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DNA damage and related modifier genes in Italian Cystic fibrosis patients

SILVIA STERPONE[1], TOMMASO CORNETTA[1], ADRIANO ANGIONI[2], ERSILIA FISCARELLI[2], VINCENZINA LUCIDI[2], ANTONELLA TESTA[3] and RENATA COZZI[1*]

[1] Dipartimento di Biologia Università degli Studi "Roma TRE"
[2] Ospedale "Bambino Gesù" Roma
[3] Sezione di Tossicologia e Scienze Biomediche ENEA Casaccia Roma

ABSTRACT

Cystic Fibrosis (CF) is an autosomal recessive multisystemic disorder showing a highly heterogeneous phenotype, even among siblings carrying identical *CFTR* mutations. Moreover, oxidative stress is of central importance in the pathogenesis of cystic fibrosis. The present study seeks to value the presence of oxidative damage in CF patients and the possible modifier effect of repair and glutathione-S-transferase genes. We analysed the presence of DNA damage in leukocytes of 63 CF patients at an Italian CF centre and 63 controls, through the alkaline Comet assay to detect DNA strand breaks. Furthermore, controls and 93 CF subjects were genotyped for 5 genes by RFLP-PCR (*XRCC1,OGG1,GSTP1*) and PCR assay (*GSTM1, GSTT1*). No difference in Comet assay values was observed comparing controls to CF patients, although CF subjects showed slightly higher mean values. The crude Odds-Ratio (OR) was higher than one for *XRCC1* and *GSTP1* genotypes and liver status and for *XRCC1* and *OGG1* genotypes and pancreatic insufficiency, but in all cases the p-values were not significant. In this case-control study, neither DNA damage nor gene polymorphisms seem to influence CF manifestation.

Key terms: Comet assay/ cystic fibrosis/ DNA damage/ genetic polymorphisms/ modifier genes

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disorder that affects approximately one in 2500 births among most Caucasian populations, though its frequency may vary in specific groups (Rowntree and Harris, 2003). The disease gene, encoding Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) was cloned about twenty years ago (Riordan et al., 1989); it is located on chromosome 7q31.2, contains 27 exons and spans over 230 kb of genomic DNA (Zielenski et al., 1991). The CFTR multi-protein is a small chloride channel with complex function involving the transport of ions and molecules in addition to Cl. Currently about 1,600 mutations are listed in the Cystic Fibrosis Mutation Database (CFMDB http: //www.genet.sickkids.on.ca/cftr/), which can be subdivided into five classes based on the molecular alteration at the protein level. The most common mutation, accounting for around 70% of mutations worldwide, is *ΔF508* (class II), a base triplet deletion encoding a phenylalanine residue at position 508 of *CFTR* resulting in a mis-folding and mis-localization of the protein (Cystic Fibrosis Genetic Analysis Consortium 1994; Rowntree and Harris, 2003). The majority of the other *CFTR* mutations are very rare, only a few mutations having overall frequencies above 1%. In addition, over 200 polymorphisms have been identified within *CFTR* itself, which do not cause CF but may alter CFTR protein production and/or

* Corresponding author: RENATA COZZI, Dipartimento di Biologia V.le G.Marconi 446 00146 Roma Italy, Tel. +39 6 57336333, Fax: +39 6 57336321, E-mail: cozzi@uniroma3.it

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function. These polymorphisms may influence disease phenotype in patients with coexisting mutations.

The CF phenotype is highly heterogeneous among individual patients, even among siblings carrying identical *CFTR* mutations. The so-called 'severe' mutations (class I-II-III) are those where the absence of functional CFTR correlates with pancreatic insufficiency, liver disease and diagnosis at an early age (Zielenski, 2000). 'Mild' mutations (class IV-V) that may still produce a small amount of functional CFTR, are generally associated with pancreatic sufficiency and milder pulmonary disease and diagnosis at a later age (Rowntree and Harris, 2003; Slieker et al., 2005).

Patients homozygous for the *ΔF508* gene or compound heterozygous for *ΔF508* and another 'severe' mutation have lung disease, pancreatic insufficiency, obstructive azospermia, but express discordant liver phenotype. So it appears that other factors (environment, diet, genetics) besides the *CFTR* genotype modulate the liver expression in CF patients as Castaldo and colleagues (2001) suggested. Respiratory disorders account for about 95% of mortality in CF patients, but the severity of respiratory expression is highly heterogeneous among CF patients (Salvatore et al., 2002). CF patient homozygotes for *ΔF508* invariably have severe pulmonary phenotype; the severity differs in patient compound heterozygotes for *ΔF508* (Johansen et al., 1991). These data suggest that also the pulmonary phenotype could be modulated by other factors in addition to *CFTR* genotype, e.g. genetic factors inherited independently (Salvatore et al., 2002).

It is now well established that oxidative stress is of central importance in CF pathogenesis and the reactive oxygen species (ROS) contribute both to the decline of lung function (Mahadeva and Lomas, 2000) and to the onset and progression of hepatic disease (Rozmahel et al., 1996). Increased oxidative stress in CF patients is due to many factors, such as the precocious imbalance between antioxidant defence and the production of oxidant species.

Many findings indicate that the CFTR channel is not only permeable to chloride anion but also to larger organic anions, including reduced glutathione (GSH) (Linsdell and Hanrahan, 1998). Recently in vitro and in vivo studies (Gao et al., 2001; Velsor et al., 2001) have shown that CFTR dysfunction causes altered permeability to GSH, causing a chronic and progressive deficiency of extracellular GSH in CF patients.

Given the role of GSTs (Glutathione-S-Transferases) in the detoxification of ROS, genes encoding the GSTs should be considered candidates for association studies on several diseases, Cystic Fibrosis included. Up to now two studies have been published on pediatric patients from French CF medical centres: Henrion-Caude and co-workers (2002) found that the frequency of the GSTP1-Ile^{105}/Ile^{105} genotype was significantly higher in CF patients with liver disease. They concluded that the GSTP1-Ile^{105}-encoding allele contributes to hepatic dysfunction in children with CF and that *GSTP1* could be a modifier gene of CF; on the other hand, Flamant and colleagues (2004), by investigating the influence of *GSTM1*, *M3*, *P1* and *T1* polymorphisms on severity of lung disease, found that variant of the *GSTM3* gene is associated with clinical severity in CF.

The other important class of proteins enrolled in antioxidant defence are those involved in DNA repair systems, as they recognize and repair the damage induced on the DNA molecule by ROS and other chemical and physical agents (Hu et al., 2002). These proteins are encoded by more than 100 genes and many of them display single nucleotide polymorphisms (SNPs). Recently increasing interest has been dedicated to the study of the influence of DNA repair gene polymorphisms on diseases (such as neurodegenerative disorders), characterized by oxidative imbalance, where the influence of genetic factors, the so called 'susceptibility genes', other than the 'disease gene' may contribute to modulate individual risk. Among these, particularly important are the genes coding glycosylases (such as *OGG1*) or the scaffolding proteins involved in Base

and Nucleotide Excision Repairs (such as *XRCC1* and *XRCC3*). All these genes are polymorphic and the variant alleles frequency varies from 20 to 37% in Caucasians.

In this work, we analysed the presence of DNA damage in a cohort of CF patients gathered from a medical centre in Italian Hospital Bambino Gesù (Rome, Italy). The analysis was conducted through the Alkaline Comet assay, which allows the detection of DNA strand breaks in individual cells. Furthermore, the influence of GSTs and DNA repair genes on the phenotypic variations displayed by a larger cohort of these patients was investigated. The aim of this work was both to verify the presence of DNA damage and to identify genetic variations in genes that could be used as an aid at prognosis and provide the possibility of new therapeutic interventions.

METHODS

Patients

Ninety-three Caucasian CF patients (48 females, 45 males; mean age: 14; range: 1-33 years) were recruited from an Italian CF centre (Hospital Bambino Gesù, Rome, Italy). CF disease was defined as a combination of typical clinical features (e.g. persistent pulmonary problems, meconium ileus, failure to thrive, steatorrhoe), abnormal sweat test (chloride > 60mM) and *CFTR* gene mutation identification in all patients. Definitive diagnosis of Cystic Fibrosis was based on a genotype with 2 identifiable mutations consistent with CF and /or a sweat chloride concentration that is greater than 60 mmol/L.

Clinical data were retrospectively obtained from hospital records, blind to the result of genotyping. Data included age, sex, *CFTR* mutations, liver and pancreatic insufficiency diagnosis.

Pancreatic insufficiency (PI) documented by a faecal elastase (FE) level of less than 100 μgr/gr stool.

For diagnosis of liver disease (LD), we used the following criteria: presence of portal hypertension and/or multilobar biliary cirrhosis.

Diagnosis of cirrhosis and of portal hypertension was based on the following ultrasonographic criteria: presence of irregular margins on nodular pattern, increase in the longitudinal length of the spleen in relation to age, enlarged portal vein diameter (measured at the portal hepatic section) in the absence of respiratory variations, presence of portal-systemic collateral veins.

The study was approved by the Ethics Committees of the participating hospital and informed consents were obtained from the study subjects and/or their parents.

In a sub-group of sixty-three CF patients (32 females, 31 males) the presence of DNA damage was analysed through the Alkaline Comet assay.

We also enrolled sixty-three healthy (not CF) Caucasian volunteers matched for sex and age (32 females, 31 males; mean age 12.34; range 1-20 years).

Each subject contributed to the study with a single blood drawing. Blood (approximately 5 ml) was taken by venipuncture into heparinized tubes.

Alkaline Comet Assay

The alkaline Comet assay was performed as previously described (Singh et al., 1988). 20 ml of whole blood was embedded in 180 ml of 0.7% low melting point agarose in PBS (Ca and Mg free) at 37°C, and immediately pipetted onto a frosted glass microscope slide pre-coated with a layer of 1% normal melting point agarose, similarly prepared in PBS. Two slides were prepared for each experimental point. The agarose was allowed to set at +4°C for the necessary time and the slides incubated in a lysis solution (2.5 M NaCl, 10 mM Tris-HCl, 100 mM Na_2EDTA, NaOH to pH=10, 1% Triton, 10% DMSO) for 50 min. After lysis, the slides were placed on a horizontal electrophoresis unit containing fresh buffer (1 mM Na_2EDTA, 300 mM NaOH, pH=13) for 20 min to allow DNA unwinding. Electrophoresis was conducted for 15 minutes (25 V, 300 mA) at +4°C. Subsequently, slides were gently washed in

neutralisation buffer solution for 5 min (0,4 M Tris-HCl pH= 7.5), fixed in 100% freshly methanol for 3 min and stained with ethidium bromide (2 mg/ml). Slides were analysed using a fluorescence microscope (Leica) equipped with a camera. Sixty comets on each slide, coded and blindly scored, were acquired using "I.A.S." software automatic image analysis system purchased from Delta Sistemi (Rome-Italy). To quantify the induced DNA damage, Tail DNA (TD) was used, which is a measure of the percentage of migrated DNA in the tail (Collins, 2004).

Genotyping of OGG1 and XRCC1 genes

DNA was isolated from the blood sample of the study participants using the Gentra Puregene extraction kit. Polymerase chain reaction (PCR) followed by enzymatic digestion was used for the analysis of XRCC1-Arg399Gln (a G→A transition in exon 10) and OGG1-Ser326Cys (a C→G transition in exon 7) polymorphisms in accordance with published methods (Cornetta et al., 2006).

Genotyping of GSTM1, GSTT1 and GSTP1 genes

Genetic polymorphism analysis for the *GSTM1* and *GSTT1* genes was conducted with PCR-based assays. The *GSTM1* and *GSTT1* amplification reaction was carried out according to published methods (Zhong et al., 1993; Pemble et al., 1994). *GSTP1* genotyping analysis was performed by the RFLP-PCR based method. *GSTP1**B polymorphism was determined through the method described previously (Harries et al., 1997).

Statistical Analysis

Statistical analysis of the data was carried out using the GraphPad InStat version 3.00, GraphPad Software, San Diego, CA, USA. The nonparametric Mann-Whitney U-Test was used to compare Tail DNA means between the two groups, CF patients and controls. To value the association between the COMET parameter and each single genotype, we employed a Kruskal-Wallis nonparametric ANOVA and a nonparametric Mann-Whitney U-Test for SNPs polymorphic genes (*XRCC1*, *OGG1* and *GSTP1*) and null polymorphic genes (*GSTM1* and *GSTT1*), respectively.

We used the Chi-squared test to verify the Hardy–Weinberg equilibrium of the polymorphic alleles analysed. Furthermore, we calculated crude odds ratios (OR) and its 95% confidence interval. We considered $p<0.05$ significant for all analyses.

RESULTS

The study population was composed of 93 subjects with CF and 63 sex- and age-matched healthy controls. The clinical description of patients is summarized in Table I. According to data presented by the CF Genetic Analysis Consortium (1994) about Caucasian patients, most CF subjects (66% worldwide vs. 70% in our study) present *ΔF508* mutation in homozygosis or heterozygosis. The Consortium reports patients with the other more frequent mutations (*G542X*) as 2.4%, lower than our 8%. The other mutations were always less frequent, lower than 4 % (Table I). Most patients presented pancreatic insufficiency (78%); among these, 90% presented "severe" mutations bearing homozygote *ΔF508* genotype or compound heterozygote genotype (*ΔF508*/other severe mutation). Liver disease (LD) was diagnosed in 20% of our CF population, with prevalence in females (13 females versus 6 males) and in 18 cases it is associated with pancreatic insufficiency (PI). Only 5 patients bearing homozygote *ΔF508* genotype presented both pancreatic insufficiency and liver disease. In relation to siblings, we examined 3 pairs of siblings who showed a high consistency between genotype and clinical manifestations (Table I).

All the subjects (both CF patients and controls) were genotyped for 5 genes (*XRCC1*, *OGG1*, *GSTT1*, *GSTM1* and *GSTP1*) and the allele frequencies are presented in Table II. The χ^2 analysis of the distribution of all genotypes indicated that the distribution of alleles were within the

TABLE I

Clinical description of patients with CF

Study population (n°)			93
Current age (years)			14±2,8
Sex			
Male			45
Female			48
CFTR mutation (%)			
ΔF508 het			54
ΔF508 homo			16
G542X het			8
other			22
Pancreatic			
Insufficiency (PI) n°			73
Liver Disease (LD) n°			19
Couples of siblings		PI	LD
Couple 1			
Subject. 1	N1303K/G85E	Yes	No
Subject. 2	N1303K/G85E	Yes	No
Couple 2			
Subject. 1	N1303K/G1244E	Yes	No
Subject. 2	N1303K/G1244E	Yes	No
Couple 3			
Subject. 1	ΔF508/G542X	Yes	No
Subject. 2	ΔF508/G542X	Yes	No

Hardy-Weinberg equilibrium, both in patients and in controls.

DNA damage was measured in 63 patients and 63 controls (Table III). No difference in Tail DNA values was observed comparing controls with CF patients, although CF patients showed a slightly higher mean value than did the controls (0.97 vs. 0.88). When all subjects were subdivided according to the DNA repair gene (*XRCC1* and *OGG1*) and glutathione-S-transferases gene (*GSTT1*; *GSTM1* and *GSTP1*) genotypes, no significant difference was observed among CF patients. In the same way, the distribution of Tail DNA values among controls according to all considered genotypes was not significantly different (Table III).

Table IV and V show the crude Odds-Ratio (OR) calculated to value associations between genetic polymorphisms and clinical phenotype (liver and pancreatic status respectively).

TABLE II

Distribution of genotypes in controls and CF cases

Genes	CONTROLS (63)		CF PATIENTS (93)		p value*
	Genotype	Variant allele frequency	Genotype	Variant allele frequency	
XRCC1		0,37		0,40	0,73
Arg/Arg	27 (43%)		36 (39%)		
Arg/Gln	25 (40%)		39 (42%)		
Gln/Gln	11 (17%)		18 (19%)		
OGG1		0,25		0,20	0,50
Ser/Ser	35 (56%)		58 (62%)		
Ser/Cys	24 (38%)		33 (36%)		
Cys/Cys	4 (6%)		2 (2%)		
GSTT1		0,35		0,29	0,55
Pos	41 (65%)		66 (70%)		
Null(GSTT1*0/GSTT1*0)	22 (35%)		27 (30%)		
GSTM1		0,54		0,43	0,24
Pos	29 (46%)		53 (60%)		
Null(GSTM1*0/GSTM1*0)	34 (54%)		40 (40%)		
GSTP1		0,26		0,32	0,70
Ile/Ile	34 (54%)		46 (50%)		
Ile/Val	26 (41%)		34 (40%)		
Val/Val	3 (5%)		13 (10%)		

* Chi-squared Test

TABLE III

DNA damage detected by alkaline Comet assay in control and CF blood samples

	TAIL DNA (%) ± ES					
N°	Controls	p value*	N°	CF	patients	p value*
All	63	0,88±0,29	NS	63	0,97±0,3	NS
XRCC1 genotype						
Arg/Arg	27	1,15±0,36		24	0,73±0,22	
Arg/Gln	25	0,28±0,06		28	1,44±0,39	
Gln/Gln	11	1,59±0,37	NS	11	0,30±0,04	NS
OGG1 genotype						
Ser/Ser	36	0,79±0,23		40	1,03±0,3	
Ser/Cys	24	1,09±0,37		23	0,86±0,29	
Cys/Cys	3	0,43±0,1	NS	0		NS
GSTT1 genotype						
Pos	41	0,96±0,31		48	1,09±0,33	
Null (GSTT1*0/GSTT1*0)	22	0,73±0,25	NS	15	0,59±0,13	NS
GSTM1 genotype						
Pos	29	1,13±0,38		37	0,75±0,27	
Null (GSTM1*0/GSTM1*0)	34	0,67±0,16	NS	26	1,28±0,33	NS
GSTP1 genotype						
Ile/Ile	34	0,75±0,23		38	0,93±0,29	
Ile/Val	26	1,08±0,36		21	0,41±0,1	
Val/Val	3	0,62±0,12	NS	4	4,27±0,62	NS

* Mann-Withney U-Test/ Kruskal Wallis Test

We also performed a logistic regression analysis and the model did not show significant p-value (data not shown). Therefore, we did not calculate the OR adjusted for other potential confounders (age, sex).

Analysis of the data showed an OR value higher than 1 for the *XRCC1* and *GSTP1* genotypes, and liver status [OR=1.2 (0,43-3,33); OR=1.17 (0,43-3,22)] and for repair genes *XRCC1* and *OGG1*, and pancreatic insufficiency [OR=1.22 (0.44-3.43); OR=1.14 (0.41-3.13)]. Nevertheless, in all cases the p value was not significant (p=0.94; p=0.95; p=0.9 and p=0.8 respectively). Notably, we found more homozygote GSTP1-Val^{105} patients with liver disease (21% vs. 12%) (Table IV) and CF subjects with OGG1-326Cys/Cys genotype showed PI (Table V). There was no impact of the other genotypes (*GSTT1* and *GSTM1*) on pancreatic and liver phenotype.

DISCUSSION

Cystic Fibrosis (CF) is an autosomal and multisystemic genetic disorder that affects the respiratory, digestive and reproductive tracts. Nowadays more than 1600 mutations in the *CFTR* gene have been described, although the different clinical manifestations of the disease can be explained on the basis of allelic heterogeneity in only a few cases. In fact studies on siblings and twins with the same CFTR alteration have shown that other factors - genetic, environmental - can modify the CF phenotype.

TABLE IV

Association between genotype and Liver Status (LD) in patients with CF

	CF Patients without LD (n=74)	CF Patients with LD (n=19)	Crude OR * (95% CI)	p value**
XRCC1 genotype			1,2 (0,43-3,33)	0,94
Arg/Arg	0,38	0,42		
Arg/Gln	0,43	0,37		
Gln/Gln	0,19	0,21		
OGG1 genotype			1 (0,37-2,96)	0,93
Ser/Ser	0,62	0,63		
Ser/Cys	0,37	0,32		
Cys/Cys	0,01	0,05		
GSTT1 genotype			0,64 (0,22-1,84)	0,6
Pos	0,73	0,63		
Null (GSTT1*0/GSTT1*0)	0,27	0,37		
GSTM1 genotype			1 (0,38-2,91)	0,93
Pos	0,57	0,58		
Null (GSTM1*0/GSTM1*0)	0,43	0,42		
GSTP1 genotype			1,17 (0,43-3,22)	0,95
Ile/Ile	0,49	0,53		
Ile/Val	0,39	0,26		
Val/Val	0,12	0,21		

* heterozygous and mutated homozygous were pooled
** Chi-squared Test

While a high correlation (>95%) between pancreatic insufficiency and severe *CFTR* mutations has been demonstrated (Kristidis et al., 1992), the effect of possible modifier genes has been hypothesized in relation to other disease features. Several studies have shown that hepatic manifestations, as well as the severity of respiratory expression, can be modified by genetic variations in several genetic determinants like mannose binding lectin (*MBL2*), human leucocyte antigens class II (*HLA*), antiprotease α1-antitrypsin (*α1AT*), glutathione-S-transferase (*GSTs*), *TNFα*, *ADRB2* and *TGFα* genes (Cutting, 2005; Duthie et al., 1995; Flamant et al., 2004; Gabolde et al., 2001).

This implies that relatively common genetic variations, with little or no overt phenotypic effect on the general population, can have a significant effect in the context of CF. As well, polymorphisms in genes involved in antioxidant defences could contribute to the variability of the patients' phenotypes. In fact, mutations in the *CFTR* gene cause an imbalance between oxidant and anti-oxidant species: in particular the extracellular levels of reduced GSH decrease. We have hypothesized that the resulting oxidative stress could cause an increase in DNA damage in CF patients and that variant alleles in genes involved both in DNA repair pathways and in oxidative species metabolism could contribute to the variability of clinical CF phenotype.

TABLE V

Association between genotype and Pancreas Status in patients with CF

	CF Patients with PS (n=20)	CF Patients with PI (n=73)	Crude OR * (95% CI)	p value**
XRCC1 genotype			1,22 (0,44-3,43)	0,9
Arg/Arg	0,35	0,4		
Arg/Gln	0,45	0,41		
Gln/Gln	0,2	0,19		
OGG1 genotype			1,14 (0,41-3,13)	0,8
Ser/Ser	0,6	0,63		
Ser/Cys	0,4	0,34		
Cys/Cys	0	0,03		
GSTT1 genotype			0,77 (0,25-2,4)	0,86
Pos	0,75	0,7		
Null (GSTT1*0/GSTT1*0)	0,25	0,3		
GSTM1 genotype			0,85 (0,31-2,34)	0,96
Pos	0,6	0,56		
Null (GSTM1*0/GSTM1*0)	0,4	0,44		
GSTP1 genotype			0,58 (0,21-1,60)	0,42
Ile/Ile	0,6	0,47		
Ile/Val	0,25	0,4		
Val/Val	0,15	0,13		

* heterozigous and mutated homozigous were pooled
** Chi-squared Test

In the present study CF patients did not show any significant increase in DNA damage in comparison to healthy control subjects, as evaluated through the alkaline Comet assay. This result is partially in contrast to Brown and co-workers (Brown et al., 1995) who showed that an increase of urinary 8-oxo-deoxyguanosine (8-oxodG) concentration positively correlated with an increase of plasma α-tocopherol concentration in CF patients. 8-oxodG is considered a marker of DNA damage since its elimination is consistent with an adequate DNA repair mechanism where vitamin E is favourably involved. Although our results might be unexpected, we can assume that the daily administration of anti-oxidant drugs to our CF patients, such as vitamin E and β-carotene, could protect them against an excess of DNA damage measurable with the Comet assay.

The analysis of the variant allele frequencies of the five considered genes showed that they were in agreement with the literature data, both for the patients and the control population, and the genotype distributions were in Hardy-Weinberg equilibrium. Furthermore, we did not find any significant association between pancreatic status and all the considered genotypes, according to the strong correlation between severe *CFTR* mutations and pancreatic insufficiency (Kristidis et al., 1992). In fact, the vast majority (90%)

of our CF patients who showed PI were homozygous or heterozygous for *ΔF508* mutation. As far as liver phenotype, we found that the frequency of CF patients who were homozygous for GSTP1-Val^{105} was higher among those showing hepatic disease, but there was not a significant association with *GSTP1* genotype and liver disease.

These data are not in agreement with those presented by Henrion-Caude and co-workers (2002), who found a significant association between the GSTP1-Ile^{105}/Ile^{105} genotype and liver disease. This is probably due to the total absence of CF homozygote GSTP1-Val^{105} patients with liver disease in the French study. Moreover, the two studies showed other differences in mean age (11.5±5.25 vs. 14±2.8), CFTR mutations (Δ*F508* homozygotes: 56/106 vs. 16/93) and clinical classification for liver status that could explain discrepancies in the results.

As recently suggested by Bartlett and co-workers (2009), differences in the age range would also be important to understand discrepancies among studies since liver disease usually begins at age 10 or 12 years and younger patients without LD may be not completely informative.

Current findings support the role of GSTs in hepatic cytoprotection and an alteration in their catalytic function may compromise defence in the injured liver. *CFTR* is expressed in the biliary epithelial cells of the liver and mutations of this gene cause impairment in ductal bile secretion (Zsembery et al., 2002). As well, *GSTP1* expression is highly predominant in liver biliary epithelial cells so that variant alleles could contribute to the liver injury.

In conclusion, in this case-control study, polymorphisms in repair and glutathione-S-transferase genes seem not to assume prognostic significance, probably due to the meagre number of CF subjects gathered in one year (February 2006-March 2007). It is our hope to increase the number of CF patients in the Italian and European register in order to verify more profoundly the role of the *GSTP1* gene in liver disease (Accurso and Sontag, 2008).

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