8 Phenotyping the 711+1 G>T mutation in cystic fibrosis patients

A. Masson[1], P. Daigneault[2]. [1]*Centre Mère-Enfant du CHU de Québec – Université Laval, Département de Pédiatrie, Quebec, Canada;* [2]*Centre Mère-Enfant du CHU de Québec – Université Laval, CHUL – Département de Pédiatrie, Québec, Canada*

Objectives: To describe the phenotype associated with the 711+1 G>T mutation when associated with various other common CFTR mutations compared to the most common DF508 mutation in a similar setting.
Methods: We have retrospectively reviewed the clinical charts of adult and pediatric patients with a single 711+1G>T mutation combined with another CFTR mutation in patients with CF over a 15 year period (1997–2012) in Quebec City, Canada to compare them to patients with a DF508 mutation but similar other characteristics. We have evaluated variables for nutritional, respiratory and infectious outcomes as well as common CF complications.
Results: All measured variables for respiratory (FEV1), nutritional (BMI and BMI%), infectious (age at first *Pseudomonas* colonization, presence of other pathogens) status and presence of complications (diabetes, significant liver disease, nasal polyps) as well as initial characteristics at diagnosis (sweat test value, age at clinical diagnosis, pancreatic status) were similar ($p > 0.05$) between patients with a 711+1G>T mutation and a DF508 mutation when other initial factors were taken into account including the mutation on the second allele.
Conclusion: Patients with a X/711+1G>T genotype are expected to have a similar clinical course as patients with a X/DF508 genoytype. Genetic counseling for these patients can therefore be done in a similar way.

9 CFTR rearrangements in Serbian patients with classical cystic fibrosis

A. Divac Rankov[1], J. Deretić[1], M. Petrić[1], M. Ljujic[1], A. Nikolic[1], D. Radojkovic[1]. [1]*Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Biology, Belgrade, Serbia*

Objectives: Although majority of over 1600 mutations identified in CFTR gene are point mutations or small deletions/insertions, large gene rearrangements have been reported with a relatively high frequency in various ethnic groups. The aim of our study was to define the incidence of CFTR gene rearrangements in Serbian CF patients in which one or no mutation was identified after the detection of 7 most common mutations (c.1521_1523delCTT, c.489+1G>T, c.1624G>T, c.1652G>A, c.1657C>T, c.1585–1G>A, c.3909C>G) or after the sequencing of the whole CFTR gene.
Methods: Out of 154 patients referred to our Laboratory for molecular analysis, the clinical diagnosis was not confirmed in 27 patients. Seven CFTR rearrangements (dele1: c.4_53+69del53+4192_53+4489invinsG; dele2: c.54–5811_164+2186del273+6780_237+6961inv; dele2_3: c.54–5490_273+10250del; dele14b_17b: c.2620–674_3367+198del; dele17a_18: c.2988+1173_3468+2111del; dele22_23: c.3964–78_4242+577del; dele22_24: c.3964–3890_3143delinsTAACT) were detected by duplex PCR assay. The breakpoints were confirmed by sequencing.
Results: We identified CFTR gene rearrangements in 3/27 patients. In one patient dele2 was identified (genotype c.1521_1523delCTT/dele2), and in two dele2_3 was identified (genotypes: c.1521_1523delCTT/dele2_3 and c.1753G>T/dele2_3) thus confirming clinical CF diagnosis.
Conclusions: These results are in concordance with our previous findings that the molecular basis of CF in Serbia is highly heterogeneous. It would be beneficial if the detection of these rearrangements would be incorporated in routine diagnostic analysis of CF since their incidence was relatively high and the method was reliable and inexpensive.

10 Complex alleles in CFTR gene in Venezuelan patients with cystic fibrosis

K. Sanchez[1], E. de Mendonca[1], L. Mindiola[2], X. Matute[2]. [1]*IVIC, Caracas, Venezuela;* [2]*J. M. de los Ríos, Hospital, Caracas, Venezuela*

Cystic Fibrosis (CF) is caused by combination of two mutant allels at the cystic fibrosis conductance regulator gene (CFTR). There has been a steady increase in the number of reports of polymutant variants on the same allele (complex alleles). The aim of this study was to determine if polymutant alleles are present in Venezuelan patients. For this purpose, 100 patients was studied all of which are included in the national program of CF and have clinical symptoms of CF. Detection of the mutation was performed by PCR amplification of 27 exons of CFTR gene which contains the codon that encodes for CFTR protein, and sequenced by automated sequencing at ABI3130XL. The allelic frequency was 38% vs. 62% WT allele, distributed in 18% two alleles mutated and 40% only one allele mutated. Patients which were diagnosed with two mutations, previously reported as complex alleles in the cystic fibrosis database (http://www.genet.sickkids) were verified by family testing. We found only two patients with allele complex, Allele 1: p.P508del/W–p.I1027T/WT in heterozygous condition and Allele 2: p.G628R–p.S1235R in homozygous condition, these alleles were confirmed with genetic characterization of both parents for each patient. It is important to detect the presence of complex alleles in our population, in order to be taken into consideration when performing genetic studies for molecular diagnostic purposes, preventing the reports of false positives. The report of the frequency of these cases and to know their clinic presentation would increase the knowledge of the phenotypic consequences of CFTR mutations and help to better understand the relationship between CFTR structure and function.

11 Disease related *CFTR* variants found in the Norwegian screening program

H.J. Gaup[1], E. Lundman[1], A.M. Bjørnæs[1], O.-T. Storrøsten[2], T. Rootwelt[1,3], R.D. Pettersen[1]. [1]*Oslo University Hospital, The National Neonatal Screening Unit, Oslo, Norway;* [2]*Oslo University Hospital, Norwegian Resource Centre for Cystic Fibrosis, Oslo, Norway;* [3]*Oslo University Hospital, Department of Pediatric Research, Women and Children's Division, Oslo, Norway*

Objectives: Cystic Fibrosis (CF) is one of the 23 disorders included in the Norwegian national neonatal screening program. Testing is performed using a three-tier Immunoreactive trypsinogen (IRT)/DNA/DNA protocol. We present the frequencies of disease related *CFTR* mutations identified in 2nd and 3rd tier and the corresponding IRT values from the first 22 months of screening.
Methods: IRT was measured using GSP™ (Perkin Elmer) and samples >60 ng/mL were included for 2nd tier. This involved Luminex-based analysis of 71 mutations as well as Sanger sequencing of a local common mutation. If only one mutation was found, 3rd tier testing included sequencing of an additional panel of clinically rare alleles found among Norwegian CF patients.
Results: DNA assessments revealed 23 children with 2 *CFTR* mutations and 85 with only one *CFTR* mutation. Only 17 different *CFTR* variants in our DNA panel were identified. Table 1 shows the distribution of the most frequent alleles and the corresponding range of IRT values.

Table 1. The 6 most frequent CFTR variants and IRT values

Variant	n	Allele frequency, %	Min IRT, ng/mL	Max IRT, ng/mL
p.R117H	43	32.8	60.2	162.5
p.F508del	39	29.8	60.0	134.4
p.R117C	17	13.0	61.1	155.7
c.3873+2T>C	6	4.6	70.0	124.3
c.262_263delTT	6	4.6	63.7	155.7
c.3528delC	3	2.3	68.6	91.4

Conclusion: The screening program identified a limited numbers of the *CFTR* variants previously reported clinically. Some carriers revealed IRT values significantly above the cut-off level. The most frequent *CFTR* mutations were p.R117H, p.F508del, and p.R117C. Taken together, this suggests a review of the IRT/DNA protocol.