

Association Between Carrier Screening and Incidence of Cystic Fibrosis

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CYSTIC FIBROSIS (CF) IS AN AUTOSOMAL RECESSIVE DISEASE caused by mutations in the CF transmembrane regulator (*CFTR*) gene (RefSeq NM_000492).¹ In Europe, the median birth incidence of CF is 1 in 3500.² Fluctuations in the number of annual CF births have been repeatedly reported,³⁻⁵ and some studies suggest a progressive decrease in CF birth incidence.^{3,6} However, these trends were recorded in populations diagnosed because of symptoms and are subject to ascertainment bias. The number of diagnoses may be incomplete for recent years, because patients may not yet be diagnosed or some patients could be lost before they are diagnosed. Incidence may be better appreciated when the analysis is performed on CF populations diagnosed through neonatal screening programs.

Analyses of CF birth rate trends in areas in which neonatal screening has been performed for a sufficient number of years have given contradictory results. Decreases in birth incidence have been reported in East Anglia⁷ and Massachusetts.⁸ Conversely, in Colorado, no temporal trend was observed in the incidence of CF.⁵ Contrasting re-

Context A downward trend in cystic fibrosis (CF) birth incidence has been reported in some areas.

Objective To evaluate the association between carrier screening and CF birth incidence.

Design, Setting, and Participants In northeastern Italy, CF birth incidence is monitored by means of a long-standing neonatal screening program. In the same area, 2 sections using different carrier detection approaches were identified—the western region, in which CF carrier tests are offered only to relatives of patients or to couples planning in vitro fertilization; and the eastern region, in which carrier testing is offered to relatives and carrier screening to infertile couples and to couples of reproductive age. A total of 779 631 newborns underwent CF neonatal screening between January 1993 and December 2007, of whom 195 had CF detected.

Main Outcome Measure Cystic fibrosis birth incidence in the 2 regions.

Results A time-related decrease in birth incidence was found, with a mean annual percentage decrease of 0.16 per 10 000 neonates ($P < .001$). In the western region, 2559 carrier tests were performed, 314 carriers were identified, and 9 carrier couples were detected. In the eastern region, 87 025 carrier tests were performed, 3650 carriers were identified, and 82 carrier couples were detected. The birth rate decrease was greater in the eastern region (decrease rate, 0.24; 95% confidence interval [CI], 0.12-0.36) than in the western region (decrease rate, 0.04; 95% CI, -0.16 to 0.08; $P = .01$). 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; $P = .003$).

Conclusion In northeastern Italy, carrier screening was associated with a decrease in the incidence of CF.

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sults were obtained when CF newborn screening cohorts from Italy and France were compared.⁴

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 Health⁹ and the American College of Medical Genetics¹⁰ 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.^{11,12}

In northeastern Italy, CF neonatal screening has been performed for many years¹³ and is regularly used to monitor CF birth incidence. Since the early

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and questions on p 2605.

1990s, a significant progressive decrease of CF birth rates has been recorded.⁴ At the same time, CF carrier screening has been performed in the eastern part of northeastern Italy. Our goal was to evaluate the association between CF carrier screening and birth incidence in northeastern Italy.

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.¹⁴ 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.¹⁵ 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.

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,¹⁶ 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.

Modalities and Data Collection

Newborn Screening. In the area under study, CF neonatal screening has been performed since the 1970s, initially measuring meconium proteins and later immunoreactive trypsinogen (IRT). Since 1993, a combination of IRT, meconium lactase, and mutation analysis has been adopted.¹³ Adherence to CF neonatal screening is high—an analysis of 2002-2007 birth records showed that 98.7% of all newborns were screened.

Because CF neonatal screening programs can detect infants with atypical forms of CF or *CFTR*-related disorders, only newborns with an undisputable diagnosis of CF, defined by increased IRT levels and sweat chloride of more than 60 mEq/L in the first 2 months of life, were included in the study.^{16,17} Children with CF not detected by neonatal screening who were diagnosed at a later age because

of symptoms (false-negative screenings) were excluded.

Partitioned data on the number of neonates screened per year in both regions were available for the 2002-2007 period. During that period, the ratio of the number of newborns in the western region to the number in the eastern region was stable, ranging from 1 in 1.608 to 1 in 1.633. Given the low variability (0.21% coefficient of variation), the mean ratio of 1:1.62 (95% confidence interval [CI], 1.61-1.63) was used to calculate the western and eastern regions' screened populations for the years 1993 to 2001.

Carrier Tests. A preliminary survey of regional health authorities identified 2 laboratories in the western region and 18 in the eastern region performing *CFTR* molecular genetics analysis. A questionnaire was sent to the 20 laboratories. The information collected through the questionnaire included target (relatives of affected or heterozygous individuals, infertile couples, general population), number of tests performed, and number of single carriers and carrier couples detected per year between 1993 and 2007. Specific genotype information was requested for carriers of mutations not included in the revised American College of Medical Genetics panel,¹⁸ and only CF-causing mutations were considered for analysis.^{16,17}

Prenatal Diagnosis. Prenatal diagnosis is routinely offered to carrier couples in both regions. If a fetus carries 2 parental mutations, the couple may choose to terminate the pregnancy. Two genetic laboratories in the area perform CF prenatal diagnosis (Verona and Padua, Italy). Both contributed data on number, time, and results of the prenatal diagnoses performed.

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 χ^2 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 bootstrap. 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 Ve-

rona 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 144 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 neonates (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

Year	Total		Western Region		Eastern Region		Relative Risk (95% CI) ^a
	No. of Screened Neonates	No. of CF Cases	No. of Screened Neonates	No. of CF Cases	No. of Screened Neonates	No. of CF Cases	
1993	46 698	16	17 846 ^b	6	28 852 ^b	10	0.97 (0.29-2.94)
1994	46 985	17	17 956 ^b	5	29 029 ^b	12	0.67 (0.19-2.05)
1995	46 812	20	17 890 ^b	3	28 922 ^b	17	0.29 (0.05-0.99)
1996	48 647	15	18 591 ^b	6	30 056 ^b	9	1.08 (0.32-3.39)
1997	49 297	9	18 839 ^b	5	30 458 ^b	4	2.02 (0.43-10.10)
1998	50 378	16	19 252 ^b	6	31 126 ^b	10	0.97 (0.29-2.94)
1999	51 355	16	19 626 ^b	8	31 729 ^b	8	1.61 (0.52-4.94)
2000	51 660	14	19 742 ^b	5	31 918 ^b	9	0.90 (0.23-2.98)
2001	53 314	14	20 375 ^b	9	32 939 ^b	5	2.90 (0.87-11.05)
2002	52 293	6	19 993	2	32 300	4	0.80 (0.07-5.63)
2003	54 691	14	20 768	3	33 923	11	0.45 (0.08-1.63)
2004	56 327	11	21 592	7	34 735	4	2.81 (0.72-13.11)
2005	56 444	10	21 572	3	34 872	5	0.97 (0.15-5.03)
2006	57 111	9	21 766	6	35 345	3	3.25 (0.69-20.07)
2007	57 619	10	21 989	7	35 630	3	3.78 (0.86-22.66)
Total	779 631	195	297 797	81	481 834	114	1.15 (0.85-1.54)

Abbreviations: CF, cystic fibrosis; CI, confidence interval.

^aWestern vs eastern region.

^bEstimated data.

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

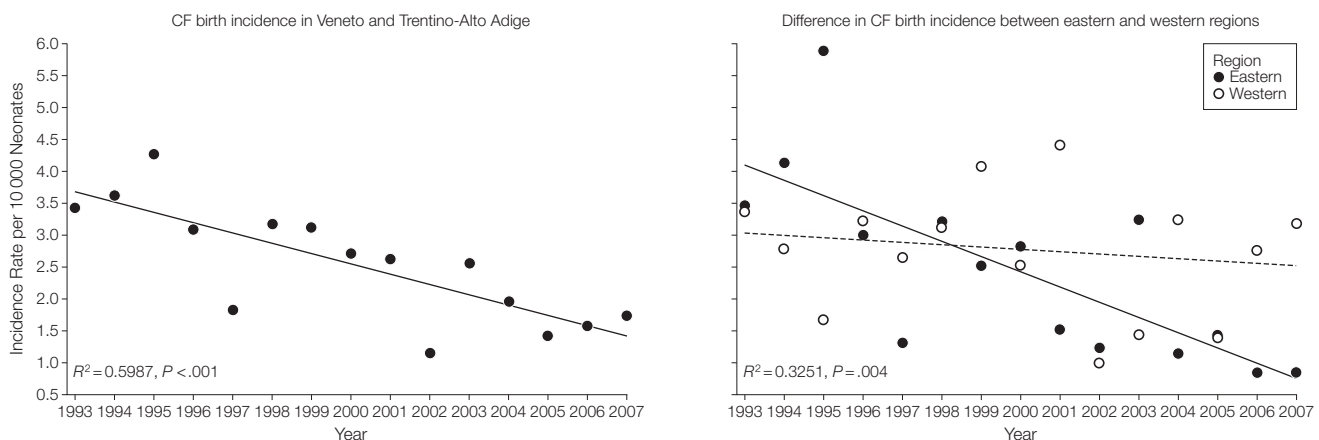
Twenty-six of the 91 heterozygous couples opted for prenatal diagnosis, 9 underwent prenatal diagnoses twice, for a total of 35 prenatal diagnoses (5 in the western region and 30 in the eastern region). Ten fetuses with CF were detected (3 in the western region and 7 in the eastern region) and 8 pregnancies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

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.

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

Year	No. of Carrier Tests Performed		No. of Single Carriers		No. of Carrier Couples	
	Eastern Region	Western Region	Eastern Region	Western Region	Eastern Region	Western Region
1993	27	19	10	7	NA	NA
1994	37	21	8	9	1	NA
1995	782	82	52	19	2	NA
1996	1615	111	60	22	2	1
1997	2193	54	109	15	5	1
1998	2987	38	121	8	4	NA
1999	3529	51	156	16	7	NA
2000	5050	75	232	24	13	2
2001	6649	76	266	15	10	NA
2002	7702	74	308	11	4	1
2003	8363	109	338	13	11	1
2004	9943	452	404	48	4	NA
2005	11 065	331	477	23	7	NA
2006	12 830	481	515	39	8	2
2007	14 253	585	594	45	4	1
Total	87 025	2559	3650	314	82	9

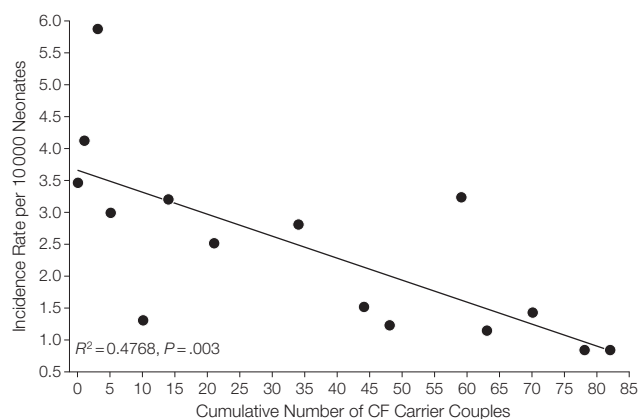
Abbreviation: NA, not applicable.

COMMENT

Our results confirm previous findings of decreasing CF birth rates in north-eastern Italy⁴ 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.^{3,6,7} However, other studies^{4,5} 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.^{16,17,19}

Our study goal was to evaluate birth rate trends of indisputable CF, diagnosed following the most recent consensus recommendations.^{16,17} Newborns with elevated IRT values may

Figure 2. CF Incidence vs Cumulative Number of CF Carrier Couples Discovered in the Eastern Region of Italy

CF indicates cystic fibrosis. The solid line represents the fitted linear regression curve for CF incidence. R software (R Foundation for Statistical Computing, <http://www.r-project.org/>) was used to create the fitted linear regression curve. The adjusted R^2 and test for significance of regression for the linear regression are shown.

have intermediate sweat test results (30-60 mEq/L) or carry *CFTR* mutations of unclear phenotypic consequences, which are not clearly linked to CF.²⁰ The diagnosis of CF in these infants is equivocal¹⁹ 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.

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 1.39.²¹ 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 repro-

ductive 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, Rizzotti, Assael.

Statistical analysis: Girardi.

Study supervision: Castellani, Picci, Tamanini, Rizzotti, Assael.

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