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Screening Oocyte Donors for Cystic Fibrosis Using Multiplex PCR Amplification of the CFTR Gene from Buccal Cell DNA

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INTRODUCTION

For the past decade, oocyte donation has been used successfully to treat a variety of female reproductive disorders. Presently, more than 3000 cases are being performed in the United States and the number of cases continues to grow annually (1). The increased demand for services is due in part to extending care to women of advanced reproductive age (>40 years).

Essential to establishing a registry is designing a protocol for the screening and selecting of women to enlist as suitable donors. Guidelines have been published by the American Society of Reproductive Medicine to help direct clinics with donor screening (2). Minimal genetic testing for autosomal recessive diseases known to be prevalent in different ethnic groups is recommended, but in general, genetic health is largely determined by history alone.

Cystic fibrosis (CF) represents one of the most prevalent genetic illnesses in the population, occurring in 1 in 2500 live births, with a carrier frequency of 1 in 25 Caucasians (3,4). CF is a chronic, progressive, and debilitating disease that ultimately runs a protracted course with a median survival of 29 years. Inheritance is along autosomal recessive lines and typically results from mutations in a single gene, the cystic fibrosis transmembrane conductance regulator (CFTR), located on the long arm of chromosome 7. Presently, more than 400 mutations of this gene have been identified, with changes in $\Delta F508$ being the most common (68–85%).

Because of the relatively high frequency of CF carriers in the general Caucasian population, many women interested in being gamete donors may be identified to carry CF prior to participation. Determination of the carrier status of sperm donors has been published elsewhere (4), but attempts at screening oocyte donors have not been reported. We determined the frequency of CF carrier status in our oocyte donation program

and surveyed the attitude of recipients of donor oocytes regarding genetic screening of our donor pool.

MATERIALS AND METHODS

The study was approved by the Columbia University College of Physicians & Surgeons Institutional Review Board, and informed consent was obtained. From September 1996 to July 1997, 69 consecutive ovum donors were screened and enrolled in the program. In general, guidelines for oocyte donation endorsed by the American Society of Reproductive Medicine were followed (2). These included a thorough history and physical examination, a screen for infectious diseases, and a family history for diseases with a major genetic component such as severe hypertension. In addition, these young and healthy donors were screened using a three-generation genetic history for common hereditary disorders and malformations. Included were inquiries with respect to a history of CF or chronic lung ailments.

Each donor consented to a cheek brush specimen to obtain buccal cells for genetic analysis. DNA isolated from buccal cells was used to test for 32 CF mutations (including $\Delta F508$) representing 90% of known mutations. Regions of the CFTR gene were amplified enzymatically, then hybridized to specific CF mutation oligonucleotide pools. Results for each pool were then characterized as positive or negative, and specimens with positive results were tested for specific mutation identity.

During this same period, recipients ($n = 60$) enrolled in the program were questioned regarding their desire for the genetic testing of donors, how they would react to the results of positive screens, and how much additional cost they would be willing to assume for donor testing.

RESULTS

Two (2.9%) of 69 donors were found to be heterozygous for the $\Delta F508$ mutation. Twelve samples (17.4%) did not amplify on the first run and required repeat testing. Upon questioning, all but one recipient (98%) desired their donors to be screened for CF. Although during the study period CF testing was provided without charges, recipients stated a willingness to pay for services over a range of costs (\$100–\$1000; mode, \$250). Five recipients (8%) stated that they would not use the donor if she tested positive, regardless of the husband's CF status. In the case of the two recipients

whose donors tested positive, both elected to proceed with the selected donors because their husbands screened negative for carrier status. In addition, the two donors who tested positive were counseled with respect to their results. They understood the medical significance and stated that this would not affect their future reproductive choices.

DISCUSSION

Buccal smear for determining CF carrier status is an easy and simple diagnostic test to perform. It allows rapid screening of large populations. We discovered a carrier status frequency of 2.9% within our donor group, which is comparable to the incidence within the general population. This figure is not precise because there is a large number of mutations that occur at low frequencies and 10% of CF mutations cannot be detected by current techniques. According to Lemna *et al.* (5), given a 90% detection using current screening methods, the potential for detection of couples at risk is approximately 81%. If only one partner screens positive for CF heterozygosity, the risk of CF in the offspring is estimated to be 1 in 984. If neither partner is positive, the risk is 1 in 964,400. Therefore, if the oocyte donor is discovered to be a heterozygote and the recipient's husband screens negative, the risk of CF in the offspring is not zero but actually higher than the generally quoted pretest risk of 1 in 2500. It is not surprising that recipient couples strongly desire to know their donors' status and are willing to pay for this information. What is surprising, however, is that only 8% of couples would reject a selected donor if she tested positive for a CF mutation.

The implications of false results should be additionally considered. In theory, CF screens have a sensitivity and specificity of 100%. However, false results may occur secondary to laboratory error or failure to identify all CF mutations. A false-negative result has the

potential to grant a false sense of security. On the other hand, women who screen positive may have increased feelings of fear or guilt and may even be unwilling to reproduce in the future. Given these concerns, a well-developed educational program must be in place to counsel both the infertile couple and the oocyte-donor with respect to test results, prior to initiating any population-based genetic screening.

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