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DELETIONS OF COLLAGEN TYPE 4 ALPHA 5 GENE IN 4 FAMILIES WITH ALPORT'S SYNDROME M.Sanak, R.M'Rad, G.Dechennes, M.C.Gubler, S.Heuertz, J.P.Grunfeld, M.Broys, Jing Zhou, K.Tryggvason, M.C.Hors-Cayla; INSERM U12, U192, Dept. Nephrology Hosp. Enfants Malades, Paris; Biocenter, Univ. Oulu; Med. Genetics Dept., Pediatrics Institute, Krakow

Alport's syndrome (AS) is manifested by progressive renal disease with typical basement membranes morphology and frequent association with ocular signs and deafness. This hereditary disease has been assigned to the collagen type IV alpha 5 gene (COL4A5) located on X chromosome and several mutations have been reported. We present 4 families in which gross deletion of COL4A5 were found as a cause of AS. Using standard Southern blot technique and cDNA probes of COL4A5 absence of restriction fragments or presence of 'junction' fragments proved a mechanism of the mutation. The span of deletion varied at least from 1.6 to 94 kb. Nonetheless the basic clinical features of the families were indifferent. Deletion of promoter region of COL4A5 in family TR, which might affected another gene, was associated with generalized leiomyomatosis in AS patients - a rare syndromal phenomenon.

| | | | | | | |
|---|---|---|---|---|---|---|
| Family FR | deafness | + | ocular s. | + | deletion | 94 kb |
| Family FE | | + | | + | at least | 5.5 kb |
| Family PO | | ? | | + | | 2.7 kb |
| Family TR | | + | | + | | 1.6 kb |

**Conclusions.**
The wide range of deletion of COL4A5 gene has rather uniform and complete phenotypic manifestation of AS. The correlation between type of a gene rearrangement and clinical picture of AS needs further evaluation. Molecular study of CO4A5 gene and RFLP offers a new tool for genetic counselling.

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**Hyperoxaluria in Phosphate-Treated X-linked Familial Hypophosphataemic Rickets (XLH)**

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1990 Reusz and his colleagues reported that hyperoxaluria during phosphate supplementation in XLH might contribute to the development of nephrocalcinosis in this disorder. We analysed 18 random urine samples from 11 children with XLH (age:3.0-11.0 years) who were treated with 1-4g elemental phosphorus/day, given in four to six single doses, and 30-60 ng 1.25(OH)2D3/kg/day given in two divided doses. There were no signs of severe nephrocalcinosis or renal stones. Urines were collected at about 9 AM after an overnight fast and after the bladder was emptied at about 6 AM. Oxalate excretion was analysed by a enzymatic method and the results were compared with oxalate excretion in random urines from 19 healthy children (age: 4.0-17.0 years).

The oxalate excretion was significantly higher (< 0.01) in XLH (15,9-187,2 µg/mg creatinine) compared to our control group (1,3-27 µg/mg crea).
There was a positive relationship between the oxalate and phosphate excretion, both values being relative to creatinine excretion ($r = 0.71 p < 0.01$).

In conclusion, we found an elevated oxalate excretion in patients with XLH with appears to be dependent on the phosphate excretion, which in turn is known to be dependent on phosphate intake in XLH. Further long-term studies are necessary to determine whether hyperoxaluria is of clinical relevance for development of nephrocalcinosis in XLH.

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INDUCTION OF CONGENITAL CATARACT IN THE OFFSPRING OF DIABETIC RATS. Martin Simán, Peter Naeser, and Ulf J. Eriksson. Department of Medical Cell Biology, University of Uppsala, Uppsala, Sweden.

In previous experimental studies we have found 47 % incidence of congenital cataracts among the fetuses of manifestly diabetic (MD) rats, and 4 % in the offspring of normal (N) rats. The aim of the study was to clarify the role of the sorbitol shunt in the pathogenesis of this congenital defect. Light microscopical evaluation of the lenses of day 16-22 fetuses revealed excessive formation of vacuoles in offspring of MD rats compared to N offspring at all time points. On gestational day 16 we found a doubled aldose reductase (AR) activity in the fetuses of MD rats compared to the N fetuses. This difference as well as the net activity of AR decreased in both groups during subsequent development. The sorbitol concentration was increased more than tenfold in the MD fetal lenses compared to N fetal lenses at all time points. The sorbitol concentration in both the MD and the N group decreased from day 16 to day 20 and increased again slightly on day 22.

We conclude that the diabetic uterine milieu induces AR activity and sorbitol formation in the lens. This over-activity of the sorbitol shunt may produce metabolic and osmotic imbalance in the fetal lens, resulting in excessive vacuole formation and subsequent development of congenital cataract.

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**ODD-NUMBERED LONG-CHAIN FATTY ACIDS IN ERYTHROCYTE MEMBRANE LIPIDS AND PROPIONIC ACID IN PLASMA FOR METABOLIC CONTROL OF PATIENTS WITH PROPIONIC ACIDAEMIA**

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In patients with propionic acidaemia (PA) and methylmalonic acidaemia (MMA) the increased concentration of propionyl-CoA in cells leads to a relative abundance of odd-numbered long-chain fatty acids (OLCFA) in body lipids.
During a period of 5 years we used the OLCFA content in erythrocyte membranes for long-term metabolic control in 5 patients with PA.
The determination of OLCFA in erythrocyte membrane lipids was done by capillary column gas liquid chromatography and the sums of the individual OLCFA- C15:0, C17:0 and C17:1- were calculated and expressed as a percentage of the total C14-C22 fatty acids.
In 3 patients OLCFA are usually below 1.9% indicating a good metabolic control. In one patient, metabolic control is fair (OLCFA < 3.1%) and one neonate with severe course of PA showed a decrease of OLCFA with age and an increase during metabolic decompensation before death. In general, during acute metabolic crisis we only found an elevation of propionic acid in plasma. After crisis there was a delayed increase of OLCFA. In one patient a decrease of OLCFA could be seen after therapy with metronidazole.
OLCFA seem to be a valuable parameter for long-term metabolic control in patients with PA.

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MOLECULAR ANALYSIS OF PKU IN POLAND

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To investigate the molecular basis in phenylketonuria (PKU) in the population of southern Poland we characterized the restriction lenght polymorphism (RFLP) haplotypes at the phenylalanine hydroxylase gene locus, screened for mutations with allele-specific oligonucleotides by amplification of the genomic DNA with the polymerase chain reaction and sequenced the exons of the PAH gene from 23 PKU families. 71.7% of all PKU alleles are characterized by the following five mutations: codon R408W, R261Q, R158Q, R252W and the splicing defect in intron 10. The splice mutation in intron 10 was detected by DdeI restriction analysis. In the investigated families the overall frequency for predictions in regard to the genotype of additional offspring was about 96%. With an overall frequency of about 98% the DNA diagnostic approach could be used to predict a phenotypically normal child (genotype: either normal/normal or normal/mutant).

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BROADBAND ULTRASOUND ATTENUATION (BUA) MEASUREMENTS IN NEONATAL BONE AND CORRELATION WITH GESTATIONAL AND POSTNATAL AGE. Patricia A. Hamilton, Darius Nassiri and Fiona J. Weir. St George's Hospital Medical School London U.K.

The frequency dependence of attenuation of broadband ultrasound and the velocity of ultrasound passage through bone have been used as indicators of bone mineral content. BUA in adults has been shown to correlate well with established methods of measuring bone mass or density. Ultrasound has the advantages of safety, economy and portability over other techniques.

We have modified this technique for use in neonates whereby the BUA coefficient (BUAC), which normalises the BUA for the thickness of the bone, and the velocity may be measured.

The aim of this study was to see how BUAC and velocity change with gestational age (GA) and postnatal age (PNA). BUAC and velocity were measured once in 26 infants <72 hours old. Two infants were studied sequentially at twice weekly intervals.

| Results | median | range |
|---|---|---|
| Birthweight (Kg) | 1.81 | 0.83-3.65 |
| Gestation (w) | 34 | 25-40 |
| BUAC (dB/MHz/cm) | 24 | 5-38 |
| Velocity (M/s) | 1700 | 1550-1884 |

Regression analysis showed BUAC correlated with GA ($r=0.57$ $p<0.005$) and with PNA ($r=0.95$ $p<0.05$) but not with birthweight ($r=0.33$). Velocity did not correlate with GA ($r=0.25$) or birthweight ($r=0.01$).

BUAC can be used to monitor bone development in neonates.