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The influence of CFTR complex alleles on precision therapy of cystic fibrosis

**Author names and affiliations.**

Benoit Chevalier<sup>1,2</sup> and Alexandre Hinzpeter<sup>1,2</sup>

1 INSERM, U1151, Institut Necker Enfants Malades, INEM, Paris, France

2 Université Paris Descartes, Paris, France

**Corresponding author**

Alexandre Hinzpeter: [alexandre.hinzpeter@inserm.fr](mailto:alexandre.hinzpeter@inserm.fr)

**Keywords**

Cystic fibrosis, CFTR, complex alleles, cis-mutation, pathogenic variant, Splicing defects, Orkambi

## **Abstract**

*CFTR* is an extensively studied gene and multiple sequence variants have been identified, many of which still need to be defined as neutral or disease causing. Complex alleles are defined when at least two variants are identified on the same allele. Each pathogenic variant can affect distinct steps of the *CFTR* biogenesis. As *CFTR* modulators are being developed to alleviate specific defects, pathogenic variants need to be characterized to propose adequate treatments. Conversely, *cis*-variants can affect treatment response when defects are additive or if they alter the binding or efficacy of the modulator. Hence, complex alleles increase the complexity of *CFTR* variant classification and need to be assigned as neutral, disease causing or modulating treatment efficacy. This review was based on a symposium session presented at the 16th ECFS Basic Science Conference, Dubrovnik, Croatia, 27 to 30 March, 2019.

## Main text

Cystic fibrosis (CF, MIM# 219700) is an autosomal recessive disease caused by pathogenic variants within the *CFTR* gene. To date, more than 2000 variants have been identified in patients with CF and milder monosymptomatic or oligosymptomatic diseases called CFTR-related disorders (CFTR-RD). They have been categorized in six classes depending on their effect on protein production, maturation, folding, activity, conductance, stability at the cell surface [1,2].

Recent advances have enabled to propose pathogenic variant -based personalized therapy in CF, with molecules which alleviate specific defects, such as potentiators (e.g. Ivacaftor) which activate CFTR gating mutants such as p.Gly551Asp (legacy name G551D) [3,4] and correctors (e.g. Lumacaftor) which favor cell surface expression of misfolded CFTR such as p.Phe508del (legacy name F508del)[4]. In this case, p.Phe508del functional restoration can be enhanced by combining Lumacaftor and Ivacaftor (Orkambi) [5]. However, Orkambi was found to be suboptimal in clinical trials; new correctors, alone or in combination, expected to enhance treatment efficacy, are under evaluation [6]. These variant based therapies led to the re-classification of *CFTR* pathogenic variants depending on their sensitivity to treatments, referred to as theratyping [7].

These informations are implemented in the CFTR2 database (cftr2.org) which enables to define the appropriate treatment for the considered variant [8]. While being continuously implemented, CFTR2 contains information for only a subset of variants. In fact, many of the 2000 pathogenic and non-pathogenic variants referenced in the CF Mutation Database (<http://www.genet.sickkids.on.ca/app>) still need to be assigned as CF-causing or not and may be referred to as variants of unknown significance (VUS). These variants can be located within exons, introns, 5' or 3' untranslated regions. With the advances in sequencing technologies, it is now possible to sequence the *CFTR* locus with high-throughput, encompassing regions containing distant regulatory elements which control the spatio-temporal expression levels of CFTR. This has led to the identification of a new set of variants [9,10] which need to be further characterized to propose efficient therapies.

Pathogenicity or non-pathogenicity of cis variants can be assessed using clinical features [11] or by measuring CFTR activity in relevant tissues when available, such as nasal epithelial cells [12] or colon biopsies [13]. As many of these variants have been identified in discrete cases, clinical data is scarce for many variants and assessment of their pathogenicity usually requires functional assays. Characterization can be achieved using in vitro assays to evaluate the effect of the considered variants on specific cellular processes, such as gene expression

level, mRNA splicing, protein folding or channel activity. Luciferase based in vitro assays have been developed to measure effects on transcriptional expression and stability: minigenes measure effects on mRNA splicing, while biochemical assays evaluate protein folding, maturation or stability and electrophysiological assays measure the effect on channel activity. Each variant-can be neutral or affect independently a key step in the CFTR biogenesis. Some pathogenic variants have been shown to affect multiple steps, with defects being additive. This can also occur in the case of complex alleles. The actual impact of additive defects however appears difficult to apprehend using in vitro assays and the final outcome on CFTR function can only be assessed in patient derived samples.

Complex alleles are defined as the presence on the same parental allele of at least two sequence variants. Additive defects were shown for different complex alleles, such as the p.[Arg74Trp;Val201Met;Asp1270Asn] (legacy name [R74W;V201M;D1270N]) allele which displays partial exon 3 skipping due to p.Arg74Trp [14], and a protein with reduced function due to p.Asp1270Asn [15] and p.Val201Met [16]. p.Arg74Trp and p.Asp1270Asn alone may not be sufficient to cause a CFTR disease in combination with a CF-causing variant in the other parental allele [16]. Similarly, p.Leu997Phe (legacy name L997F) was identified in two distinct allelic contexts, either alone or in combination with p.Arg117Leu (legacy name R117L). Clinical evaluation of patients showed that p.Leu997Phe could be associated to CFTR-RD while the p.[Arg117Leu;Leu997Phe] is associated with a mild CF phenotype [17]. Another example is the p.Arg117His pathogenic variant (legacy name R117H) which is associated with CF when in cis with the [TG12T5] intronic splicing variant, while both variants are associated with CFTR-RD [18]. For these alleles, the identification of the proper therapy could be challenging. Indeed, variants affecting different processes would need combinatory therapies addressing these distinct defects, e.g. targeting splicing and protein folding.

On the other hand, some variants were shown to be neutral and in this case only one variant is disease-causing, e.g. p.[Ile148Thr;Ile1023\_Val1024del] (legacy name [I148T;3195del6]) where p.Ile148Thr was shown to be neutral and p.[Ile1023\_Val1024del] is the CF-causing variant [16]. Indeed, variants referenced in the CF Mutation Database as CF-causing because they were observed in CF patients could in fact have residual or normal function and be in cis of a deleterious pathogenic variant that still needs to be identified. Such pathogenic variants could be located in another exon or in distant regulatory elements. As many of these variants have been identified in discrete cases, it would be challenging to perform retrospective genetic analysis of the complete locus to identify these cis variants.

Finally, c.-234T>A (legacy name -102T>A) was shown to enhance *CFTR* promoter activity which reduced the severity of the phenotype associated to the p.Ser549Arg CF-causing variant (legacy name S549R)[19]. This latter example illustrates the possibility to enhance *CFTR* expression to reduce disease severity. *CFTR* exon 10 could represent a good target to increase *CFTR* transcripts as it is known to be associated to high basal exon skipping. Alternatively, strategies targeting the 3'UTR of *CFTR* which contains destabilizing elements could also be beneficial. These strategies could be combined to modulators to enhance the amount of targetable CFTR, hence increasing treatment efficacy.

Treatments aiming at enhancing the activity of p.Phe508del-CFTR such as Orkambi combination therapy resulted in a modest efficacy and very important variability of the response [5]. This could be linked to the destabilization of Lumacaftor rescued p.Phe508del-CFTR by chronic Ivacaftor treatment [20,21], the action of Orkambi on mucociliary clearance [22] or the presence of cis variants which could either reduce the amount of full-length CFTR available for the corrector or modify the folding of the channel and therefore the activity of the corrector. This last hypothesis was recently illustrated for complex alleles involving p.Phe508del, which have been described in the literature and in CF mutation databases (CFTR1, CFTR2 and *CFTR*-France; summarized in Table 1). As soon as p.Phe508del has been identified in a diagnostic setting after a first screening of the frequent CF-causing variant, other possible cis variants are not routinely searched and their frequency is probably underestimated. Nonetheless, their frequency turned out to be low [10], but higher in regional cohorts, e.g. the p.[Ala238Val;Phe508del] in the South of Italy [23] or p.[Phe508del;Ile1027Thr] in Brittany, France [24].

Cis pathogenic variants can be associated to multiple defects, as illustrated in Figure 1. While variants altering the transcriptional activity of CFTR or the stability of the transcript have not been described yet, enhanced exon skipping was observed with c.609C>T (p.Ile203) and c.2770G > A (p.Asp924Asn), two defects that would in turn reduce the amount of correctible p.Phe508del-CFTR available in cells [25]. Additionally, p.Leu467Phe was found to reduce CFTR maturation by half and, when associated to p.Phe508del, prevented the response to corrector treatments [25]. These results illustrate additivity of the folding defects, rendering the double mutant resistant to Lumacaftor rescue. Complex alleles were also in part involved in the absence of response to Orkambi in a cohort of p.Phe508del homozygous patients [26]. Indeed, three complex alleles were identified in non-responders while none could be identified in responders. While c.609C>T (p.Ile203) was previously identified as a splicing mutant, p.Phe87Leu was found to prevent Lumacaftor rescue without altering protein folding.

Interestingly, this amino acid is in the vicinity of the proposed binding site of VX809 [27] and could affect Lumacaftor binding. Hence, cis pathogenic variants could reveal important regions involved in the sensitivity of CFTR to modulators.

### **Future directions**

The use of next generation sequencing (NGS) techniques enables to sequence the *CFTR* locus in CF patients, leading to a better characterization of the patient's genotype and the identification of novel sequence variants. The impact of these variants will need to be evaluated using in vitro assays and novel cellular models both patient-derived or generated using the CRISPR/Cas9 technology. The identification of additional variants in cis with disease-causing variants could enable to predict response to current therapies and propose combinatory treatments targeting distinct defects.

### **Summary**

CFTR complex alleles carry at least two sequence variants on the same allele. Each pathogenic variant can affect distinct steps of the CFTR biogenesis. Cis variants can affect treatment response when defects are additive or if they alter the efficacy of the modulator. Hence, complex alleles increase the complexity of the pathogenic variant landscape and need to be taken into account to propose an optimal treatment.

**Conflict of interest**

Authors have no conflicts of interests to declare

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**Table 1. CFTR p.Phe508del complex alleles reported in the literature.**

The name of the complex allele is indicated and the exon number carrying the cis variant.

	Mutation name		Exon number
	<i>HGVS</i>	<i>Legacy name</i>	
<b>1</b>	c.[157T>G;1521_1523delCTT]	p.[Leu53Val;Phe508del]	[L53V;ΔF508] 2
<b>2</b>	c.[259T>C;1521_1523delCTT;3080T>C]	p.[Phe87Leu;Phe508del;Ile1027Thr]	[F87L;ΔF508 ;I1027T] 3, 19
<b>3</b>	c.[390C> G;1521_1523delCTT]	p.[Leu130;Phe508del]	[L130;ΔF508] 4
<b>4</b>	c.[609C>T;1521_1523delCTT]	p.[Ile203;Phe508del]	[I203;ΔF508] 6
<b>5</b>	c.[713C>T;1521_1523delCTT]	p.[Ala238Val;Phe508del]	[A238V;ΔF508] 6
<b>6</b>	c.[1399C>T;1521_1523delCTT]	p.[Leu467Phe;Phe508del]	[L467F;ΔF508] 11
<b>7</b>	c.[1521_1523delCTT;1658G>A]	p.[Phe508del;Arg553Gln]	[ΔF508;R553Q] 12
<b>8</b>	c.[1521_1523delCTT;1784T>C]	p.[Phe508del;Met595Thr]	[ΔF508;M595T] 14
<b>9</b>	c.[1521_1523delCTT;1897C>T]	p.[Phe508del;Leu633Thr]	[ΔF508;L633T] 14
<b>10</b>	c.[1521_1523delCTT;2562T>G]	p.[Phe508del;Thr854]	[ΔF508;T854] 15
<b>11</b>	c.[1521_1523delCTT;2719A>G]	p.[Phe508del;Ile907Val]	[ΔF508;I907V] 17
<b>12</b>	c.[ 1521_1523delCTT;2770G>A]	p.[Phe508del;Asp924Asn]	[ΔF508;D924N] 17
<b>13</b>	c.[1521_1523delCTT;3080T>C]	p.[Phe508del;Ile1027Thr]	[ΔF508;I1027T] 19
<b>14</b>	c.[1521_1523delCTT;3080T>C;4264C>T]	p.[Phe508del;Ile1027Thr;Arg1422Trp]	[ΔF508;I1027T;R1422W] 19, 27
<b>15</b>	c.[1521_1523delCTT;3199G>A]	p.[Phe508del;Ala1067Thr]	[ΔF508;A1067T] 20
<b>16</b>	c.[1521_1523delCTT;3443A>G]	p.[Phe508del;Asn1148Ser]	[ΔF508;N1148S] 21
<b>17</b>	c.[1521_1523delCTT;3988C>G]	p.[Phe508del;Gln1330Glu]	[ΔF508;Q1330E] 25
<b>18</b>	c.[1521_1523delCTT;4212A>T]	p.[Phe508del;Ile1404]	[ΔF508;I1404] 26
<b>19</b>	c.[1521_1523delCTT;4312C>T]	p.[Phe508del;Arg1438Trp]	[ΔF508;R1438W] 27
<b>20</b>	c.[1521_1523delCTT;4389G>A]	p.[Phe508del;Gln1463]	[ΔF508;Q1463] 27
<b>21</b>	c.[1521_1523delCTT;4423G>A]	p.[Phe508del;Val1475Met]	[ΔF508;V1475M] 27

**Figure 1 Effect of cis variants identified in combination with p.Phe508del.**

The most common mutation p.Phe508del produces a misfolded CFTR protein. Functional restoration can be achieved by combining Lumacaftor and Ivacaftor (Orkambi), but a variability of the response is observed. Complex alleles, defined by the presence of another mutation on the same parental allele could explain part of the variability in treatment efficacy.

A. At the DNA level, variants located in regulatory elements could reduce the amount of *CFTR* transcripts.

B. At RNA level, variants can destabilize *CFTR* transcripts or enhance exon skipping, such as c.609C> T (p.Ile203) and c.2770G> A (p.Asp924Asn).

C. At protein level, variants can affect protein folding or maturation, such as p.Leu467Phe which reduces protein maturation by half, rendering p.[Leu467Phe;p.Phe508del] resistant to Lumacaftor treatment. Resistance to treatment was also observed with p.Phe87Leu which could alter Lumacaftor binding as CFTR amino acid 87 is close to a suggested binding site of Lumacaftor.

