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Highly preferential association of NonF508del CF mutations with the M470 allele

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Abstract

Background: On the basis of previous findings on random individuals, we hypothesized a preferential association of CF causing mutations with the M allele of the M470V polymorphic site of the *CFTR* gene.
Methods: We have determined the M/V-CF mutation haplotype in a series of 201 North East Italian and 73 Czech CF patients who were not F508del homozygotes, as F508del was already known to be fully associated with the M allele.
Results: Out of 358 not F508del CF genes, 84 carried the V allele and 274 the less common M allele. In the N-E Italian population, MM subjects have a risk of carrying a CF causing mutation 6.9× greater than VV subjects when F508del is excluded and 15.4× when F508del is included. In the Czech population a similar, although less pronounced, association is observed.
Conclusions: Besides the possible biological significance of this association, the possibility of exploiting it for a pilot screening program has been explored in a local North East Italian population for which CF patients were characterized for their CF mutation. General M470V genotyping followed by common CF mutation screening limited to couples in which each partner carries at least one M allele would need testing only 39% of the couples, which contribute 89% of the total risk, with a cost benefit.

Keywords: M470V polymorphism; Haplotype; CFTR; CF risk

1. Introduction

Cystic Fibrosis (CF) is the most common severe autosomal recessive disease among Europeans (*ca.* 1/2500). To date several hundred CF-causing mutations with very different frequencies in different populations have been described. However, in all European countries, the combined (=total) frequency of the CF-causing alleles is 0.02 [1]. Therefore, the proportion of the 'at risk' couples (+/CF × +/CF) is $\approx (2 \times 0.02)^2 \approx 0.00016$, a figure comparable with the prevalence of Down syndrome for which screening procedures are commonly carried out. Thus, considering the severity of the CF disease and its expected prevalence, a general prevention program would be justified.

Since no simple and reliable method to identify at the phenotypic level the heterozygotes for CF alleles is available, only the more challenging method of mutation detection at the DNA level has to be used. CFTR is a large gene [2], therefore, if the CF alleles were represented by many different extremely rare alleles, even retrospective molecular genetic analysis would be tremendously difficult. Fortunately, this is not the case because a single CF allele,

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F508del, accounts for a large portion of all the CF mutations, about 90 and 50% in Northern and Southern Europe, respectively [1]. Clearly then, for Northern Europe, a screening restricted to this CF allele would be effective enough for prospective counselling, whereas this strategy would be far from sufficient for Southern Europe. In this case such a goal would require a screening of the several tens common CF mutations that account for approximately 85% of all the CF mutations of the area [1], an extensive task in cost-benefit terms.

It has been shown that the variability of European random *CFTR* genes is almost completely restricted to the *CFTR* genes carrying the M allele of the M470V polymorphic site [3]. This finding has been tentatively interpreted as an example of sweeping selection caused by the birth of an advantageous mutation in a V gene; from that moment onwards this hypothetical gene would have rapidly spread [3] while accumulating some variability through recombination and recent mutational events. This 'allele-restricted' variability has been reported also for some CF mutations, and particularly for F508del (hereafter sometimes designated simply F mutation), in various European countries [4–6], thus allowing one to subdivide the general population into classes of individuals having a different risk of being carriers of a CF mutation. We decided to quantify this differential risk in one Southern European country, and to test this finding in a second Central European country, by determining the frequency of the M and the V alleles in a sample of NonF508del CF genes (hereafter designated as NonF CF mutations).

Indirect CF prevention through haplotype analysis has been utilized since long (see, for example, Ref. [7]); here we have explored the possibility of utilizing a single polymorphism instead of a complex haplotype.

2. Materials and methods

2.1. The sample

Both the Italian and the Czech samples consisted of CF patients not homozygous for F508del (201 Italians and 73 Czechs) and of their parents. The Italian patients (almost exclusively from the North-Eastern Italy regions Veneto and Trentino Alto Adige) were selected from a cohort of 268 patients: 67 F/F homozygotes; 130 F/NonF and 71 NonF/NonF (clearly in HW equilibrium); equivalent to 264 F and 272 NonF genes, in good agreement with the 1:1 F *vs* NonF subdivision of the CF alleles in this area [1]. The Czech patients were selected from a cohort of 247 patients among which the frequency of the F mutation was 0.719. All patients had typical clinical findings of pulmonary and/or gastrointestinal disease together with sweat chloride levels greater than 60 mEq/l.

2.2. Molecular genetics analysis

The selected 274 CF patients and their parents have been specifically examined for the M470V polymorphism as previously described [8]. The CF mutations have been searched for as follows:

2.2.1. Italian sample

All the 201 CF patients were analyzed [9] for F508del and for 15 other CF mutations (1st group in Table 1).

Thirty one additional CF mutations (ref. nos. 5 and 6 of the 1st group and all those of the 2nd group in Table 1), instead, have been detected not examining all samples.

The CF mutation was not identified in 39 genes and not assigned to M or V allele in 4 cases. These 43 CF genes are here reported as 'not defined'.

2.2.2. Czech sample

All the 73 CF patients were examined with the "Roche molecular system kit" which detects 38 CF mutations. The specimens negative with this 'standard' analysis were examined by DGGE [10]. The 13 'not defined' CF mutations refer to not identified CF mutations.

2.3. Classification of the M/V-CF haplotypes

For the present study a NonF CF mutation is informative if it has been unambiguously assigned either to the M470 or to the V470 CFTR allele. Such assignment has not been possible for 36 CF mutations of the Italian sample (18 CF M/V patients with both parents M/V heterozygotes), and 6 CF mutations of the Czech sample (3 CF M/V patients). The simple exclusion of these CF mutations from the sample would have preferentially affected the CF mutations

Notes to Table 1:
(1)It cannot be excluded that very few of these mutations actually are not CF-causing mutations. Even if this were the case it would not affect the overall results to an appreciable extent.
(2)CF mutations which, when tested, were examined in all the specimens.
(3)The distance is from the 3' end of the deletion.
(4)CF mutations not searched for in all individuals.
(5)In one case this mutation has been found in cis with the R1070Q variant.
(6)DEFINED = mutations identified AND assigned either to M or to V allele; NOT DEFINED = mutations not identified OR not assigned; n.t. = not tested.
° = mutations screened in all specimens in both populations.
♦ = CF mutations not searched for in all the Italian specimens.
The grey boxes refer to CF mutations found both in the M and the V alleles.

Table 1
A list of the CF mutations found in the two samples and their association with the M or the V allele

Ref. No.	CF mutation[1]	Kb from the M470V site	Italian sample			Czech sample		
			n	M	V	*n*	M	V
1	**F508del**	**0**	**130**	**130**	**0**	**60**	**60**	**0**
	Non F508del 1st group[2]							
2	CFTRdele2,3	53[3]	n.t.			16	0	16
°3	G85E	51	7	4	3	0		
4	711+5G>A	29	11	11	0	n.t.		
5	♦R334W	20	1	1	0	1	1	0
6	♦R347P	20	1	0	1	3	0	3
°7	I507del	0	8	8	0	0		
°8	1717-1G>A	+28	21	21	0	2	2	0
°9	G542X	+28	20	20	0	7	7	0
°10	G551D	+28	1	0	1	12	12	0
11	Q552X	+28	3	3	0	n.t.		
°12	R553X	+28	8	5	3	0		
13	1898+1 G>A	+30	n.t.			4	2	2
14	2143delT	+32	n.t.			5	5	0
°15	2183AA>G	+32	22	22	0	1	1	0
°16	2789+5G>A	+33	5	5	0	1	1	0
17	3132delTG	+51	3	0	3	n.t.		
°18	R1162X	+68	35	35	0	0		
°19	3849+10 kbC>T	+78	6	6	0	6	3	3
°20	W1282X	+83	6	6	0	1	1	0
°21	N1303K	+93	26	25	1	10	10	0
	TOTAL		**184**	**172**	**12**	**69**	**45**	**24**
	2nd group[4]							
22	CFTRdele1	80	1	0	1	n.t.		
23	185+1 G>A	80	n.t.			1	1	0
24	Q39X	56	3	3	0	n.t.		
25	E92X	29	n.t.			1	1	0
26	K114X	29	1	1	0	n.t.		
27	R117H	29	1	1	0	0		
28	406-1C>T	29	1	1	0	0		
29	E193K	26	1	0	1	n.t.		
30	R352W	20	1	0	1	n.t.		
31	R352Q	20	1	1	0	n.t.		
32	1497delGG	11	2	2	0	n.t.		
33	S466X[5]	0	2	2	0	n.t.		
34	1564delCA	0	1	0	1	n.t.		
35	S549R	+28	1	1	0	n.t.		
36	1845delAG	+30	1	0	1	n.t.		
37	1874insT	+30	1	0	1	n.t.		
38	S589N	+30	1	0	1	n.t.		
39	1898+3A>G	+30	2	0	2	n.t.		
40	2184delA	+32	2	2	0	0		
41	2184 insA	+32	n.t.			1	0	1
42	2790-2A>G	+44	2	2	0	n.t.		
43	S945L	+44	n.t.			1	1	0
44	3120+1 Kbdel8.6 kb	+48	3	3	0	n.t.		
45	R1066H	+51	1	1	0	n.t.		
46	3272-26 A>G	+52	1	0	1	n.t.		
47	W1145X	+55	1	0	1	n.t.		
48	D1152H	+55	3	0	3	n.t.		
49	R1158X	+68	4	4	0	n.t.		
50	3878delG	+83	1	0	1	n.t.		
51	4006-8T>A	+93	1	0	1	n.t.		
52	4016insT	+93	1	0	1	n.t.		
53	4382delA	+107	1	0	1	n.t.		
54	Q1476X	+107	3	0	3	n.t.		
	TOTAL		**45**	**24**	**21**	**4**	**3**	**1**
	TOTAL "DEFINED"[6] 1st+2nd group		**229**	**196**	**33**	**73**	**48**	**25**
	TOTAL "NOT DEFINED"[6]		**43**	**25**	**18**	**13**	**5**	**8**
	GRAND TOTAL		**272**	**221**	**51**	**86**	**53**	**33**

associated with the V allele, thus causing a highly biased underestimate of the CF-V haplotype frequency. Therefore we decided not to discard them. Sixteen of the Italian patients and the three Czech patients were heterozygotes for a CF mutation always found on the M allele in the specific population under study and another CF mutation, thus the latter was assigned to the V allele. In particular, 12 Italian and the 3 Czech CF patients carried the F508del (that has been unambiguously assigned to the M allele 118/118 and 57/57 times in the present two samples, respectively, in agreement with previous reports); 1 carried the 2183*AA→G* (21/21); 1 carried the I507del (7/7), 1 carried the 1717-1*G→A* (20/20) and 1 carried the G542X (19/19). The CF mutations of the remaining two Italian patients have been assigned to the class of the 'not defined' CF mutations.

3. Results

3.1. The Italian sample

Table 1 reports the 47 CF mutations found in the present survey. Twenty five were associated only with the M allele, 19 (all uncommon) only with the V allele and 3 with both.

The here observed frequencies of the first group CF mutations only can be considered reliable estimates of their relative contribution to the overall CF mutation frequency, whereas the frequencies of the F508del and of all the CF mutations of the second group are underestimates.

Table 2
Frequencies of the M and the V alleles in (a) the Italian and (b) the Czech CF samples

CF mutations	*n*	M		V	
		Obs	Exp[a]	Obs	Exp[a]
(a)					
F508del	130	130	50.1	0	79.9
		1.00	0.385	0.00	0.615
Other					
Defined[b]	229	196	88.2	33	140.8
		0.856	0.385	0.144	0.615
Not defined[b]	43	25	16.6	18	26.4
		0.581	0.385	0.419	0.615
Total	272	221	104.7	51	167.3
		0.813	0.385	0.187	0.615
(b)					
F508del	60	60	23.1	0	36.9
		1.00	0.385	0.00	0.615
Other					
Defined[b]	73	48	28.1	25	44.9
		0.658	0.385	0.342	0.615
Not defined[b]	13	5	5.0	8	8.0
		0.385	0.385	0.615	0.615
Total	86	53	33.1	33	52.9
		0.616	0.385	0.384	0.615

[a] computed by utilizing the frequencies of the M and V alleles in Europe (0.385 and 0.615, respectively: see Modiano et al., Ref [11])
[b] see caption (6) of Table 1.

Table 3
Absolute frequencies of the 6 possible couples in the Italian sample of 71 couples of parents of NonF/NonF CF patients

Couples	Exp1	Obs	Exp2
MM × MM	1.6	10	7.0
MM × MV	9.9	24	25.4
MM × VV	8.0	5	5.1
MV × MV	15.9	25	23.2
MV × VV	25.4	7	9.4
VV × VV	10.2	0	0.9
	$\chi^2_{3df}=93.9$		$\chi^2_{2df}=3.0$
	$P\approx 0$		$P=0.22$

For each type of couple the expected absolute frequencies were computed by multiplying its expected relative frequency in the general population by 71 (total number of couples of the sample) and by 1 (uniform risk: Exp1) or by the here estimated specific relative risk (Exp2, see text).

The complete association of the F508del with the M allele is confirmed.

Among the 229 defined CF mutations other than F508del 196 (=0.856) turned out to be associated with the M allele and only 33 (=0.144) with the V allele (Table 2a).

For the 43 not defined CF mutations (the least common ones) the degree of the preferential association with the M allele (0.581, instead of 0.385, which is the frequency of this allele in Europe, ref. 11; see Table 2a), though statistically significant ($P\approx 0.01$), is fourfold less than that found among the defined CF mutations (0.581/0.419 *vs* 0.856/0.144; see Table 2a). This result suggests that the (rarest) not defined CF mutations are largely due to recent mutational events which should have occurred randomly on the *CFTR* genes (see Introduction).

On the whole, among the 272 NonF CF mutations which cumulative frequency in the general population is 0.01 (namely half of 0.02), 221 (=0.813) were found among the CFTR-M genes (which frequency is 0.385). Thus, in the random population, the overall frequency of the haplotypes M-NonF CF mutations is $0.813\times 0.01=0.0081$. Therefore, in a sample of *CFTR* genes carrying the M allele the frequency of the NonF CF mutations is $0.0081/0.385=0.0211$. The complementary frequency of the haplotype V-NonF CF mutations is $0.187\times 0.01=0.0019$, which corresponds to a frequency within the V CFTR alleles of $0.0019/0.615=0.0031$. The strong association of the CF mutations with the M allele has been quantitatively proved also at the couple level (Table 3). Clearly, since the F508del mutation is only associated with the M allele, the overall frequency of the CF alleles among this allele is $(0.01+0.0081)/0.385=0.047$.

3.2. The Czech sample

It consists of 60 F508del and 86 NonF CF alleles (73 defined and 13 not defined) (Tables 1 and 2b). Also in this case the frequency of the F allele and of the CF mutations of the second group are to be considered underestimates. A preferential association of the NonF CF

mutations with the M allele is observed, though to a much lesser extent than in the Italian sample (0.616 *vs* 0.813: Table 2b *vs* Table 2a). This is mainly due to the occurrence at a relatively high frequency in this population, of the CFTRdele2,3 CF mutation (5.5% of the CF alleles, ref. 12), which is always associated with the V allele.

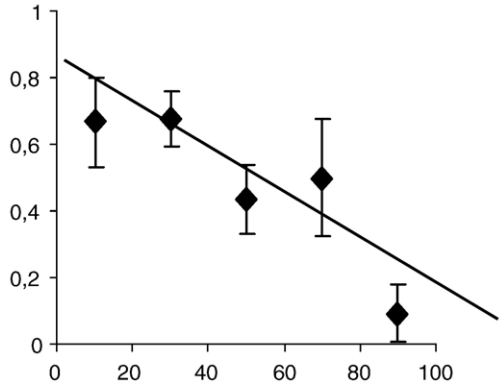
4. Discussion

A high heterogeneity among different European populations for CF alleles is known [12], and it has been confirmed by present data. In particular, out of 12 NonF CF mutations studied in all the 272 Italian and 86 Czech NonF CF alleles, 3 showed very different frequencies: G551D ($q_{Italy}=0.004$, $q_{Czech}=0.139$; $P<10^{-6}$); 2183 AA>G ($q_{Italy}=0.081$, $q_{Czech}=0.012$; $P\approx 0.03$); R1162X ($q_{Italy}=0.129$, $q_{Czech}=0.00$; $P\approx 5\times 10^{-4}$).

By far the most common of the CF alleles in both populations is the F508del allele (50% and 70% of the CF alleles in Italy and in Czech Republic, respectively). Its exclusive association with the M allele observed in the present two samples, was already known (e.g. ref. 4;5), though exceptions were reported [6].

Among NonF CF mutations, G85E, R553X and N1303K were found both with the M and the V allele within the Italian sample; 1898+1 G>A and 3849+10 Kb C>T were found both with the M and the V allele within the Czech Republic; and G551D was found in Italy with the V allele (1/1) and in the Czech Republic with the M allele (12/12). Thus at least 6 of the 53 NonF CF mutations are certainly associated with both M and V. All the other 47 CF mutations were associated with only one of these two alleles: 27 (amounting to a total of 177 CF chromosomes) with the M allele (ref. nos. in Table 1: 4, 5, 7, 8, 9, 11, 14, 15, 16, 18, 20, 23, 24, 25, 26, 27, 28, 31, 32, 33, 35, 40, 42, 43, 44, 45 and 49), and 20 (for a total of 44 CF chromosomes) with the V allele (ref. nos. 2, 6, 17, 22, 29, 30, 34, 36, 37, 38, 39, 41, 46, 47, 48, 50, 51, 52, 53 and 54), even though V is more frequent than M (0.615 *vs* 0.385). Obviously, however, some of these CF mutations may well be associated with both, thus the present ratio of 6/53 is clearly an underestimate. At any rate, since this source of error should have affected the M and the V alleles to the same extent, it makes sense to compare these two alleles even for the CF mutations detected only once.

The preferential association with the M allele turned out to be correlated with the distance (hence with the recombination rate) between the CF mutation and the M470V site ($P_{one\ tail}=0.0135$, see Fig. 1, Section (a) of the Table). These data allow



y = a + bx
a = 0.838 ± 0.107
b = – 0.007 ± 0.002
r = 0.92
$P_{one\ tail}$ = 0.0135

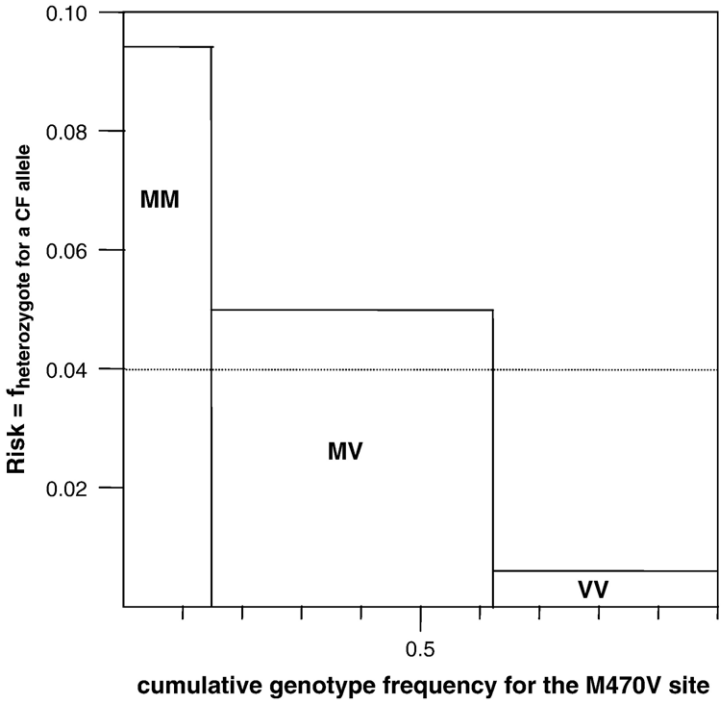
abscissa: d= distance from the M470V site

ordinate: proportion of sites only associated with the M allele

	CF mutations[1] (a)		random variants[2] (b)		pooled data (c)	
d	**only with M**	**the others**	**only with M**	**the others**	**only with M**	**the others**
0-20 Kb	6	3	2	1	8	4
20-40 Kb	13	9	10	2	23	11
40-60 Kb	5	6	5	7	10	13
60-80	3	2	1	2	4	4
> 80 Kb	1	6	0	4	1	10

[1] present data; [2] Pompei et al., 2006, ref.3

Fig. 1. Correlation between the proportion of variable sites where the minor allele is only associated with the M allele and their distance from the M470V site.

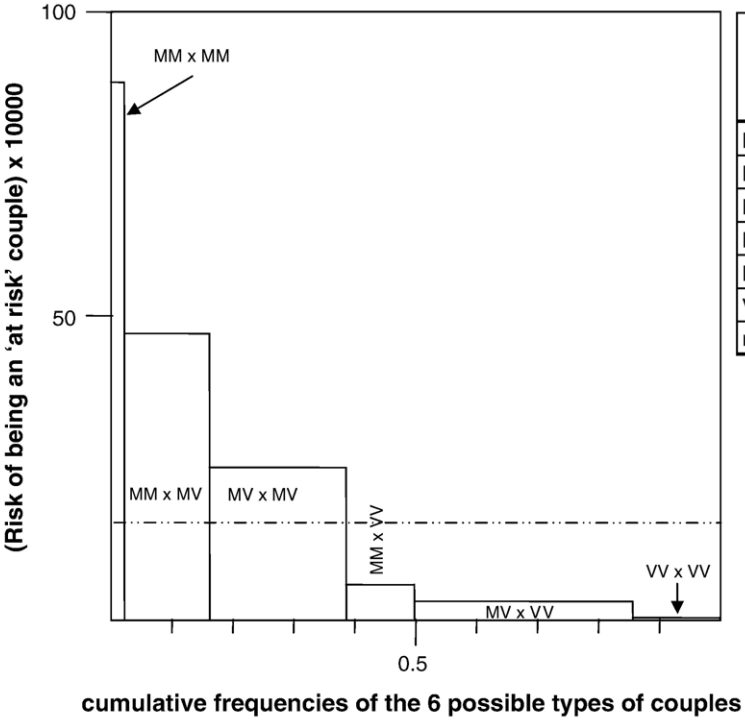


Genotype	Risk (a)	Frequency (b)	Relative contribution to TOTAL RISK (a x b)/0.04
MM	2 x 0.04708 = 0.09416	0.148	0.349
MV	0.04708 + 0.00305 = 0.05013	0.474	0.593
VV	2 x 0.00305 = 0.00610	0.378	0.058
random	0.04		

The level of the dotted line indicates the frequency of heterozygotes for a CF allele in the random population (4 %)

Fig. 2. Contribution of M/V genotypes to the overall individual risk of being heterozygous for a CF allele in the Italian population.

one to extend the findings of Pompei et al. [3] on the CFTR variable sites obtained on a sample of random individuals also to the CF mutations (Fig. 1, Section (b) and (c) of the Table) thus strengthening the evidence for a rapid amplification of the V allele possibly due to a recent strong selective advantage favouring the V allele itself or a variant born in a V



couples	risk x 10^4 a	frequency b	relative contribution to the total risk (a x b)/16
MM x MM	88.7	0.022	0.122
MM x MV	47.2	0.140	0.414
MV x MV	25.1	0.224	0.352
MM x VV	5.7	0.112	0.040
MV x VV	3.1	0.358	0.068
VV x VV	0.4	0.143	0.003
random x random	16		

	odd ratio	
couples	vs random x random	vs VV x VV
MM x MM	5.54	238.44
MM x MV	2.95	126.94
MV x MV	1.57	67.58
MM x VV	0.36	15.44
MV x VV	0.19	8.22
VV x VV	0.02	1
random x random	1	43.03

The level of the dotted line indicates the risk of the random couples (16 x 10^{-4})

Fig. 3. The partition of the risk of being an 'at risk couple' among the 6 possible M/V couples in the Italian population.

Table 4
CF mutations found in the 53 CF patients of the Bolzano province

CF mutation	Absolute and relative (%) frequencies	Associated with[(1)]
F508del	56 (52.8)	M
711+5 G>A	10 (9.4)	M
R347P	3 (2.8)	V
S466X	1 (0.9)	M
1717-1 G>A	1 (0.9)	M
G542X	1 (0.9)	M
G551D	2 (1.9)	V
1874insT	1 (0.9)	V
2183AA>G	3 (2.8)	M
2789+5G>A	1 (0.9)	M
R1162X	24 (22.6)	M
N1303K	2 (1.8)	M

[(1)]Based on data of Table 1.

haplotype. The function shown in Fig. 1 suggests that at the beginning of this process, *ca.* 85% of the variability was restricted to the M alleles. Then, such proportion decreased through recombination with an estimated slope equal to 0.007. Since no evidence for the occurrence of an HSR (Hot Spot of Recombination) in the *CFTR* gene does exist and present data also exclude such an occurrence, it appears reasonable to assume that the Recombination Rate is uniformly distributed in the here explored region and equal to the commonly accepted overall mean value of 1 cM=1 Mb. On these bases the estimate of the time 0 is approximately 1100 generations (≈25,000 yrs).

4.1. Implication for a prospective screening strategy in Northern Italy

The strong association between the M allele and the CF mutations implies that individuals differing for the M/V genotype have a different risk to be a CF carrier. Figs. 2 and 3 compare individuals and couples, respectively, with different M/V genotypes for their specific risk and for their contribution to the total risk. For each genotype or couple, the contribution is the area corresponding to the product of its frequency (abscissa) by its specific risk (ordinate). A very good agreement was found for the six types of couples between their observed proportions and those inferred from the CF/MV haplotype frequencies (see Table 3). The specific risk is the value that matters for genetic counselling, whereas the values relevant for public health are the relative contributions of the different individual or couple genotypes to the overall risk. As shown in Fig. 3, the 6 possible types of couples can be subdivided into two classes: couples in which both partners have at least one M allele, and the remaining couples.

First class couples, which amount to the 39% of the total, have risks ranging from 1.6 and 5.8 fold greater than the random risk of 16×10^{-4}. Second class couples, 61%, have risks ranging between one third and one fiftieth of the weighted mean risk. On this basis, a reasonable prospective screening option would consist in examining only the 1st class couples for the CF mutations most common in the area under study, considering not at risk the 2nd class couples.

4.2. A pilot screening program

The expected results of a pilot screening program as that here proposed have been computed for the Bolzano province population (Alto Adige, North East Italy).

All the CF patients of this local population have been fully characterized (Table 4); the prevalence of the disease and the M/V allele frequencies have been assumed to be those observed in other European populations, namely 1/2500; 0.385 and 0.615. Table 5 shows the estimated residual

Table 5
Inferred individual and couple risks for the Bolzano province population with two different screening strategies

Level	Frequency (%)	One-phase screening strategy	Two-phase screening strategy	
		Residual risk after the use of the CF mutation kit[a]	1st phase risk ($\times10^4$) based on the M/V genotype[b]	2nd phase residual risks[c]
Individual		**Equal for all genotypes $0.02\times0.04=8\times10^{-4}$**	**Weighted mean=400**	
MM	14.8		980 (36.3)	**8×10^{-4}**
MV	47.4		508 (60.2)	**8×10^{-4}**
VV	37.8		37 (3.5)	**37×10^{-4}**
Couple		**Equal for all couples $(8\times10^{-4})^2\approx0$**	**Weighted mean 16**	**Weighted mean[d] 1.18×10^{-4}**
1st class				
MM×MM	2.2		96 (13.2)	**≈0**
MM×MV	14.0		50 (43.7)	**≈0**
MV×MV	22.4		26 (36.2)	**≈0**
2nd class				
MM×VV	11.2		4 (2.5)	**4×10^{-4}**
MV×VV	35.8		2 (4.2)	**2×10^{-4}**
VV×VV	14.3		0.1 (0.1)	**0.1×10^{-4}**

1st and 2nd class couples=couples with a risk higher or, respectively, lower than the weighted mean risk (see text).
[a] CF mutation detection rate: 98%.
[b] Bracketed figures are the relative percent contributions to the weighted mean risk: (percent frequency × risk)/weighted mean risk.
[c] Following the use of the CF mutation kit on the 1st class individuals or couples only.
[d] Obtained by adding the risk of each type of couple multiplied by the corresponding frequency: (4×0.112)+(2×0.358)+(0.1×0.143)=1.18.

risks with two different prospective carrier screening strategies at the individual or at the couple level:

(a) the one-phase screening strategy would consist in examining all individuals or couples with a home-made kit which detects 98% of the CF mutations. This strategy would lead to the identification of almost all the CF carriers and the residual individual risk would be 8×10^{-4} (i.e. 2% of 400×10^{-4}, the population carrier frequency);
(b) the two-phase screening strategy would consist in first typing all the individuals or couples for the M/V genotype. This phase would lead to the subdivision of the general population into classes of individuals or couples having different risks of being CF carriers or an at risk couple. The at risk individuals or couples only would then be examined with the CF mutation kit in the second phase.

Both strategies would attain a substantially full prospective prevention of CF. Being almost equal the benefits, the difference would then depend only on cost: mutation screening in all individuals *vs* M470V genotyping in all individuals and mutation screening in 62% of the individuals, or 39% of the couples.

For the Czech sample, the contribution of the F508del to the CF total frequency (=0.02) is equal to the 70% (12), namely to 0.014, and the combined contribution of the four most common NonF CF mutations (ref. nos. 2, 9, 10 and 21 in Table 1) is half of the residual CF alleles frequency, namely 0.006/2=0.003. Thus, two options are available: that of screening for only the F508del and that of screening also for the other four 'common' CF mutations.

It appears clearly that, at the couple level, the screening of F508del would be effective enough to reduce more than tenfold the standard random risk (from 16×10^{-4} to 1.5×10^{-4}) for the couples where none of the parents turned out to be heterozygous for that mutation.

The great difference between the strategies convenient for prospective genetic counselling on Italians *vs* Czechs highlights the necessity of a preliminary detailed information on the CFTR genetic structure of any population to be subjected to a general prospective genetic counselling.

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