25 Extensive genetic analysis in non-classic CF patients

Y. Fichou[1,2], M.P. Audrézet[1,2], P. Trouvé[1], A. Chauvin[1], C. Le Maréchal[1,2], C. Férec[1,2]. [1]*Inserm, U613, Génétique Moléculaire et Génétique Epidémiologique, Brest, France;* [2]*Laboratoire de Génétique Moléculaire, CHU Morvan, Brest, France*

Although more than 1,500 mutations and polymorphisms have been reported within the cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene, the genetic basis of the disease remain obscure in some CF patients. Moreover, it has been suggested that other genes may be involved in the pathogenesis of the disease. A cohort of unambigously characterized CF patients (n = 16), who carry at least one *CFTR* mutation-negative allele after the complete scanning by modern, potent techniques, was investigated by alternative strategies: quantitative multiplex PCR of short fluorescent fragments (QMPSF) of evolutionarily conserved regions (ECRs) within both the distal *CFTR* promoter and introns, as well as sequencing of *CFTR* 3′-UTR and the epithelial Na^+ channel (ENaC) subunit-encoding sequences. QMPSF analysis and sequencing revealed a specific, atypical haplotype that may be associated to the genetic defect in one patient. While a total of two and thirteen polymorphisms were respectively identified within the *CFTR* 3′-UTR and ENaC-encoding genes, major disease-causing mutations could not be detected in the other patients. In spite of an extensive analysis of the CF chromosomes in our cohort, genetic factors of the disease in these subjects remain to be found and additional studies must be carried out. Nevertheless, our strategy may have applications for the study of both distal promoter and intronic variations in numerous genes.

26 Molecular, cellular and functional study of seven rare mutations of CFTR

F. Fresquet[1], A. Sterlin[1], P. Melin[1], M. Baudis[1], M.C. Pasquet[1], F. Becq[1], A. Kitzis[1], F. Bilan[1]. [1]*Institut de Physiologie et de Biologie Cellulaires UMR CNRS 6187, Poitiers, France*

To date, about 1500 mutations have been found within human CFTR sequence. These mutations could be classified according to their degree of severity in CF disease (severe, mild or polymorphic). While the most common mutations (F508del and the 30 mutations routinely screened in CF patients), are well characterized, few data are available for rarer mutations. So genetic counseling is particularly difficult when fetuses or CF patients present these orphan variations. We have developed in vitro biology assays to characterize mutation impact upon CFTR maturation process, trafficking and activity in order to establish a genotype/phenotype correlation. We present here our results obtained with seven rare mutations which have been isolated in our lab.

We have used a GFP-tagged CFTR construct to generate these mutations by site-directed mutagenesis. Each plasmid was transfected in COS-7 cells to express the mutated proteins. We visualized CFTR trafficking by confocal microscopy and its cellular localization was determined using several markers. By western blot, we studied CFTR maturation process by quantifying the relative amount of mature and non mature CFTR (C and B bands). We evaluated CFTR channel activity by efflux assays, using notably pharmacoperones (MPBs or Miglustat).

Our results indicate a clear physiopathological effect of the studied CFTR mutations (retainment in ER, abnormal maturation, null CFTR activity). These data, in relation with clinical survey of the patients should be useful for genetic counseling. Moreover, this work could contribute to the improvement of pharmacological studies of CFTR mutations. In conclusion, we recommend that each new or orphan CFTR mutation is subjected to this type of study.

27 Identification and characterization of three CFTR gene partial duplications

M. Legendre[1], A. LeFloch[1], F. Niel[1], C. Gameiro[1], J. Martin[1], D. Grenet[2], J. Derelle[3], V. Izard[4], C. Costa[1], M. Goossens[1], E. Girodon[1]. [1]*Laboratoire de Génétique, CHU Henri Mondor, Créteil, France;* [2]*Pneumologie, Hôpital Foch, Suresnes, France;* [3]*Médecine Infantile, Hôpital d'Enfants, Vandoeuvre-les Nancy, France;* [4]*Urologie, CHU Bicêtre, Le Kremlin-Bicêtre, France*

Background: CF and CFTR-related disorders are mainly due to point mutations scattered over the whole CFTR gene. Search for large CFTR rearrangements using semi-quantitative fluorescent multiplex (QFM) PCR assays is now part of the molecular diagnosis and allows to identify 2% of CF alleles. Rearrangements mainly comprise single or multiple exon deletions; duplications are rare and are indeed more difficult to detect and characterize. Of the four CFTR duplications reported, three were detected in our laboratory by QFM-PCR, in two CF patients and a CBAVD patient. They involved exons 4–8, 10–18 and 11–13, respectively, in trans of another CFTR mutation.

Methods: The duplications were characterized by using a combination of long-range (LR) PCR, digestion of LR-PCR products and sequencing.

Results: Two duplications were fully characterized, in direct tandem each: dup10_18 (~70kb long), and dup11_13 (~17kb long). Characterization of the remaining dup4_8 is in process. However, given the classical CF phenotype of the patient, we hypothesize that the duplicated region is located inside the CFTR gene and interferes with the transcription, translation or maturation process, thus resulting in a null mutation.

Conclusion: Effective tools are required to detect duplications, which may indeed be under-diagnosed. Refinement of the breakpoints is important to confirm a deleterious effect and should contribute to understand the duplication mechanism.

28 Chronic sinusitis in CF child carrying two missense mutations S519G and G576A in the NBF1 domain of CFTR

M.N. Feuillet-Fieux[1], J.P. Bonnefont[2], M. Ferrec[1], I. Sermet[3], B. Lacour[1], G. Lenoir[3]. [1]*Biochemical laboratory, Hôpital Necker, Paris, France;* [2]*Medical Genetics, Hôpital Necker, Paris, France;* [3]*Pediatric Department, Hôpital Necker, Paris, France*

Chronic sinusitis invariably affects Cystic Fibrosis (CF) patients. Certain genotypes may be more likely associated with sinus symptoms and the main allele was F508del (76%) (Jorissen MB 1999 Am J Resp Crit Care Med 159:1412).

We reported a case of eleven-year-old girl, from South of France, with digital hippocratism, presenting in childhood pansinusitis with Pneumococcus lung infection, episodes of asthma and a borderline elevated sweat test (56 mmol/l). The CFTR gene was entirely screened for mutations by DGGE-sequencing of all exons and flanking intronic regions. The genotype associated two missense CFTR mutations in the NBF1 region, namely: (i) 1687A>G (S519G exon 10), once reported in a non CF patient, and (ii) 1859G>C (G576A exon 12) previously considered as a neutral polymorphism, later detected in classical CF, likely to affect the CFTR mRNA, lacking exon 12 (Pagani F. Hum Mol Genet 2003; 12,10: 1111), found in cis on the paternal allele; (infertile biological father needed in vitro fecundation). Five additionnal polymorphisms were found: 3 intronic, namely (GATT)6/(GATT)7, IVS6a, 1001+11C>T, IVS6b, and (TG)10T9/(TG)10T7, IVS8, 2 exonic, 2694T>G (T854T exon 14a) and 4521G>A (Q1463Q exon 24) at heterozygous state. The 470V was not present.

Previously reported, CF carrier status might predispose to chronic sinusitis without nasal polyps (Wang XJ 2000 JAMA 11;284:1814). Although greater series of CF with pansinusitis would be necessary to verify these clinical symptoms-mutations-polymorphisms associations, that constitutes an approach in genotype-phenotype relation in such mild CF disease with chronic rhinosinusitis.

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