WS8.5 Help for the interpretation of unclassified variants: example of the UMD-*CFTR*-France Locus Specific Database

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Exhaustive research to identify genetic disorders in the *CFTR* gene leads to the detection of constantly increasing number of sequence variations of unknown clinical significance (UV), specially with the event of new sequencing technologies. In 2009 the collaboration of 9 French specialized laboratories led to collect molecular and minimal clinical data not only in CF but also in *CFTR*-RD patients and "compound heterozygous" unaffected parents. These manually-curated data are integrated into a database: UMD-*CFTR*-France. Currently, it contains genotypes and haplotypes of 3473 subjects: 68.7% CF, 24.16% *CFTR*-RD, 7.14% analysed in another context (NBS, fetal bowel anomalies . . .). 581 different variants have been reported (12507 entries): 62.3% disease-causing mutations, 8.4% non-pathogenic alterations and 29.3% UVs (mostly rare missense variations). To assess UV pathogenicity we have worked on a classification (UV1 to 4) based on frequency, first description in the Cystic Fibrosis Mutation Database, familial segregation, results of *in silico* studies (e.g SIFT, PPH2, HSF, MaxEnt) and data from the literature (epidemiology, functional studies). The collection of data from the general population is in process. Pooling of these data should significantly help the interpretation of rare variants, the analysis of correlations between genotypes/haplotypes and phenotypes, and participate in the improvement of diagnosis and genetic counseling.

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WS8.6 Decision algorithm and scoring method for the classification of variants of unknown clinical significance in the *CFTR* gene

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Molecular diagnosis of cystic fibrosis and CFTR-related disorders led to the worldwide identification of nearly 1900 sequence variations in the *CFTR* gene. Except for a set of 21 frequent disease-causing mutations, the majority of them are private point mutations or microinsertions/deletions. Thus, it is difficult to establish *a priori* the effect of rare intronic, synonymous or missense variants on the function of the encoded protein and therefore their involvement in the disease, which directly impacts genetic counselling. In this context we developed a model for the classification of variants of unknown clinical significance according to international guidelines (*Clinical Molecular Genetics Society*, 2007) and specifically adapted to the *CFTR* gene. We built a decision algorithm and defined a rating scale for a series of major and minor criteria, including thorough *in silico* and *in vitro* functional studies at mRNA and protein level. We first applied this model to 15 unclassified variants (10 intronic and 5 exonic) found in our cohort of patients. Six variants were classified as probably non-pathogenic considering their impact on splicing while nine, including 3 apparent missense variants, were considered as likely or probably pathogenic. This preliminary study was designed to test and refine our model, which is being validated on a wide range of *CFTR* variants. Finally, our data provide strong arguments to select variants that require transcript analysis in nasal epithelial cells to confirm *in vitro* results and establish genotype/phenotype correlation.

WS8.7 Preimplantation genetic diagnosis for cystic fibrosis using multiplex fluorescent PCR

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Objectives: Cystic Fibrosis (CF) is one of the most frequent indications for preimplantation genetic diagnosis (PGD), allowing couples at-risk of bearing offspring with CF to reproduce without fear of having an affected child. We present here a fluorescent multiplex PCR approach for PGD for CF as well as the results of our large experience over a 8-year period.

Methods: In order to provide a reliable PGD procedure for CF, we have developed an efficient fluorescent multiplex PCR protocol allowing the simultaneous analysis of the p.F508del mutation together with eight polymorphic sequences either located within or on each side of the *CFTR* gene. We have applied this PCR protocol in 66 PGD cycles from 32 couples, including

i. couples with both members heterozygous for a severe or large spectrum *CFTR* gene mutation (n = 23),
ii. a CF-affected member (n = 4) and
iii. CBAVD in males (n = 5).

All (but one) cycles with embryo biopsy resulted in embryo transfer. One to 3 genetically unaffected embryos (healthy or carrier) were selected for transfer in 47 cycles resulting in 17 clinical pregnancies (36.2% per transfer). Sixteen babies were born and no child was diagnosed with CF after the newborn screening test that is systematically performed in France for CF. One pregnancy is still ongoing.

Conclusion: These results show that PGD is presently a practical approach for prevention of CF in affected families. The multiplex PCR protocol we have developed is higly reliable enabling an accurate diagnosis in all cases. It may be applicable to all the couples requesting PGD for CF analysed in our PGD program, broadening the range of prenatal testing options for these families.

WS8.8 The diffusion processes of the *CFTR* mutations in Brittany (France)

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The aim of this study is to show that historical data can help understanding the spreading process of CFTR mutations on a specific area that is particularly affected by the disease.

The population at the root of this study is composed of patients clinically diagnosed as suffering from CF and having lived in Brittany (705 ind). Their ancestry was traced back and brought together more than 250 000 kinspeople.

The carriers who share the same CF mutation are kindreds. The mapping of their common ancestors' living places show a differential distribution, depending on specific CF mutations.

At the ancestors' level, we observed marital unions at an early age, particularly women's, and frequent remarriage, particularly men's. In addition, married couples were prolific, thus allowing more genetic transmissions. The geographical stability that prevailed at the time of the wedding does not seem to produce genetic diversity. Moreover, we reckoned that in terms of life expectancy there might be some selective advantage to being a healthy carrier.

Inbreeding was in no way a key factor in this study. Only 0.8% were born from first or second cousin unions.

At the ancestors' level, we must go back to the 7th generation to see a higher proportion of close kinship. Therefore, more often than consanguinity, it is endogamy which tends to carry on a certain degree of genetic homogeneity.

CF frequency of occurrence and its Breton distribution today can be accounted for by the presence of a harmful gene combined with high fertility, a relatively settled population with a limited availability of possible partners, and the selective advantage this harmful gene was for healthy carriers.