A novel loss of function mutation in OTX2 is associated with phenotypically variable anophthalmia and isolated growth hormone deficiency

Galia Gat-Yablonski [1,2], Liat Ashkenazi-Hoffnung [1] , Yael Lebenthal [1], Alexander W Wyatt [4], Nicola K Ragge [4,5], Sumito Dateki [6,7], Maki Fukami [6], Tsutomu Ogata [6], Moshe Phillip [1,2,3,]

[1] *The Jesse Z and Sara Lea Shafer Institute for Endocrinology and Diabetes, National Center for Childhood Diabetes, Schneider Children's Medical Center of Israel*
[2] *Felsentein Medical Research Center, Petah Tikva 49202*
[3] *Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel*
[4] *Department of Physiology, Anatomy and Genetics, University of Oxford, Le Gros Clark Building, Oxfor*
[5] *Moorfields Eye Hospital NHS Foundation Trust, London EC1V 2PD, UK*
[6] *Department of Endocrinology and Metabolism, National Research Institute for Child Health and Develo*
[7] *Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Jap*

Introduction: Heterozygote mutations of the gene encoding transcription factor OTX2 were recently shown to be responsible for ocular as well as pituitary abnormalities. Objective: To identify the genetic cause of anophthalmia and IGHD in a Sephardic Jewish family

Patients/ Methods: . Patients and Methods: The index case presented with unilateral anophthalmia and short stature underwent hypothalamic-pituitary axis evaluation as well as brain MRI. DNA was analyzed for mutations in the HESX1, SOX2 and OTX2 genes. Laser-scanning microscope was used to identify subcellular localization of the mutant protein. EMSA was performed to follow correct promoter binding and transactivation analysis was performed using the Luciferase assay

Results: Results: MRI in the proband revealed a small anterior pituitary gland, invisible stalk, ectopic posterior lobe and right anophthalmia. Endocrine evaluation showed IGHD. Molecular analysis yielded a novel heterozygous OTX2 mutation (c.270A>T, p.R90S) within the homeodomain. The paternal family has 4 other male cases of bilateral anophthalmia. Functional analysis revealed that the mutation inhibited the DNA binding activity of the protein and that the mutant OTX2 protein barely retained transactivation activities.

Conclusions: Conclusion: A novel loss-of-function mutation R90S in OTX2 is associated with familial anophthalmia and IGHD and is characterized by phenotypic variability

Familial Isolated Hypogonadotropic Hypogonadism caused by a novel homozygous mutation in a splice site of the GPR54 Gene

Maha Abdulhadi-Atwan [1,2], Pinchas Renbaum [3] , Hila Friedman [3], Sharon Zeligson [3], Efrat Levy-Lahad [3], David Haim Zangen [1]

[1] *Division of Pediatric Endocrinology, Hadassah Hebrew University Medical Center*
[2] *Dep. of Pediatrics, Al Yamama Medical Center*
[3] *Medical Genetics Institute, Shaare-Zedek Medical Center,*

Introduction: Isolated hypogonadotropic hypogonadism (IHH) is characterized by low gonadotropin levels and normal other pituitary hormones. Although familial IHH is mostly attributed to mutations in the gonadotropin-releasing hormone receptor (GnRHR) gene, the recently identified Kisspeptin/GPR54 signaling pathway gene mutations have become a significant etiology of IHH.

Methods: Clinical, endocrine, imaging, and molecular genetic characterization was performed in two IHH patients.

Results: A 16 y old Palestinian girl and her 20y old brother born to consanguineous parents presented with no pubertal development, infantile uterus on ultrasound and prepubertal descended testes, respectively. Both were normosmic and basal and stimulated gonadotrophins were prepubertal. Basal estradiol levels (for the girl) were low at 50 pmol/l and failed to rise in response to hCG. Her brain CT scan was normal. The brother had low basal testosterone (1.87 nmol/l). Combined therapy of hCG and HMG (LH and FSH) normalized testosterone levels but failed to increase testes size. Replacement with exogenous sex steroids achieved development of secondary sexual characteristics. DNA was extracted from 3 affected siblings (another affected sister) and other family members. Initial homozygosity studies using microsatellite markers (located in proximity to candidate genes: GnRHR, GPR54, GnRH, and Kiss1 did not yield a possible molecular etiology. SNP array studies thereafter revealed a relatively small area of homozygosity at the telomeric end of chromosome 19. Sequencing the GPR54 gene revealed a novel homozygous G>A mutation at the nt -1 canonical acceptor splice site of intron 1 in all 3 affected siblings. The mutation results in skipping of exon 2, leading to a frameshift which results in an altered protein from residue 82, with a premature stop codon at residue 151. (p. A82GfsX151). To assure the aberrant transcript formation we extracted RNA from transformed lymphocytes of the 3 affected siblings. Sequencing the reversed transcribed RNA at the exon 1 to 3 segment revealed that exon 2 was indeed missing with the expected frame shift resulting in a premature stop codon. The mother (menarche at 14y) was heterozygous, while a healthy sister and 5 normal Jerusalem Palestinians had the normal sequence.

Conclusions: A novel IVS1-1G>A mutation in GPR54 results in a severe IHH phenotype with failure to exhibit any pubertal maturation. Carriers of the heterozygous mutation may manifest a subtle phenotype. The subnormal gonadal response to hCG in patients may implicate a direct effect of kisspeptin/GPR54 on gonadal function. Further expression studies are currently performed.

Preimplantation genetic diagnosis (PGD) for inherited endocrine diseases: an ounce of prevention is worth a pound of cure.

Orit Barenholz [1], Gheona Altarescu [2] ,Ehud J Margalioth [1], Irit Varshaver [1], Pinchas Renbaum [2], Baruch Brooks [1], Ephrat Levy-Lahad [2], Talia Eldar-Geva [1],

[1] *Shaare Zedek Medical Center, IVF unit*
[2] *Shaare Zedek Medical Center, Medical Genetics Institute, Zohar PGD lab*

Introduction: Genetically inherited endocrine diseases may be severe, debilitating and sometimes fatal. Some of them carry a predisposition to cancer, and therefore in addition to the actual medical risk, they pose a significant mental burden on the known carriers. Preimplantation Genetic Diagnosis (PGD) enables the carriers of a known mutation to avoid a delivery of an affected child. Here we present our experience in PGD for some endocrine diseases.

Patients/ Methods: Five families were referred to the IVF-PGD Unit after being diagnosed as carriers of an autosomal recessive disease following the birth of an affected child: 3 couples were found to be heterozygotes for Persistent Hyperinsulinemic Hypoglycemia of infancy (SUR1), one couple was diagnosed as carriers for Congenital Adrenal Hyperplasia (CYP21B), and one couple diagnosed as heterozygotes for Hypoparathyroidism- Retardation- Dysmorphism syndrome (HRD). None of the patients was diagnosed by screening. One other patient was a female carrier of a RET mutation with MEN2 phenotype

Results: The patients were managed with standard IVF protocols, followed by polar body/ blastomere biopsy, single-cell PCR and transfer of the non-mutant embryo(s). Among the carriers of autosomal recessive diseases, average clinical pregnancy rate per patient per cycle was 53.2% (similar to the outcomes of first cycles in other IVF patients). All patients achieved an ongoing pregnancy/ live birth. The RET mutation carrier had 8 IVF cycles: in two cycles there were no healthy embryos for transfer, in another two cycles there was only one embryo available for transfer, and the desirable pregnancy was achieved only following the 8th cycle- an ongoing twin pregnancy

Conclusions: Some inherited endocrine diseases are preventable by PGD. Keeping in mind the possibility of referring patients for IVF with PGD may, in the long run, make the incidence of these diseases lower. For couples with an affected child, both the chance of having another affected child, and, on the other hand, termination of a pregnancy with an affected fetus, may be unacceptable. Fortunately for those, PGD is feasible, safe and effective, and therefore should be considered.

Multiple endocrine neoplasia type 1 (MEN-1), the hadassah-hebrew university medical center experience

Sameer Kassem [1], Benjamin Glaser [1] , Dganit Barak [1], Merav Fraenkel [1], David Gross [1]

[1] *Endocrinology Service, Hadassah Medical Center*

Introduction: MEN-1 is an autosomal dominant genetic disorder with a prevalence of 2-4 per 100,000. The main manifestations are parathyroid (PT), gastro-enteropancreatic (GEP) and pituitary tumors, but may affect other organ systems as well. MEN-1 is associated with significant morbidity and mortality with up to 50% dying before the age of 50. Treating MEN-1 affected subjects presents a unique diagnostic and therapeutic challenge. In the current study, we present our experience with MEN-1 affected patients, including clinical and genetic information.

Patients/ Methods: Clinical data was obtained for patients followed at Hadassah Medical Center between the years 2003-2009. Genetic analysis was carried out in the laboratory of Prof. A Calender, France, and the NIH, USA, and included direct sequencing and quantitative multiplex short fragment PCR of exons 2-10 of the menin gene. Clinical diagnosis of MEN-1 was defined as the presence of at least 2 out of the 3 main manifestations of MEN-1 (PT, GEP, pituitary).

Results: Our cohort included 25 subjects from 20 families. Clinical information was available on 22. Average age at presentation was 36 years (range 17-75). Initial presentation was hyperparathyroidism in 12 (55%), GEP tumor in 6 (27%) and pituitary tumor in 4 (18%). During evaluation and follow up, 19 (86%) developed hyperparathyroidism, 15 (68%) GEP tumors (7 non-secreting , 6 gastrinomas, 2 insulinomas) and 12 (55%) developed pituitary tumors (9 prolactin, 2 ACTH and 1 null). Two patients had metastatic carcinoid tumor, and 1 thymic carcinoid. Genetic testing was performed in 18 subjects. MEN-1 mutations were found in 12 (67%), and a genetic variant of unknown significance in one. Eleven (50%) patients underwent abdominal surgery for resection of tumor, 7 are treated with somatostatin analogs and 2 patients underwent peptide receptor radionuclide therapy (PRRT). Eight patients underwent parathyroid surgery, 6 treated with cabergoline for prolactinoma and 3 underwent pituitary surgery. Twenty two subjects are alive (age 45.4±10 years), whereas 3 died of metastatic GEP tumors at the ages 41, 45 and 56.

Conclusions: MEN-1 is a complex genetic disorder. Hyperparathyroidism is the most common and earliest manifestation. GEP tumors cause most of the morbidity and mortality associated with MEN-1. Menin mutations or variants were found in 72% of patients with clinically defined MEN-1.

Congenital IGF-1 deficiency protects from cancer development - additional support

Rachel Steuerman [1, 2], Orit Shevah [1, 2] , Zvi Laron [1, 2], ,

[1] *Endocrinology and Diabetes Research Unit, Schneider Children's Medical Cebter*
[2] *Tel Aviv University*

Introduction: In a previous study (1) we have found that homozygous isolated congenital IGF-I deficiency (such as in Laron Syndrome) protects patients from future development of malignancies. This was confirmed by another group (2). Aim: a) To find out whether congenital IGF-I deficiency combined with other pituitary hormone deficiencies also confers protection from cancer. b) To enlarge the population of patients with isolated congenital IGF-I deficiencies.

Patients/ Methods: By survey among endocrinologists in Israel and other countries, using a pre-structured questionnaire for patients and first and second degree family relatives.

Results: The main data obtained until Jan 15, 2010 are sunnerized in the following table. The data reinforce our previous findings (1) and those of Guevara-Aguirre in 75 LS pts. (2) that cong. IGF-1 deficiency protects from the development of malignancy. In the present study we found in addition that pts. with cong. IGHD, IGF-1 R mutation and IGF-1 gene deletion also fall into this category. Out of 6 pts. with a GHRH-R defect and out of 97 pts. with cong. MPHD including GH def. we found 1 pt. with cancer in each. Among the first degree family members (most heterozygotes) we found 27 cases of cancer. In addition, 30 out of 131 second degree relatives also reported malignancies.

Conclusions: a) The present survey underlines the important role cong. IGF-1 deficiency has on the development of malignancies. b) It is premature to define which pts. with GHRH-R defect or cong. MPHD are not protected from cancer.

References: (1) Shevah O., Laron Z., Growth Hormone & IGF Res. 2007,17: 51-57. (2) Guevara-Aguirre et al., Hormone Research 2007,68 (Suppl 1):175.

Peptide receptor radioligand therapy (PRRT) is an effective treatment for the long-term stabilization of malignant gastrinomas

Simona Grozinsky-Glasberg [1], Dganit Barak [2] , Merav Fraenkel [2], Jan Müeller [3], Ilan Shimon [1], David J. Gross [2]

[1] *Institute of Endocrinology, Beilinson Hospital, Rabin Medical Center, and Sackler Faculty of Medicine, Tel Aviv, Israel*
[2] *Endocrinology and Metabolism Service, Department of Medicine, Hadassah Medical Center*
[3] *Institute of Nuclear Medicine, University Hospital, Basel, Switzerland*

Introduction: Gastrinomas represent a rare group of neuroendocrine tumors usually located in the duodenum or pancreas. They secrete gastrin, being responsible for the clinical picture of severe acid-related peptic disease and diarrhea, known as the Zollinger-Ellison syndrome (ZES). While symptomatic control may be achieved with proton-pump inhibitors (PPIs) and somatostatin analogues (SSAs) treatment, little data is known regarding the possible anti-tumor effect of the peptide receptor radioligand therapy (PRRT) in gastrinomas patients. Aims: To assess the effect of PRRT on symptoms control, gastrin secretion and tumor load in patients with malignant gastrinomas, with progressive disease.

Patients/ Methods: We have retrospectively studied 11 consecutive patients with metastatic gastrinomas followed at two referral centers in Israel for a mean period of 5 years. The patients were symptomatically treated with PPIs (n=8), and/or with monthly injections of octreotide LAR (30 mg/month) (n=8) or lanreotide Autogel (120 mg/month) (n=1), all patients presented with an ECOG score of 0-1 (1), and received PRRT (90Yttrium- or 177Lutetium-DOTATOC) for progressive disease. Patients had serum gastrin measurements performed pre- and post-treatment, as well as radiological assessment before and every 3-6 months following PRRT, using the RECIST criteria for tumor response (2).

Results: The dosage of PRRT was 432.45±223.29 mCi (mean±SD), with a mean number of 2±1.26 courses/patient (range 1-4), depending on tumor uptake. PRRT was well tolerated in all patients, without serious side effects: 8/11 patients (73%) experienced a transient decrease in their blood counts, while in one patient (9%) a temporarily increase in creatinine levels appeared. Following PRRT, symptomatic improvement was observed in all patients, as well as significant suppression in gastrin levels, which decreased from 4831 mI/L to 932.6 mI/L ($p<0.001$) (normal 40-108 mI/L). Periodic radiological surveillance showed partial tumor response in 5/11 (45%). Tumor stabilization has been achieved in 6/11 (55%) patients, with no complete response. During the follow-up, 4/11 patients (36%) died due to tumor progression (mean time to progression of 8±2.8 months), in this group, the mean survival time following last PRRT reached 14±6.9 months. In 7/11 patients, the anti-tumor effect of PRRT persisted for a mean period of 11±3.16 months (ongoing).

Conclusions: In patients with malignant gastrinomas, PRRT has a valuable effect in reducing/stabilizing the tumor load, with a concomitant decrease in serum gastrin levels. Our data indicate an important anti-proliferative effect of PRRT on gastrinoma tumor cells, together with a significant clinical benefit with respect to disease symptoms, with minimal adverse effects.

Impaired arterial properties in active acromegaly are reversed by effective therapy

Marianna Yaron [1], Elena Izkhakov [1] , Jessica Zak [1], Ibrahim Azzam [1], Maya Ish-Shalom [1], Susan Gilad [1], Naftali Stern [1], Yona Greenman [1]

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

Introduction: Active acromegaly is associated with increased cardiovascular and cerebrovascular morbidity and mortality. Normalization of GH and IFG-1 levels has been shown to improve some of the cardiac function abnormalities. The aim of the present study was to evaluate parameters of arterial function in active and controlled disease.

Patients/ Methods: The arterial properties of 18 subjects with acromegaly (7 males and 11 females) were studied by repeat non-invasive measurements of arterial properties using applanation tonometry and pulse wave analysis and assessing parameters of arterial stiffness: pulse wave velocity (PWV), central blood pressure, augmentation index (AIx), and large/small artery compliance (C1 and C2). By ultrasonography, common carotid artery far-wall intima-media thickness (IMT) and flow-mediated dilatation (FMD) of the brachial artery was measured. Nine subjects with active acromegaly (GH 7.76 ± 12mU/L, IGF-1 469.9 ± 246 ng/ml) and nine subjects with controlled or cured disease (GH 1.59 ± 0.1mU/L, IGF-1 160 ± 0.3ng/ml) were studied.

Results: Mean age was 48.3 ± 19 years in subjects with active disease and 56.4 ± 13years in those with controlled acromegaly, p=NS). Weight, waist circumference, body mass index, HDL- and LDL-cholesterol and triglycerides were similar in the two groups Glucose tolerance abnormalities were found in 5 (50%) patients with active disease and in two (27%) cured subjects, with significantly higher fasting plasma insulin level in the active group (21.9 ± 11.17 vs. 14.8 ± 3.8 MIU/mL, respectively, p=0.0075). Systolic blood pressure (SP) but not diastolic blood pressure (DP) was significantly higher in patients with active disease (128 ± 17 vs. 113.1 ± 12mmHg, p=0.005). Central (aortic) blood pressure was higher in the active disease group (SP: 118 ± 12 vs. 106 ± 12mmHg, p=0.04, DP: 74 ± 8 vs. 66 ± 6mmHg, p=0.02, respectively) as was systemic arterial resistance (1427 ± 323 vs. 1299 ± 215dynes/sec/cm(-5), p=0.006). Hypertension was found in three patients with active disease and two in cured subjects. Analysis of arterial function parameters were performed with adjustment for age, gender and SBP. Small artery elasticity index (C2) was decreased in the active disease group (6.71 ± 4.06 vs. 8.5 ± 4.2 ml/mmHg x 100, p= 0.03). There were no significant differences in other arterial stiffness parameters PWV, Alx, C1, IMT and measurement of endothelial function (FMD).

Conclusions: Patients with active acromegaly have decreased small artery elasticity index, increased systemic systolic BP, increased central SP and DP as well as increased systemic arterial resistance. These vascular derangements could contribute to the increased propensity for cardiovascular disease in acromegaly but are apparently reversed by effective treatment.