Print ISSN 0391-4097
Online ISSN 1720-8386

JOURNAL OF ENDOCRINOLOGICAL INVESTIGATION

Vol. 34, Suppl. to No. 3, 2011

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Società Italiana Endocrinologia

XXXV NATIONAL CONGRESS OF THE ITALIAN SOCIETY OF ENDOCRINOLOGY

Montesilvano (Chieti - Pescara)
May 18-21, 2011

On-line submission
http://mc.manuscriptcentral.com/jei
Electronic versions of articles on www.jendocrinolinvest.it

Editrice Kurtis
Published by
Editrice Kurtis
Via Luigi Zoja, 30
20153 Milano, Italy
Visit Editrice Kurtis journals online at www.kurtis.it

OC001

IDENTIFICATION OF X-LINKED GENETIC FACTORS INVOLVED IN MALE INFERTILITY WITH A HIGH RESOLUTION ARRAY-CGH

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Background: X and autosome linked candidate genes have been studied only sporadically in relationship with male infertility and none have turned out to be significant contributor to spermatogenic failure. A substantial advancement is expected with the use of array based whole genome assays which already have lead to the identification of genetic risk factors for various other complex diseases. The X chromosome is of special interest since contains many testis specific genes. Moreover, men are hemizygous for genes located on this chromosome and "de novo" mutations in a X-linked gene will have an immediate impact since compensation by a second, normal allele is not possible. Our aim was to identify novel X-linked genetic factors involved in male infertility using an innovative approach (high resolution X chromosome specific array-CGH).
Materials and methods: 54 azoospermic, 9 cryptozoospermic and 33 oligozoospermic men were analyzed with a high resolution customized array-CGH platform for the X chromosome. Selected CNVs found with array-CGH were validated by PCR plus/minus or TaqMan® Assay and subsequently screened in a case/control setting.
Results: We found a total of 71 CNVs mapping on both Xp and Xq. A total of 31 CNVs (14 gains and 17 losses) were selected for the case-control study. 20/31 were specifically present in patients and may represent causative variants for impaired spermatogenesis. A recurrent deletion in Xq27.3 was significantly more frequent in patients (5,9%) with respect to controls (2,2%) (p=0,005; OR: 2,96 95%CI 1,3-6,6) and thus represents a new genetic risk factor for male infertility.
Conclusions: Our approach, based on high resolution customized array-CGH platform specific for the X-chromosome appears to be a valid tool for the identification of new X-linked risk factors for impaired spermatogenesis.

OC002

A ROLE OF VITAMIN D 25-HYDROXYLASE IN HUMAN TESTIS

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25-hydroxyvitamin D concentration is regarded as the most reliable indicator of vitamin D status. Mutations in the *CYP2R1* gene, highly expressed in the testis and encoding vitamin D 25-hydroxylase, results in a vitamin D deficiency and a defective calcium homeostasis leading to rickets.
Aim of our study was to investigate the role of testicular CYP2R1 in vitamin D production.
CYP2R1 mRNA expression and protein production were evaluated by quantitative RT-PCR, western blot analysis and immunofluorescence from testis samples. Hormonal and bone-marker levels were determined in 15 male radically orchiectomized patients compensated by testosterone-replacement therapy at follow-up, 21 patients with Sertoli cell-only syndrome (SCOS), 36 patients with severe hypospermatogenesis and 41 healthy controls aged-matched at Padova's Center for Male Gamete Cryopreservation.
Compared to normal human testis, a lower gene and protein expression of CYP2R1 could be found in testis samples with diagnosis of hypospermatogenesis and SCOS ($P<0.05$). Moreover, at immunofluorecence, CYP2R1 shows a co-localization with INSL-3, a Leydig cell marker.
Despite all blood sample were performed in the same season, both orchiectomized patients and testiculopathic patients had significantly lower levels of 25-hydroxyvitamin D compared to controls (respectively 30.2±16.3 and 45.8±22.05 vs. 74.9±38.0 nmol/L, $p<0.05$).
Our results suggest an association between testicular function and vitamin D reduction, despite the same season of measurement, same age and no nutritional derangements.

OC003

SPERM MITOCHONDRIAL DNA INTEGRITY AND COPY NUMBER IN PATIENTS WITH ASTHENOZOOSPERMIA

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Introduction: Mitochondria in spermatozoa supply the energy necessary for sperm motility. Thus, an abnormal mitochondrial DNA (mtDNA) may have a detrimental effect of this fundamental parameter for male fertility. Therefore, this study was undertaken to appraise the relationship between mtDNA deletions and copy number and sperm motility. We also evaluated sperm mitochondrial function, sperm apoptosis and chromatin/DNA integrity by fluo-cytometry. Finally, semen basal and stimulated radical oxygen species (ROS) production was evaluated. **Materials and methods:** To accomplish this, 11 healthy donors with normal sperm parameters and 24 patients with progressive motility ≤20% and non-progressive motility >50% were selected. Sperm mtDNA deletions were evaluated by long-range PCR technique following amplification of a 8.7 kb fragment which contains genes encoding respiratory complex subunits. Its deletion generates 4.9 and 7.4 sub-fragments associated with abnormal sperm parameters. Quantitative real-time PCR was used to determine the mtDNA copy number, using two sets of primers for the mitochondrial 16S rRNA gene and the nuclear GAPDH gene. By flow cytometry, we also evaluated: sperm mitochondrial membrane potential (MMP) by JC-1 staining; sperm phosphatidylserine (PS) externalization (a marker of early apoptosis) by annexin V and propidium iodide (PI) staining; sperm chromatin condensation by PI staining; and DNA fragmentation by TUNEL assay. Semen ROS production was evaluated by chemiluminescence both basally and following stimulation with fMLP or PMA. **Results:** We found that 23 asthenozoospermic patients (95.8%) had multiple mtDNA deletions, whereas all donors showed an intact mtDNA. mtDNA copy number (18.4±3.8) was significantly higher in patients compared to donors (5.7±0.9). Asthenozoospermics had a significantly higher percentage of spermatozoa with low MMP (29.7±5.1%) compared to donors (4.8±1.3%), whereas no significant difference was observed for PS externalization, chromatin compactness and DNA fragmentation. Finally, a significantly higher basal and fMLP- and PMA-stimulated ROS production was found in asthenozoospermic semen samples. A significant positive correlation was found between mtDNA copy number and fMLP-stimulated ($r=0.63$, $p<0.005$) and PMA-stimulated ($r=0.67$, $p=0.001$) ROS production. **Conclusions:** Asthenozoospermic patients had poor sperm mtDNA integrity, higher mtDNA copy number and a worse mitochondrial function as well as an increased oxidative stress. This suggests that men with poor motility have sperm mitochondrion genetic and functional abnormalities regardless to the cause of asthenozoospermia.

OC004

A NOVEL TREATABLE FORM OF MALE INFERTILITY LINKED TO POLYMORPHISM OF FSH BETA SUBUNIT PROMOTER

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Background High follicle stimulating hormone (FSH) plasma levels indicates severe male factor infertility due to a primary testicular disorder. However, about half of oligozoospermic men have inappropriately low-normal FSH levels. A single nucleotide polymorphism in the *FSHB* gene promoter modulates the transcription of the gene for the beta subunit of FSH. We tested whether this variant is associated with male infertility, sperm count and FSH plasma levels, and whether it could be a pharmacogenetic tool for the treatment of male infertility with FSH.

Methods 248 subjects with normozoospermia, 79 with azoospermia (absence of sperm in the ejaculate even after semen centrifugation) and 435 with oligozoospermia (total sperm count <40 million/ejaculate) were evaluated for semen parameters, reproductive hormone levels and *FSHB* -211 G/T polymorphism (rs10835638).

Results *FSHB* -211 TT genotype was associated with significantly lower FSH levels (mean ± SD, 3.3±2.5 IU/L *vs* 9.1±8.9 IU/L in GG homozygotes, P=0.0002). TT homozygotes were 25% (5/20) of subjects with azoo-oligozoospermia and low FSH levels (≤1.5 IU/L). We did not observe this genotype in men with high FSH levels (>8 IU/L). TT homozygous men (13 subjects) had a primary testicular disorder but low or inappropriately normal FSH levels. Treatment with FSH in these subjects induced a dramatic improvement in sperm count and quality, significantly higher with respect to carriers of the G allele.

Conclusions *FSHB* -211 TT genotype represents a novel treatable form of male infertility characterized by severe spermatogenic impairment and low or inappropriately normal FSH plasma levels. This genetic marker can be used as a molecular diagnostic tool for male infertility and might represent a valid pharmacogenetic approach for identification of potential responders to FSH treatment.