54 Complete screening of the CFTR gene in idiopathic chronic pancreatitis

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Introduction: Chronic pancreatitis (CP) is an inflammatory disease characterized by a progressive and irreversible loss of exocrine and endocrine pancreatic function. In developed countries, an excessive consumption of alcohol explain the majority of the CP cases, followed by "idiopathic" causes which account for about 20% of the cases. In 1998, the CFTR gene has been suggested to play a role in idiopathic chronic pancreatitis (ICP). Moreover, some CFTR mutations have been shown to selectively disrupt bicarbonate conductance and therefore selectively target the pancreas for CFTR-associated injury. In order to determine whether or not different types of CFTR mutations affect the risk of developing CP, we recruited 244 patients with ICP who have developed the disease before or at the age of 20 years.
Methods: The 27 exons and all the intron/exon junctions of the CFTR gene were screened by denaturing high-performance liquid chromatography (DHPLC) technique or by High-resolution melting analysis. The intron 8 poly (T) variants were analyzed by using a fluorescent multiplex PCR. Finally, quantitative fluorescent multiplex PCR (QFM-PCR) was performed to screen genomic rearrangements.
Results: About 30% of ICP patients are carrying at least one mutation of the CFTR gene including the T5 allele in intron 8. This frequency appears about 10-fold higher that the expected carrier rate in French population. Finally, it appears that about 10% of ICP patients are compound heterozygotes (mild/mild or severe/mild mutations).
Conclusion: These data show that not only compound heterozygosity, but also CF carrier status for different types of CFTR mutations, increase the risk of developing CP. So, the idiopathic CP could be classified as so called CFTR related disease.

55* Identification of CFTR rearrangements by a CGH locus specific array

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Mutation in the CFTR gene is responsible of a large spectrum of clinical phenotype from severe CF to male sterility due to congenital bilateral aplasia of the vas deferens (CBAVD). More than 1500 CFTR mutations have already been described and new semi quantitative approaches have been developed to evidence gross rearrangements in the 27 exons of the gene. We have shown that these deletions account for about 1 to 3% of the CF disease causing mutations worldwide. We have described 8 new rearrangements and characterized their breakpoint junctions. However this technique missed the intronic region as well as the 5′ and the 3′ UTR part of the gene. In this work, we designed an Agilent 15K custom array on the locus CFTR to carefully analyse deletions or duplications. This oligonucleotide array enable us to cover 2 Mb encompassing the CFTR locus as well as the gene itself. We tested 114 patients with CF in this study and evidenced the 11 deletions collected in our laboratory from five countries worldwide. About the 109 DNA samples for whom the genotype was incomplete, we observed 5 duplications and 2 deletions of several exons. We also screened 160 patients with CBAVD and 2 deletions were observed in intronic region. This analysis was useful to determine accurately the 5′ and 3′ breakpoints of the variation. This allowed a rapid design of primers to amplify the breakpoint junction and to obtain the sequence permitting the characterisation of the defect at the molecular level. Our custom array is an excellent tool allowing a rapid detection and a characterization of the rearrangements not only the 27 exons of the gene but also those located throughout the 200 kb genomic region of the CFTR gene.
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56 A two-days full-scanning of the CFTR gene for simultaneous detection of point mutations and large rearrangements using High Resolution Melting analysis (HRM) in combination with quantitative real-time PCR

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Scanning technique of the CFTR gene is usually required when the mutated alleles, about 20%, are not identified by commercial genotyping assays. Distinct methods such as D-HPLC or MLPA are routinely used to detect either point mutations or large rearrangements. We report here a new one-step method for detecting both types of gene modifications.
All 27 coding/flanking regions of the CFTR gene and 2 reference genes were amplified under identical fluorescent conditions. Amplicons were then analysed using the Lightcycler 480 (Roche) according to a closed-tube methodology not requiring a post-PCR processing. Results of melting analysis (HRM) and relative quantification were compared with those of reference methods, DGGE/D-HPLC and QMPSF/MLPA respectively.
A total of 344 different point variations, 11 various deletions or duplications spanning 21 exons were successfully assessed in two separated laboratories. In this series, the sensitivity of our method was 99.7% for heterozygous variants and 50% for homozygous variants. Thus, this one-step method is at least as efficient as the reference methods. Results were strongly reproducible, not operator dependant and did not vary using DNA from different extraction methods (including DNA from Guthrie cards). Moreover, PCR products were fully compatible for subsequent restriction or sequencing analysis.
In conclusion, this combined HRM-real-time PCR method provides a robust, faster, and low-cost alternative tool for complete CFTR scanning for diagnostic purpose and can be easily transposable to other gene analysis.

57 Strategy for prenatal diagnosis of cystic fibrosis in Russia

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Twenty different CF mutations (CFTRdele2,3(21kb), 394delTT, 604insA, L138ins, 621+1G>T, R334W, R347P, F508del, 1677delTA, G542X, 2143delT, 2184insA, K598ins, 3821delT, S1196X, 3677insTCAA, 3849+10kbC>T, W1282X, 3944delTG, N1303K) were accounted for 77% of CF alleles in Russian patients. We have performed 123 prenatal diagnoses for 98 couples. Two cases included twin pregnancies: one homozygous and one heterozygous. 74 families with 17 different genotypes were fully informative for direct DNA analysis performed by using multiplexPCR, heteroduplex analysis and restriction analysis. In 19 families only one parent mutant allele was known and in 5 families none of CF mutations were identified, so prenatal analysis was done by indirect DNA analysis using haplotype analysis of four dinucleotide repeats, one tetranucleotide repeat and four biallelic restriction polymorphic sites. Materials used for fetal DNA analysis were CVS samples. 30 fetuses were affected, 66 – heterozygous carriers and 28 were healthy with two normal alleles of CFTR gene. In all cases when prenatal diagnosis indicated that the fetus had CF the couples chose pregnancy termination.
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