.

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A CRITICAL EVALUATION OF COPPER METABOLISM IN INDIAN WILSON'S CHILDREN WITH SPECIAL REFERENCE TO THEIR PHENOTYPES AND RELATIVES. *R. PRASAD, G.KAUR AND B.N.S. WALIA.* DEPARTMENT OF BIOCHEMISTRY AND PAEDIATRICS* PGIMER, CHANDIGARH, INDIA

WILSON'S DISEASE IS AN AUTOSOMAL RECESSIVE DISORDER OF COPPER ACCUMULATION LEADING TO LIVER, KIDNEY AND/OR BRAIN DAMAGE. SERUM COPPER AND CERULOPLASMIN IN CONTROL SUBJECTS (141 CASES OF DIFFERENT TYPES OF LIVER CIRRHOSIS) WERE SIGNIFICANTLY HIGHER AS COMPARED TO WILSON'S DISEASE (51) AND THEIR RELATIVES (58) WHILE MARKED HYPERCUPRIURIA (145+/-7 ug/24Hrs.)WAS OBSERVED IN WILSON'S CHILDREN ONLY. THERE WAS A GOOD CORRELATION (R=0.92) OBSERVED BETWEEN COPPER NOT BOUND TO CERULOPLASMIN AND URINARY COPPER EXCRETION IN WILSON'S PATIENTS. INTERESTINGLY, 24 HOUR URINARY EXCRETION OF COPPER AND C-AMP WERE SIGNIFICANTLY (P<0.01) ELEVATED IN WILSON'S CHILDREN ASSOCIATED WITH RENAL TABULAR ACIDOSIS AS COMPARED TO PATIENTS WITH HEPATOLOGICAL AND/OR NEUROLOGICAL MANIFESTATIONS. 20% CASES OF WILSON'S DISEASE AND 25% OF RELATIVES OF WILSON'S DISEASE HAD SERUM CERULOPLASMIN BETWEEN 14-20 mg./100ml. THEREFORE, 13 CASES OF WILSON'S WERE CONFIRMED BY MEASURING HEPATIC COPPER (90+/-8ug/g WET TISSUE: MEAN +/- SD). DURING THE FAMILY SCREENING BY SERUM COPPER, CERULOPLASMIN AND URINARY COPPER AND HEPATIC COPPER, 10 SIBLINGS WERE DIAGNOSED TO HAVE PRESYMPTOMATIC WILSON'S DISEASE. THESE SUBJECTS WERE THEN STARTED THE D-PENICILLAMINE THERAPY, BECAUSE PRESYMPTOMATIC TREATMENT PREVENTS PROGRESSION OF THE DISEASE AND ITS COMPLICATIONS.

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Neonatal Hemochromatosis. *G. Serra, W. Bonacci, C. Bellini.* Servizio di Patologia Neonatale, Universita' di Genova, Italia.

The female infant was the product of an uneventful 36-week pregnancy. Parents were non-consanguineous and healthy. At birth the child was jaundiced and had hepatomegaly with ascites. Laboratory studies revealed the following: total bilirubin 14.5 mg/dl (direct 0.5), albumin 1.7 mg/dl, prothrombin time 30", Factor II 16%, V 22%, VII 16%, X 22%, fibrinogen 114 mg/dl; ammoniemia was normal. Serum ferritin concentration was 1,915 mg/ml. Urinary succinylacetone was absent. Alpha1-antitrypsin deficiency was excluded. All viral and serologic studies and cultures were negative. The patient's condition progressively deteriorated and despite intensive management the child died on day 21 of life of diffuse uncontrolled cutaneous and mucous bleeding. Post mortem evaluaton revealed significant iron deposits in the liver as well as in other main organs; extensive loss of parenchyma was evident; residual hepatocytes showed iron overload; giant cell transformation was also found. The pathologic picture was compatible with the diagnosis of neonatal hemochromatosis (NH). NH (OMIM 231100) is an uncommon polyvisceral iron storage disorder of prenatal onset. It is a phenotypically defined disease and it is believed that various insults during fetal life may result in the NH phenotype. NH is determined on the basis of a specific pathological diagnosis. Its genetic or environmental bases are still unknown. NH is usually considered an autosomal recessive disorder. Parents and sibs of patients with NH are not necessarily at increased risk of iron storage disease. NH is not genetically related to hereditary hemochromatosis.

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Identification of two novel polymorphisms in the glucocerebrosidase gene region. *E. Sidransky*[1], *E. K. Lau*[1], *S. Winfield*[1], *B. K. Stubblefield*[1], *A. Zimran*[2], *N. Tayebi*[1], *and E. I. Ginns*[1]. [1]Clinical Neuroscience Branch, IRP, NIMH, NIH, Bethesda, MD; [2]Shaare Zedek Medical Center, Jerusalem, Israel.

Gaucher disease, an inherited glycollpid storage disorder, is caused by a deficiency of the catabolic enzyme glucocerebrosidase. The gene for glucocerebrosidase is located on chromosome 1q21 and has a highly homologous pseudogene situated 16kb downstream. We now report two novel polymorphisms in the glucocerebrosidase gene region: the first one consists of a tetranucleotide (AAAT) repeat upstream to the glucocerebrosidase gene, and the second is a series of a dinucleotide (CT) repeat in the intergenic region between the glucocerebrosidase gene and its pseudogene. These two polymorphisms, along with the previously reported *Pvu* II polymorphism in intron 6 of the glucocerebrosidase gene, were analyzed in Gaucher patients (n=106) and two control populations (Askenazi n=72 and non-Jewish n=46). Strong linkage disequilibrium was found between the common N370S mutation and particular haplotypes, but no significant linkage disequilibrium was found in patients carrying the L444P or 84GG mutations. We also found exceptions to previous reports that the N370S/84GG genotype is always associated with a Pv1.1-/Pv1.1+ genotype. Several unusual cases of patients with unexpected haplotypes led to the recognition of novel complex alleles, and contribute to our understanding of the origin of glucocerebrosidase mutations. The study of these markers may reveal possible ancestral chromosomes which led to affected alleles, and that may be diagnostically useful in Gaucher patients when the specific mutations have not been identified.

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Maternal hyperhomocysteinemia and occurrence of orofacial clefts in offspring. *R.P.M.Steegers-Theunissen*[1,2], *W.Y.Wong*[1], *A.Kuijpers-Jagtman*[4], *P.H.M.Spauwen*[4], *B.C.J.Hamel*[5], *H.J.Blom*[6], *C.M.G.Thomas*[7], *T.K.A.B.Eskes*[1]. Department of Obstetrics/Gynaecology[1], Epidemiology[2], Orthodontics and Dentistry[3], Plastic and Reconstructive Surgery[4], Clinical Genetics[5], Laboratory of Pediatrics and Neurology[6], Laboratory of Endocrinology and Reproduction[7], University Hospital St Radboud, Nijmegen, The Netherlands.(Intro.by M.M.Tolarova).

Orofacial cleft (OFC), i.e. cleft lip with or without cleft palate, is a classical example of a multifactorial disorder. Evidence has been accumulated over the past decade showing that majority of OFC results from an interaction between environmental factors, including nutritional deficiency or toxicity, and genetic factors. Results from case-control and intervention studies suggest that periconceptional vitamin supplementation, including folic acid, reduces the recurrence risk of OFC. However, the fundamental biological processes that underly the preventive action of folic acid supplementation are as yet unknown. Folate and the vitamin B_{12} and B_6 are involved in the metabolism of homocysteine.

In order to invesigate the folate-dependent homocysteine metabolism, a standardized methionine loading test was carried out in 29 mothers of a child with OFC and 56 control women.

Surprisingly, in 8 mothers of a OFC child and 2 controls - in the absence of liver and kidney dysfunction - hyperhomocysteinemia was established. In general, the folate, vitamin B_{12} and B_6 levels were within the normal ranges. Therefore, this preliminary finding suggests a disorder in the enzymes involved in remethylation of homocysteine or in the metabolism of folate and/or vitamin B_{12}.

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Spectrum of Mutations in 21-hydroxylase deficient form of Congenital adrenal hyperplasia in Singapore. *Agnes Tay*[1], *Kah-Yin Loke*[2], *Larry Poh*[2]. [1]Institute of Molecular and Cell Biology, [2]Dept of Paediatrics,National University of Singapore

Congenital adrenal hyperplasia is due to a deficiency in cytochrome P450 enzymes, the most common of which is 21-hydroxylase. This enzyme is encoded by the CYP21 gene on chromosome 6p. Our aim was primarily to determine the spectrum of genetic abnormalites responsible for this disease; such analysis has not been previously reported in South-East Asia. In addition, we hope to develop rapid screening assays for mutations common in our local population.

Fourteen unrelated patients from the Endocrine outpatient clinic were studied with a view to characterising the specific mutations. DNA was extracted from peripheral leukocytes. The CYP21 gene amplified from genomic DNA using the polymerase chain reaction and the products of amplification sequenced. Sequencing of six exons and one intron where mutations have previously been described revealed mutations in 6 out of the 14 individuals. These included: intron 2 splice site mutation (3 patients), 8-bp deletion in exon 3 (1 patient), I172N missense mutation in exon 4 (1 patient), and Q318X nonsense mutation in exon 8 (1 patient). For the intron 2 mutation, allele-specific oligonucleotide hybridization proved to be a reliable and rapid screening technique.

Sequencing of the remaining exons is ongoing and we hope to infer genotype-phenotype correlations when we have catalogued the mutations in all the affected patients.