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WS8.1 The first 1,000 CFTR genes sequenced in the Mayo Clinic Clinical Molecular Genetics Laboratory

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Introduction: The Mayo Clinic Molecular Genetics Laboratory has completed whole CFTR gene analysis for 1,154 clinical samples. Here we report our findings for a large set of samples analyzed in an academic reference laboratory setting.
Methods: All exons of the CFTR gene were sequenced using an automated, laboratory developed fluorescent Sanger sequencing workflow. Whole exon deletion/duplication testing was done using MLPA (MRC Holland).
Results: 43% of samples were positive for the detection of at least one potentially disease causing allele (including 5T). 140 individuals had 2 known mutations detected, 271 were carriers of one known mutation; 57 had one mutation and one variant of uncertain significance (VUS), 100 had one VUS, and 17 had 2 or more VUS's. Of the cases with 2 mutations, 25 had one copy of the 5T allele (13 dF508/5T); and, 41 had 2 copies of ACMG mutations (16 homozygous dF508). Homozygous results were seen for 6 additional mutations: 2954delT, G27X, I1234V, S945L, R75X, and R117H-7T in a 30 y/o female with pancreatitis. The majority of cases (125/140) with 2 known, disease causing mutations were seen in patients whose samples had a reason of referral (RFR) of possible or definite CF (or Unknown RFR) versus those with RFR's for pancreatitis (9) or male infertility (6).
Conclusions: The high positivity rate indicates that the test is being ordered appropriately. However, the high number of cases with 2 ACMG panel mutations indicates that testing first with a panel test may be more cost effective. The number of VUS's is higher in individuals over 21 y/o and with a reason of referral of pancreatitis that in individuals being tested at under 21 years of age.

WS8.2 Evaluation of next generation sequencing as a diagnostic tool for CFTR rare mutation screening

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The laboratory offers screening for CFTR mutations using commercial kits and Sanger sequencing. Amplicon sequencing on a Roche GS Junior Next Generation Sequencing (NGS) platform was evaluated for its potential to replace the current Sanger sequencing method for rare mutation screening. The GS Junior offers up to 70 000 reads per run for amplicon sequencing and read lengths around 400 bases. Eight compound heterozygote CF affected patients were selected with 13 unique mutations in 10 separate exons. Various mutation types were included to test the system's sensitivity.
Library preparation was performed using the Multiplicom CFTR MASTR kit, which amplifies all CFTR coding regions in 2 multiplex reactions, and MID barcoding was performed using the Multiplicom 454 MID kit which also incorporates the 454 adaptor sequences for downstream processes. Libraries were picogreen quantified, mixed and sequenced using the Roche Titanium sequencing kit.
Data was analysed on the Roche GS Amplicon Variant Analyser. A total of 28 variants were identified (>10%), 17 of which were exonic. Only the c.1329_1330ins4 mutation in exon 9 was not called by the software although visible in the sequence. A further 8 confirmed CF patients with only one identified mutation were subsequently sequenced. Analysis of this data has to date identified 4 potentially pathogenic changes. Confirmation by Sanger sequencing will be performed. Further sequencing of patients is planned and data analysis with the Roche package will be compared to the Softgenetics NextGENe software package.
NGS has the capacity to transform diagnostic testing for CF with the potential to speed up mutation detection and reduce costs.

WS8.3 Co-implication of the 5′ and 3′ regions of *CFTR* exon 10 in its alternative splicing: prediction to pathology

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A CF patient presents a high level of sweat chloride concentrations and expressed a severe phenotype with pancreatic insufficiency, chronic cough, and bronchial infection (*Haemophilus influenzae* and *Staphylococcus aureus*). Moreover, measurements of changes in nasal potential difference in response to chloride-free isoproterenol show an absence of functional CFTR protein. This patient carries c.3659delC and c.[1210–12T[5]; 1392G>T] in trans.
In this work, we investigated the implications of c.1392G>T mutation (last nucleotide of exon 10) and c.1210–12T[5] (5′ of exon 10) on splicing. The effect of the c.1392G>T mutation was studied in cis with the polymorphism 11TG 5T (the patient's genotype) or 11TG 7T (control) since the polymorphism c.1210–24TG[m]–12[T]n located at the 3′ end of intron 9, is known to modulate the splicing. For this purpose, we have used the technique of hybrid minigene construction. After transfections, the mRNAs were extracted, and after RT-PCR, the amplified products were sequenced.
Our results show that the complex allele c.[1210–12T[5]; 1392G>T] affects the splicing of exon 10 and induces the production of many transcripts: one missing the exon 10 in high quantity, and two other, not yet described, inducing a frameshift. The complex allele c.[1210–12T[7]; 1392G>T] induces two alternative splicings: one with exon skipping and another with frameshift, both in a low quantity. Most of mRNA has the normal length, including the c.1392G>T mutation. We demonstrate here that the complex allele c.[1210–12T[5];1392G>T] induces CF in patient by alternative splicing. These results enhance the crucial role of complex alleles in CF and in our case, on RNA splicing.

WS8.4 Consequences of partial duplications of the human *CFTR* gene on CF diagnosis: mutations or ectopic variations

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CFTR exon 10 and its flanking regions are duplicated in human genome. At least 8 ectopic sequences are found in the genome. These duplicated regions present mutations in respect to the normal CFTR exon 10 sequence.
Due to the polymorphic sequence of the 3' intron 9 sequence, it may appear difficult to sequence exon 10 and some mutations described in this exon could, in fact, be variation observed in an ectopic duplicated exon 10.
In a previous work (A. el Seedy *et al., 2009)* we described a methodology to realise PCR only of exon 10 and not of ectopic regions. We then shown that the previously described c.[1392+6insC; c.1392+12G>A] mutations are in fact a sequence present in an ectopic duplicated exon 10 and not in CFTR gene.
In this work, we analysed mutations described in the CF data base as being CFTR mutations but also found in ectopic exon 10 regions: c.1392G>T (p.Lys464Asn), c.1338_1339delAT (p.Ile444X), c.1235delC (p.Ala412GlufsX30), and c.1247A>G (p.Asn416Ser). We show that these mutations appear to be authentic mutations in *CFTR* exon10 and not ectopic variations in analysed patients. These mutations validate the usefulness of our new strategy in the mutation analysis of this region of *CFTR*.