VARIED CLINICAL PRESENTATIONS OF SIX PATIENTS WITH MUTATIONS IN CYP11A1 ENCODING THE CHOLESTEROL SIDE-CHAIN CLEAVAGE ENZYME, P450SCC

Neta Loewenthal[1,2], Michal Abramsohn[3], Meng Kian Tee[4], Walter Miller[4], Ruti Parvari[3], Eli Hershkovitz[1,2]

1 Pediatric Endocrinology Unit, Soroka Medical University Center, Beer Sheva, Israel
2 Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel
3 Department of Developmental Genetics and Virology, Faculty of Health Sciences and the National Institute of Biotechnology Negev, Ben Gurion University of the Negev, Beer Sheva, Israel
4 Department of Pediatrics, University of California, San Francisco, San Francisco, Clifornia, USA

Context: Steroidogenesis is initiated by converting cholesterol to pregnenolone by the cholesterol side-chain cleavage enzyme, P450scc, encoded by*CYP11A1*. P450scc deficiency disrupts adrenal and gonadal steroidogenesis, and is clinically and hormonally indistinguishable from congenital lipoid adrenal hyperplasia; only 11 such patients have been reported previously.

Objective: We aim to expand published experience with P450scc deficiency, facilitating its diagnosis and treatment.

Patients and Methods: We studied six children with adrenal insufficiency; DNA sequencing identified P450scc deficiency in all. A novel missense mutation was recreated in vector F2, expressing the fusion protein P450scc-FerredoxinReductase-Ferredoxin and transfected into COS-1 cells; production of pregnenolone was assayed and Michaelis-Menten kinetics were calculated. Previously described P450scc mutants were assayed in parallel.

Results: In a Bedouin kindred,four children presented with adrenal insufficiency at 1-4 years; all were compound heterozygotes for c.849C>T (Arg232Stop) and c.799T>C (Phe215Ser). Two were 46,XX and two were 46,XY; all had appropriate genitalia. Two patients, presented during the neonatal period, were homozygous for the mutant c.849C>T (Arg232Stop). As assayed in the F2 system, the Phe215Ser mutant retained 3.5% of wild-type activity, whereas the previously described Leu141Trp and Ala269Val mutants had 2.9%, and 10% of wild-type activity respectively, and Val415Glu and c.835delA lacked detectable activity.

Conclusions: Although P450scc is required to produce placental progesterone that is needed to maintain pregnancy, severe mutations in P450scc are compatible with term gestation; milder P450scc mutations present later. Only DNA sequencing distinguishes P450scc, StAR and congenital adrenal hypoplasia

NORMAL RANGES OF BASAL AND ACTH-STIMULATED FREE CORTISOL IN CHILDREN

Ori Eyal[1,3], Naomi Weintrob[1,3], Asaf Oren[1,3], Tali Taxir[1,3], Irina Serdukov[1,3], Anita Schachter Davidov[1,3], Naftali Stern[2,3], Rona Limor[2,3]

1 Pediatric Endocrinolgy Unit, Dana-Dwek Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
2 Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel
3 Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Background: Standard assays for serum cortisol measurements determine total cortisol (TC) concentrations but not the unbound biologically active serum free cortisol (sFC). Measurement of TC would be greatly influenced by alteration in cortisol-binding globulin (CBG) concentrations. It is, therefore, important to determine sFC levels when CBG levels are either decreased or increased

Objectives: To determine basal and ACTH-stimulated sFC levels in healthy children and to assess their relationship to TC, age, gender and Tanner stage

Subjects and methods: Baseline and stimulated serum TC and FC concentrations were measured before and after IV administration of 250 mcg Synacthen (Defiante Farmaceutica,S.A.,Portugal) in healthy children referred for exclusion of adrenal dysfunction. Serum TC was determined by chemiluminescence (Rosh, Cobas A 411), and serum FC was measured by the same methods following equilibrium dialysis. A TC response of 20 mcg/dl was considered normal.

Results: The study group consisted of 55 subjects (38 girls, 17 boys) whose median age was 8.5 years (range, 0.6-16.9). Mean baseline TC and sFC levels were 11.7 ± 6.1 mcg/dl (95 CI, 10.0-13.3) and 0.35 ± 0.28 mcg/dl (95 CI, 0.27-0.42), respectively. Mean peak TC and sFC levels were 31.9 ± 5.1 mcg/dl (95 CI, 30.5-33.3) and 1.6 ± 0.5 mcg/dl (95 CI, 1.46-1.73), respectively. Mean fractions of sFC at baseline and at peak were 3.1 ± 1.2% (95 CI, 2.8-3.4) and 5.88 ± 1.66% (95 CI, 5.4-6.3) ($p<0.001$), reflecting a lower increase in TC (247%) compared to sFC (647%). Baseline and peak TC and sFC levels were positively correlated ($r=0.81$, $p<0.001$ and $r=0.43$, $p<0.001$, respectively). Peak TC levels and age were negatively correlated ($r=-0.38$, $p=0.005$). There was a significant difference between TC at baseline and at peak between children aged <10 years compared to those >10 years ($p=0.04$ and 0.01, respectively).

Summary: Based on these findings, we suggest normal ranges for basal and ACTH-stimulated sFC. The finding that TC, but not sFC, is age-dependent indicates that sFC may be more reliable than TC for measuring adrenal reserve in children

LOSS OF LENGTH IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA IS ASSOCIATED WITH ELEVATED HYDROCORTISONE DOSAGE DURING THE FIRST YEAR OF LIFE

Abdulsalam Abu-Libdeh, Yaarit Ribak[1], Yardena Rakover, Naomi Weintrob[3], Eli Hirshkovitz, Amnun Zung[5], Haim Pinhas, David Zangen[1]

1 Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel
2 Pediatric Endocrine Unit, Ha'Emek Medical Center, Afula, Israel
3 Pediatric Endocrine Unit, Dana Children Hospital, Israel
4 Pediatric Endocrine Unit, Soroka University Medical Center, Beer Sheva, Israel
5 Pediatric Endocrine Unit, Kaplan Medical Center, Rehovot, Israel
6 Pediatric Endocrine and Diabetes Unit, Edmond and Lily Safra Children's Hospital, Sheba, Israel

Background: Decreased final height in congenital adrenal hyperplasia is mainly caused by advanced bone age and compromised pubertal growth. Elevated glucocorticoid levels are associated with decreased chondrocyte proliferation and linear growth. We studied the correlation between hydrocortisone (HC) dosage and growth velocity during the fastest growth period -1sty of life.

Methods: Ethnicity, mutation, clinical phenotype, HC dosage, and growth parameters at 1, 3, 6 and 12 months of age were assembled from 71 patients with salt-wasting CAH at 5 pediatric endocrine centers in Israel. Normal 1sty growth data (from Israeli Ministry of Health) was used for comparison.

Results: Six-months-old CAH males and females were significantly shorter than controls (2.23cm ; $p= 0.001$, 1.64 cm; $p <0.05$, respectively). This deficit increased further at 1y of age. A strong negative correlation was found in males between the HC dosage at 3 months and the length at 6 and 12 months and between the HC dosage at 6 months and the length at 12 months of age ($r = -0.609$, $r = -0.517$, respectively). A weaker yet statistically significant relation was found between HC dosages at 3-6 months, and length at 6-12 months for the entire group ($r = -0.3$, $p <0.05$).

Conclusion: Patients with CAH may lose significant height already at 6-12 months of age. Higher dosages of glucocorticoids are associated with a slower growth velocity during the 1sty of life mainly in boys. Intensive infantile optimization of the HC dosages may improve 1sty growth and final height in CAH patients.

A HUGE ADRENAL MYELOLIPOMA WITH EXTRAMEDULLARY HEMATOPOIESIS IN A THALASSEMIC PATIENT

Mohammad Sheikh-Ahmad[1], Leonard Saiegh[1], Maria Reut[1], Ali Abid[1], Gabriel Dickstein[1], Carmela Shechner[1]

1 Division of Endocrinology, Bnai-Zion Medical Center, Haifa, Israel

Introduction: Myelolipoma of the adrenal gland is a benign tumor, which seldom produces symptoms. Histologically, the tumor is composed of varying proportion of fat and bone marrow elements. We describe a case of huge adrenal myelolipoma with extramedullary hematopoiesis in a thalassemic patient.

Case report: A 26-years-old man with thalassemia major was referred to our clinic for evaluation of an adrenal mass, discovered incidentally during routine hematologic follow up. The patient was asymptomatic. On 2003, an abdominal CT demonstrated an 8x8x8.8 cm left adrenal mass, measuring 9.7x8.2x10 cm on repeated CT on 2011. Radiologic findings were consistent with myelolipoma. Investigation for hypercorticolism was negative and urinary 24-hour excretion of catecholamines was within the normal range. There was no history of arterial hypertension. Heat damaged Tc-99m labeled RBC SPECT scan showed high uptake on the anatomic region of the left adrenal mass, confirming the presence of extramedullary hematopoiesis. We recommended left adrenalectomy, but the patient refused surgery.

Discussion: The etiology of myelolipoma is not clear. The estimated incidence of myelolipoma at autopsy is 0.08-0.2%. Symptoms are rare, and may be caused by mechanical compression, hemorrhage or necrosis. Most myelolipomas are inactive hormonally. Apart from thalassemia, large adrenal myelolipomas have also been reported in other patients with chronic hemolysis, including sickle cell anemia and hereditary spherocytosis. Such an association suggests that tumor growth may be influenced by the extrinsic stimulation of erythropoietin. The most well recognized complication of myelolipoma measuring >10 cm is spontaneous rupture with retroperitoneal hemorrhage.

Adrenal extramedullary hematopoiesis is very rare; 6 similar cases were reported in the literature. The diagnosis of extramedullary hematopoiesis of an adrenal myelolipoma should be included in the differential diagnosis of adrenal incidentaloma in patients with thalassemia or chronic hemolytic anemia. Surgery for large myelolipoma may be considered to prevent rupture and life-threatening hemorrhage.

A SYSTEMS APPROACH TO STEROID METABOLOMICS

Edward Vitkin[1], Amir Ben-Dor[3], Michael Shmoish[2], Michaela Hartmann F.[4], Yakhini Yakhini[2,3], Stefan Wudy A.[4], Ze'ev Hochberg[1]

1 Faculty of Medicine, Technion, Haifa, Israel
2 Faculty of Computer Science, Technion, Haifa, Israel
3 Agilent Laboratories, Agilent, Tel Aviv, Israel
4 Steroid Research and Mass Spectrometry Unit, Center of Child and Adolescent Medicine, Justus Liebig University of Giessen, Giessen, Germany

We describe a novel bioinformatics approach to analysis of high throughput steroid metabolomics data comprising four consecutive stages: (a) data collection; (b) data normalization; (c) statistical interpretation; and (d) results visualization. We present analysis of data from a monogenic disorder – the adrenocortical enzyme defect 21-OHase deficiency (CAH) and from a complex disease - obesity.

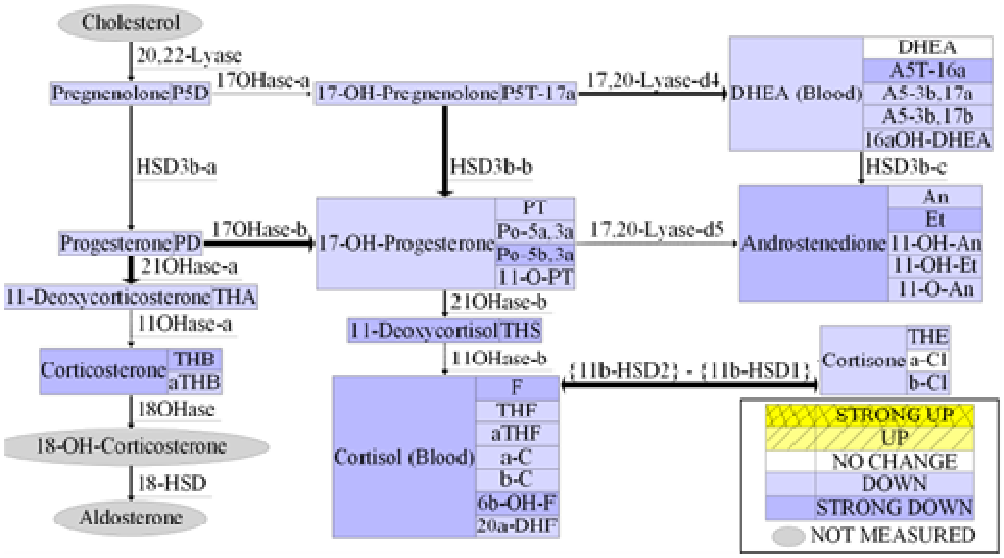
Metabolic Concentrations of 31 urinary steroid metabolites (measured by GC-MS) were analyzed in 29 untreated CAH patients (classical and non-classical), 70 obese and 324 healthy children aged 2-18 yrs. Blood precursor steroids were reflected by their typical urinary metabolites. Activity of 12 enzymatic reactions was estimated by considering product-to-substrate ratios.

We first normalized the measured steroid concentrations to control for the effects of known factors (e.g., age, sex and BMI). To this end, for each subject, we identified its reference group – the set of normal subjects with similar sex, age and BMI combination (only sex and age for the obese). Each urine, blood and reaction measurement was Z-score normalized according to the subject's reference group and compared by TNoM and TTest.

This novel method easily identified reduced 21-OHase activity in CAH. For obese subjects (Figure) following tendencies were discovered: (1) decrease in levels of urinary steroids, suggesting reduced activity of cholesterol delivery, which may point to malfunction of STAR protein; (2) increased 17,20-liase-δ4 activity, which may explain androgenization in obese women; and (3) decreased 11-OHase activity, which may explain hypertension in obesity.

The developed visualization tool color-codes the metabolic network thus enabling the examination and interpretation of individual subjects and of classes of subjects. The analysis results are depicted according to p-value scores and to directionality (Figure).

Thus, our novel systems approach for steroid metabolomics powerfully discriminates CAH patients from control and provides hypothesis-generating insights into metabolic pathways in obesity - a complex steroid-related disease.


Cholesterol
20,22-Lyase
Pregnenolone
P5D
17OHase-a
17-OH-Pregnenolone
P5T-17a
17,20-Lyase-d4
DHEA (Blood)
DHEA
A5T-16a
A5-3b,17a
A5-3b,17b
16aOH-DHEA
HSD3b-a
HSD3b-b
HSD3b-c
Progesterone
PD
17OHase-b
17-OH-Progesterone
PT
Po-5a,3a
Po-5b,3a
11-O-PT
17,20-Lyase-d5
Androstenedione
An
Et
11-OH-An
11-OH-Et
11-O-An
21OHase-a
11-Deoxycorticosterone
THA
21OHase-b
11-Deoxycortisol
THS
11OHase-a
Corticosterone
THB
aTHB
11OHase-b
{11b-HSD2} - {11b-HSD1}
Cortisone
THE
a-Cl
b-Cl
18OHase
18-OH-Corticosterone
Cortisol (Blood)
F
THF
aTHF
a-C
b-C
6b-OH-F
20a-DHF
18-HSD
Aldosterone
STRONG UP
UP
NO CHANGE
DOWN
STRONG DOWN
NOT MEASURED

ALDOSTERONE INCREASES OXIDATIVE STRESS IN DIFFERENTIATED, BUT NOT IN UNDIFFERENTIATED 3T3-L1 ADIPOCYTES VIA ACTIVATION OF MINERALOCORTICOID AND GLUCOCORTICOID RECEPTOR

Rona Limor[1], Uda Moataz[1], Esther Knoll[1], Naftali Stern[1]

1 Institute of Endocrinology, Metabolism and Hypertension, Tel Aviv Sourasky Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

In the cardiovascular system, aldosterone promotes inflammation, tissue remodeling and endothelial dysfunction. In diet-induced obesity, lipid accumulation involves acceleration of preadipocyte differentiation into mature adipocytes. Mutually exerted control mechanisms may exist between aldosterone secretion and fat: circulating aldosterone is increased in proportion to fat mass in humans and fat tissue expresses gluco and mineralocorticoid receptors (GR, MR). Here we tested whether or not aldosterone affects oxidative stress in 3T3-L1 cells, a rodent line of preadipocytes which undergoes differentiation to mature fat cells under the combined influence of serum, dexamethasone and insulin. First, both undifferentiated and differentiated 3T3-L1 cells expressed MR as well as GR as quantified by Real Time PCR. Second, aldosterone (at a physiological concentration-1nmol/l) had no effect on cell differentiation as assessed by the expression of fat cell differentiation markers, including AP2 and PPARγ. Third, aldosterone (1nmol/l) induced a~3.5 increase in the formation of intracellular reactive oxygen species (ROS) as determined by oxidative conversion of cell-permeable chloromethyl-2",7"-dichlorodihydroflurescein diacetate to fluorescent dichlorofluorescein (DCF) in both differentiating and fully differentiated adipocytes but not in undifferentiated 3T3-L1 preadipocytes. This effect was fully blocked by the specific MR antagonist eplerelone (100nmol/l) and also by the Ruthenium 486, an antagonist of glucocorticoid receptor. We identified three isoforms of lipoxygenase (LO), dioxygenase enzymes which incorporate molecular oxygen into unsaturated fatty acids such as arachidonic acid and linoleic acid in these cells, platelet 12(S)-LO, leukocyte type (12/15)- and an epidermal LO, of which the expression of the platelet and leukocyte type increased with aldosterone treatment. One LO product, 12 hydroxyeicosatetraenoic acid (12HETE) was able to increase ROS formation in differentiated 3T3-L1 cells (X2-3 folds). These results suggest that aldosterone can increase oxidative stress in differentiated, but not undifferentiated adipocytes, indicating that fat accumulation in mature fat cells predisposes adipose tissue to the pro-oxidative effect of aldosterone, and perhaps serves as a means to increase ROS through oxidation of fatty acids such as 12HETE.

POTENT ANTI-APOPTOTIC ROLE FOR EXTENSIVE INDUCTION OF STEROIDOGENIC ACUTE REGULATORY (STAR) PROTEIN REVEALED IN CARDIAC MYOFIBROBLASTS FOLLOWING EXPERIMENTAL INFARCTION IN MURINE HEART

Eli Anuka[1], Natalie Yivgi-Ohana[1], Sarah Eimerl[1], Naomi Melamed-Book[1], Yelena Chepurkol[2], Asher Shainberg[3], Edith Hochhauser[2], Joseph Orly[1]

1 Department of Biological Chemistry, The Alexander Silberman Institure of Life Sciences, The Hebrew University of Jerusalem, Jerusalem, Israel

2 The Cardiac Research Laboratory of the Department of Cardiothoracic Surgery, Felsenstein Medical Research Center, Tel Aviv University, Rabin Medical Center, Petah Tikva, Israel

3 Mina and Everard Goodman Faculty of Life Sciences, Bar Ilan University, Ramat Gan, Israel

Steroid hormones affect a plethora of heart functions. In search for potential *de novo* steroid hormones synthesis in the heart, we discovered that expression of StAR, known as indispensable for high steroidogenic output in cells of the classical steroidogenic tissues, is also activated in vimentin positive fibroblasts observed in mouse heart following experimental myocardial infarction (MI). Intriguingly, such StAR induction is not associated with any *de novo* ability of the fibroblasts to make steroids, thus strongly suggesting a novel role for this protein. We show that 3 days after MI, StAR expression rise in fibroblasts congregating in the injured myocardium of the left ventricle, where the tissue suffers apoptosis and necrosis resulting from hypoxia, ischemia and oxidative stress. Recapitulating stress insults in cultured heart fibroblasts, using widely used pro-apoptotic agents such as H2O2 or staurosporine, caused 25-35% apoptosis. Intriguingly, however, this also activated StAR expression in some of the cells that remained intact, suggesting a protective role for this protein against apoptotic death; confocal quantification of fragmented nuclei showed that StAR expression reduced the incidence of apoptosis by nearly 75%. Additionally, siRNA mediated knockdown (60%) of StAR in the heart fibroblasts doubled the number of annexin V positive apoptotic cells assessed by FACS. Similar results were obtained with ovarian granulosa cells, or HeLa cells transiently transfected with StAR construct, suggesting that StAR can confer an anti-apoptotic effect in any cell type. Collectively, these findings revise our understanding of StAR biology suggesting that StAR expression is not necessarily restricted to steroidogenic cell types; it can be induced by non-hormonal stress-related stimuli and; the protein activity in such case is anti-apoptotic. Physiologically wise, the StAR response to MI insults seems to spare the myofibroblast precursor cells during infarct and paves the way for initiation of a post-MI myocardium tissue repair process.