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COMMENTARY

A commentary on the novel complex allele [A238V; F508del] of the *CFTR* gene: clinical phenotype and possible implications for cystic fibrosis etiological therapies

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The paper of Diana *et al.*[1] concerns the identification of a new c.[713C>T; 1521_1523delCTT] p.[Ala238Val;Phe508del] (legacy name: [A238V;F508del]) complex allele of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. An allele is defined complex when 2 (or more) mutations are arranged *in cis* (i.e., on the same allele of a gene). *CFTR* complex alleles is a usually neglected topic. However, it is highly relevant for both a better comprehension of the genotype–phenotype relationship and for the effectiveness of the emerging personalized therapies for cystic fibrosis (CF). Systematic studies have not yet been performed and we are in the early phase of isolated and scattered observations. Nevertheless, about 50 complex alleles of *CFTR* have been so far described. For a list (probably non-exhaustive) of published *CFTR* complex alleles, see ref. 2 integrated by refs 3–10.

In this case, the authors found *in cis* the A238V and the F508del mutations. Although the A238V is an already known CFTR mutation, this is the first time it is described within a complex allele. The authors analyzed a case series made up of 218 patients. At a first analysis, 63 patients resulted to be homozygous F508del/F508del and 155 patients resulted to be compound heterozygous for the F508del and another CFTR mutation. The complex allele was found in 18 patients, which represents a rather high prevalence: overall allelic prevalence, 0.04 (18 out of 436 alleles); prevalence in F508del alleles, 0.06 (18 out of 281 alleles); overall prevalence in patients, 0.08 (18 out of 218 patients); prevalence in compound heterozygous patients (F508del/other mutation), 0.07 (11 out of 155 patients); prevalence in F508del/F508del homozygous patients, 0.11 (7 out of 63 patients).

The authors also highlight possible functional consequences of this newly discovered complex allele. It is suggested that CF patients with the complex allele have a higher systemic inflammation, possibly reflecting lung inflammation, than patients with similar mutated genotypes without the complex allele. In turn, the enhanced inflammation seems to be linked to a worsening of pulmonary function indicators.

From functional point of view, when the mutations of a complex allele are never found separately, it is impossible to evaluate the specific clinical effects of each mutation, as well as it is hard to ascertain if the complex allele produces some modulated (worsened or ameliorated) clinical presentation in respect to the single mutations. In their work, the authors could distinguish the effect of the [A238V;F508del] at least from that of the F508del alone, although the absence in their case series of patients with A238V alone did not allow the reciprocal comparison. Advisable future prospects of the studies on complex alleles may be their systematic experimental functional characterization. *In vitro* studies based on specific constructs transfected in suitable recipient cells should be performed. They would be aimed to the evaluation, at biochemical and cellular level, of the overall effect of each known complex allele, as well as to distinguish the relative functional contribution of each mutation. This approach would complement the clinical findings. Awaiting the time for such a massive, and not easy, experimental approach, every study linking biochemical, microbiological and clinical aspects of CF to specific complex alleles are welcomed.

Complex alleles may have diagnostic, prognostic and therapeutic consequences (Figure 1). From the diagnostic point of view, the common approach of ending the mutational search after the first two mutations on different alleles have been found, appears as a severe limitation of the accuracy of *CFTR* genotyping.[2,10] This may be seen as an unavoidable consequence of the use of mutational panels, as extended they are. To be meaningful, mutational search protocols should include complex alleles at least when one mutation already known to be *in cis* with another is found. In addition, an extended mutational search should be planned whenever clinical discrepancies arise in probands with apparently identical genotypes.

The prognostic ability depends on a predictable genotype–phenotype relationship. A first step within the path from genotype to phenotype is the transition from the *CFTR*-mutated genotype to CFTR protein residual function. This step is influenced by *CFTR* intragenic variability, including the combination of mutations *in trans* and *in cis*. The

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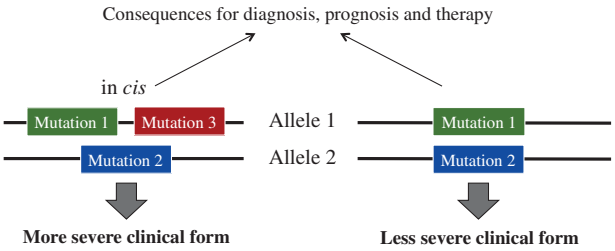

Figure 1 Schematic representation of genotypes with or without a complex allele. Mutation 1 and Mutation 2 are *in trans* arrangement and are present in two patients. The patient on the right has only these two mutations whereas the patient on the left has Mutation 3 also on the same allele of Mutation 1 *in cis* arrangement. In this case, it is assumed that the effect of Mutation 3 worsen that of the Mutation 1. The presence of the complex allele has diagnostic, prognostic and therapeutic consequences. If the Mutation 3 remains undetected, an inaccurate genotyping will result with the two patients showing the same apparent genotype. This may have as a consequence an apparent poor genotype–phenotype relationship.

disclosure of complex alleles is crucial to ameliorate the comprehension of the genotype–phenotype relationship and, consequently, the prognostic skill.

At least 2 mutational class-specific treatments are already in clinical use for CF.[11] It should be evident that an inaccurate mutated genotype may severely hamper the therapeutic response to these kind of approaches, due to the presence of a second and possibly undetected mutation. From this point of view, the [A238V;F508del] may have a considerable impact on the CF-personalized therapy. In fact, it is one of the few complex alleles found *in cis* with the F508del mutation that, due to the fact that it is the most frequent CF mutation, is a primary target of personalized therapies. The high prevalence of this complex allele, may have as a consequence that up to 11% of the patients homozygous for F508del mutation and up to 7% of patients compound heterozygous for it, may have a reduced therapeutic answer due to the presence of A238V also on the same allele. Great attention should be paid to this subject, also taking into account the high costs of these new mutation class-specific therapies. Obviously, similar considerations may be posed also for other complex alleles, as well as for a future application of CF gene therapy based on gene targeting approaches.

Taking together the scattered experimental data available, complex alleles seem to account for a greater degree of variability than usually acknowledged with consequent diagnostic, prognostic and therapeutic spin-offs. For this reason, each description of a new complex allele and the corresponding phenotypic information may aid to compose the puzzle. All researchers working on complex alleles invariably deal with their relatively low abundance if taken individually. This complicates both the statistical analysis and clinical functional characterization. It appears to be mandatory to organize international multicentric research projects that can collect case series sufficiently large to overcome these limitations.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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