GENETIC COMPLEMENTATION ANALYSIS OF MEVALONATE KINASE DEFICIENCY IN CULTURED FIBROBLASTS

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Mevalonate kinase deficiency (MKD), an inborn error of sterol synthesis, presents with clinical variability. To define a genetic basis for this variability, we determined MK activity in fibroblast heterokaryons obtained from polyethylene glycol fusion. Fusion of control fibroblasts with 6 MKD cell lines yielded positive complementation (37% of control activity). None of the fusions between the 6 MKD cell lines yielded detectable MK activity. Michaelis constants in an MK assay optimized for use in biopsied chorionic villi (BCV) were 0.06 and 0.7 mM for mevalonate and ATP, respectively. MK activity in control BCV increased linearly (0.8-4.3 nmol/min/mg protein) with gestational age from 7 to 14 weeks. MK activity in BCV from a pregnancy at risk for MKD was 430 pmol/min/mg protein, suggesting an unaffected fetus. We conclude there is no evidence for genetic heterogeneity in MKD which would explain the variability in clinical expression. The availability of an optimized MK assay for use in BCV allows reliable 1st trimester prenatal diagnosis for families at risk.

LONGITUDINAL LUMBAR BONE MINERAL MEASUREMENTS BY DUAL ENERGY X RAY ABSORPTIOMETRY IN PREMATURE NEWBORNS.

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Dual energy X Ray absorptiometry (DXA) was used to measured the bone mineral content (BMC) and bone mineral density (BMD) of the lumbar spine in 29 premature newborns during the first year of life. Mean birth weight was 1290 ± 150 g, and mean gestational age was 30.4 ± 0.5 weeks. There were studied at day 40 ± 4, 59 ± 1, 187 ± 2 et 365 ± 5 postnatal age. The data compared to normal value obtained in infants less than 2 years old are shown in table (mean ± SEM).

Investigation number	1	2	3	4
Age (d)	40.8 ± 1.9	89 ± 0.8	184 ± 2	365 ± 5
Weight (g)	1974 ± 20	3580 ± 106	6017 ± 295	7483 ± 607
Length (cm)	42.8 ± 1.9	51.7 ± 2.5	59.9 ± 0.9	71 ± 1
BMC (g)	0.90 ± 0.06	1.35 ± 0.6	2.68 ± 0.18	4.41 ± 0.16
	(2.3 ± 0.04)	*(2.9 ± 0.05)*	*(3.9 ± 0.05)*	*(5.1 ± 0.04)*
BMD (g/cm2)	0.13 ± 0.01	0.15 ± 0.006	0.20 ± 0.01	0.25 ± 0.009
	(0.20 ± 0.002)	*(0.22 ± 0.002)*	*(0.25 ± 0.002)*	*(0.30 ± 0.002)*

(mean ± SEM) normal values in infants

Mean BMC and BMD were significant lower in premature babies than in normal infants during the first 6 months of life. At one year of age, BMC and BMD were not significant different between the two groups.

ANALYSIS OF MUTANT PKU ALLELES IN THE POPULATION OF SOUTHERN POLAND

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A sample of 22 PKU probands were screened for six mutations by polymerase chain reaction (PCR) amplification of their genomic DNA and hybridization with allele-specific-oligonucleotides. The following mutations were tested: exon 5,codon 158 (CGG-CAG); exon 7,codon 261 (CGA-CAA),codon 281 (CCT-CTT); exon 12,5' splice donor site (GT-AT), codon 414 (TAC-TGC) and codon 408 (CGT-TGG). Dot-blot hybridization with 32P labelled ASO was used to detect the presence of the mutations. Results: The analysis covered 44 mutant chromosomes from 22 unrelated PKU children. About 66% of PKU alleles studied were characterized by three distinct mutations: in codon 408, 158 and 261, with codon 408 (56.8%) being the most prevalent and tightly linked to haplotype (HT) 2. The codon 158 and 261 mutations were found in 6.8% and 2.3% of all PKU alleles, respectively. The missence mutation in codon 158 was detected in 2 patients who suffered from the classical form of PKU, and was linked to HT 4. The G to A transition in codon 261 was only found in a single chromosome and was linked to HT 1. No mutant PAH gene exhibiting the splicing defect in intron 12, neither the mutations in codon 414 and 281 was identified. Our results confirm molecular heterogeneity of PKU and show that the majority (66%) of all PKU alleles are characterized by three different mutations.

INCREASED CONCENTRATION OF 25-HYDROXYVITAMIN D (25D) AND 1,25 DIHYDROXYVITAMIN D (1,25) RECEPTORS IN KIDNEYS OF RATS TREATED WITH PHENOBARBITAL (PB).

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Chronic administration of PB and other anticonvulsant drugs can induce hypocalcemia and osteopenia. A decreased plasma 25D concentration can be responsible, and maybe other derangements of 1,25D. The study of receptor concentration in a target tissue (kidney) will inform on the performance of vitamin D metabolites. Ten wistar rats were treated with PB (6 mg/Kg/d) from the 21st day of life for ten weeks. At this time, whole blood was obtained, both kidneys were removed for 25D and 1,25D receptor quantitation (Reinhart, Hollis 1986). Control group included 5 rats.

	25 D			1.25 D		
	Control		PB	Control		PB
Kd(nM)	1.8(1.4)		2.0(1.6)	0.5(0.1)		0.5(0.2)
Bmax(pm/mg)	4.3(0.6)	*	6.4(1.4)	78.6(9.2)	*	134.6(62.8)

m(sd); *p < 0.02

Plasma values showed no differences apart from 25D which was respectively 8.3(2.4) and 5.2(2.3) ng/ml ($p < 0.05$). Raised concentration of 25D receptors is in agreement with low hepatic output. Converseley raised concentration of 1,25 receptors suggests a normal response for calcium reabsorption, and up-regulation response or a direct induction of PB over 1 α-hydroxilase, requiring further studies. CAICYT PM: 89-0018.

SECONDARY HYPERPARATHYROIDISM IN A PRETERM INFANT CAUSED BY HIGH PLASMA PROTEIN CONCENTRATION

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The intrauterine bone mineral accretion rate can be achieved in preterm infants by supplementation with calcium (Ca) and phosphorus (Pi) up to the point where both ions are simultaneously excreted with urine. We report on a female preterm infant (birth weight 1030g, gestational age 29 weeks) who unexpectedly developed bone mineral deficieny, measured by single photon absorption densitometry at right mid humerus (67 mg cm^{-1} at 1530 g body weight on day 60), in spite of rather high plasma concentrations of Ca (2.6-2.9 mmol/l) and Pi (2.2-3.0 mmol/l) as well as constant urinary excretion of Ca and Pi. The explanation was found to be an unusual high plasma total protein concentration (82 g/l at birth decreasing to 74 on day 54) leading to low ionized Ca (0.85-0.95 mmol/l). This subsequently caused secondary hyperparathyroidism (330 pg/ml, 1-84 assay, on day 50) and osteopenia. Oral Ca gluconate (1-2 mmol kg^{-1} day^{-1}) increased ionized Ca (1.1 M), normalized plasma PTH (27 pg/l) and allowed good bone mineralization (27 mg cm^{-1} per 520 g weight gain), which equaled to the intrauterine rate (4.4 mg cm^{-1} per 100 g weight gain). **Conclusion:** Ionized, not total, plasma Ca is relevant to mineralization. In case of high protein concentration calciuria does not reliably indicate calcium surplus as usually and Ca supplementation should be increased until ionized Ca is normal to achieve normal mineralization.

PLASMA OSTEOCALCIN LEVELS IN VITAMIN D DEFICIENCY RICKETS

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In order to evaluate the interest of osteocalcin, which is synthetized in bone concomitant with the appearance of mineralized bone, we studied 20 infants aged 7 to 15 months with vitamin D deficiency rickets, before and after treatment (1000 mg/m² calcium and 2.5 mg vitamine D). Serum alkaline phosphatases (ALP) levels, bone and liver isoenzymes, were measured by Bio Merieux Kit, isoenzymes by cellulose acetate electrophoresis ; 25 and 1.25 dihydroxy vitamin D were measured according to Shepard method ; intact parathyroïd hormon by Nichols kit. Before treatment to the serum osteocalcin and vitamin D metabolites levels were lower in infants with rickets than in control subjects. A significant change and rise was observed as soon as 1 week after treatment. In contrast, ALP and its bone isoenzyme levels were elevated before treatment and did not change significantly over 4 weeks, and secondary hyperparathyroïdism was reduced after 2 or 3 weeks. To conclude osteocalcin appears to be an early indicator of bone response to treatment in vitamine D deficiency rickets and its low level before treatment might be due to decreased vitamine D metabolites levels rather than hyperparathyroïdism.