Association Between Carrier Screening and Incidence of Cystic Fibrosis

Carlo Castellani, MD
Luigi Picci, PhD
Anna Tamanini, PhD
Paolo Girardi, PhD
Paolo Rizzotti, MD
Baroukh Maurice Assael, MD

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane regulator (CFTR) gene (RefSeq NM_000492). In Europe, the median birth incidence of CF is 1 in 3500. Fluctuations in the number of annual CF births have been repeatedly reported, and some studies suggest a progressive decrease in CF birth incidence. Contrasting results were obtained when CF newborn screening cohorts from Italy and France were compared.

A circumstance that might influence CF birth rates is the detection of carrier couples. Heterozygote couples can be identified following the birth of an affected child or through the offer of CFTR mutation analysis to relatives of patients, but also by carrier screening. In fact, guidelines from the US National Institutes of Health and the American College of Medical Genetics recommend that the CF carrier test be offered to couples planning a pregnancy. Once couples are made aware of their carrier status, they could modify their reproductive plans and thus affect CF birth incidence. Carrier screening programs for thalassemia and Tay-Sachs disease have been successful in reducing the incidence of these diseases in selected populations.

In northeastern Italy, CF neonatal screening has been performed for many years and is regularly used to monitor CF birth incidence. Since the early years, it has been performed for a sufficient number of years have given contradictory results. Decreases in birth incidence have been reported in East Anglia and Massachusetts. Conversely, in Colorado, no temporal trend was observed in the incidence of CF. Contrasting results were obtained when CF newborn screening cohorts from Italy and France were compared.

For editorial comment see p 2595.

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was started in the early 1990s by the carrier test to the general population. There is evidence of infertility are not offered with no family history of CF and no fertilization. Individuals and couples infertile couples undergoing in vitro fertilization. Multidisciplinary teams involving geneticists, genetic counselors, obstetricians, and nurses are responsible for providing genetic counseling and prenatal diagnosis. The approach to CF mutation analysis in individuals of reproductive age. Individuals with no CF family history have access to the test at a nominal cost.

We define carrier screening for CF as CFTR gene mutation analysis on a person who is not known to be at increased risk of having an affected child (ie, who has no family history of CF). In the western region, CF carrier screening is offered only to a relatively small population of infertile couples planning assisted reproduction, whereas in the eastern region, it is widely offered to couples from the general population. Because the incidence of CF can vary in different populations, the ethnic background from a sample of the population was collected from 2 hospital maternity departments in the western region and 2 in the eastern region, and the distribution of neonates of non-Italian origin was compared. The audit was performed on all infants born in 2 consecutive months in 2007.

Methods
Cystic fibrosis birth incidence in northeastern Italy was determined from newborn screening in the years between 1993 and 2007. Incidence trends were compared in 2 sections, the eastern region where population carrier screening is performed and the western region with a more limited carrier testing program.

Area and Population Under Study
Our study was conducted in Veneto and Trentino Alto-Adige, Italy. All patients with CF who were residents in northeastern Italy have been followed up since the early 1970s by a single CF center located in Verona, Italy. The Verona Cystic Fibrosis Center has traditionally been cautious in the use of the CF carrier test, reserving it for individuals with a positive family history and their partners. Heterozygous parents identified through neonatal screening are requested to alert their relatives of reproductive age to contact the Genetic Counselling Service, where they can be carrier tested for free. More recently, the CF carrier test has also been performed on infertile couples undergoing in vitro fertilization. Individuals and couples with no family history of CF and no evidence of infertility are not offered CFTR mutation analysis in the Verona Cystic Fibrosis Center. A similar policy has been adopted in areas close to the center, located in the western part of Veneto and in Trentino-Alto Adige. In this study, these areas are referred to as the western region. The approach to CF mutation analysis is different in the eastern part of Veneto, where a campaign offering the CF carrier test to the general population was started in the early 1990s by the University of Padua, Padua, Italy. Following that initiative, a steadily growing number of laboratories in the area, referred to as the eastern region, have routinely performed CF mutation analysis in individuals of reproductive age. Individuals with no CF family history have access to the test at a nominal cost.

Termination data enabled calculation of adjusted incidence rates of CF corresponding with the birth incidence that would have been observed if CF fetuses had not been aborted. Adjusted CF incidence was determined in the 2 regions, for each year and for the whole study period. Seven months were
added to the date of the prenatal diagnosis in the case of chorionic villus sampling and 5 months in the case of amniocentesis.

**Statistical Analysis**

The statistical analysis for comparison of incidences was performed by using parametric tests such as χ² test, t test, and ratio test of Poisson for comparison in small proportions. The association between variables was measured by Pearson correlation in quantitative data and Fisher independence test for contingency tables. Relative risk was estimated to compare CF incidence between the regions. The regression analysis was performed by linear and Poisson models (generalized linear models). Correlation between the number of CF cases and the number of carrier tests in both regions was analyzed using bootstrapping. Tests had α level for significance set at P < .05. Statistical analysis was performed by using the R 2.9.1 for Windows statistical package under x86 machine (R Foundation for Statistical Computing, http://www.r-project.org/).

The study was approved and informed consent was waived by the Verona Hospital Ethical Committee (Comitato Etico per la Sperimentazione).

**RESULTS**

**Neonatal Screening Population**

Between January 1993 and December 2007, 779,631 newborns were screened for CF, with an average per year of 51,975. Partitioned data on the number of neonates screened per year in both regions are shown in **Table 1**.

The proportion of parents of non-Italian origin was equally distributed in both regions. Nationality data reported in a sample from the neonatal screening program showed 797 Italian, 223 non-Italian, and 147 non-European parents out of 1020 individuals from the western region. In the eastern region, there were 754 Italian parents, 246 non-Italians, and 147 non-Europeans out of 1000 individuals (P < .54).

**CF Population and Birth Incidence**

One hundred ninety-five newborns with CF were detected through neonatal screening (Table 1). The neonatal screening procedure did not detect 9 children with CF, who were later diagnosed because of clinical symptoms (sensitivity = 0.955). Two newborns with false-negative test results were born in 1993, 1 newborn in 1994, 3 newborns in 1995, and 1 newborn each in 1996, 1997, and 2002. Their mean age at diagnosis was 49.5 months (range, 2-139 months). The overall incidence of CF was 1 in 3998, or 1 in 3821 including false-negative test results.

A time-related decrease in birth incidence was confirmed, with a mean annual percentage decrease of 0.16 (95% CI, 0.09-0.23) per 10,000 newborns (Poisson regression analysis, P < .001) (Figure 1). The rate of decrease was greater in the eastern region (eastern region: decrease rate, 0.24; 95% CI, 0.12-0.36; western region: decrease rate, 0.04; 95% CI, -0.16 to 0.08), as suggested by the existence of a statistically significant interaction term between time and region in the Poisson regression model (P < .01) (Figure 1).

In 2008, 7 neonates with CF were identified, out of 58,879 screened at birth. Five neonates were from the

**Table 1. Screened Populations, CF Cases, and Relative Risks for Total and Both Regions by Each Year**

<table>
<thead>
<tr>
<th>Year</th>
<th>Total No. of Screened Neonates</th>
<th>No. of CF Cases</th>
<th>Western Region No. of Screened Neonates</th>
<th>No. of CF Cases</th>
<th>Eastern Region No. of Screened Neonates</th>
<th>No. of CF Cases</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>46,698</td>
<td>16</td>
<td>17,846b</td>
<td>6</td>
<td>28,852b</td>
<td>10</td>
<td>0.97 (0.29-2.94)</td>
</tr>
<tr>
<td>1994</td>
<td>46,985</td>
<td>17</td>
<td>17,956b</td>
<td>5</td>
<td>29,029b</td>
<td>12</td>
<td>0.67 (0.19-2.05)</td>
</tr>
<tr>
<td>1995</td>
<td>48,812</td>
<td>20</td>
<td>17,890b</td>
<td>3</td>
<td>30,922b</td>
<td>17</td>
<td>0.29 (0.05-0.99)</td>
</tr>
<tr>
<td>1996</td>
<td>48,647</td>
<td>15</td>
<td>18,591b</td>
<td>6</td>
<td>30,056b</td>
<td>9</td>
<td>1.08 (0.32-3.39)</td>
</tr>
<tr>
<td>1997</td>
<td>49,297</td>
<td>9</td>
<td>18,839b</td>
<td>5</td>
<td>30,458b</td>
<td>4</td>
<td>2.02 (0.43-10.10)</td>
</tr>
<tr>
<td>1998</td>
<td>50,378</td>
<td>16</td>
<td>19,252b</td>
<td>6</td>
<td>31,126b</td>
<td>10</td>
<td>0.97 (0.29-2.94)</td>
</tr>
<tr>
<td>1999</td>
<td>51,355</td>
<td>16</td>
<td>19,626b</td>
<td>8</td>
<td>31,729b</td>
<td>8</td>
<td>1.61 (0.52-4.49)</td>
</tr>
<tr>
<td>2000</td>
<td>51,660</td>
<td>14</td>
<td>19,742b</td>
<td>5</td>
<td>31,918b</td>
<td>9</td>
<td>0.90 (0.23-2.98)</td>
</tr>
<tr>
<td>2001</td>
<td>53,314</td>
<td>14</td>
<td>20,375b</td>
<td>9</td>
<td>32,939b</td>
<td>5</td>
<td>2.90 (0.87-11.03)</td>
</tr>
<tr>
<td>2002</td>
<td>52,293</td>
<td>6</td>
<td>19,993</td>
<td>2</td>
<td>32,300b</td>
<td>4</td>
<td>0.80 (0.07-5.63)</td>
</tr>
<tr>
<td>2003</td>
<td>54,691</td>
<td>14</td>
<td>20,768</td>
<td>3</td>
<td>33,923b</td>
<td>11</td>
<td>0.45 (0.08-1.63)</td>
</tr>
<tr>
<td>2004</td>
<td>56,327</td>
<td>11</td>
<td>21,592</td>
<td>7</td>
<td>34,735b</td>
<td>4</td>
<td>2.81 (0.72-13.11)</td>
</tr>
<tr>
<td>2005</td>
<td>56,444</td>
<td>10</td>
<td>21,572</td>
<td>3</td>
<td>34,872b</td>
<td>5</td>
<td>0.97 (0.15-5.03)</td>
</tr>
<tr>
<td>2006</td>
<td>57,111</td>
<td>9</td>
<td>21,766</td>
<td>6</td>
<td>35,345b</td>
<td>3</td>
<td>3.25 (0.69-20.07)</td>
</tr>
<tr>
<td>2007</td>
<td>57,619</td>
<td>10</td>
<td>21,889</td>
<td>7</td>
<td>35,830b</td>
<td>3</td>
<td>3.78 (0.86-22.66)</td>
</tr>
</tbody>
</table>

Total: 779,631 screened, 195 CF cases, 297,797 total cases, 481,834 eastern region cases, 114 western region cases.
western region and 2 were from the eastern region, thus confirming the previous year’s trend. These newborns were not included in the analysis, because incidence in that year could not be compared with carrier screening data, which were not collected for 2008.

**Carrier Tests**

All 20 laboratories answered the study questionnaire. The carrier test was offered to CF relatives by 13 laboratories (1 in the western region), infertile couples by 18 laboratories (2 in the western region), and the general population by 15 laboratories (none in the western region). Altogether in the western region, 2559 carrier tests were performed; 314 carriers and 9 carrier couples were detected. In the eastern region, 87,025 carrier tests were performed; 3650 carriers and 82 carrier couples were detected (Table 2). Carrier rate (number of carriers/number of tests) was 1/8.1 in the western region and 1/23.8 in the eastern region. A significant negative correlation of −0.68 (bootstrap 95% CI, −0.98 to −0.38) between the number of tests and CF cases was found in the eastern region (P=.02), but not in the western region (correlation coefficient=0.17; bootstrap 95% CI, −0.32 to 0.62; P=.59). There is statistically significant evidence of a difference in the correlations between the 2 regions (bootstrap 1-side Fisher Z test, P=.02).

In the whole area under study (both regions), the increase in the number of screened carriers over time was significantly correlated with the decrease in CF birth incidence (correlation coefficient=−0.53; 95% CI, −0.20 to −0.74; P=.003). In the eastern region, a significant negative correlation between the annual CF birth incidence and the cumulative number of carrier couples was found (correlation coefficient=−0.73; 95% CI, −0.90 to −0.32; by Pearson test, P=.003) (Figure 2).

**Prenatal Diagnoses and Terminations**

Twenty-six of the 91 heterozygous couples opted for prenatal diagnosis, 9 underwent prenatal diagnoses twice, for a total of 35 prenatal diagnoses (5 in the western region and 30 in the eastern region). Ten fetuses with CF were detected (3 in the western region and 7 in the eastern region) and 8 pregnancies were terminated (3 in the western region and 5 in the eastern region). Two pregnancies resulted in the birth of infants with CF.

Between 1993 and 2007, 90 additional prenatal diagnoses were detected by testing couples at high risk who had an older CF child and not following carrier screening. Thirty of these prenatal diagnoses were in couples from the western region and 60 were in couples from the eastern region. The fetuses had CF in 21 cases (6 from the western region and 15 from the eastern region) and the pregnancies were terminated in 16 cases (4 from the western region and 12 from the eastern region). Furthermore, 1 termination in the western region followed the finding of hyperechogenic bowel on fetal ultrasound imaging during pregnancy and subsequent mutation analysis in the parents.

Birth incidence trends calculated adjusting for pregnancy terminations confirmed a time-related decrease, with a mean annual percentage decrease of 0.145 (95% CI, 0.05-0.24) per 10,000 neonates (Poisson regression, P<.001 for time parameter). Again, the rate of decrease was greater in the eastern region (Poisson regression, P=.048 for interaction term between time and region).

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**Figure 1.** CF Birth Incidence in Veneto and Trentino-Alto Adige, Italy, and the Difference in CF Birth Incidence Between the Eastern and Western Regions by Study Year

CF indicates cystic fibrosis. For both scatterplots, the solid lines represent the fitted linear regression curves for CF birth incidence in Veneto and Trentino-Alto and the eastern region, respectively, and the dotted line represents the fitted linear regression curve of the western region. R software (R Foundation for Statistical Computing, http://www.r-project.org/) was used to create the fitted linear regression curves in both scatterplots. The adjusted R² and test for significance of regression for the linear regressions are shown.
COMMENT

Our results confirm previous findings of decreasing CF birth rates in northeastern Italy and find an association with CF carrier screening. The overall negative trend was due to a decrease in the area where the CF carrier test is offered to the general population.

Diagnoses of CF decreased significantly during the 15-year study period, in a fashion similar to previous studies. However, other studies have not detected such a downward trend. Factors that may contribute to discordance in the literature results include the percentage of births from non-white population subsets or variations in the percentage of couples of reproductive age undergoing CFTR mutation analysis. Also, CF populations identified through symptoms may not include late diagnoses or severe cases in which the patient died before diagnosis. Neonatal screening programs avoid this kind of bias, but may end up including very mild cases, which do not reach the minimum diagnostic criteria for CF.

Our study goal was to evaluate birth rate trends of indisputable CF, diagnosed following the most recent consensus recommendations. Newborns with elevated IRT values may have intermediate sweat test results (30-60 mEq/L) or carry CFTR mutations of unclear phenotypic consequences, which are not clearly linked to CF. The diagnosis of CF in these infants is equivocal and including them in the analysis could have fictitiously modified birth incidence.

Children who tested negative at neonatal screening (IRT levels at birth less than the cutoff) and were diagnosed because of symptoms were not included. Because all patients with CF in the area are followed up by the Verona Cystic Fibrosis Center, we were aware of all such cases. Eight of the 9 false-negative case results were born before 1998, and their average age at diagnosis was 4 years. There may be other children with CF who remain undetected, especially in the later years, and including the possibly incomplete false-negative data would misleadingly accentuate the downward CF birth rate trend.

Table 2. Carrier Tests Performed, Single Carriers, and Carrier Couples Detected in the Western and Eastern Regions in 1993-2007

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Carrier Tests Performed</th>
<th>No. of Single Carriers</th>
<th>No. of Carrier Couples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eastern Region</td>
<td>Western Region</td>
<td>Eastern Region</td>
</tr>
<tr>
<td>1993</td>
<td>27</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>1994</td>
<td>37</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>1995</td>
<td>782</td>
<td>82</td>
<td>52</td>
</tr>
<tr>
<td>1996</td>
<td>1615</td>
<td>111</td>
<td>60</td>
</tr>
<tr>
<td>1997</td>
<td>2193</td>
<td>54</td>
<td>109</td>
</tr>
<tr>
<td>1998</td>
<td>2987</td>
<td>38</td>
<td>121</td>
</tr>
<tr>
<td>1999</td>
<td>3529</td>
<td>51</td>
<td>156</td>
</tr>
<tr>
<td>2000</td>
<td>5050</td>
<td>75</td>
<td>232</td>
</tr>
<tr>
<td>2001</td>
<td>6649</td>
<td>76</td>
<td>266</td>
</tr>
<tr>
<td>2002</td>
<td>7702</td>
<td>74</td>
<td>308</td>
</tr>
<tr>
<td>2003</td>
<td>8363</td>
<td>109</td>
<td>338</td>
</tr>
<tr>
<td>2004</td>
<td>9943</td>
<td>452</td>
<td>404</td>
</tr>
<tr>
<td>2005</td>
<td>11065</td>
<td>331</td>
<td>477</td>
</tr>
<tr>
<td>2006</td>
<td>12830</td>
<td>481</td>
<td>515</td>
</tr>
<tr>
<td>2007</td>
<td>14253</td>
<td>585</td>
<td>594</td>
</tr>
<tr>
<td>Total</td>
<td>87025</td>
<td>2559</td>
<td>3650</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not applicable.
Our study also provides the first evidence, to our knowledge, of a long-term negative correlation between CF carrier screening practice and CF birth incidence monitored by an accurate neonatal screening program. Although CF birth incidence decreased over time in the whole area under study, the number of new CF cases identified each year decreased only slightly in the western region, with its limited carrier testing, and decreased significantly in the eastern region, with its intensive screening of couples of reproductive age. The overall negative trend in northeastern Italy is mainly due to a reduction of CF births in the eastern region. The reduction appears to be connected with the extensive use of mutation analysis in the general population—as the number of individuals screened with the CF carrier test progressively increased, CF birth incidence gradually and constantly decreased. The smaller nonsignificant reduction in birth rates in the western region suggests that there are other factors acting on CF birth incidence (ie, the changing ethnic background of the population or the possibility that parents with a CF child diagnosed early through neonatal screening alter their reproductive planning). The effect of the detection of heterozygous couples on CF birth rates depends on how the knowledge of their carrier status influences a family’s reproductive attitudes.

A limitation of our study was the lack of information about the reproductive choices of the 82 carrier couples in the eastern region. In the future, a clearer picture of the attitudes and conduct of these families should be obtained by means of structured interviews or questionnaires.

The 82 couples chose to be tested because either they were planning to have children or a pregnancy was already ongoing. According to the Italian Institute for Statistics, the average number of children per woman in northeastern Italy in the 2005-2007 period was 1.39. Thus, an estimate of the total number of children that should have been born to these carrier couples would be 113 children (82 × 1.39), of whom 1 in 4 (ie, 28 infants) would be expected to have CF. By adding these 28 infants to the 114 children with CF detected in the eastern region, birth incidence in the eastern region would become 1 in 3393, similar to 1 in 3676 in the western region.

The inclusion of terminated fetuses in the analysis would not significantly change the analysis results, and the observed trends in CF birth incidence cannot be explained by the reported terminations alone. This is consistent with other carrier screening experiences, which highlights the fact that factors other than prenatal diagnoses and terminations may influence CF incidence.22,23 Carrier couples have various reproductive options. They can accept the 25% risk and have a child with no prenatal diagnosis, have no further biological children, have a child by heterologous fertilization, or have a child by preimplantation genetic diagnosis. In Italy, the last 2 options are forbidden by law and frequently couples of CF carriers move to other European countries for assisted reproduction.

Studies in families who had a child with CF confirm wide differences in reproductive attitudes.24-26 In the area where our study took place, it had been previously shown27 that before prenatal diagnosis was available, most couples chose not to have other children. When prenatal diagnosis became available, birth rates increased, but fecundity was still lower than in equivalent couples with no affected children. These findings may not be generalizable to heterozygote couples with no personal experience of CF. However, couples from our study area who already had a child with CF chose to terminate 18 of 22 pregnancies with CF, similar to the study carrier couples with no family history.

A shortcoming inherent in the ecological design of our study is that there may be factors unaccounted for that are responsible for the results. Although this cannot be unquestionably ruled out, it is improbable. The populations of both regions are culturally, socially, and economically homogeneous, whereas they differ with respect to the number of carrier tests performed and carrier couples detected.

Another limitation is the calculation rather than direct measurement of the 1993-2001 neonatal populations in both regions. The low variability of the 2002-2007 ratio of the number of newborns in the western region to the number in the eastern region suggests that the calculated approximation for the 1993-2001 period is precise. Moreover, by restricting the study to the 2002-2007 period, the rate of decrease would still be greater in the eastern region (P = .03 interaction term between time and region).

Alternative explanations for the downward CF birth incidence are population mixing or partial detection by the neonatal screening system. Both were analyzed and excluded. In northeastern Italy, a relevant contribution to the increasing natality trend of the latest years comes from neonates of non-Italian descent. Parents of these infants often belong to ethnic groups in which CF is less common than among white populations. However, the homogeneous distribution of different ethnicities in both regions seems to exclude any effect of population mixing on the study results.

The performance of the neonatal screening system has not deteriorated over the years. The neonatal screening laboratory participates regularly and successfully in international quality control schemes for the IRT assay and mutation analysis.28,29 The only updates in the protocol during the study period concerned the mutation panel, which has been gradually widened, a change that would be expected to increase sensitivity as opposed to decreasing it. False-negative test results were concentrated in the 1993-1997 period, further highlighting the sensitivity of the neonatal screening procedure. Furthermore, false-negative test
results were not included in the analysis. Lastly, any errors in the neonatal screening procedure should have consequences not just in the eastern region, but in all the screened areas.

Our study was designed to examine the relationship between extensive use of CF mutation analysis in the general population and CF birth rates, not to analyze costs and benefits of carrier screening. Further research concerning this issue is needed.

In conclusion, our analysis shows that the decreased rate of CF births in northeastern Italy is associated with a reduction in CF births in its eastern part, where a negative correlation was found between incidence and the number of carrier tests performed. Screening couples of reproductive age may result in a reduction in CF incidence.

**Author Contributions:** Dr Castellani had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Castellani, Assael. Acquisition of data: Picci, Tamanini, Rizzotti. Analysis and interpretation of data: Castellani, Girardi, Assael. Drafting of the manuscript: Castellani. Critical revision of the manuscript for important intellectual content: Picci, Tamanini, Girardi, Assael.

**Statistical analysis:** Girardi. Study supervision: Castellani, Picci, Tamanini, Rizzotti, Assael.

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**REFERENCES**