Genetics

P007

CFTR genotypes and spermatology in cystic fibrosis patients without CBAVD

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Objectives: Evaluate *CFTR* genotypes and spermatology in non-azoospermic men with cystic fibrosis.
Methods: The cohort consisted of 10 Russian men, aged from 17 to 29 (21.7 ± 4.3) years, which were selected during reproductive study of CF patients. Semen analysis was performed in accordance with the WHO Guidelines (2010). Sperm DNA fragmentation was evaluated by the TUNEL. Quantitative karyological analysis of immature germ cells from the ejaculate, QKA IGCs (Russian patent №2328736, L.F. Kurilo) and TEM were also performed.
Results: All patients developed PS-CF. The following *CFTR* genotypes were found: F508del/3849+10kbC>T (n = 8), 3849+10 kb C > T/Q493R (n = 1), CFTRdele2.3/3849+10 kb C > T (n = 1). No genital abnormalities, endocrine diseases, varicocele, cryptorchidism, sexually transmitted infections were found. Sperm count varied from 7.2 to 243 (89.8 ± 84.2) million/ml. Teratozoospermia was detected in 6 patients (F508del/3849+10kbC>T). Normal sperm motility was in 1 (normozoospermic) patient (3849 +10kbC>T/Q493R), sperm motility (PR<32%) was found in 9 patients: astenozoospermia (n = 2), oligoastenozoospermia (n = 1), astenoteratozoospermia, AT (n = 3), and oligoastenoteratozoospermia, OAT (n = 3), including necrozoospermic men with total asthenozoospermia. The ejaculate volume varied from 0.3 to 6 (2.3 ± 1.8) ml. Oligospermia/increased semen viscosity and leukospermia was found in 3 and 4 patients, respectively. The ejaculate pH was normal in 9 samples. Various sperm morphology abnormalities were detected by TEM: atypical heads (66%), reacted acrosome (50%), immature chromatin (33%), fibrous membrane flagellum (16%). Sperm DNA fragmentation was evaluated in 2 samples: 32% and 4% (N ≤1 5%). Signs of incomplete meiotic arrest were found by QKA IGCs in 4 of 6 samples.
Conclusion: 3849+10kbC>T variant of *CFTR* gene is characteristic for CF patients without CBAVD, at that semen parameters are variable. Oligospermia detected in some patients indirectly indicates seminal vesicles hypoplasia.

P008

L138ins is a common *CFTR* gene mutation in infertile Russian men

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Objectives: The aim of study was to investigate the frequency of common *CFTR* gene variants in infertile Russian men.
Materials and methods: A large cohort of 6,978 infertile Russian men was evaluated. The analysed panel of *CFTR* gene variants included 30 common mutations and the IVS8Tn polymorphism. AFLP-PCR, MLPA methods, and DNA sequencing by Sanger and MPS/NGS were used.
Results: Pathogenic *CFTR* gene variants were detected in 276 (3.96%) patients. The following mutations were found: F508del (n = 172), CFTRdele2,2 (n = 20), L138ins (n = 18), W1282X (n = 15), 1677delTA (n = 8), 2143delT (n = 7), 3849+10kbC>T (n = 8), E92K (n = 8), G542X (n = 6), 2184insA (n = 5), others (n = 18). Heterozygous mutations were revealed in 267 (3.83%) patients. Two pathogenic *CFTR* gene variants were found in 9 azoospermic patients presenting signs of CBAVD syndrome with 'atypical CF' (pancreatitis, sinusitis). The L138ins mutation was detected in 6 of them (in 7 of 18 chromosomes). In 5 patients, L138ins was found in a compound heterozygote with another *CFTR* mutation (F508del, n = 4; N1303K, n = 1), in 1 patient – in a homozygote, in other one in a compound heterozygote with the 5T allele. The L138ins variant was detected in 6.16% patients with CFTR gene mutations.
Conclusion: F508del, CFTRdele2,3, and L138ins are the 3 most frequent CFTR mutations in infertile Russian men. The L138ins variant is a common mutation for infertile Russian men, which have 2 pathogenic CFTR mutations without 'classic CF'.

P009

The spectrum of *CFTR* mutations in newly diagnosed cases of cystic fibrosis through newborn screening in the Republic of North Macedonia

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Objectives: The spectrum and frequencies of *CFTR* mutations varies considerably between populations and regions of the world. The Balkan region is known for greater heterogeneity, attributed to a long history of population migration in these countries from the Middle East and Europe. The aim of the study was to analyse the genotype in newly diagnosed cases of cystic fibrosis (CF) in the last 3 years, since newborn screening (NBS) for CF was introduced, including a 6 month pilot study.
Methods: A 2-step IRT-IRT algorithm is performed, and then a sweat test (Macroduct sweat collection system followed by chloridometer chloride analysis) for confirmation/exclusion of the CF diagnosis when IRT values were both over the cut off: 70 ng/ml and 45 ng/ml, respectively. In cases of positive or borderline sweat tests, mutation analysis of *CFTR* gene is performed: snapshot reaction for 11 most common regional *CFTR* mutations or extended gene analysis: multiplex ligation-dependent probe amplification (MLPA), for detection of deletions/duplications, Sanger DNA sequencing or next generation sequencing.
Results: During the study period, 24 cases were diagnosed with CF, 22 after positive NBS for CF and 2 cases with meconium ileus that were screening negative. Concerning their CFTR genotype, 11 cases were homozygote for F508del, 10 were compound heterozygotes for F508del and other CFTR mutation and 3 were without F508del mutation. The most common *CFTR* disease-causing mutation of F508del was found with an overall incidence of 66.7%. Other more frequent mutations were G542X (8.3%), N1303 K (6.2%) and 457TAT>G (4.2%). Six mutations were found in 1 CFTR allele each: G1349D, G126D, V456F, G551D, 621+1G->T and CFTRdupexon22. The last one is a newly discovered mutation with unknown consequences, found in a pancreatic-sufficient case. In only 1 patient did the second mutation remain to be proven by gene sequencing.
Conclusion: Our region shows marked genetic heterogeneity for CF.

P010

Genotype features in patients with cystic fibrosis in the North Caucasus Federal District of the Russian Federation

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Objective: The North Caucasian Federal District (NCFD) is located in the southern European part of the Russian Federation (RF). It includes 7 sub–federal entities and more than 60 nationalities (Russians –30.3%, Chechens –14.2%, Avars –9.2%, Ossetians, Dargins, Kabardins, Ingushes –5% each, other nationalities are 0.5–4.5%); thus it determines the genetic diversity of the population as a whole, and the patients with CF as well. Our aim is to determine the features of the genotype in CF patients in NCFD.