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Published in:
Gastroenterology

DOI:
10.1053/j.gastro.2022.06.019

Publication date:
2022

Cirrhosis and portal hypertension in compound heterozygous people with CF harboring one F508del *CFTR* gene mutation

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Conflict of interests: Sanne S. Duursma, Henkjan J. Verkade, and Frank A.J.A. Bodewes have no conflict to disclose.

Author contributions to manuscript: Sanne Suzan Duursma, MSc (Writing – original draft: Lead); Henkjan J. Verkade Prof MD PhD (Conceptualization: Equal; Data curation: Supporting; Formal analysis: Supporting; Funding acquisition: Equal; Supervision: Equal; Writing – review & editing: Equal); Frank A.J.A. Bodewes, MD PhD (Conceptualization: Lead; Data curation: Lead; Formal analysis: Lead; Funding acquisition: Lead; Supervision: Lead; Writing – review & editing: Lead)

Funding: The authors thank the C&W De Boer Foundation for generous financial support for these studies.

Study material: Data is provided by the European Cystic Fibrosis Society Patient Registry (ECFSPR).

Word count: 986 words

Cystic Fibrosis (CF) is a multi-organ, genetic disease with a European prevalence of ~0.7 per 10,000 people.(1) CF is associated with a decreased life expectancy, primarily related to progressive pulmonary failure.(2) Approximately 5% of people with CF develop a severe hepatic phenotype that comprises cirrhosis, portal hypertension, splenomegaly, and hypersplenism and can lead to variceal bleeding, ascites, hepatopulmonary syndrome or liver failure. In people with CF, cirrhosis with portal hypertension is usually diagnosed in the first two decades of life, with a reported peak incidence at ~10 years of age, and is associated with an increased mortality risk at younger age.(2,3)

CF is caused by mutations in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) protein gene, causing a reduced or absent protein function. Over 2000 *CFTR* mutations have been identified, which, to varying degrees, negatively influence protein function and phenotype severity.(4)

A widely accepted method to classify *CFTR* mutations is based on their consequence on intracellular CFTR endocytic trafficking.(5) Class I mutations include nonsense and splice site mutations, in which no functional CFTR protein is translated. Class II mutations impair intracellular CFTR protein folding. Class III mutations are characterized by impaired gating function of the transport protein and class IV by decreased conductance. Finally, class V mutations result in the production of functional CFTR protein, however, in insufficient quantities.(6) Classes I to III mutations are generally associated with more severe phenotypes characterized by declining pulmonary function and exocrine pancreatic insufficiency. Classes IV and V have been associated with a relatively mild phenotype.(7)

In Europe, approximately 85% of the people with CF carry the *CFTR* F508del mutation, a class II mutation, on at least one allele. Around 60% of F508del carriers are homozygotes (F508del/ F508del). ~40% are compound heterozygotes, in which a different disease-causing *CFTR* mutation accompanies one F508del mutation on the other allele (F508del/ other).(7)

The relationship between specific *CFTR* gene mutations and the prevalence of cirrhosis and portal hypertension in people with CF and their mortality risk is unknown. In people with CF, who develop clinically significant cirrhosis with portal hypertension, the homozygote F508del mutation is more prevalent compared to people with CF without this severe hepatic phenotype.(3,8) Data on the effects

and contribution of more rare *CFTR* mutations on the severe hepatic phenotype is lacking due to the relatively low number of patients with these *CFTR* mutations.

The European Cystic Fibrosis Society Patient Registry (ECFSPR) collects yearly data on ~50,000 European people with CF, including information on *CFTR* mutations and clinical and biochemical characteristics. Due to a large number of patients included, the registry provides the unique opportunity to study the genotype-phenotype relationship in a rare patient population: people with CF harboring a compound heterozygote mutation. Compound heterozygosity, in this study is defined as one F508del mutation and one other disease-causing mutation, classified by severity.

To assess the contribution of these rarer *CFTR* mutations on severe hepatic phenotype, we collected registry data regarding *CFTR* mutations, reported liver disease, and mortality of patients harboring at least one F508del mutation and one other disease-causing mutation. We performed a retrospective analysis of ECFSPR patient data collected between 2008 and 2016.

We included data from 17,947 compound heterozygote people with CF. We identified 544 (3%) patients with clinically significant cirrhosis with portal hypertension (CFCPH) in this patient group. We were able to classify the second mutation of 438 patients (~80%) according to the CFTR protein defect class of their second mutation (see supplementary data). People with CF harboring a second mutation, which we could not classify, were excluded.

The prevalence of CFCPH in compound heterozygote people with CF corresponded with the functional CFTR protein defect class of the second mutation in a stepwise fashion from 5.2% in class I to 0.3% in class V (fig 1).

Subsequently, we assessed the mortality rate during the 8-year study observation window. The mortality rate in the F508del compound heterozygote CFCPH patients was almost twofold higher compared to the F508del compound heterozygote people with CF without this severe hepatic phenotype (10% vs. 4%, $p<.05$). This observation underlines the significantly increased mortality risk of CFCPH.

When compound heterozygote patients developed CFCPH, the observed mortality rate ranged between 8% and 15%. However, there was no significant association between the severity of the mutation (i.e., second mutation class) and the observed mortality risk.

Hereafter, we compared F508del compound heterozygotes to F508del homozygotes with CFCPH patients. We found a similar mortality rate in the two groups (F508del/other vs. F508/F508del mutations; 10% vs. 12%, respectively, $p>.05$).

Our results conclude that the risk of developing CFCPH in F508del compound heterozygote people with CF is significantly associated with the *CFTR* functional mutation class of their second *CFTR* mutation and, subsequently, with the severity of CFTR protein dysfunction. The mortality risk of F508del compound heterozygote people who developed CFPCH is almost twice compared to patients without this severe hepatic phenotype. However, once CFCPH had been diagnosed, the subsequent mortality risk was not associated with the mutation class of the second *CFTR* mutation in F508del compound heterozygotes. The latter indicates that the increased risk of death is mainly determined by the sheer presence of CFCPH and is similar for the different genotypes.

This study provides novel insights into the association between the second *CFTR* gene mutation severity class in F508 del compound heterozygote people with CF and the prevalence of CFCPH and subsequent mortality. The characterization of this genotype-phenotype relationship may contribute to an earlier identification of patients at risk and allows for an early and improved prognostication for CFCPH patients.

Acknowledgments

The authors would like to thank the people with CF, and their families, for consenting to their data being included in the European CF Society Patient Registry (ECFSPR). We thank the centers and individual country representatives for allowing the use of the data and the ECFSPR for providing access to anonymized patient data. The authors thank the C&W De Boer Foundation for the generous financial support for these studies.

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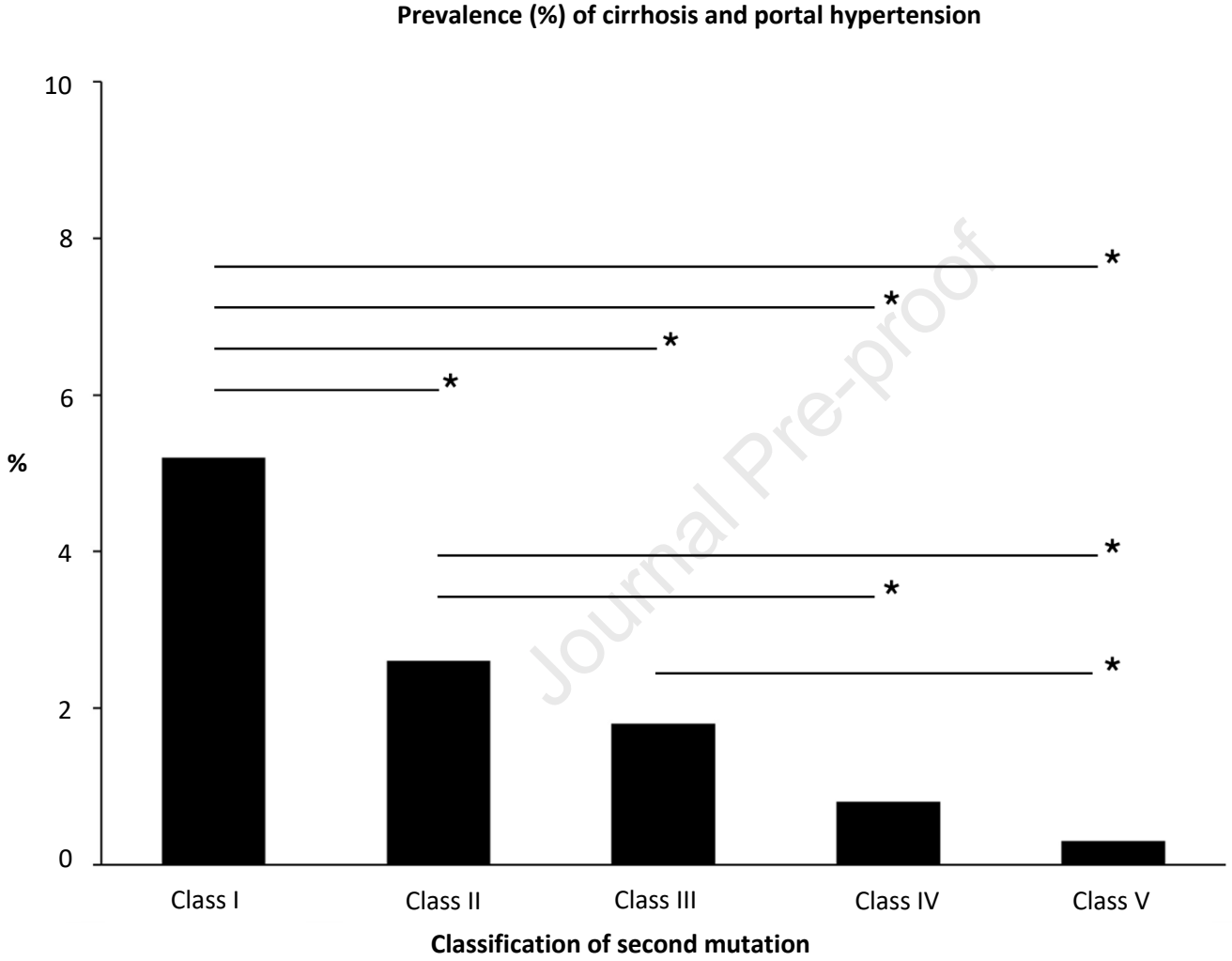

Figure 1. Prevalence of cirrhosis and portal hypertension in CF patients with one F508del *CFTR* mutation and a different second *CFTR* mutation, which is categorized according to the functional class (class I to V, based on the consequence of the mutation on intracellular CFTR endocytic protein trafficking). * $p<0.05$.

Supplementary data

Classified mutations

Class	Mutation	Source	Class	Mutation	Source
I	1078delT	1	II	E92K	8
	1154insTC	2		F508del	1
	1717-1G->A	1		G550X	1
	1811+1G->C	3		G628R(G->C)	1
	1898+1G->A	2		G85E	1
	2143delT	4		I336K	9
	2183AA->G	5		I507del	1
	2184delA	2		L1065P	10
	2184insA	2		L927P	11
	3659delC	1		N1303K	1
	3905insT	2		R1070Q	12
	394delTT	2		R560T	1
	4016insT	2		S945L	13
	4326delTC	2	III	G1069R	5
	621+1G->T	1		G1244E	1
	711+1G->T	2		G551D	1
	CFTRdele17a,17b	1		G970R	1
	CFTRdele2,3	6		L1077P	2
	E585X	2		R347H	2
	E60X	2		S1251N	1
	E822X	2		S549R	1
	G542X	2	IV	D1152H	2
	L732X	1		P205S	14
	Q493X	2		R117H	1
	Q552X	2		R334W	1
	R1066C	2		R347P	1
	R1162X	2	V	2789+5G->A	1
	R553X	2		3272-26A->G	2
	V520F	2		3849+10kbC->T	1
	W1282X	2		A455E	1
	Y1092X	2			
	Y1092X(C->G)	1			
	Y849X	7			

Mutations which could not be classified

1080delA(p.W361GfsX7	549C/T	polyT7
1112delT	557delT	Q220X
1161delC	574delA	Q39X
1248+1G->A	604insA	Q890X
1249-1G->A	61X	R1158X
1259insA	624delT	R75X
1461insAGAT	c.1021-1022DUP	R851X
1465insTAAT	c.1117-1g>A	S1196X
1491-1500del	c.1130_1131insA	S18I
1609delCA	c.1243-1247del	S549R(A->C)
1677delTA	c.1585-2A->G	SPLICMUTEX20
1811+1G>A	C.1725 delC	TC1844EXON12
182delT	c.3300_3014del	W1098X(TAG)
1833delTT	c.3468 2dupT	W1274X
185+1G->T	c.3889_90insT	W57G
1898+1G->C	c.4197_4198del	W57R
1898+1G->T	c.50delT/p.(Phe17Ser	Y84X
1949del84	c.54-?_489+?del(dele	Y913S
2184del	c.580-2a>G	
2185insC	C15851GA	
2634insT	C276X	
2694T/G (c.2562T>G)	CFTRdele14b-17b	
2711delTp.Phe861Leuf	CFTRdele17a17b18(c.3	
2869insG	CFTRdele22-24	
2948delA	CFTRdele22,23	
295ins8(p.Arg55Asnfs	E1308X	
3056delGA	E692X	
3121-1G->A	IVS18+2dupT(c.3468+2	
3750delAG	K710X	
3821delT	L206Trp	
3Polymorph	L327P	
4015delA	L558S	
405+1G->A	L571S	
406-1G->C	M1137R	
4108delT	M470V	
4271delC	M9611	
457TAT->G	NPY1092X	
494T>C: p.Leu1655Ser	PAsp1152His	

Mutation Classification Source List

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