5 Large scale, high throughput, cystic fibrosis screening using a new kit for CFTR mutation detection

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Cystic fibrosis (CF, OMIM 219700) is an autosomal recessive disorder caused by the presence of mutations in both alleles of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.
Until now, more than 1,900 CFTR gene mutations have been described. The most widespread mutation found is the c.1521_1523delCTT (p.Phe508del, F508del), with a high relative frequency in different populations. The frequency of the DeltaF508 mutation is estimated to about 50% of all CFTR mutations in southern Europe and up to 80–90% in the Northern European Countries. Other mutations typically have a population frequency below 5 percent, or are specific in certain ethnic groups.
This project aims to analyze high number of consecutive anonymous newborn blood spots, from Tuscany and Umbria, Italy, for the detection of the most frequent mutations in the CFTR gene as well as the polythymidine and TG repeats of intron 9.
The qualitative, high throughput, screening is performed using the Devyser CFTR Core Kit, based on PCR allele specific technology and capillary electrophoresis.
The method has been optimized for automated processing to ensure the accuracy and the reliability of the results while minimizing the time and costs.

6 The difficulty of the diagnosis of cystic fibrosis when a new mutation is present

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Introduction: Cystic Fibrosis is the most common hereditary disease with autosomal recessive character in Caucasians. It is characterized by a mutation in the transmembrane conductance regulator protein – CFTR – and its manifestations are essentially respiratory and digestive.
Case report: Girl 3 years old, father and mother smokers; sister 5 years healthy. First hospitalization from 2 to 4 months of age with acute Bronchiolitis with severe distress. Since then multiple hospitalizations, medicated with 125mcg fluticasone 12/12 h, salbutamol 100 mcg SOS and daily respiratory physiotherapy. Esophagus contrasted X-ray and gastric pH monitoring normal, two sweat tests of 55 and 48 mmol/L and chest X-ray with diffuse bilateral infiltrates. Kept persistent wheezing and tachypnea with severe impact on the height-weight evolution. Oriented to Pediatric Pulmonology in July 2010 (10 months), presenting perioral cyanosis, Sp O2 75–80% and bilateral scattered crackles. Fecal elastase normal and pulmonary CT with hypo dense bands at bases in relation to infectious complications and impaction. CF molecular study was negative and *Staphylococos aureus* and *Pseudomonas aeruginosa* were detected in respiratory secretions. Given the persistence of clinical symptoms, was performed extended molecular CF study. R75Q mutation in heterozygosity and sequence variation c.3140–92C>T heterozygosity in the CFTR gene were detected. Treatment was started with evident clinical improvement.
Comments: In this case we want to stress the importance of extended molecular study of CFTR gene when there is a very suggestive clinic of Cystic Fibrosis, since the basic molecular study detects only 80% of mutations.

7 It's a family affair – A diagnostic dilemma of cystic fibrosis within a family

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Objectives and Methods: We present how the diagnosis of CF was made in a family initially through non-identical twins.
Twin 2 presented at 5 weeks with poor feeding, vomiting and offensive stools. Follow-up found she had loose stools, chronic cough and failure to thrive.
Twin 1 was admitted at 7 weeks with respiratory distress and ventilated for 48 hours secondary to a viral respiratory tract infection. She continued to have abnormal stools and a persistent cough.
Failure to thrive blood and stool testing was normal.
Newborn screening IRT and faecal elastase were normal. Sweat tests were equivocal. CF genotyping eventually found 2 mutations: dF508– I1027T and TG12T5 compound heterozygotes.
Standard CF management including Creon was commenced at 4 months. They are thriving and symptoms have settled, followed up by the CF team. Sweat tests became positive at 2 yrs 3 months.
Their 7 year old brother had recurrent lower respiratory infections and abnormal stools. Sweat test and faecal elastase was normal. Genetic testing showed a TG12T5 heterozygote. He responded well to CF treatment.
Their maternal half brother is 12 years old. He has respiratory symptoms, normal stools and is thriving. IRT, sweat test and initial genetic testing are normal. Extended screening is ongoing.
Their father has a chronic productive cough, positive sweat test and is a dF508-I1027T heterozygote, under the care of the adult CF team.
Their mother is an asymptomatic TG12T5 heterozygote.
Discussion: Sometimes the diagnosis of CF is difficult if tests are normal. Some CF genes will cause atypical phenotypes. The diagnosis allows testing of family members from which others may be diagnosed and treated.

8 The *in cis* effect of c.2562T>G and c.2657+5G>A *CFTR* splice variants

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Cystic fibrosis (CF) is caused by mutations in the CFTR gene, approximately 1,900 have been described and most are presumed to be pathogenic. A significant fraction of CF-causing mutations affects pre-mRNA splicing. The potential pathogenic nature of many non-obvious splicing variants has been neglected.
Here, we aimed to provide insights on a new CFTR complex allele carrying the coding SNP c.2562T>G (exon 15) and the splicing mutation c.2657+5G>A (intron 16) in *cis*, thus testing if c.2562T>G has an effect on *CFTR* splicing. We investigated for the occurrence of abnormal splicing using an adequate *in vitro* minigene model mimicking the *in vivo* situation. The splicing products were analysed both at the RNA and protein levels. Our results show that the minigene carrying the c.2562T>G SNP alone showed a similar behaviour, at the RNA and protein level, as that of a WT minigene. A minigene carrying the c.2657+5G>A mutation alone, produced three transcripts: a WT transcript, a transcript lacking exon 16 and a transcript lacking both exons 15 and 16. Exon 16 skipping is the main aberrant transcript resulting from this mutation. The double mutant (c.2562T>G and c.2657+5G>A) produced the same transcripts as the single mutant c.2657+5G>A. According to these *in vitro* data, the c.2562T>G SNP does not seem to have any splicing effect and/or a functional impact on CFTR expression when occurring in *cis* with the c.2657+5G>A splicing mutation.
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