Genetic counselling after carrier detection by newborn screening when one parent carries $\Delta F508$ and the other R117H

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Newborn screening (NBS) for cystic fibrosis (CF) has been carried out in Victoria, Australia since 1989. The primary screen is immunoreactive trypsinogen (IRT) followed by $\Delta F508$ mutation analysis. As part of this process, carrier babies are detected and their parents are routinely offered carrier testing as part of their follow up. The $\Delta F508$ parent is identified and the other parent has an extended mutation analysis performed in case they are also a carrier. One of the mutations in the extended analysis is R117H which is associated with a broad phenotypic range, from CF with suppurative lung disease, to no clinical disease. We present four healthy $\Delta F508$ carrier babies identified by our NBS service with both parents identified as carriers, one $\Delta F508$ and the other R117H. Owing to the variable phenotype associated with R117H we have developed an approach to this difficult genetic counselling situation. Centres offering or considering NBS for CF will need an approach to this problem.

Newborn screening (NBS) for cystic fibrosis (CF) has been carried out in Victoria, Australia since 1989.¹ This is to offer early diagnosis and facilitate genetic counselling for affected families. Newborn screening for CF involves a day 3 heel prick blood specimen to measure immunoreactive trypsinogen (IRT) as the primary screen. Babies with an IRT >99th centile of values have $\Delta F508$ mutation analysis from the heel prick specimen. Babies who are homozygous for $\Delta F508$ are considered to have CF while those with one $\Delta F508$ allele are referred for sweat testing to determine whether they have CF (sweat chloride >60 mmol/l) or are carriers only (sweat Cl <40 mmol/l).²

We routinely offer genetic counselling to families of babies with CF and also to families of CF ($\Delta F508$ heterozygote) carriers. The parents of babies who are $\Delta F508$ carriers are offered carrier testing, the $\Delta F508$ parent is identified, and the other parent tested for 10 CFTR mutations. This accounts for approximately 85% of all CF mutations in the Australian Caucasian population. This approach provides useful information, reassuring parents where only one is a carrier and providing the opportunity for prenatal testing in subsequent pregnancies if both parents are identified as carriers.

One of the mutations in the extended mutation analysis is R117H, which is considered to be a mild CF mutation associated with a broad phenotype, ranging from no clinical disease, to CF with suppurative lung disease.³ Subjects who are compound heterozygotes for $\Delta F508$ /R117H may have raised (Cl >40 mmol/l) or normal (Cl <40 mmol/l) sweat electrolytes.

In the course of routine carrier testing following NBS, we have identified four couples in which one partner carried the $\Delta F508$ mutation, and the other R117H. The findings from the carrier testing raised the question of how to counsel these families regarding the carrier infant who could be a compound heterozygote ($\Delta F508$ /R117H) with a normal sweat test and their risk of subsequent children being affected with CF. Furthermore, this raised the issue of whether genotyping should be offered for each of their other children who could be asymptomatic compound heterozygotes and therefore potentially at risk of developing CF.

The aim of this paper is to present the details of NBS and carrier testing offered to these four families and to discuss the

implications for genetic counselling when both parents of $\Delta F508$ carrier babies are found to be carriers, one with $\Delta F508$ and the other with R117H. We believe that this information is extremely important for centres that are already screening or considering the introduction of CF newborn screening so that appropriate genetic counselling is offered and an approach to the discovery of well infants who are compound heterozygotes with $\Delta F508$ /R117H is developed.

CASE SUMMARIES

Infant 1: A term male infant was detected by newborn screening with an IRT >99th centile and found to be a $\Delta F508$ heterozygote. He had a sweat test (Wescor macroduct) with an adequate volume of sweat collected, Cl 28 mmol/l and Na 21 mmol/l. He was considered to simply be a $\Delta F508$ carrier and his parents were referred for genetic counselling and carrier detection. His father was found to be the $\Delta F508$ carrier and his mother carried R117H. Intron 8 polythymidine sequencing showed a 7T/7T sequence. After genetic counselling (LC and JM) the parents elected to test the infant for R117H and he was found to be a compound heterozygote for $\Delta F508$ /R117H (9T/7T).

The parents also elected to test their other two children aged 6 and 4 who were well, and one was also a $\Delta F508$ /R117H (9T/7T) compound heterozygote and the other a carrier of R117H (7T/7T). Both compound heterozygote children are well with no features of CF. The parents have elected to bring these two children for review by a CF physician (JM) once a year.

Table 1 summarises the results of the other three infants and their families.

DISCUSSION

In the course of newborn screening for CF we have identified four infants who were $\Delta F508$ heterozygotes with normal sweat electrolytes and whose parents were both identified as carriers, one with $\Delta F508$, and one with R117H. After genetic counselling, all four couples elected to test their infants to see if they were compound heterozygotes, and their other children who were asymptomatic. In each case, the infants were found to be compound heterozygotes for $\Delta F508$ /R117H. This is a

Table 1 Details of four infants identified by newborn screening as $\Delta F508$ carriers with both parents identified as cystic fibrosis carriers, one parent with $\Delta F508$ and the other with R117H

	Newborn screening results	Sweat test	Parents' genotype	Infant's genotype	Siblings
Infant 1	IRT 99th centile $\Delta F508/-$	Cl 28 mmol/l Na 21 mmol/l	Mother: R117H/ Father: $\Delta F508/-$	$\Delta F508/R117H$ (7T/7T)	F, 10 y, asymptomatic, $\Delta F508/R117H$ (7T/9T) M, 12 y, asymptomatic, R117H
Infant 2	IRT 99th centile $\Delta F508/-$	Cl 18 mmol/l Na 17 mmol/l	Mother: R117H/ Father: $\Delta F508/-$	$\Delta F508/R117H$ (9T/7T)	M, 5 y, asymptomatic, R117H
Infant 3	IRT 99th centile $\Delta F508/-$	Cl 33 mmol/l Na 27 mmol/l	Mother: R117H/ Father: $\Delta F508/-$	$\Delta F508/R117H$ (9T/7T)	F, 6 y, asymptomatic, $\Delta F508/R117H$ (9T/7T) M, 4 y, asymptomatic, R117H (7T)
Infant 4	IRT 99th centile $\Delta F508/-$	Cl 29 mmol/l Na 29 mmol/l	Mother: R117H/ Father: $\Delta F508/-$	$\Delta F508/R117H$ (9T/7T)	Nil

challenging counselling situation and we will present the available information from the literature that guided the counselling process.

R117H a class IV cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation, which is known to produce a CFTR protein with reduced chloride transport.^{4,5} The intron 8 polythymidine sequence in the gene influences the severity of the CF phenotype when R117H is found in conjunction with a severe CF mutation.⁶ The thymidines are found in sequences of 5 (5T), 7 (7T), or 9 (9T) repeats with the fewer number of thymidines associated with less efficient mRNA splicing at the splice acceptor site with greater skipping of exon 9 in the CFTR protein. As a result, there is lower than normal level of full length CFTR mRNA and a decrease in mature, functional CFTR protein.⁷ R117H on a 5T background is acknowledged as a disease producing CFTR mutation and in combination with a severe mutation (for example, $\Delta F508$) generally results in pancreatic sufficient CF.⁸ There is less known about R117H on a 7T background. In one series, subjects were asymptomatic or had absent vas deferens only.⁹ A more recent series of patients known to CF clinics through clinical presentation or NBS included a small number of adults with adult onset CF disease.³ It is possible, however, that compound heterozygotes with R117H on a 7T background remain asymptomatic and are never tested. The range of possible phenotypes associated with the R117H mutation highlights the importance of obtaining the intron 8 polythymidine sequence prior to predicting the severity of the phenotype in an R117H compound heterozygote.¹⁰ The difficulty lies in the certainty of the information provided to parents and patients; in particular those with R117H on a 7T background.

The four couples were informed of their carrier testing results by phone and counselled in person by the genetic counsellor responsible for NBS and a respiratory physician. It was explained that the infant detected by newborn screening may be a carrier of $\Delta F508$ only or could be a compound heterozygote with $\Delta F508/R117H$, but that it was not possible to determine which outcome was likely from the results of the sweat test alone. The possible outcomes were explained to the parents. If the infant was a $\Delta F508$ carrier alone, there would be no health risk at all (similar to both parents who were healthy carriers), the only implication being that the infant's future partner should be tested for CFTR mutations when pregnancy became relevant to enable accurate assessment of the risk of having a child with CF. This outcome would be expected to "clear the air" and provide reassurance to the parents about the future health of their child. If the infant was tested and found to be a compound heterozygote, there was a range of possible outcomes, from no CF disease, congenital bilateral absence of the vas deferens (in a male), or late onset cystic fibrosis lung disease. Unfortunately there is no way of predicting with any certainty these outcomes. What was made clear was that these well infants, with no symptoms of CF, should not be labelled with a diagnosis of CF even if found to

be compound heterozygotes.¹¹ The parents understood that they would be supported in their decision. With this information, the families all made the choice to test their infants for R117H.

Several reasons were given by the parents for making this decision, the most common being that a "negative" result would relieve their anxieties, as the parents felt that now they had been informed of the situation, any cough or wheezing illness would particularly concern them in the future. In addition, each felt that, as the phenotype of a compound heterozygote (with a 7T background) is still unclear, it would be important to have access to a respiratory physician should there be any medical concerns.

The next decision was whether to test the healthy siblings of the carrier infant to clarify their genotypes as it was possible that they too may be compound heterozygotes with $\Delta F508/R117H$. This proved an ethical dilemma, as guidelines from the Human Genetics Society of Australasia (HGSA) recommend that carrier testing not be offered to children.¹² This recommendation exists because there are no proven health benefits to knowing CF carrier status for a child, and that it is preferable that individuals are given the opportunity to make autonomous decisions regarding genetic testing. We felt that this situation fell outside the HSGA guidelines because of the possibility of CF disease developing in compound heterozygotes and the parents' right to be aware of this. The following options for the siblings were discussed with the parents: do nothing further at the moment but test for carrier status when it was likely to be meaningful in the future; sweat test the children to exclude current CF; or genotype the siblings and deal with the information as discussed above. The parents were made aware that they would be supported in their decision whatever it was. With this information all four couples chose to genotype their other healthy children.

The result of the R117H testing revealed that all four infants were compound heterozygotes for $\Delta F508/R117H$ on a 7T background as were a number of their healthy siblings. Given that three of the four infants had sweat chlorides ranging from 29 to 33 mmol/l, this result is perhaps not surprising. Investigators from the Wisconsin NBS service have reported the mean sweat chloride in 184 healthy infants (mean age 9.3 weeks) with no $\Delta F508$ mutation to be 10.6 (5.3) mmol/l (95% CI 9.9 to 11.3) and in 128 $\Delta F508$ heterozygotes (carriers detected with an IRT >99th centile, mean age 8.8 weeks) to be 14.9 (8.4) mmol/l (95% CI 13.4 to 16.4).¹³ Similar figures were reported from NSW with $\Delta F508$ carriers (IRT >99th centile) having a mean sweat chloride of 15.5 (6.2) mmol/l.¹⁴ This means that a sweat chloride of 30 mmol/l is 4 standard deviations above healthy control infants and 2 standard deviations above $\Delta F508$ carriers.

It is not clear how many infants detected by NBS with an increased IRT and one $\Delta F508$ allele might actually be compound heterozygotes with normal sweat electrolytes. In one series of 57 $\Delta F508$ infants detected by NBS, five were

found to be compound heterozygotes with R117H(7T) and 10 with 5T.¹⁵ The frequency of R117H has been reported as 1% of CFTR mutations in CF patients who have been genotyped, but may be higher in the community.¹⁶ Witt *et al* found 0.6% of pregnant women to be R117H carriers, but this paper did not include intron 8 polythymidine sequences.¹⁷

The final issue for the four couples was to determine the frequency of follow up of healthy compound heterozygote children with $\Delta F508/R117H(7T)$. This depended on the parents' level of concern and ability to present to an appropriate service if symptoms developed. It would be inappropriate for these children to be seen in a CF clinic and to be seen too often, as this might send mixed messages about the inevitability of symptoms. After discussion with the parents of our four infants, all chose an annual check up with a physician familiar with CF, but in a non-CF clinic setting.

Given the uncertain nature of the outcome of asymptomatic infants detected with R117H, it could be questioned as to the value of including it in a CFTR mutation panel. The current technology used for the extended CFTR mutation analysis uses multiplex testing for a number of severe exon 4 mutations, and R117H is also detected. With multiplex testing it is not possible to suppress this result without withholding the information, and we feel it is our obligation to inform patients/parents of their results. In the future, gene sequencing may solve this problem; however, it can be argued that finding R117H on a 5T background is worthwhile as it is a disease producing mutation.

We have developed an approach to the counselling of couples who have an infant detected by newborn screening as a $\Delta F508$ heterozygote with a normal sweat test but who are both CFTR mutation carriers, one $\Delta F508$ and the other R117H. It will take many years to know whether the asymptomatic compound heterozygotes with R117H on a 7T background develop features of CF to justify their early detection. It is vital that centres already screening for CF or considering the introduction of a CF NBS programme develop an approach to this problem that is an unavoidable part of the screening process.

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