**Original Article**

# The Incidence of Cystic Fibrosis in Central Anatolia Region of Turkey in 2015 and 2016

Hangül et al. Incidence of Cystic Fibrosis in Middle Anatolia

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**Background:** Cystic fibrosis is the most prevalent metabolic chronic disease in Caucasian children in the Europe. Cystic fibrosis has seen approximately 1 in 3000 births in northern European countries. Birth prevalence varies worldwide.
**Aim:** The aim of this study to determine the incidence of cystic fibrosis in Central Anatolia Region of Turkey by using the new born screening program data
**Study Design:** Cross-sectional study.
**Methods:** We used the records of the new born screening program which is implemented by Konya and Kayseri Provincial Health Directorates
**Results:** There were total 119006 live births in Konya and Kayseri, between January 2015 and December 2016. New born screening test was applied to all these babies. In this two years period, there were 22 live born babies diagnosed with cystic fibrosis in Konya with an incidence of 2,9 per 10 000 live births and 13 live born babies diagnosed with cystic fibrosis in Kayseri with an incidence of 2,8 per 10 000 live births.
**Conclusion:** We found the incidence of cystic fibrosis 2.9 per 10 000 live births in Central Anatolia which is similar to Northern European Countries.
**Keywords:** Cystic fibrosis, incidence, Middle Anatolia, Turkey, newborn screening

Cystic fibrosis (CF) is the most prevalent inheritable chronic disease in Caucasian children, characterized mainly by obstruction and infection of airways and by maldigestion (1). It is an autosomal recessive disorder, and the most common mutation of CF is DF508 (2). The gene encoding the CFTR protein is located on chromosome 7 (3).
The incidence of CF is variable worldwide (4). In northern European countries, CF is seen approximately 1 in 3000 births (5). The frequency of CF in Turkey is not clearly known and is agray zone in Europe. It will be possible to obtain data on the actual frequency of the disease in Turkey by using the National newborn screening (NBs) program, which is conducted by the Child and Adolescent department of the Public Health General Directorate. In this paper, we describe the method and result of the CF NBs program for the first 2 years, present the first CF incidence using NBs data of two cities, and determine the live-birth incidence, demographic, and clinical features of CF in two large cities in Turkey.

**Materials**

In this retrospective cross-sectional study, we evaluated the records of the newborn NBs program, between January 1, 2015 and December 31, 2016 for CF. The program is run by Konya and Kayseri provincial health care services. Their demographic data, clinical features, symptoms at first admission, and genetic analysis results were assessed. According to these records, patients who were immunoreactive trypsinogen (IRT) positive were evaluated. First IRT blood values > 90 ng/ml babies are evaluated a second time. If the second IRT blood values >70 ng/ml, the babies are directed to CF centers (XXXX University, School of Medicine and XXXX University, School of Medicine) where sweat tests are performed. These two university hospitals are the only CF centers in these cities.

The diagnosis of CF is based on sweat chloride levels (>60 mmol/L) and/or two identified CF mutations, as well as characteristic symptoms of CF (1,6). We also used the CF records of these two universities between January 1, 2015 and December 31, 2016 to find CF patients diagnosed without positive NBs results.

Genetic analysis of the patients was begun by obtaining genomic DNA from peripheral blood with the MagPurix Blood DNA Extraction Kit 200. The CFTR gene library was constructed using the Nextflex Cystic Fibrosis Amplicon Panel (Austin, Texas.) according to the manufacturer's instructions. This kit contains 61 double primers and is a kit that allows the analysis of 27 exon and exon-intron connections of the CFTR gene. It covers all pathogenic variants in the NCBI ClinVar database. Sequencing was performed with the MiSeq Illumina (San Diego, California.) platform. The obtained data was evaluated with Seq Genomize (Istanbul, TURKEY) and Integrative genomics viewer software. The mutation names, type, genomic region, genomic localization, and legacy protein nomenclature were determined according to the nomenclature of the Human Genome Variation Society.

This study was approved by XXXX University ethics committee (number: 2016/642) and informed written consent was obtained from the parents of the participating CF patients. The publication of the national screening program data was approved by Public Health General Directorate, Child and Adolescent Department.

**Statistical analysis**

Birth incidence was calculated by dividing the number of new cases of CF by the number of live births.

**Results**

There were 119,006 live births in the provinces of Konya and Kayseri, between 2015 and 2016. NBs test was performed on all of these babies. At the first IRT screening, 3,325 newborn babies were found. In the second examination, 808 babies were examined with higher IRT blood values. They were referred to CF centers for further evaluation. All the babies were given a sweat test. Twenty (57%) of them had a sweat test result> 60 mmol/L consistent with CF, eight (22.9%) of them were found to have a suspicious sweat test (30-59 mmol/L), and 5 (14.3%) of them were negative (<30 mmol/L). We could not collected sweat in 2 patients (5.8%). Although the IRT was negative, three patients were diagnosed as having CF with complaints of Pseudo-Bartter's syndrome and genetic results. Two patients who underwent surgery for meconium ileus died before their follow-up. Three patients did not come to the hospital for follow-up. Therefore, genetic analysis was performed in 30 patients. A total of 35 patients were diagnosed as CF with newborn screening, sweat test results, clinical findings, and genetic screening (Figure 1).

Demographic and clinical characteristics of patients are as follows: 45.8% of the patients are female and 54.2% are male. Patients were diagnosed at an average of 45 (minimum;3-maximum; 300) days. At the time of diagnosis, the most common symptom was Pseudo Bartter syndrome (36.9%). Thirteen (34.3%) patients were asymptomatic, and 22 (65.7%) patients had one or two symptoms (Table 1).

Genetic analyses were performed in 30 patients. We could not managed genetic test in five patients because 2 of them died and 3 of them were dropped out from the study. Fifteen patients were homozygous and 15 patients were compound heterozygote. Twenty-one different gene variants were detected in 30 patients. The most common mutation in our patients was F508del (17/30), followed by G85E, N1303K, G542X, and W1282X (Table 2).

There were 10 live births of patients with CF (from 37,567 live births in Konya) giving an incidence of 2.6 per 10, 000 (i.e. 1/3,756) live births in 2015 and 12 live births of patients with CF (from 35,962 live births in Konya) giving an incidence of 3.3 per 10 000 (i.e.1/2,996) live births in 2016. There were 8 live births of patients with CF (from 23,077 live births in Kayseri) giving an incidence of 3.4 per 10, 000 (i.e. 1/2,884) live births in 2015 and 5 live births of patients with CF (from 22,400 live births in Kayseri) giving an incidence of 2.2 per 10,000 (i.e. 1/4480) live births in 2016. Consequently, the records obtained over two year period were evaluated and there were 35 live births of patients with CF (from 119,006 live births in Konya and Kayseri) giving an incidence of 2.9 per 10,000 (i.e. 1/3,400) live births between January 1, 2015- December 31, 2016.

**Discussion**

In this study, we found the frequency of the disease to be1 in 3,400 live births in this region. This study is the first incidence study for Turkey based on NBs. The NBs program, which started in January 1, 2015, makes it possible to recognize the disease early. Many studies have shown that early diagnosis of CF has an important role in increasing the average life duration. The NBs program diagnoses CF patients earlier. In the later years of

life, they have better nutritional status, growth parameters, and healthier lungs than patients diagnosed later (7, 8).

The NBs program facilitated us to be able to record patients better and to obtain information about the frequency of the disease. However, the frequency of CF in Turkey is not clearly known and for this reason, we do not have enough knowledge about the size of CF. Only a small study in 1973, before the NBs programs and with limited data, the study of Gurson et al. found the incidence of CF to be 1 in 3,000 live births in Turkey (9). Our results were similar with this study. The differences between the study of Gurson et al. (9) and our study; their study was not multicentric, it was local study only including the patients from Istanbul. And also, the first aim of Gurson et al.(9) was to compare the sweat test method and the neutron activation method which can be used in the screening of CF. In this study, 6061 patients were screened to investigate the efficacy of these tests for CF, and they found two patients were diagnosed with CF. They calculated CF incidence as 1/3000 at 1973(9). Other than this study, there is no any report showing the incidence of CF by this time. The first aim of our study was to determine the incidence of CF in central Anatolia region of Turkey.

The highest CF incidence is seen in northern European countries as 1/3,000 live births. In the USA, the disease occurs in roughly 1 in 3,000 white Americans, 1 in 4,000–10,000 Latin Americans, and 1 in 15,000–20,000 African Americans (10). In Africa and Asia CF is very rare. In Japan it is as rare as 1/350,000 live births (11). Konya and Kayseri are the oldest settlements of Turkey and are located in the middle Anatolian region. Due to the location, it is influenced by both Western and Eastern cultures. These cities have more than 3,500,000 inhabitants and are leading industrial and trade centers, which receive emigrants from other parts of Turkey, especially from neighbor cities for employment. These two cities have the potential to reflect the entire middle Anatolia. Our results showed that CF is as prevalent in middle Anatolia as in northern European countries.

In this study, eight patient's sweat test was suspicious and 5 were negative. In Turkey's CF centers, infants whose second IRT results are positive, even though they had a negative or suspicious sweat test, are followed clinically. Patients with a negative and suspected sweat test have repeated one month later. Hereby the false negative results that may be caused by the sweat test are reduced. When sweat test results are evaluated, sometimes-false negative results can be obtained (12). In addition to biological variability and some CFTR mutations, false negative sweat testing may occur due to technical concerns (13).For this reason; we think that patients with positive IRT results should be monitored for a certain period even though the sweat test is negative. The IRT is a screening test, not a diagnostic test, which can lead to false negative results. Despite this, screening tests have great significance in terms of early identification of diseases. In our study, 3 (0.2%) patients, who have CF symptoms, were diagnosed as having CF even though their IRT was negative. We reviewed the results of other countries NBs tests, the false negative rate reported for Australia 5% (14), the United States (1.7%). and France (3.4%) (8, 15). Even though the NBs test can have false negative results, they can identify most of the patients. For this reason, the screening test has a significant contribution to the health care system.

In false positive individuals, serum IRT is reduced much more rapidly than in children with CF and for this reason, a higher second IRT has a stronger positive predictive value (16). Interpretation of IRT tests is complex, due to confounding factors such as age-related declines in IRT blood levels, prematurity, and perinatal stress, fecal contamination of blood spots, trisomy 13 and 18, other serious congenital abnormalities, renal failure, bowel atresia, nephrogenic diabetes insipidus, some congenital infections, and neonatal blood transfusions (17, 18, 19). The false positive IRT value leads to over follow-up of the patient and increases the anxiety of their families. Even if the sweat test of these infants is normal, the anxiety of the families is not reduced (20, 21). This is a disadvantage of the NBs test. New studies can be performed to reduce false positive results without reducing the sensitivity of the test.

The median time for diagnosing infants was 45 days. The earliest diagnosis was 3 days and the latest diagnosis was 300 days. The infants who were diagnosed the latest had negative NBs results. All of them had pseudo-Bartter's syndrome and were diagnosed as having CF according to the genetic results. At the time of diagnosis, 36.9% of the patients had a pseudo-batter and 34.3% of the patients were asymptomatic while other patients had one or more CF related symptoms. The patients who were asymptomatic could not be diagnosed previously and were recently diagnosed by the NBs test. Early diagnosis of CF has an important role in increasing average life duration and this shows us the importance of the screening test.

We requested genetic counseling for35 patients diagnosed as having CF. However, 5 (14.3%) patients were lost to follow up. CF mutation was detected in 30 (85.7%) patients. The frequency of the most common mutation, DF508, is 40% in the Middle Anatolia population. Seven of these patients were homozygous and 10 were heterozygous. Other common mutations in our study were G85E (8.3%), N130K (6.6%), G42X (5%), and W128X (5%) (Table 2). In the study by Yılmaz et al. (22) in Turkey, the frequency of the DF508 mutation was 28.4% and in a study by Onay et al. (23)the frequency was 25%.In our study result, the frequency of DF508 (40%) was higher than these studies. Sample numbers were different and the presence of the regional ethnic differences may explain this situation in Turkey. Turkey has many different ethnic groups due to historical migration routes. Several studies have shown that the frequency of the DF508 mutation differs in different countries: with frequencies of 86.9 % in Denmark (24), 73% in Scotland (25), 55% in Italy (26), 54% in Greece

(27), 17.8%, inIran (28), and 17.9% in Tunisia (29). Our study result was lower than Europe but higher than eastern countries. In our study, 21 different mutations were detected in 30 patients. This data confirms the considerable molecular heterogeneity of CF among the Turkish population. To illustrate Turkey's genetic structure better, further studies with larger samples are necessery.
One of the limitation of this study is the absence of all regions of Turkey's NBS program data. Another limitation is that the diagnosis of newborn babies who died without being screened, are not known. This problem affects many incidence studies as our study too.
In conclusion, this is the first study to determine the incidence of CF in middle Anatolia and according to our data, the incidence of CF is similar to northern European countries.

**Conflict of Interest:** The authors declare that they have no conflict of interest.
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**References**

1.Kose M, Pekcan S, Kiper N, et al. Doll-like face: is it an underestimated clinical presentation of cystic fibrosis? Pediatr Pulmonol 2008;43:634-637.
2. Castellani C, Cuppens H, Macek M Jr, et al. JS Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros2008; 7:179-196.
3. Rogan MP, Stoltz DA, HornickDB. Cystic fibrosis transmembrane conductance regulator intracellular processing, trafficking, and opportunities for mutation-specific treatment. Chest 2011; 139:1480-1490.
4. Hamosh A, FitzSimmons SC, Macek MJ, Knowles MR, Rosenstein BJ, Cutting GR. Comparison of the clinical manifestations of cystic fibrosis in black and white patients. J Pediatr1998; 132:255-259.
5.Farrell PM. The prevalence of cystic fibrosis in the European Union. J Cyst Fibros 2008; 7:450-453.
6.Farrell PM, White TB, Ren CL, et al.Diagnosis of cystic fibrosis: consensus guidelines from the Cystic Fibrosis Foundation. J Pediatr 2017; 153:4-14.
7.Accurso FJ, Sontag MK, Wagener JS. Complications associated with symptomatic diagnosis in infants with cystic fibrosis. J Pediatr 2005; 147:37–41.
8.Farrell PM, Kosorok MR, Rock MJ, et al.Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. Pediatrics 2001; 107:1-13.
9. Gürson CT, Sertel H, Gürkan M, Pala S. Newborn screening for cystic fibrosis with the chloride electrode and neutron activation analysis. Helv Paediatr Acta 1973; 28:165-174.
10.Walters S, Mehta A. Epidemiology of cystic fibrosis. In Hodson M, Geddes DM, Bush A eds. Cystic fibrosis, 3rd edn London: Edward Arnold Ltd 2007:21-45.
11.Yamashiro Y, Shimizu T, Oguchi S, Shioya T, Nagata S, Ohtsuka Y. The estimated incidence of cystic fibrosis in Japan. J Pediatr Gastroenterol Nutr1997;24:544-547.
12.Cinel G. Ter testi. In Arslan AT, Kiper N editors. Çocuk göğüs hastalıklarında tanı yöntemleri 1st ed. Probiz Ltd İstanbul, 2016:118-124.
13. Beauchamp M, Lands LC. Sweat-testing: a review of current technical requirements. Pediatr Pulmonol 2005; 39:507-511.
14. Massie RJ, Olsen M, Glazner J, Robertson CF, Francis I. Newborn screening for cystic fibrosis in Victoria: 10 years' experience. Med J Aust 2000; 172:584-7.
15.Munck A, Dhondt JL, Sahler C, Roussey M. Implementation of the French nationwide cystic fibrosis newborn screening program. J Pediatr 2008; 153:228-233.
16.Castellani C, Southern KW, Brownlee K, et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros 2009;8:153-173.
17.Therrell BL, Hannon WH, Hoffman G, Ojodu J, Farrell PM. Immunoreactive trypsinogen (IRT) as a biomarker for cystic fibrosis: challenges in newborn dried blood spot screening. Mol Genet Metab 2012; 106:1-6.
18.Massie J, Curnow L, Tzanakos N, Francis I, Robertson CF. Markedly elevated neonatal immunoreactive trypsinogen levels in the absence of cystic fibrosis gene mutations is not an indication for further testing. Arch Dis Child 2006; 91:222-225.
19. Green A, Isherwood D, Pollit R. A laboratory guide to newborn screening in the UK for cystic fibrosis. UK National Screening Committee publication. UK: PHE Publications 2014 p. 1-48
20.Tluczek A, Mischler EH, Farrell PM, et al.Parents' knowledge of neonatal screening and response to false-positive cystic fibrosis testing. J Dev BehavPediatr1992; 13:181-186.
21. Mischler EH, Wilfond BS, Fost N, et al. Cystic fibrosis newborn screening: impact on reproductive behavior and implications for genetic counseling. Pediatrics 1998; 102:44-52.
22.Yilmaz E, Erdem H, Ozgüç M, et al. Study of 12 mutations in Turkish cystic fibrosis patients. Hum Hered1995; 45:175-177.

23.Onay T, Zielenski J, Topaloglu O, et al. Cystic fibrosis mutations and associated haplotypes in Turkish cystic fibrosis patients. Hum Biol 2001; 73:191-203.
24.Schwartz M, Johansen HK, Kock C, Brandt NJ. Frequency of the DF508 mutation on cystic fibrosis chromosomes in Denmark. Hum Genet 1990; 85:427-428.
25. McIntosh I, Curtis A, Lorenzo ML et al. The haplotype distribution of the delta F508 mutation in cystic fibrosis families in Scotland. Hum Genet 1990; 85:419-420.
26.Novelli G, Gasparini P, Savonia A, Pignatti PF, Sangiuolo F, Dallapiccola B. Polymorphic DNA haplotypes and DF508 deletion in 212 Italian CF families. Hum Genet 1990; 85:420–421.
27.Balassopoulou A, Loucopoulos D, Kollia P, et al.Cystic fibrosis in Greece: Typing with DNA probes and identification of the common molecular defect. Hum Genet 1990; 85:393-394.
28.Alibakhshi R, Zamani M. Mutation analysis of CFTR gene in 70 Iranian cystic fibrosis patients. Iran J Allergy Asthma Immunol 2006; 5:3-8.
29.Messaoud T, Verlingue C, DenamurE,etal.Distribution of CFTR mutations in CF patients of Tunisian origin: identification of two novel mutations. Eur J Hum Genet 1996; 4:20–24.

| Table: 1: Demographic and clinical features of CF patients | | |
|---|---|---|
| Age of diagnosis (day)<br>(Minimum-Maximum) | 45<br>(min:3-max:300) | |
| Gender | Female | Male |
| | 45.8% (n=16) | 54.2% (n=19) |
| Symptoms at first admission | Pseudo Bartter | 36.9% (n=14) |
| | Asymptomatic | 34.3% (n=13) |
| | Nutritional Problem | 10.5% (n=4) |
| | Meconium İleus | 10.5% (n=4) |
| | Dyspnea | 5.2% (n=2) |
| | Prolonged Jaundice | 2.6 % (n=1) |

| Table 2: Genetic results for children with CF mutations | | |
|---|---|---|
| Genotype features | Homozygous (n) | Compound heterozygous (n) |
| | 15 | 15 |
| Genotype (Legacy Name) | Number of patients | One Alleles frequency % (n) |
| F508del | 17 (7*) | 40 (24) |
| G85E | 3 (2*) | 8.3 (5) |
| N1303K | 3 (1*) | 6.6 (4) |
| G542X | 3 | 5 (3) |
| W1282X | 2 (1*) | 5 (3) |
| R334W | 2 | 3,3 (2) |
| D110H | 1* | 3.3 (2) |
| DI507 | 1 | 1.6 (1) |
| Y515 | 1* | 3,3 (2) |
| S1455x | 1* | 3,3 (2) |
| c.1126_1127 ins A | 1* | 3.3 (2) |
| L467F | 1 | 1.6 (1) |
| 1677delTA | 1 | 1.6 (1) |
| E831X | 1 | 1.6 (1) |
| Q2P | 1 | 1.6 (1) |
| R170C | 1 | 1.6 (1) |
| exon2del | 1 | 1.6 (1) |
| P5L | 1 | 1.6 (1) |
| E217G | 1 | 1.6 (1) |
| exon22dup | 1 | 1.6 (1) |
| 711+3A->G | 1 | 1.6 (1) |
| *Homozygous patientsnumber | | |

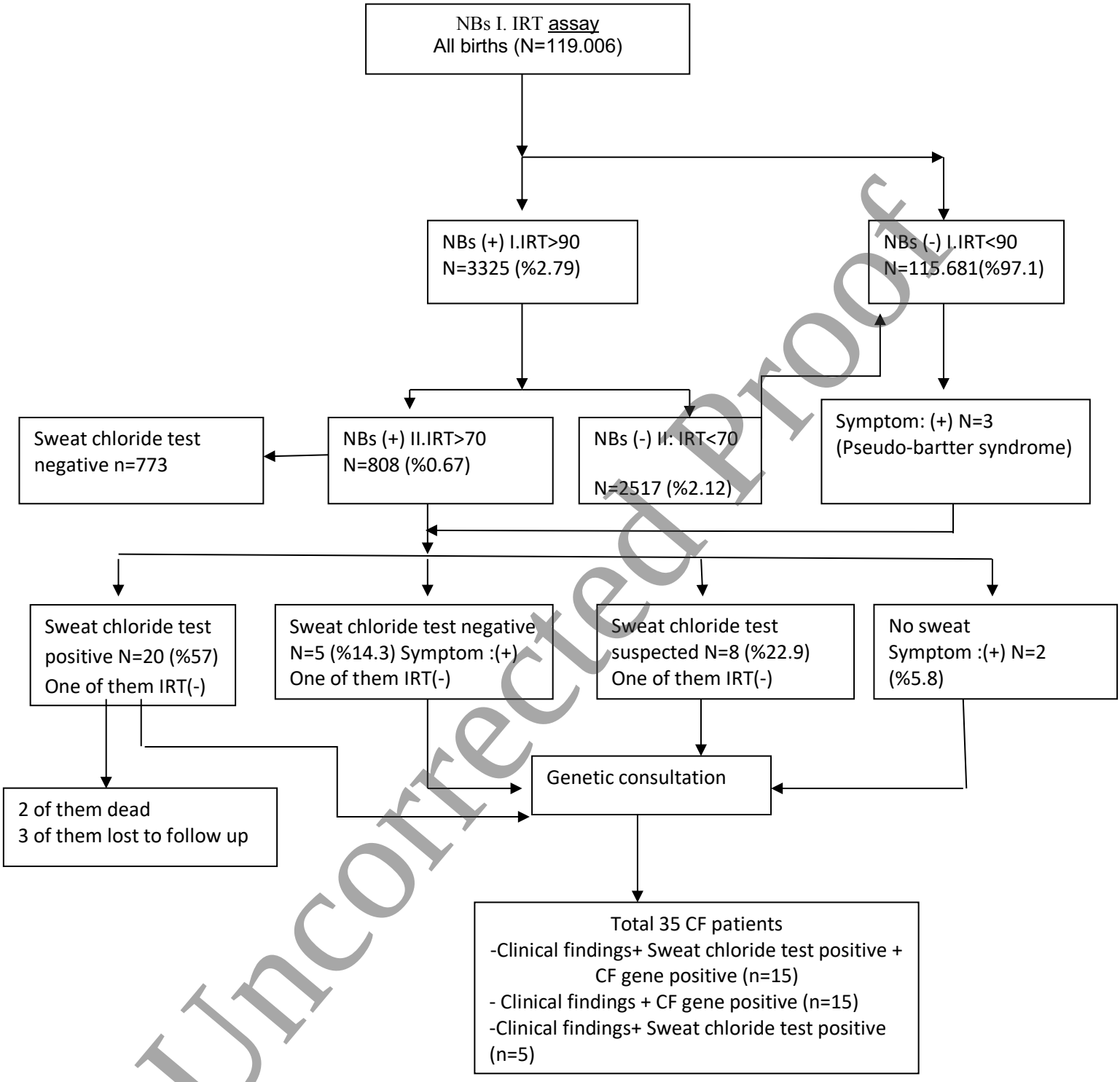




**FIG. 1.** Cystic fibrosis newborn screening algorithm and overview of results
CF: cystic fibrosis; NBs: newborn screening; IRT: immunoreactive trypsinogen