The dangers of including nonclassical cystic fibrosis variants in population-based screening panels: p.L997F, further genotype/phenotype correlation data

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Purpose: Recently, a major CLIA-certified commercial laboratory began offering an extended cystic fibrosis (CF) carrier screening panel containing 103 variants including p.L997F. Our laboratory has already received two invasive prenatal diagnostic samples where one parent carries a classic CF mutation and the other carries p.L997F. One fetus inherited both variants. **Methods:** We queried our databases containing >2500 CF sequencing analyses to find all individuals with the p.L997F variant. For all compound heterozygous patients, clinical information was obtained by a genetic counselor telephoning the medical provider. **Results:** There were four compound heterozygous patients carrying the p.L997F variant and a second pathogenic CF allele. Three patients were discovered by newborn screening and were asymptomatic at ages 28, 40, and 60 months, respectively. The fourth individual is currently aged 10 years and has the diagnosis of atypical CF with recurrent pancreatitis, sinusitis with nasal polyps, and mild lung disease. His length and weight are in the 90th and 75th centile, respectively. The fifth patient was a compound heterozygote for p.F508del and a complex allele containing p.L997F and a deletion of exons 2–9. This patient has the diagnosis of classical CF. **Conclusion:** The p.L997F variant is not a classical CF mutation, and its inclusion in population-based carrier screening panels is a disservice to couples who may make poorly informed reproductive decisions based on incorrect assumptions. ***Genet Med* 2011:13(12):1042–1044.**

Key Words: *cystic fibrosis, carrier screening, mild variants*

Cystic fibrosis (CF) is the most common life-limiting inherited disease among whites.[1–3] Patients suffering from classical CF exhibit a constellation of symptoms including exocrine pancreatic insufficiency, chronic lung disease, and congenital bilateral absence of the vas deferens in males. There are many forms of milder CF that range from lung disease without pancreatic disease (pancreatic sufficient CF), isolated pancreatitis, chronic sinus, and/or pulmonary disease in adults, to isolated congenital bilateral absence of the vas deferens in males.[1–3]

The CF transmembrane regulatory (CFTR) protein regulates chloride flux.[4] Thus, CF is one of the channelopathies. Almost all patients with classical CF will have diagnostic elevations of chloride ion in their sweat following the newborn period.[3] The diagnosis of CF relies on both clinical observation and laboratory testing.[1–3]

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Recommendations for population-based carrier screening for CF were published in 2001 by the American College of Medical Genetics[5] in combination with the American College of Obstetricians and Gynecologists[6] using a panel of 25 mutations. Initial technical guidelines for population-based CF screening were published in 2002.[6] This initial 25-mutation panel was modified to remove two mutations in 2004 when it became clear that p.I148T (c.443T>C) was a polymorphism and c.1078delT(c.948delT) was found to be present in <0.1% of CF chromosomes.[7] The recommendations targeted mutations believed to cause classical CF, while purposefully avoiding mutations causing mild CF by specifically addressing the p.R117H(c.350G>A) mutation, which alone causes mild CF but when in *cis* with a 5T intron 8 variant allele becomes a classical CF mutation. In the original recommendations and in the revision, the screening committee stressed that only when p.R117H(c.350G>A) is present with the 5T variant should the individual be labeled a CF carrier.[5,7]

Soon after the initial recommendations were published, some commercial laboratories began offering "expanded panels" of mutations and making claims of increased sensitivity of carrier detection, especially in Hispanics and African Americans.[8] This announcement ushered in a decade of the "CF arms race," when commercial laboratories and reagent manufacturers began to offer an increasing number of mutations often with little or no literature support for their selections.[8,9] In fact, almost all expanded panels include two CF variants, p.D1152H(c.3454G>C), and p.L206W (c.617T>G) that are clearly mild or variable alleles.[10–16] A recent publication from a laboratory on its experience with expanded panel screening using a 97-mutation panel described three mild mutations: p.R117H(c.350G>A) without 5T, p.D1152H (c.3454G>C), and p.L206W(c.617T>G) to account for 8.88%, 3.93%, and 1.20% of CF alleles, respectively.[17] Therefore, the inclusion of these variants is likely responsible for the exaggerated claims of increased sensitivity made by various laboratories and vendors for their extended panels (see "Discussion").[9,17,18]

Recently, a major commercial clinical laboratory has escalated the arms race into triple figures by offering a 103-mutation panel. Not only does this panel include the mild variants p.D1152H (c.3454G>C) and p.L.206W(c.617T>G) but also adds p.L997F (c.2991G>C), another allele with a mild and variable phenotype.[19] Several publications support the conclusion that the p.L997F(c.2991G>C) allele is not a classical CF allele. A child homozygous for p.L997F was asymptomatic at 3 years of age, had normal sweat chlorides on multiple occasions, and normal electrophysiologic testing.[20] A compound heterozygote for p.L997F(c.2991G>C) had normal measured sweat gland CFTR activity.[21] A compound heterozygote for p.R117H and p.L997F had normal sweat chloride values results.[22] In a series of adult patients with mild bronchiectasis, p.L997F(c.2991G>C) was determined to be a mild CF mutation.[23] In a study of 18 patients with idiopathic pancreatitis who had CFTR complete sequence analysis, nine patients were compound heterozygotes for p.L997F and another CF mutation. The authors conclude that newborns with p.L997F(c.2991G>C) and another CF mutation

should be classified as having atypical and not classical CF.[24] Two other publications describe patients with pancreatitis who are compound heterozygotes for p.L997F(c.2991G>C) and a classic CF mutation without other symptoms of CF.[25,26] The most comprehensive study of this variant appeared in this journal last year and examined 12 individuals heterozygous for p.L997F and another CF mutation. The only individual diagnosed with classic CF had a complex allele of p.L997.F (c.2991G>C) in *cis* with p.R117L(c.350G>T). The other eight individuals had either no symptoms or mild symptoms. The authors concluded "The eight subjects without the complex allele showed the most varied biochemical and clinical outcome and were diagnosed as having mild CF, CFTR-related disorders or even no disease."[27]

Despite the fact that the 103-mutation panel has only been available for a limited time, our laboratory has already received two prenatal diagnostic samples from couples with one parent being a carrier of p.L997F(c.2991G>C). Both couples received screening from the laboratory in question. When our analysis determined a fetus to be a compound heterozygote for a classical CF mutation and p.L997F(c.2991G>C), we embarked on a review of our data from our comprehensive sequencing database and investigation of the four individuals with compound heterozygosity for the p.L997F(c.2991G>C) mutation and another deleterious CF mutation.

METHODS

Extensive sequencing analysis

Sanger sequencing of all coding exons and intron-exon boundaries was performed as described previously.[28]

Data analysis

All sequencing data are stored in a Microsoft Excel™ file that included the clinical indication for the study, sweat test results, symptoms, and genotype data including the intron 8 poly T tract status.

Clinical information

One of the authors (J.B.R.), a genetic counselor, contacted the ordering physician's office and obtained the relevant clinical information.

RESULTS

The p.L997F(c.2991G>C) variant was found in 23 individuals including four who were compound heterozygotes for p.L997F and a second CF mutation and a fifth individual who had a compound allele consisting of p.L997F(c.2991G>C) and deletion of exons 2–9 determined by quantitative fluorescent PCR. As we cannot determine the breakpoints of this deletion, it is not possible to provide an exact nucleotide position for the deletion. Of these six individuals, three were asymptomatic children who were discovered by elevated newborn trypsinogen. Their ages were 28 months, 40 months, and 60 months, respectively. Their second CF mutations were p.F508del(c.1520-1522delTCT), p.R1162L, and c.71201G>T, respectively. An individual with the diagnosis of classical CF had p.F508del (c.1520–1522delTCT) and a complex allele consisting of p.L997F (c.2991G>C) and a large deletion of exons 2–9. As that deletion includes p.F508del, the p.L997F(c.2991G>C), and the deletion must be in *cis*. The only other symptomatic patient was a compound heterozygote for p.G551D(c.1652G>A), a classic CF mutation, and p.L997F(c.2991G>C). This patient is now aged 10 years and has had recurrent pancreatitis, sinusitis with polyps, and two episodes of pneumonia. His current height is in the 90% and weight in the 75% and carries the diagnosis of atypical CF. This patient had two negative sweat tests performed at a CF center. Deletion/duplication analysis was not available when this individual was evaluated, so it is possible he carries a large rearrangement in *cis* with the p.L997F (c.2991G>C) variant.

There were 18 individuals in the database with p.L997F (c.2991G>C) as their only potential disease-causing CFTR mutation. Of these, eight had also had negative deletion/duplication analyses. In 16 cases, the referring physician had provided an indication for testing. These were atypical CF (11 patients), pancreatitis (two patients, age 13 and 22 years), elevated newborn trypsinogen, and two asymptomatic adults. It is difficult to come to any conclusions regarding the effect of the p.L997F(c.2991G>C) allele as these patients apparently have a functional CFTR allele in *trans*.

We did not find a single instance of the complex allele p.L997F/pR117L described by Lucarelli et al.[27]

DISCUSSION

We confirmed the several previous reports of asymptomatic infants and toddlers who carry p.L997F(c.2991G>C) as at least one CFTR variant allele. In our series of six compound heterozygous patients, three were asymptomatic toddlers. A single 10-year-old patient had recurrent pancreatitis, nasal polyps, sinusitis, mild lung disease with normal growth and development and was classified as atypical CF. The only patient diagnosed with classical CF had a compound allele with p.L997F in *cis* with a large deletion of exons 2–9. Although we did not discover any p.L997F/p.R117L alleles, this confirms the observation of Lucarelli et al.[27] that the p.L997F allele is not, in and of itself, a classical CF allele.

It is possible that the three toddlers in our series may develop pancreatitis or other symptoms at later ages, but it is highly unlikely they will ever develop classical CF if they are asymptomatic at their current ages. The argument could be made that pancreatitis can be a serious disorder, and therefore, screening is appropriate to give parents the opportunity to avoid the birth of an affected child. However, the penetrance of the p.L997F in combination with a classic CF allele cannot be established at this time due to the paucity of data. The decision to recommend population-based carrier screening was based on the accumulated knowledge from thousands of patients, confirming that two classic CF mutations caused a devastating life-limiting disease >99% of the time.[5,6] However, the inclusion of the polymorphism, p.I148T(c.443T>C) in the initial panel demonstrates that even with large amounts of data, mistakes can be made. In addition, there is no consensus that recurrent pancreatitis should rise to the level of population-based carrier screening, even should it be possible. Population-based carrier screening is currently restricted to the prevention of devastating life-limiting disease with known phenotype-genotype correlations.

We reviewed the carrier testing result for both parents who carry the p.L997F(c.2991G>C) variant in our prenatal cases. The results stated that this parent was a carrier of CF without any qualifications. Although it is optimal that couples receive genetic counseling before undergoing an invasive procedure, in this case, the couple was under the assumption that they were at risk for having a child with classical CF and had chosen to undergo an invasive prenatal diagnosis based on that faulty assumption.

The initial recommendations from the American College of Medical Genetics specifically excluded the inclusion of variants causing atypical CF from population-based carrier screening. The specification that p.R117H only be reported as a CF classical mutation when in *cis* with a 5T allele reflects this mandate.[5] The population-based carrier screening program was designed to include only confirmed classical CF mutations and exclude those with any question of being atypical variants because there is no current method to confirm a diagnosis of CF in a fetus even after a pregnancy termination.

A recent publication from Genzyme Genetics Laboratory described a 2-year experience of more than 300,000 CF carrier screens performed with a 97-mutation panel. The mild mutations p.R117H(c.350G>A) without 5T, p.D1152H(c.3454G>C), and p.L206W(c.617T>G) accounts for 8.88%, 3.93%, and 1.20% of CF alleles, respectively.[17] They report that during this 2-year period, 36 presumably healthy adults were found to have two CF mutations. Extrapolating to 8 years of population-based carrier screening, these data lead to prediction that approximately 144 potentially healthy fetuses would have been diagnosed with CF had their parents undergone the expanded panel screening.[17] Thirty of the 36 healthy patients with two CF mutations had at least one copy of p.R117H(c.350G>A) without 5T, p.D1152H(c.3454G>C), or p.L206W(c.617T>G). These mutations account for 14% of all CF mutations discovered by the laboratory during this time. Therefore, the increased detection rates claimed from expanded panel screening are due almost entirely from these mild mutations that have high prevalence in certain populations. Also of note is that 11 mutations on the panel were not seen in a 2-year period with more than 350,000 carrier screens.[17]

There are many reasons to decry the CF arms race. As summarized in an editorial in this journal, they include a false sense of security, a false sense of danger, uncertain allele frequencies and arbitrary selection of rare variants, paucity of genotype-phenotype correlation data, problems with ethnic mutation data, added cost, potential for monopolization, dwindling predictive value, and law of diminishing returns.[8] This study adds another reason, the inclusion of mild or atypical CFTR variants, which may result in couples making poorly informed reproductive decisions.

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