Human Genetics Society of Australasia Position Statement: Population-Based Carrier Screening for Cystic Fibrosis

Martin B. Delatycki,[1,2] Jo Burke,[3] Louise Christie,[4] Felicity Collins,[5] Michael Gabbett,[6] Peter George,[7] Eric Haan,[8] Liane Ioannou,[2,9] Nicole Martin,[10] Fiona McKenzie,[11] Peter O'Leary,[12] Nicole Scoble-Williams,[13] Gillian Turner,[14] and John Massie[2,15]

[1]Clinical Genetics, Austin Health, Melbourne, Victoria, Australia
[2]Murdoch Childrens Research Institute, Melbourne, Victoria, Australia
[3]Tasmanian Clinical Genetics Services, Hobart, Tasmania, Australia
[4]Hunter Genetics, Newcastle, New South Wales, Australia
[5]Clinical Genetics, The Children's Hospital at Westmead, Sydney, New South Wales, Australia
[6]Genetic Health Queensland and The University of Queensland, Brisbane, Queensland, Australia
[7]University of Otago, Christchurch, New Zealand
[8]South Australian Clinical Genetics Service, SA Pathology (at Women's and Children's Hospital), and University of Adelaide, School of Paediatrics and Reproductive Health, Adelaide, South Australia, Australia
[9]Monash University, Melbourne, Victoria, Australia
[10]Queensland Fertility Group, Brisbane, Queensland, Australia
[11]Genetic Services of Western Australia, Perth, Western Australia, Australia
[12]Curtin University, Bentley, Western Australia, Australia
[13]IVF Australia, Bondi Junction, New South Wales, Australia
[14]University of Newcastle, Newcastle, New South Wales Australia
[15]Respiratory Medicine, Royal Children's Hospital, Melbourne, Victoria, Australia

Since the discovery in 1989 that mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*) underlie cystic fibrosis (CF), the most common life shortening genetic disorder in Caucasians, it has been possible to identify heterozygous mutation carriers at risk of having affected children. The Human Genetics Society of Australasia has produced a position statement with recommendations in relation to population-based screening for CF. These include: (1) that screening should be offered to all relatives of people with or carriers of CF (cascade testing) as well as to all couples planning to have children or who are pregnant; (2) the minimum *CFTR* mutation panel to be tested consists of 17 mutations which are those mutations that are associated with typical CF and occur with a frequency of 0.1% or higher among individuals diagnosed with CF in Australasia; (3) that genetic counselling is offered to all couples where both members are known to have one or two *CFTR* mutations and that such couples are given the opportunity to meet with a physician with expertise in the management of CF as well as a family/individual affected by the condition.

∎ **Keywords:** cystic fibrosis, heterozygote, carrier, carrier testing, genetic counselling

Background

Population-based carrier screening refers to genetic testing in order to identify heterozygous carrier status of individuals who do not have an increased risk of being a carrier (i.e., they have no family history of the condition). Carriers of autosomal recessive and most X-linked recessive conditions are healthy. The main health implications of being a carrier are for the children of that individual. In Australia, the main conditions for which population carrier screening is undertaken are hemoglobinopathies (e.g., β- thalassemia) and autosomal recessive diseases are more common in the Ashkenazi Jewish community, including among others, Tay Sachs disease.

Since the cloning of the *CFTR* gene in 1989, it has been possible to identify carriers of CF. The offer of carrier screening for CF was introduced into obstetric practice in

the United States in 2001 and there have been a number of local programs, in Scotland, Denmark and England, which have provided useful data regarding uptake of screening and use of reproductive interventions by carrier couples.

The basic principles of population screening were developed in 1968 by Wilson and Jungner (1968). In the era of genetic screening there have been additions to these principles. Importantly, appropriate education and counseling should be provided so that individuals can make informed decisions about being tested: the individual's decision is respected and the individual is protected from stigmatization and discrimination (Khoury et al., 2003).

Cystic Fibrosis

CF is an autosomal recessive disorder caused by mutations in the *CFTR* gene, which encodes CFTR, a chloride channel gene. Homozygosity or compound heterozygosity for mutations in the *CFTR* gene can be associated with a broad range of phenotypes. Most (85–90%) patients with CF have progressive suppurative lung disease and pancreatic exocrine insufficiency (classical CF). Other features include neonatal meconium ileus, multifocal biliary cirrhosis, elevated sweat electrolytes and congenital bilateral absence of the vas deferens (CBAVD). Most individuals with classical CF have two *CFTR* gene mutations that result in no functional *CFTR* activity. There are, however, some *CFTR* mutations that are associated with residual *CFTR* activity, resulting in milder disease that includes a range of presentations from pancreatic sufficient CF (which in general is associated with milder lung disease) through to isolated organ system involvement such as CBAVD, chronic bronchiectasis or chronic pancreatitis, and in some cases no identifiable problems. The daily therapies for classical CF are rigorous and include chest physiotherapy, mucolytic agents, frequent or continuous courses of antibiotics, pancreatic enzyme replacement and nutritional supplementation. Hospital admissions become increasingly frequent and prolonged as the disorder progresses. Individuals with CF generally receive their medical care in specialist multi-disciplinary clinics. The median survival for individuals with classical CF with pancreatic insufficiency is 37 years while it is approximately 56 years for those with pancreatic sufficiency (American College of Obstetricians and Gynecologists (ACOG) Committee on Genetics, 2011). Lung transplantation has also proven effective for some individuals with end-stage lung disease, but median survival at 5 years post transplantation is approximately 60% (Diso et al., 2013).

There is active research to identify therapies to further improve quality of life and longevity for individuals with CF. A number of approaches to restoring *CFTR* function have been investigated, including gene replacement therapy and medications to activate *CFTR*. In 2012, the first such therapy, ivacaftor, was licensed for use in Australia. Ivacaftor is a so-called *CFTR* potentiator and has only been proven

to be effective for individuals with the p.Gly551Asp mutation (representing 4.7% of mutations in Australia; Ramsey et al., 2011). Clinical trials of ivacaftor for other class III–VI mutations (see below) are planned and trials of other medications to activate p.Phe508del *CFTR* are underway.

The prevalence of CF varies in different populations. In Caucasians and Ashkenazi Jews it affects around 1 in 2,500, with a carrier frequency of approximately 1 in 25. The incidence among South East Asians is believed to be less than 1 in 100,000, with a carrier frequency about 1 in 160. CF is rare in Australian Aboriginals, Torres Strait Islanders and New Zealand Maoris.

CFTR Mutations

More than 1,900 alterations have been identified in the *CFTR* gene (http://www.genet.sickkids.on.ca/app). One mutation, p.Phe508del, accounts for about 70% of the mutations worldwide, although the frequency varies in different populations. The next most common mutations together account for 5–8% of mutations, while most of the other 1,900 *CFTR* alterations have been identified in only one or a few individuals or families. Only a relatively small number of the alterations have been definitively shown to be associated with disease through in-vitro studies of *CFTR* activity (http://www.cftr2.org/). *CFTR* mutations have been divided into six classes, depending on the anticipated functional outcome of the mutation: class I (no *CFTR* production), class II (*CFTR* degraded within cytosol), class III (no functional *CFTR* activity), class IV (reduced *CFTR* activity), class V (splicing defects), and class VI (mutations decreasing stability of *CFTR* that is present or affecting the regulation of other channels) (http://www.umd.be/CFTR/W˙CFTR/gene.html). As a general rule, class I, II, III and VI mutations are associated with classical CF while class IV and V mutations are associated with a broader phenotype that ranges from pancreatic sufficient CF to CBAVD or no disease.

Guidelines from the ACOG and American College of Medical Genetics (ACMG) recommend that CF-causing mutations with an allele frequency of ≥0.1% in people with classical CF are included in their population screening panel (ACOG Committee on Genetics, 2011). Currently, a panel of 23 mutations is recommended. While most of these mutations are associated with classical CF, the decision to include them is based purely on population frequency and not on either CFTR dysfunction or phenotypic outcome. One of the mutations included in the ACOG/ACMG panel is p.Arg117His which may, when present in compound heterozygosity with a class I, II, III or VI mutation, have a broad phenotype that includes no disease, isolated CBAVD or pulmonary disease. It has been found that when p.Arg117His occurs in *cis* (in the same copy of *CFTR*) with the intron 8 polythymidine repeat sequence, 5T, it usually causes pulmonary disease, whereas, when it is in *cis* with 7T or 9T

it generally does not. The overall clinical penetrance of p.Arg117His/p.Phe508del is in the order of 3% (Thauvin-Robinet et al., 2009). In the ACOG/ACMG guidelines, reflex testing is recommended such that when p.Arg117His is identified by a screening laboratory, testing is performed to assess whether it is in *cis* with a 5T, 7T or 9T. Only those who have p.R117H in *cis* with 5T are notified that they are carriers and have carrier testing recommended for their partner. The ACOG/ACMG guidelines are clear that testing for 5T should only occur when p.Arg117His is found and not be tested routinely.

Cystic Fibrosis Screening in Australasia

Newborn screening for CF by immunoreactive trypsinogen and genetic testing is undertaken in all Australian states and New Zealand. This results in early diagnosis of most affected individuals. Newborn screening also enables parents of affected individuals to be identified as carriers and to be offered genetic counseling in relation to subsequent child bearing. Reproductive options include prenatal testing, pre-implantation genetic diagnosis (not available in all centers), donor gamete/embryo, to have no further children, and adoption. Newborn screening also identifies some infants who are CF carriers and, by implication, at least one of their parents as a carrier.

In most Australian states and New Zealand, carrier testing is offered free of charge to close relatives of individuals with CF and relatives of carriers. The exception is in Victoria where free testing is only offered to the parents of an individual with CF. The degree of relatedness required for access to free testing in other Australian states and New Zealand differs in different jurisdictions but is generally first +/- second degree relatives of an affected individual or carrier. This is called cascade testing. Importantly however, the vast majority of individuals born with CF have no family history of the condition.

International Practice

The ACOG, ACMG and the US National Institutes of Health (NIH) recommend that all individuals who are planning a pregnancy or are in the early stages of pregnancy be offered CF carrier screening (ACOG Committee on Genetics, 2011). In contrast, population carrier screening is not currently recommended in the United Kingdom, France and Canada.

Carrier Detection

The sensitivity of a screening test depends on the a priori risk of an individual being a carrier, as well as the screening test offered. Among the non-Ashkenazi Jewish Caucasian population, about 88% of carriers will be identified by the ACOG/ACMG recommended 23 mutation panel, and thus about 77% of carrier couples will be detected (Haddow &

Palomaki, 2002). For Ashkenazi Jewish couples, the figures are around 94% and 88% respectively, and among Asian couples they are 49% and 24% respectively. The residual carrier risk after a negative test result on the 23 mutation testing panel in Caucasians is about 1 in 200, 1 in 400 in Ashkenazi Jews, and 1 in 180 in Asians.

Screening Models

There are two main screening models:

1. **Two-step screening**. In this model one member of the couple (usually the female) is tested initially. If she is found to be a carrier then her partner is tested. If he is also found to be a carrier, formal genetic counseling is offered and reproductive options are discussed. Family members of carriers are also offered screening (cascade screening).

2. **One-step screening**. Here, both members of a couple are tested at once. There are two models for the way that results are handled, couple risk screening and expanded one step screening:

(a) *Couple risk screening*. In this model, samples are obtained from both members of the couple and they are treated as a single unit. If both are found to be carriers they are offered genetic counseling. If one or neither is found to be a carrier they are informed that they are at low risk for having a baby with CF and no further action is taken. The advantage of this model is that it requires less work in terms of counseling carriers and obtaining a sample from the other member of a couple when one is found to be a carrier. However, if both members of a couple are not available, screening cannot occur. Furthermore, if carriers are not informed of their individual carrier status, the opportunity for cascade screening is missed. In addition, if a member of a couple has a new partner they may not request screening in the potentially mistaken belief that because they had a low risk result with their original partner, the new partnership is also at low risk.

(b) *Expanded one-step screening*. In this model, both partners are tested at the same time and each is given their result. The benefit of this approach is that a much more accurate residual risk can be given if both partners are negative for the screened mutations. This approach can also save time and reduce anxiety by eliminating the need to recall a partner if the first one is a carrier, and maximizes the opportunity for cascade screening.

Single Disorder Versus Expanded Carrier Screening

Previously, the ACMG has defined the standards for prenatal/preconception screening for several rare disorders, including CF, spinal muscular atrophy, and a panel of recessive disorders common in the Ashkenazi Jewish

population. The advent of next generation sequencing technologies has made feasible broader based approaches to genetic testing for general population preconception screening through the testing of multiple genes 'with high fidelity, quick turnaround time and lower costs' (Grody et al., 2013). The ACMG has released a position statement acknowledging this technologically driven shift and highlighting the need for clear criteria for selection of target genes and adherence to basic ethical principles in devising any general population screening program (Grody et al., 2013).

Direct to consumer testing is already available for screening of panels of several hundred mutations in >100 different genes and the NIH is commencing a clinical trial to determine the acceptability and utility of a whole genome sequencing approach (Clinical trial: NCT01902901). While the laboratory costs of such expanded screening programs may well reduce, it is likely that the costs associated with providing information to patients and pre/post test counseling will increase.

Timing of Screening

Screening for CF carrier status can theoretically be carried out from the newborn period. In practice it is offered in high school, pre-pregnancy or during pregnancy. That is, it is offered to individuals old enough to understand the implications of screening and able to make an informed choice about whether or not to have screening. Pre-pregnancy screening is ideal, as it allows couples time to have both partners tested if a two-stage approach is being used, and gives carrier couples increased reproductive options compared to antenatal screening. These options include prenatal diagnosis, pre-implantation genetic diagnosis, donor gamete/embryo, not having children and adoption. The reality is that many couples do not plan pregnancies and even when pregnancies are planned, issues related to risks of genetic disease in children are not considered until a pregnancy occurs. Therefore any CF screening program needs to include early pregnancy screening if it is to be useful to the greatest number of people.

Outcomes of Screening

From the studies reviewed in the ACCE (**A**nalytic validity, **C**linical validity, **C**linical utility and associated **E**thical, legal and social implications) document from the US Centers for Disease Control, there were 54 couples who were both carriers of CFTR mutations, of whom 49 (91%) chose to have prenatal diagnosis (Haddow & Palomaki, 2002). Of the 18 couples with an affected fetus, 15 (83%) chose to terminate the pregnancy. An Australian study found that of 11 carrier couples identified where the female was pregnant at the time, nine chose prenatal diagnosis and the three affected pregnancies were terminated (Massie et al., 2009).

An important consideration in offering screening for CF is the psychosocial impact of screening. A systematic review of published data, which included data from Australia, found minimal evidence of adverse outcomes for women identified as carriers of CF by population carrier screening programs (Ioannou et al., 2010; 2014).

Health Economic Considerations

Economic considerations are one important factor in deciding whether or not to introduce a screening program. A review of the literature regarding economic evaluation of CF screening found much heterogeneity in study design, modeling and reporting (Radhakrishnan et al., 2008). Nevertheless, the majority of studies reported that the cost of a screening program is less than the potential healthcare costs averted through the birth of fewer individuals with CF. One study compared CF screening to other screening programs, including newborn screening for PKU and mammography, and concluded that CF screening represents good value for money by comparison (Rowley et al., 1998).

Recommendations

The HGSA CF Population Screening Committee recommends that:

1. CF carrier testing should be offered to relatives of individuals with CF and relatives of carriers of CF (cascade testing). The underlying mutation(s) in the affected/carrier family member should be sought to enable the most accurate carrier testing. If the underlying mutation(s) is not known, referral for genetic counseling is appropriate. In general, cascade testing should be offered to first-degree relatives of the affected/carrier individual in the first instance. The first-degree relatives of those found to be carriers in the first round of cascade testing should then be offered testing.
2. All couples intending to have children, or who are pregnant, should be made aware of the availability of CF carrier screening. Pre-pregnancy screening is preferable to antenatal screening because it allows more options for carrier couples, including pre-implantation genetic diagnosis and donor gamete/embryo, but it is recognized that screening in the early antenatal period is more often sought.

   Screening should be either expanded one-step or two-step. Expanded one-step screening has the advantage over two-step screening that more carriers (but *not* more carrier couples) are identified and therefore, there is greater opportunity for cascade screening. One-step couple risk screening is not recommended as most carriers will not be made aware of their carrier status and the opportunity for cascade screening will be missed. In addition, if individuals change partners, they may mistakenly believe that they are at low risk of having a child with CF because they were at low risk with their original partner. If screening is performed in pregnancy, it is

preferable to screen both members of a couple simultaneously so that results are available in time for prenatal testing to be considered if both are found to be carriers.

3. People considering CF carrier testing should receive sufficient information to allow them to make an informed choice about whether or not to proceed. Appropriate written educational material should be made available. The sensitivity and limitations of testing should be made clear. This includes information on test sensitivity in individuals with different ethnic backgrounds and the fact that testing cannot identify all carriers. The reproductive options available where both members of a couple are identified as carriers should also be included in pretest education. Informed financial consent should be obtained if testing will result in out-of-pocket expenses for the tested individual.

4. Carriers identified by CF population screening should be informed about the significance of their result. Their current and all future partners should be offered screening. Information about the risk to, and availability of cascade testing for relatives should be provided. Similarly, the partners of individuals with CF should be offered carrier testing.

5. All mutations with a frequency greater than 0.1% of CFTR mutations among individuals with clinically diagnosed CF in Australasia should be considered for inclusion in the screening panel. However, mutations that are associated with an unclear phenotype, or that lead to atypical disease without lung involvement or to no disease, should not be included in the screening panel even if they occur with a frequency greater than 0.1%. The Committee, therefore, recommends that p.Arg117His, p.Pro67Leu and the intron 8 polythymidine repeat sequence not to be included in the panel. The Committee recognizes that many commercially available kits contain these low penetrance mutations. If such kits are used, appropriate advice should be given to individuals/couples found to have a mutation associated with low penetrance and/or mild phenotype.

Twenty-eight mutations occur with a frequency greater than 0.1% in individuals with clinically diagnosed CF in Australasia (see Appendix for methodology). However, the number of alleles that were available for analysis (4,773) was such that if a number of sibs from one family were affected by CF, the mutation could appear with a frequency of greater than 0.1% and yet be very rare in the community. The Committee therefore recommends that the 17 mutations identified in at least 10 alleles be included as a minimum in the screening panel. This panel will identify a minimum of 80% of carriers in Australasia, which equates to about 650,000 individuals. The 17 mutations are:

1. p.Phe508del
2. p.Gly551Asp
3. p.Gly542X
4. p.Asn1303Lys
5. c.489+1G>T
6. c.1585–1G>A
7. p.Trp1282X
8. p.Arg553X
9. p.Ile507del
10. c.3717+12191C>T (3849+10kb)
11. p.Arg560Thr
12. p.Val520Phe
13. p.Arg1162X
14. c.3528delC
15. p.Asp1152His
16. c.1766+1G>A
17. p.Gln493X

Collection of data regarding CFTR mutation frequency in Australasia should continue and the recommended mutation panel should be revised as new information becomes available.

It is important to note that this panel alone is not sufficient for testing individuals affected by CF (including pancreatic sufficient CF and mild lung disease) or CFTR related disorders such as CBAVD and recurrent pancreatitis.

6. Sequencing of the CFTR gene for carrier testing (e.g., when one partner is found to be a carrier and the other does not carry one of the mutations tested for in the screening panel) is not recommended because of the low yield of mutations and the likelihood of identifying alteration(s) whose significance is unknown.

7. Where both members of a couple are found to be carriers of CFTR mutations, they should be offered genetic counseling. The couple should be offered the opportunity to meet with a physician with expertise in CF and a family/individual affected by CF. Where couples are at risk of having a child with CF that would have one or two copies of the p.Gly551Asp mutation, they should be informed of the effects of ivacaftor, its availability and funding status.

8. Where a pregnant woman is identified as a carrier of a CFTR mutation and her partner is unavailable for testing, she should be offered genetic counseling to discuss her options, including prenatal testing.

9. For screening to be offered, clinical and laboratory infrastructure is required. This includes educational materials to assist individuals to make informed choices about whether to have screening, and also for providers to be able to offer screening. Also required are sufficient genetic counselors and prenatal diagnosis facilities. Laboratories should be appropriately accredited to undertake genetic testing. Laboratory infrastructure requires adequate staffing to ensure timely reporting of results.

10. While it is recommended that all couples should be made aware of the availability of CF carrier screening, the Committee recognizes that most such carrier testing

in Australia is provided on a user-pays basis at present and consequently can only be obtained by those who can afford it, resulting in inequity of access. Governments should consider funding CF carrier screening.

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Appendix
Methodology of Collection of Data for Mutation Panel Recommendations

Data was collected from the following clinical services and laboratories with regard to the *CFTR* mutations in individuals with CF:

Children's Hospital at Westmead, New South Wales
National Referral Laboratory, SA Pathology, South Australia
Hunter Genetics, New South Wales
Newborn Screening, Pathology Queensland
University of Otago, Christchurch, New Zealand
Palms Pathology, New South Wales
PathWest, Western Australia
Sydney Children's Hospital, New South Wales
Victorian Clinical Genetics Services
Genetic Services of Western Australia

A total of 4,773 alleles were available for testing. The mutation was unknown for 532 (11.1%) alleles but not all were fully sequenced. Therefore, the mutation panel recommended represents a minimum number and as more alleles are sequenced, it is likely that more mutations will meet the inclusion criteria.

The Committee wishes to express its gratitude to the Directors of the above named laboratories for providing this data.