article

Genotype-phenotype correlation and frequency of the 3199del6 cystic fibrosis mutation among I148T carriers: Results from a collaborative study

K. G. Monaghan[1], W. E. Highsmith[2], J. Amos[3], V. M. Pratt[4], B. Roa[5], M. Friez[6], L. L. Pike-Buchanan[4], I. M. Buyse[5], J. B. Redman[7], C. M. Strom[7], A. L. Young[2], and W. Sun[7]

Purpose: We expect that the mutation panel currently recommended for preconception/prenatal CF carrier screening will be modified as new information is learned regarding the phenotype associated with specific mutations and allele frequencies in various populations. One such example is the I148T mutation, originally described as a severe CF mutation. After implementation of CF population-based carrier screening, we learned that I148T exists as a complex allele with 3199del6 in patients with clinical CF, whereas asymptomatic compound heterozygotes for I148T and a second severe CF mutation were negative for 3199del6. **Methods:** We performed reflex testing for 3199del6 on 663 unrelated specimens, including I148T heterozygotes, compound heterozygotes, and a homozygous individual. **Results:** Less than 1% of I148T carriers were also positive for 3199del6. Excluding subjects tested because of a suspected or known CF diagnosis or positive family history, 0.6% of I148T-positive individuals were also positive for 3199del6. We identified 1 I148T homozygote and 6 unrelated compound heterozygous individuals with I148T and a second CF variant (2 of whom also carried 3199del6). In addition, one fetus with echogenic bowel and one infertile male were heterozygous for I148T (3199del6 negative). **Conclusions:** Reflex testing for 3199del6 should be considered whenever I148T is identified. Reflex testing is of particular importance for any symptomatic patient or whenever one member of a couple carries a deleterious CF mutation and the other member is an I148T heterozygote. Further population data are required to determine if I148T, in the absence of 3199del6, is associated with mild or atypical CF or male infertility. ***Genet Med* 2004:6(5):421–425.**

Key Words: *cystic fibrosis, mutation analysis, 3199del6, I148T*

Over 1000 cystic fibrosis (CF) mutations have been identified; most are rare, having been detected in only one family.[1] In 2001, the ACMG[2] and ACOG[3] developed a panethnic panel of 25 common mutations with a frequency $\geq 0.1\%$ in the general population and recommended that all Caucasians who are either pregnant or considering pregnancy be screened. ACMG/ACOG also recommend that screening be made available to individuals in lower risk ethnic groups. We expect that the mutation panel will be modified as new information arises regarding the phenotype associated with specific mutations and allele frequencies in various populations. The I148T mutation, which is currently included in the panel, was first reported in 1990 as a severe mutation[4–7] and accounted for 9% of French-Canadian CF mutations.[8] After implementation of CF population-based carrier screening, two studies noted a $>$ 100-fold increase in the frequency of I148T among individuals undergoing carrier screening compared to patients with clinical CF.[9–10] Further studies revealed a second mutation, 3199del6, in-*cis* with I148T in affected patients,[9,11] a finding previously reported in 1998.[1] The 3199del6 mutation was not present in asymptomatic compound heterozygous individuals with I148T and a second, severe CF mutation, such as ΔF508. Initial studies reported that approximately 1.8% of I148T heterozygotes identified by carrier screening also have the 3199del6 mutation.[9] Subsequent studies identified 3199del6 in 0.9% of I148T carriers and determined the frequency of 3199del6 in the general population to be $<$ 0.1%.[12] We have summarized the cases in which I148T was detected in our laboratories and report the frequency of 3199del6 among 663 I148T-positive individuals.

From the [1]Henry Ford Hospital, Detroit, Michigan; [2]Mayo Medical Laboratories, Rochester, Minnesota; [3]Specialty Laboratories, Santa Monica, California; [4]Quest Diagnostics Nichols Institute, Chantilly, Virginia; [5]Baylor College of Medicine, Houston, Texas; [6]Greenwood Genetic Center, Greenwood, South Carolina; [7]Quest Diagnostics Nichols Institute, San Juan Capistrano, California.

Kristin G. Monaghan, PhD, Department of Medical Genetics, CFP-4, Henry Ford Hospital, Detroit, MI 48202.

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MATERIALS AND METHODS

Patient population

Reflex testing for 3199del6 was performed on 663 patients positive for I148T among the 7 participating laboratories be-

tween 6/95 and 11/03. The ethnicity/races of these individuals included African American, Caucasian, Hispanic, Middle Eastern, and Asian. One hundred forty-three adult subjects were tested for the indication of carrier screen, and 505 had an unspecified indication, although most are presumed to have been referred for carrier screening. The indications for the remaining cases were as follows: suspected diagnosis of CF (7), family history of a CF mutation (2), male infertility (3), clinical diagnosis of CF (1), and fetal echogenic bowel (2).

Some of the individuals whose data are included in this article were also included in the recently published article by Buller et al.[12] The previous study analyzed 3199del6 by a different method (Promega ReadIT), whereas the analysis reported in this study was done by DNA sequencing. Some patients were tested by both methods and therefore included in both articles. Samples previously published were tested anonymously, so there is no way to determine which specific individuals were already reported, with the exception of Case 1 (Table 2), who is known to be included in the previously published study.

I148T and 3199del6 mutation analysis

Henry Ford Hospital

CF studies for I148T were performed by PCR amplification followed by either a laboratory-developed method involving heteroduplex analysis and RFLP with *Bsr*I or oligonucleotide ligation assay (CF OLA v3.0) (Celera Diagnostics/Abbott Diagnostics/Applied Biosystems). Reflex testing for 3199del6 was performed on all individuals positive for I148T using a laboratory-developed PCR and polyacrylamide gel electrophoresis (PAGE) assay.

Mayo Clinic

CF studies were performed using either Confirmation Sensitive Gel Electrophoresis (CSGE) or the INNO-LiPA CFTR Assay System (Innogenetics). Reflex testing for 3199del6 was performed using a laboratory-developed PCR and heteroduplex assay.

Specialty Laboratories

CF studies were performed using the Linear Array CF Gold 1.0 assay (Roche Molecular Biochemicals). A laboratory-developed assay utilizing PCR and PAGE was used for the detection of 3199del6.

Quest Diagnostics

CF studies were performed by PCR amplification followed by oligonucleotide ligation assay (CF OLA v3.0) (Celera Diagnostics/Abbott Diagnostics/Applied Biosystems). Requested testing for 3199del6 was performed using a laboratory-developed direct sequence analysis of exon 17a of the *CFTR* gene.[13]

Baylor College of Medicine

CF direct mutation analysis was done using a matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry platform (Sequenom), which includes the I148T and 3199del6 mutations.

Greenwood Genetic Center

CF studies for I148T were performed by PCR amplification followed by either laboratory-developed sequencing analysis or oligonucleotide ligation assay (CF OLA v3.0) (Celera Diagnostics/Abbott Diagnostics/Applied Biosystems). Reflex testing for 3199del6 was performed with a laboratory-developed sequencing assay.

RESULTS

A summary of the 3199del6- and/or I148T-positive patients identified is shown in Table 1. Data were collected on 662 un-

Table 1
Summary of 3199del6 and/or I148T chromosomes identified among 663 individuals undergoing CF DNA testing

Indication for DNA testing	Race/Ethnicity	I148T positive	3199del6 positive
Carrier screen	Caucasian	71	0
Unspecified[a]	Caucasian	128	0
Carrier screen	Hispanic	8	0
Unspecified[a]	Hispanic	14	1
Carrier screen	Middle Eastern	7	0
Unspecified[a]	Middle Eastern	6	0
Carrier screen	Other	2	0
Carrier screen	Asian	5	0
Unspecified[a]	Asian	14[b]	0
Carrier screen	African American	3	0
Carrier screen	Unspecified	47	0
Unspecified[a]	Other	3	0
Unspecified[a]	Unspecified	341	3
Subtotal		649	4 (0.6%)
Fetus with echogenic bowel	Middle Eastern	1	0
Fetus with echogenic bowel	Asian	1	0
Male infertility	Caucasian	1	0
Male infertility	Unspecified	2	0
Rule-out CF	Unspecified	6	0
Rule-out CF	African American	1	0
Clinical CF	Caucasian	1	1
Family history of CF mutation	Armenian	1	1
Family history of a CF mutation	Caucasian	1	0
Subtotal		15	2 (13.3%)
Total		664	6 (0.9%)

[a]The majority of persons undergoing CF DNA testing for an unspecified indication are presumably undergoing carrier screening.
[b]12 individuals were heterozygous for I148T, and 1 individual was I148T homozygous.

Table 2
Summary of interesting cases

Case no.	Sex	Race	Indication	Genotype	Poly T status	Clinical information
1	Female	Asian	Carrier screening	I148T[a]/I148T[a12]	9T/9T	Asymptomatic
2	Male	Not provided	Infertility	I148T[a]/ΔF508	Not determined	CBAVD
3	Male	Not provided	Infertility	I148T[a] heterozygote	Negative for 5T	Obstructive azoospermia
4	Male	Caucasian	Infertility	I148T[a]/S1235R[20]	7T/9T	None available
5	Male	Caucasian	Family history of CF mutation	I148T[a]/ΔF508	9T/9T	Fertile male who underwent carrier screening after the identification of ΔF508 in the heterozygous form in his child during newborn screening, child's mother is negative for 25 mutation ACOG/ACMG CF panel
6	Female	Armenian	Family history of CF mutation	D110H/I148T (3199del6 positive)	Not determined	Clinical information on this individual is not available, despite multiple attempts to obtain. DNA testing on her son revealed I148T/3199del6
7	Not provided	Caucasian	Affected with CF	V520F/I148T (3199del6 positive)	Not determined	None available
8	Prenatal test	Middle Eastern	Fetal echogenic bowel	I148T[a] carrier	Not determined	Healthy male reported at age 2 years. A subsequent pregnancy of this couple was diagnosed with ΔF508/I148T (before the availability of 3199del6 reflex testing) and was terminated.
9	Prenatal test	Asian	Fetal echogenic bowel	I148T[a]/M82I	Not determined	Healthy female last evaluated at age 28 months with no signs or symptoms of CF. Sweat chloride in normal range (11 mEq/L)

[a]3199del6 negative.

related I148T heterozygotes and 1 I148T homozygote (total of 663 persons or 664 I148T chromosomes). Overall, we identified 6 unrelated individuals positive for both I148T and 3199del6 (0.9%). With the exception of Case 6 (Table 2), the phase of the I148T/3199del6 was not determined; however, they are presumed *in-cis* based on previous haplotype studies.[9] These studies demonstrated that I148T occurs on a 7T or 9T background, whereas the I148T/3199del6 occurs on a 9T background. Due to ACOG/ACMG recommendations that polyT analysis only be performed as a reflex test for R117H-positive individuals, the polyT status of most of the patients included in this report was not determined. Excluding 15 cases referred for CF DNA testing because of a known or suspected diagnosis of CF (including fetal echogenic bowel and male infertility) or a positive family history, 0.6% of I148T carriers were positive for 3199del6.

Interesting cases identified are presented in Table 2. An unaffected I148T homozygote (Case 1) was detected during routine prenatal screening. Three males (cases 2–4) with congenital bilateral absence of the vas deferens (CBAVD) (I148T/ΔF508), obstructive azoospermia (I148T carrier), and infertility (I148T/S1235R) were identified, the latter 2 negative for 5T at the intron 8 polyT locus. We cannot exclude the possibility that finding I148T in the heterozygous state in a male with obstructive azoospermia (Case 3) is merely a coincidence. Two adults underwent CF carrier testing due to a known family history of a CF mutation. Case 5 was a fertile male, compound heterozygous for I148T and ΔF508, who had a child identified by newborn screening as heterozygous for ΔF508. His wife was negative for the ACMG CF screening panel. Case 6, a female for whom no clinical information is available, was compound heterozygous for D110H and I148T/3199del6. Her son was noted to be positive for I148T/3199del6, confirming the haplotype in this family (assuming that his father is not a carrier for I148T or 3199del6). Case 7 had a clinical diagnosis of CF and was positive for V520F, a CF mutation associated with pancreatic insufficiency, and I148T/3199del6.

Two fetuses with I148T and echogenic bowel were identified, both before the availability of 3199del6 reflex testing for I148T carriers. The parents of Case 8 presented to the Genetics Clinic at 12 weeks gestation for CVS because (1) the mother was a known balanced translocation carrier and (2) advanced maternal age. Chromosome analysis revealed a male karyotype with a balanced form of the maternal translocation. Fetal echogenic bowel was subsequently noted during an ultrasound performed at 18 gestational weeks. Parental CF DNA testing identified one parent as a carrier for ΔF508, and the other had an unusual heteroduplex pattern in exon 4, which was later identified as I148T (before the availability of 3199del6 reflex testing). TORCH titers were negative. The echogenic bowel had resolved on a follow-up fetal ultrasound at 21 weeks gestation. DNA testing performed after delivery was positive for I148T, but negative for ΔF508. At age 2, this child was reportedly healthy with no symptoms of CF. CF testing for a subsequent

pregnancy, performed by CVS was positive for ΔF508 and I148T (again, prior to the availability of 3199del6 reflex testing). The parents were counseled regarding the inability to prenatally predict the course of symptoms and severity of CF, though ΔF508 and I148T were typically associated with pancreatic insufficient (PI) CF. The couple's 2 other children, then underwent CF DNA testing, and neither were compound heterozygous for the 2 mutations. After considering their options, the couple chose to terminate the pregnancy. In 2003, 3199del6 reflex testing was performed, and the I148T carrier parent was negative for 3199del6. The clinical geneticist responsible for this case was contacted regarding these results, so that the family could be counseled regarding these new findings.

The parents of Case 9 underwent CF carrier testing after the diagnosis of fetal echogenic bowel on ultrasound at 18 weeks gestation. One parent was positive for I148T. A previously unreported variant of unknown clinical significance, M82I (ATG→ATA), was identified in the other parent. M82I was incidentally detected by heteroduplex analysis of exon 3, which was used by the laboratory to screen for known CF mutations. The couple was counseled by a board-certified clinical geneticist regarding the difficulties in predicting the affect of a novel sequence change in the *CFTR* gene in the absence of functional studies. It was explained to the couple that M82I was most likely a benign polymorphism because codon 82 is not located in a critical region of the *CFTR* gene and that the substitution of methionine with isoleucine (both hydrophobic amino acids) in this region of the gene was not likely deleterious (J. Zelienski, personal communication, 2004). However, due to the presence of fetal echogenic bowel, one parent a carrier of I148T (classified as a CF mutation at that time), and the other parent a carrier of a variant of unknown clinical significance, prenatal CF testing was performed by amniocentesis. Chromosome analysis revealed a normal female karyotype. CF testing was positive for I148T and M82I. The couple was counseled regarding the possible outcomes including a normal asymptomatic child, atypical CF or mild CF, or CF with pancreatic insufficiency (due to the presence of echogenic bowel). The couple elected to continue the pregnancy. The child passed meconium within 24 hours of birth and stools were normal in the newborn period. The child was evaluated in the genetics clinic at the ages of one month and again at age 21 months. She is healthy and growing appropriately. She has no respiratory or gastrointestinal problems. A sweat chloride test performed in a CF clinic was normal (11 mEq/L). Reflex testing for 3199del6, recently performed, was negative, and the family was counseled regarding these findings.

DISCUSSION

I148T results from a T to C substitution at nucleotide 575 in exon 4 of the *CFTR* gene. This sequence change was initially reported to the CF Consortium in 1990.[1] I148T is located in the first cytoplasmic loop of the first membrane spanning domain of CFTR[14] and results in the substitution of a hydrophobic amino acid, isoleucine, for a polar amino acid, threonine. This region is conserved in humans, mouse, and bovine.[15] Despite the fact that functional studies revealed normal processing, gating, and conductance of the CFTR,[14] I148T was considered to be a severe CF mutation due to its presence in patients with classic CF and a second pathological CF mutation.[4,5,7]

3199del6, a deletion of ATAGTG from nucleotide 3199, is located in *CFTR* exon 17a and results in the deletion of isoleucine and valine at codons 1023 to 1024 within the second membrane-spanning domain. 3199del6 was reported to the CF Consortium in 1998 in a pancreatic insufficient CF patient with I148T and 3199del6 on the same chromosome and ΔF508 on the other chromosome.[1] Another study noted a patient with severe CF and 3199del6 and I148T, though it is not noted specifically whether these mutations are in-*cis* or in-*trans*.[16] 3199del6 also occurs in the absence of I148T, as a patient with severe CF has been reported with 3199del6 (negative for I148T) and G542X.[17]

Recent data suggests that 3199del6 is the deleterious mutation among I148T/3199del6 complex alleles.[9] It is puzzling that early reports of patients with classic CF and I148T/ΔF508 did not identify 3199del6, despite the fact that 2 reports described performing mutation analysis of the entire *CFTR* coding region and splice site junctions either by a screening method (DDGE) or DNA sequencing.[5,7] However, a recent report identified 3199del6 in 24 French-Canadian CF patients originally described as compound heterozygous for I148T and a severe CF mutation.[11] Though there is little doubt that 3199del6 is a deleterious CF mutation, the clinical significance of I148T in the absence of 3199del6 is unclear, as we have identified this genotype in 3 males with infertility, two of the obstructive type.

In 2001, I148T was included in the ACMG/ACOG CF panel due to its high incidence in the general population.[2,3] However, recent data revealed that I148T accounts for ≈0.06% of CF chromosomes,[9] less than the 0.1% frequency for inclusion in the CF screening panel. Therefore, based on its low frequency among CF patients and questions concerning the phenotype associated with I148T, its inclusion in the CF screening panel should be reconsidered. 3199del6 is not among the most common reported CF mutations in the world and is not routinely screened for in most diagnostic laboratories. However, as this and other studies[9,12] have identified this mutation at a low frequency among I148Tcarriers, the addition of 3199del6 to the CF panel would not significantly increase the detection rate of the panethnic CF screening panel.

In our collaborative study of 663 I148T carriers, 0.9% also had 3199del6. Excluding subjects tested because of a suspected or clinical diagnosis of CF or positive family history, the frequency of 3199del6 decreased to 0.6%. We identified 7 unrelated individuals with I148T and a second CF variant, 2 of whom also carried 3199del6. One patient with a known CF severe mutation, V520F, and I148T(3199del6) had a clinical diagnosis of CF, although no specific clinical information is available. The second individual underwent testing due to the presence of two different CF mutations in her two children and was found to have the genotype D110H/I148T(3199del6). No clinical information on this individual was available to the lab-

oratory performing the testing. One of her sons was subsequently found positive for I148T/3199del6. D110H is a rare CF sequence variant, originally reported in a mildly affected CF patient,[18] and recently in homozygous form in an infant with metabolic alkalosis.[19]

The remaining I148T compound heterozygotes were negative for 3199del6. Among these include a fertile male with the genotype I148T/ΔF508 who underwent carrier screening due to the presence of a ΔF508 in his child identified by newborn screening and an asymptomatic female, homozygous for I148T, identified by routine CF prenatal carrier screening. Three males with infertility were I148T positive. One with obstructive azoospermia was heterozygous for I148T, but no other mutation (including 5T) was detected. A male with unspecified infertility was compound heterozygous for I148T and S1235R (7T/9T)[20] and a male with CBAVD was compound heterozygous for I148T and ΔF508 (polyT status not determined).

Two fetuses with echogenic bowel and I148T were identified, both before the availability of 3199del6 reflex testing; however, both I148T carrier parents were subsequently shown to be negative for 3199del6. One fetus was compound heterozygous for I148T and M82I, a previously unreported variant of unknown clinical significance. After extensive genetic counseling the pregnancy was continued and the child is healthy, with no signs of CF and a normal sweat chloride test. The parents of the other case underwent CF carrier screening after the diagnosis of fetal echogenic bowel in a pregnancy. One parent was identified as a ΔF508 heterozygote and the other with an unknown sequence change in exon 4, later identified as I148T. Their child was positive for I148T only and at age 2 was reported as healthy. This couple underwent prenatal CF testing for a subsequent pregnancy, and the fetus was identified as positive for ΔF508 and I148T. After genetic counseling, which included the inability to prenatally predict the course of symptoms and severity of CF, although ΔF508 and I148T were typically associated with PI CF, the couple chose to terminate the pregnancy. The I148T carrier parent in this relationship is now known to be negative for 3199del6.

Our data supports previous findings that a small number of individuals with I148T are positive for 3199del6. The identification of 3 males with infertility and I148T (2 of the obstructive type and 2 who are compound heterozygous for I148Tand another CF mutation) cannot exclude the possibility that I148T alone may be associated with atypical or mild CF. Therefore, care must be taken when discussing genotype-phenotype correlations, especially during prenatal diagnosis. Unfortunately, we were unable to obtain detailed information for all patients in whom I148T was identified; therefore, we cannot make reliable predictions on the phenotype of I148T alone. Reflex testing for 3199del6 should be considered whenever I148T is identified. Such testing is of particular importance in any patient with features of CF or whenever one member of a couple carries a deleterious CF mutation and the other member carries I148T. Further studies are necessary to determine if I148T, in the absence of 3199del6, is associated with mild or atypical CF, including male infertility.

In summary, these data suggest that I148T is not an appropriate mutation for CF screening in the general population. Furthermore, the frequency of 3199del6 is $< 0.1\%$, which is below the frequency criteria for inclusion in the ACMG/ACOG CF mutation screening panel. We recommend that I148T be deleted from the CF screening panel and that 3199del6 not be added. However, if it is confirmed that 3199del6 accounts for a significant proportion of CF alleles in the French-Canadian population, laboratories may wish to offer analysis for this mutation specifically to members of this ethnic group.

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