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 polymuthidime 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.

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. Then multiple hospitalizations, medicated with 125mcg fluticasone 12/12h, 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 Staphylococcos aureus and Pseudomonas aeruginosa were detected in respiratory secretions. Given the persistence of clinical symptoms, was performed extended molecular CF study. R75Q mutation in heterozygoity and sequence variation c.3140−92C>G and c.2657+5G>A in cis with the splicing mutation 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.