The role of CFTR genetic testing in diagnosis of cystic fibrosis

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The Shwachman-Diamond syndrome (SDS), is rare disease (1/100 000 live births) caused by compound heterozygous/homozygous mutations in the SBDS gene. SDS is characterized primarily by exocrine pancreatic insufficiency, hematologic abnormalities, including increased risk of malignant transformation, and skeletal abnormalities. The pancreatic insufficiency which is observed in this syndrome, may suggests clinical observation for cystic fibrosis and may caused in delay in diagnosis of patient. A Polish girl with abnormal result of IRT during newborn screening programme for cystic fibrosis was directed for clinical and genetic evaluation for cystic fibrosis and CFTR gene analysis. Sequencing of whole coding region of the CFTR gene together with identification of frequent for Polish population mutations: dele2,3(21kb) and 3849+10kbC>T were performed. No pathogenic CFTR variants were identified. Only the IVS8+5T variant, without clinical consequence according to CF recommedation was identified. Because patient presented chronic pancreatic and liver disease, anemia, decrease concentration of stool elastase, hypertranssamm-inasemia, borderline/normal sweat test results and short stature were observed, clinical observation and genetic analysis for Shwachman-Diamond syndrome was suggested as a next diagnostic stage. Analysis of coding region of the SBDS gene was performed. Two known mutations: c.255+2T and p.C119Y, each in one SBDS allele were identified. Biparental inheritance of identified mutations was confirmed. In conclusion, because of the similarity of some symptoms in cases of atypical abdominal form of the CF, the SDS should be taken into account during diagnosis process.

Improving the sensitivity to identify CF mutations

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From the CFTR gene cloning, the scanning techniques (SSCP/HD, DGGE, DHPLC, HRM, MLPA) have been applied to detect CF mutations. Using different scanning procedures, we have identified over 250 CFTR mutations accounting for 97% of CF alleles. To evaluate this sensitivity, we have assessed the CFTR direct sequencing analysis in front of the scanning strategy. Ten samples with partial/total unknown genotype have been selected among our patients. Three out 20 alleles were already known (p.Phe508del, p.Cys592Tyr and c.1209G>A). Sequencing analysis showed one homozygous patient for the p.Ser549Arg mutation, one patient heterozygous for p.Met1101Lys and two patients bearing the p.Gly673X. Revision of our previous analysis has evidenced: (1) primers used in the early 1990s were unable to detect the p.Met1101Lys; (2) absence of heteroduplex bands in the two other mutations. It is well known the limitation of these techniques to detect homozygous changes; however, we do not find an explanation for the p.Gly673X mutation localized in the first half of exon 14. Usually, this large exon (723 nt) is analyzed in two overlapping fragments and other 23 different mutations have been identified in our series. Once again, we have taken advantage of the microsatellite haplotype (IVSSCA-IVS17bTA) associated to this mutation. Available CF samples still uncharacterized showing the 16–34 haplotype (n = 6) were sequenced specifically. The p.Gly673X mutation was identified in another patient. Our results evidence a higher sensitivity using sequencing analysis, a powerful tool now more affordable with the new equipments.

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