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## **PENETRANCE IS A CRITICAL PARAMETER FOR ASSESSING THE DISEASE LIABILITY OF *CFTR* VARIANTS**

**Running title:** Variable penetrance associated with *CFTR* variants

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## **Abstract**

**Background:** Major issues of newborn screening (NBS) for CF are the assessment of disease liability of variants and of the penetrance of clinical CF, notably in inconclusive diagnosis. The penetrance of CF is defined as the risk of a particular genotype to lead to a CF phenotype.

**Methods:** We aimed to get insight into the penetrance of CF for fifteen *CFTR* variants: 5 frequent CF-causing and 10 classified as of varying clinical consequence (VCC) or associated with a *CFTR*-related disorder (*CFTR*-RD) in *CFTR2* or *CFTR*-France databases. The penetrance was approached by: 1) comparison of variant allelic frequencies in CF patients (*CFTR2*) and in the general population; 2) estimation of the likelihood of a positive NBS test for the 14 compound heterozygous with F508del and the F508del homozygous genotypes, defined as the ratio of detected/expected number of neonates with a given genotype in the 2002-2017 period.

**Results:** A full penetrance was observed for severe CF-causing variants. Five variants were more frequently found in the general population than in CF patients: TG11T5, TG12T5, TG13T5, L997F and R117H;T7. The likelihood of a positive NBS test was 0.03% for TG11T5, 0.3% for TG12T5, 1.9% for TG13T5, 0.6% for L997F, 11.8% for D1152H, and 17.8% for R117H;T7. Penetrance varied greatly for variants with discrepant classification between *CFTR2* and *CFTR*-France: 5.1% for R117C, 12.3% for T338I, 43.5% for D110H and 52.6% for L206W.

**Conclusion:** These results illustrate the contribution of genetics population data to assess the disease liability of variants for diagnosis and genetic counseling purposes.

**Key-words:** penetrance, CF, *CFTR*, newborn screening, inconclusive cases, CFSPID

## Introduction

Newborn screening (NBS) for cystic fibrosis (CF) strives for the early detection of CF in infants to implement appropriate interventions and ultimately improve long term respiratory outcomes and survival [1,2]. Beyond the goal of NBS, almost all protocols worldwide identify infants who screen positive with an inconclusive determination [3], so called CFSPID (Cystic Fibrosis Screen Positive, Inconclusive Diagnosis) in EU or CRMS (CFTR-related metabolic syndrome) in the US. The harmonized definition published in 2017 includes infants with sweat chloride between 30-59 mmol/L and 0 or 1 CF-causing mutation, or a sweat chloride below 30 mmol/L and 2 *CFTR* mutations, at least one of which has unclear phenotypic consequences [4]. The proportion of cases with an inconclusive diagnosis further increases in programmes that include extended *CFTR* gene sequencing as a part of the NBS strategy [3,5]. It is thus a challenge to accurately assess the disease liability of variants, both for diagnosis and genetic counseling purposes. This requires to take into account available clinical information in databases and the literature, electrophysiological test results, functional analysis at the mRNA and protein level, and population genetics [6,7]. Databases may provide discordant information one should be aware of, notably because of different designs, so that the message given to the parents of a neonate may differ when referring to them. CFTR2 collects data from North American and European CF registries [8], i.e. only from patients diagnosed with CF. *CFTR*-France is dedicated to the interpretation of rare variants and is built on data from genetics laboratories and the French CF registry [9]. It collects genetic and clinical data from patients with CF and CFTR-related disorders (CFTR-RD) and from asymptomatic individuals who are compound heterozygous for two *CFTR* variants. Furthermore, the frequency of variants and genotypes in the general population should be considered to properly evaluate the risk of a particular genotype to cause

clinical CF; that is, the penetrance of clinical CF in individuals carrying a given genotype. As an illustration, taking into account both clinical observations and epidemiological data, it was estimated that the penetrance of CF in individuals compound heterozygous for R117H;T7 and F508del was as low as 0.03% and that of CFTR-RD was 3% [10]. Using variant frequencies in the general population provided by whole exome or genome sequencing (such as ExAC or gnomAD [11]), we aimed to get insight into the penetrance of CF, associated with a set of *CFTR* variants which have been identified through NBS in France [12,13]. In this study, we showed that the low penetrance associated with some variants found in inconclusive cases might help the clinician to adapt medical care and follow-up of these newborns, as well as genetic counseling given to families.

## **Methods**

In France, NBS for CF is a nationwide programme which relies on a 3-tiered strategy: i) IRT measurement at 3 days of age; ii) test for the most frequent CF-causing mutations in France (list in Table 1 caption, mutation detection rate at or above 80% in all regions) using the Elucigene® CF30v2 kit (Yourgene Health, Manchester, UK) when the IRT value is above the cut-off level; iii) retesting of IRT at 21 days of age if no mutation is identified in hypertrypsinogenemic infants with ultra-high IRT at day-3 (safetynet procedure) or if written consent for DNA testing is not obtained. Sweat testing is performed in CF care centers when one or two mutations are identified or in the absence of mutation when IRT at day-21 is above the cut-off level. Infants with a positive or intermediate sweat test are then subjected to an in-depth *CFTR* gene study, which mutation detection rate is 99.7% [12]. For the present study, we selected a set of 15 *CFTR* variants, comprising frequent CF-causing variants included in the French NBS panel as controls

and variants frequently detected in inconclusive cases of NBS in France after extended *CFTR* gene analysis and which may have been differently classified by CFTR2 and *CFTR*-France databases (Table 1). They include (i) variants classified as “CFTR-RD-causing” in *CFTR*-France, which are observed in patients with a CFTR-RD when combined in *trans* with a CF-causing variant, and (ii) variants of varying clinical consequences (VCC), which are reported as well in CF patients as in patients with a CFTR-RD, when in *trans* with a CF-causing variant. In CFTR2, as the CFTR-RD class is not stated, VCC refers to variants observed in patients with CF and in patients who do not have CF.

Variants were described according to Human Genome Variation Society recommendations and to traditional names in Table 1. Traditional names were used in the text for better readability.

Their allelic frequency ( $p$ ) in the general population was mostly taken from gnomAD in the European non-Finnish population [11]. GnomAD v2.1.1 collects exome and genome data from 125,748 and 15,708 unrelated individuals, respectively, who were enrolled in various disease-specific and population genetic studies. Allelic frequencies of the most frequent variants were taken in the French population: in a sample of 10490 alleles for F508del and R117H [10] and in a sample of 4380 alleles for the TG11T5, TG12T5 and TG13T5 variants.

As an approach to assess the penetrance of CF, in a first step, allelic frequencies in the general population were compared with those observed in CF patients, taken from CFTR2. We then estimated the likelihood of a positive NBS test for the selected genotypes, all in combination with F508del. Based on variant allelic frequency in the general population ( $p_{\text{variant}}$ ), the total number of neonates born in the 2002-2017 period ( $N$ ) and assuming Hardy-Weinberg equilibrium, the number of infants born in France in the 2002-2017 period expected to carry a given variant in *trans* with F508del was equal to  $2 \times p_{\text{F508del}} \times p_{\text{variant}} \times N$ . The expected number

of F508del homozygous newborn infants expected was equal to  $p_{F508del}^2 \times N$ . The likelihood of a positive NBS test was then defined as the ratio of detected/expected number of neonates carrying a given genotype in the same period. Reliable sweat test data was only available for diagnosis for a subset of patients. For R117H, only data for the 2002-2014 period, when the mutation was still part of the NBS panel (Elucigene® CF30, note in Table 1), were taken into account for accurate comparison. Indeed, the number of neonates carrying R117H and detected at birth dramatically decreased since the removal of the variant in January 2015 [13].

Statistical analysis was carried out using the R statistical software (version 3.2.3). All tests were performed two-tailed at a level of significance of 5%. For each genotype, the likelihood of a positive NBS was estimated as described above and its 95% confidence interval (CI) was determined. The exact binomial test was used to compare the observed genotype frequency (i.e. the number of neonates carrying this genotype divided by the total number of neonates born in the period) with the expected frequency (estimated from database frequencies and assuming Hardy-Weinberg equilibrium).

## Results

Of the 15 variants, five were classified as CF-causing and two as VCC in both databases (CFTR2 and *CFTR*-France). Discrepancies were observed for the other eight: CF in CFTR2 and VCC in *CFTR*-France (n=3); CF in CFTR2 and CFTR-RD in *CFTR*-France (n=1); VCC in CFTR2 and CFTR-RD in *CFTR*-France (n=3); and non-CF in CFTR2 and CFTR-RD in *CFTR*-France (n=1) (Table 1).

The five CF-causing variants (R334W, F508del, G542X, R553X and N1303K) were found 65 to 88 times more frequently in CF patients than in the general population. Five VCC or CFTR-RD

variants according to *CFTR*-France (R117C, T338I, L206W, D1152H and D110H) were found 3 to 13 times more frequently in CF patients than in the general population. The last five variants had an allelic frequency in the general population higher than in CF patients: all three T5 variants (TG11T5, TG12T5 and TG13T5), L997F and R117H. In particular, TG11T5 was found up to 95 times more frequently in the general population than in CF patients.

The same trend was observed with estimates of likelihood of a positive NBS test which varied greatly (Table 1 and Figure 1). A total of 12,320,023 infants were born in France in the 2002-2017 period. Of these, 2,441 had two *CFTR* variants and were diagnosed with CF or reported inconclusive. 987 were homozygous for F508del and 355 carried F508del in *trans* with one of the other 14 variants included in this study. Of the 15 variants, G542X, R553X and N1303K were fully penetrant while the most common pathogenic variant, F508del, showed a reduced likelihood of a positive NBS test, evaluated at 71.3%. Meanwhile, all three T5 variants showed a likelihood of a positive NBS test below 2%.

There were less F508del homozygous neonates observed (987) than expected (1384), providing a ratio of 71.3%. This significant difference should be regarded in the light of prenatal diagnoses and subsequent terminations of pregnancy (TOPs). Indeed, based on 2007-2017 data from the Agence de la biomédecine, who annually collects prenatal diagnosis activity from all molecular genetics laboratories in France, the number of TOPs for CF was 512 in this 11-year period [14], and extrapolated to 744 in the 2002-2017 total period. Based on a F508del allelic frequency of 67.24% in the French CF newborns [12] and thus assuming that 45.21% of the TOPs were made for F508del homozygous fetuses (that is, 336), a data which is however not available in the reports, this would bring the penetrance associated with the F508del homozygous genotype to 95.59%, which is not statistically different from 100%.



## Discussion

Referral to clinical databases is most often the first step in the process for assessing the disease liability of variants identified in patients and asymptomatic individuals. Importantly, observations in CF patients only, i.e. CFTR2, represent the ‘tip of the iceberg’ of all possible phenotypes associated with a given genotype and CFTR-France may report a more substantial part of them. Other criteria should be taken into account, such as population, computational, functional and segregation data, according to international guidelines [15,16]. We used population data to assess the penetrance associated with a panel of CFTR genotypes that have been identified in infants with a positive NBS result and an inconclusive diagnosis, and compared the results with those obtained for known CF-causing genotypes. It is important to note that the frequency of some variants found in gnomAD may not be used for accurate calculation, especially when differing from that of the local population.

As a reference, the allelic frequency of variants assessed as CF-causing in the two databases was 65 to 88 times higher in the population of CF patients than in the general population. As expected, the likelihood of a positive NBS test evaluated for CF-causing variants G542X, R553X and N1303K in *trans* of F508del was around 100%. The figure of 71.3% for the F508del homozygous genotype would be brought to 96% if considering TOPs following prenatal diagnoses, as documented above. The reduced likelihood of a positive NBS test of 58% associated with the CF-causing R334W variant could be explained by a residual function of the mutant protein [8,17], which is consistent with a mild form of CF as compared with other CF-causing genotypes.

Strikingly, the higher frequency of some variants in the general population as compared with the CF population, especially the T5 variants and L997F, is a strong argument against a severe pathogenic effect, suggesting a need for reclassification of these variants. The low penetrance of TG11T5 is consistent with that reported by Groman et al., where the variant was observed at higher frequency in fertile men than patients with congenital bilateral absence of the vas deferens (CBAVD) [18]. The description of the T5 variants in CF patients and their classification in CFTR2 as VCC, may be partly explained by the existence of other pathogenic variants located on the same parental allele (i.e., in *cis*), in the context of complex alleles, such as numerous documented in *CFTR*-France [9]. Evidence for non-penetrance had also been documented for L997F, hence its classification as non CF-causing in CFTR2, because of its description in fathers of CF patients who carried in *trans* a previously accepted CF-causing variant [19].

An intermediate penetrance was observed for R117C, D110H and L206W, which have been reported as CF-causing in CFTR2 while VCC in *CFTR*-France, and for D1152H, reported as VCC in both databases. D110H, L206W and D1152H were found around 10 times more frequently in CF alleles (CFTR2) than in the general population and the likelihood of a positive NBS test in neonates carrying these variants in *trans* of F508del was around 50% for D110H and L206W, and 12.5% for D1152H. This disparity for D1152H is likely due to normal or intermediate sweat chloride values associated with this variant [20]. The penetrance associated with R117C was even lower, being found only 3 times more frequently in CF patients than in the general population, with the likelihood of a positive NBS test being 5.5%. Although these data do not question the required follow-up of neonates detected with an inconclusive diagnosis, they could be considered to adapt infants care and frequency of medical visits, and eventually to shorten duration of follow-up [21,22]. These data should also be used to modulate the

information given to the parents, in terms of variable phenotype and of genetic counselling. They could also be considered in decision making regarding mutation-guided therapy, which is available notably for R117C, L206W and D1152H.

Moreover, although not assessed in this study, the actual risk of developing symptoms compatible with CF or CFTR-RD is probably lower, as shown for R117H;T7. Indeed, considering the 2002-2014 period before withdrawal of R117H from the NBS panel, the likelihood of a positive NBS test was assessed at 17.81%. Compared with the actual penetrance of 0.03% for CF and of 3% for CFTR-RD in individuals compound heterozygous for R117H;T7 and F508del [10], these data further show that this genotype is selected by NBS for CF, with the majority of cases expected to be asymptomatic throughout their lifetime or not symptomatic before adulthood.

In conclusion, taking into account the frequencies of variants in the general population is critical to assess the disease liability of variants, especially in asymptomatic conditions and this emphasizes the utility of population genetic databases such as gnomAD. Our study shows that a number of variants identified in inconclusive cases of NBS for CF in asymptomatic infants are associated with a low or very low likelihood of a positive NBS test. These data stress the importance to document the penetrance of variants included in NBS panels and argue against the implementation of *CFTR* gene extended sequencing as a second-tier test after immuno-reactive trypsinogen measurement. Indeed, this would lead to a huge increase of inconclusive cases who would not develop CF during their lifetime, carrying variants of VCC or of unknown significance. These data are also of utmost importance in the era of increasing requests for genetic testing in the general population, either in a preconceptional setting, i.e. CF carrier testing in partners of CF carriers and in couples without any family history of CF, or in the

perspective of extended NBS for numerous genetic diseases by using wide genome analysis [23,24].

**Conflict of interest statement:** none

## References

- [1] Castellani C, Linnane B, Pranke I, Cresta F, Sermet-Gaudelus I, Peckham D. Cystic fibrosis diagnosis in newborns, children, and adults. *Semin Respir Crit Care Med*. 2019 Dec;40(6):701-714. doi: 10.1055/s-0039-1697961. Epub 2019 Nov 3. PubMed PMID: 31679154.
- [2] Tridello G, Castellani C, Meneghelli I, Tamanini A, Assael BM. Early diagnosis from newborn screening maximises survival in severe cystic fibrosis. *ERJ Open Res*. 2018 Apr 20;4(2). pii: 00109-2017. doi: 10.1183/23120541.00109-2017. eCollection 2018 Apr. PubMed PMID: 29692998; PubMed Central PMCID: PMC5909061.
- [3] Barben J, Castellani C, Dankert-Roelse J, Gartner S, Kashirskaya N, Linnane B, Mayell S, Munck A, Sands D, Sommerburg O, Pybus S, Winters V, Southern KW. The expansion and performance of national newborn screening programmes for cystic fibrosis in Europe. *J Cyst Fibros*. 2017 Mar;16(2):207-213. doi: 10.1016/j.jcf.2016.12.012. Epub 2016 Dec 30. PubMed PMID: 28043799.
- [4] Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, Howenstine M, McColley SA, Rock M, Rosenfeld M, Sermet-Gaudelus I, Southern KW, Marshall BC, Sosnay PR. Diagnosis of cystic fibrosis: consensus guidelines from the Cystic Fibrosis

- Foundation. *J Pediatr.* 2017 Feb;181S:S4-S15.e1. doi: 10.1016/j.jpeds.2016.09.064. Erratum in: *J Pediatr.* 2017 May;184:243. PubMed PMID: 28129811.
- [5] Kharrazi M, Yang J, Bishop T, Lessing S, Young S, Graham S, Pearl M, Chow H, Ho T, Currier R, Gaffney L, Feuchtbaum L; California Cystic Fibrosis Newborn Screening Consortium. Newborn screening for cystic fibrosis in California. *Pediatrics.* 2015 Dec;136(6):1062-72. doi: 10.1542/peds.2015-0811. Epub 2015 Nov 16. PubMed PMID: 26574590.
- [6] Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, Tullis E, Assael BM, Bombieri C, Brown A, Casals T, Claustres M, Cutting GR, Dequeker E, Dodge J, Doull I, Farrell P, Ferec C, Girodon E, Johannesson M, Kerem B, Knowles M, Munck A, Pignatti PF, Radojkovic D, Rizzotti P, Schwarz M, Stuhmann M, Tzetis M, Zielenski J, Elborn JS. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros.* 2008 May;7(3):179-96. doi: 10.1016/j.jcf.2008.03.009. Review. PubMed PMID: 18456578; PubMed Central PMCID: PMC2810954.
- [7] Sosnay PR, Salinas DB, White TB, Ren CL, Farrell PM, Raraigh KS, Girodon E, Castellani C. Applying cystic fibrosis transmembrane conductance regulator genetics and CFTR2 data to facilitate diagnoses. *J Pediatr.* 2017 Feb;181S:S27-S32.e1. doi: 10.1016/j.jpeds.2016.09.063. PubMed PMID: 28129809.
- [8] US CF Foundation, Johns Hopkins University, The Hospital for Sick Children, Baltimore. The Clinical and Functional TRanslation of CFTR (CFTR2). Available from: <http://cftr2.org>; 2019 [updated 10/01/2020; cited 2011].
- [9] Claustres M, Thèze C, des Georges M, Baux D, Girodon E, Bienvenu T, Audrezet MP, Dugueperoux I, Férec C, Lalau G, Pagin A, Kitzis A, Thoreau V, Gaston V, Bieth E,

Malinge MC, Reboul MP, Fergelot P, Lemonnier L, Mekki C, Fanen P, Bergognoux A, Sasorith S, Raynal C, Bareil C. *CFTR*-France, a national relational patient database for sharing genetic and phenotypic data associated with rare *CFTR* variants. *Hum Mutat.* 2017 Oct;38(10):1297-1315. doi: 10.1002/humu.23276. Epub 2017 Jun 28. PubMed PMID: 28603918. *CFTR*-France available from: <https://cftr.iurc.montp.inserm.fr/cgi-bin/home.cgi> [updated 31/12/2019; cited 2012].

[10] Thauvin-Robinet C, Munck A, Huet F, Génin E, Bellis G, Gautier E, Audrézet MP, Férec C, Lalau G, Georges MD, Claustres M, Bienvenu T, Gérard B, Boisseau P, Cabet-Bey F, Feldmann D, Clavel C, Bieth E, Iron A, Simon-Bouy B, Costa C, Medina R, Leclerc J, Hubert D, Nové-Josserand R, Sermet-Gaudelus I, Rault G, Flori J, Leroy S, Wizla N, Bellon G, Haloun A, Perez-Martin S, d'Acremont G, Corvol H, Clément A, Houssin E, Binquet C, Bonithon-Kopp C, Alberti-Boulmé C, Morris MA, Faivre L, Goossens M, Roussey M; Collaborating Working Group on R117H, Girodon E. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. *J Med Genet.* 2009 Nov;46(11):752-8. doi: 10.1136/jmg.2009.067215. Epub 2009 Jun 29. PubMed PMID: 19880712.

[11] Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, Gauthier LD, Brand H, Solomonson M, Watts NA, Rhodes D, Singer-Berk M, England EM, Seaby EG, Kosmicki JA, MacArthur DG. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. In *BioRxiv.* 2019 p. 531210. <https://doi.org/10.1101/531210>. Available from: <https://gnomad.broadinstitute.org/> [updated 10/01/2020; cited 2019].

- [12] Audrézet MP, Munck A, Scotet V, Claustres M, Roussey M, Delmas D, Férec C, Desgeorges M. Comprehensive *CFTR* gene analysis of the French cystic fibrosis screened newborn cohort: implications for diagnosis, genetic counseling, and mutation-specific therapy. *Genet Med*. 2015 Feb;17(2):108-16. doi: 10.1038/gim.2014.113. Epub 2014 Aug 14. Erratum in: *Genet Med*. 2015 Jan;17(1):93. PubMed PMID: 25122143.
- [13] Munck A, Delmas D, Audrézet MP, Lemonnier L, Cheillan D, Roussey M. Optimization of the French cystic fibrosis newborn screening programme by a centralized tracking process. *J Med Screen*. 2018 Mar;25(1):6-12. doi: 10.1177/0969141317692611. Epub 2017 Apr 28. PubMed PMID: 28454512; PubMed Central PMCID: PMC5813881.
- [14] Rapports d'activité médicale et scientifique de l'Agence de la biomédecine, diagnostic prénatal par génétique moléculaire. Available at: <https://rams.agence-biomedecine.fr/diagnostic-sur-lembrion-et-le-foetus>.
- [15] Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PubMed PMID: 25741868; PubMed Central PMCID: PMC4544753.
- [16] Amendola LM, Jarvik GP, Leo MC, McLaughlin HM, Akkari Y, Amaral MD, Berg JS, Biswas S, Bowling KM, Conlin LK, Cooper GM, Dorschner MO, Dulik MC, Ghazani AA, Ghosh R, Green RC, Hart R, Horton C, Johnston JJ, Lebo MS, Milosavljevic A, Ou J, Pak CM, Patel RY, Punj S, Richards CS, Salama J, Strande NT, Yang Y, Plon SE, Biesecker

- LG, Rehm HL. Performance of ACMG-AMP variant-interpretation guidelines among nine laboratories in the Clinical Sequencing Exploratory Research Consortium. *Am J Hum Genet.* 2016 Jun 2;98(6):1067-1076. doi: 10.1016/j.ajhg.2016.03.024. Epub 2016 May 12. Erratum in: *Am J Hum Genet.* 2016 Jul 7;99(1):247. PubMed PMID: 27181684; PubMed Central PMCID: PMC4908185.
- [17] Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl<sup>-</sup> channels with altered pore properties. *Nature.* 1993 Mar 11;362(6416):160-4. PubMed PMID: 7680769.
- [18] Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, Guittard C, Koudova M, Fallin MD, Nemeth K, Fekete G, Kadasi L, Friedman K, Schwarz M, Bombieri C, Pignatti PF, Kanavakis E, Tzetis M, Schwartz M, Novelli G, D'Apice MR, Sobczynska-Tomaszewska A, Bal J, Stuhmann M, Macek M Jr, Claustres M, Cutting GR. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. *Am J Hum Genet.* 2004 Jan;74(1):176-9. Epub 2003 Dec 18. PubMed PMID: 14685937; PubMed Central PMCID: PMC1181905.
- [19] Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, Sharma N, Ramalho AS, Amaral MD, Dorfman R, Zielenski J, Masica DL, Karchin R, Millen L, Thomas PJ, Patrinos GP, Corey M, Lewis MH, Rommens JM, Castellani C, Penland CM, Cutting GR. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet.* 2013 Oct;45(10):1160-7. doi: 10.1038/ng.2745. Epub 2013 Aug 25. PubMed PMID: 23974870; PubMed Central PMCID:PMC3874936.
- [20] Burgel PR, Fajac I, Hubert D, Grenet D, Stremler N, Roussey M, Siret D, Languépin J, Mely L, Fanton A, Labbé A, Domblides P, Vic P, Dagonne M, Reynaud-Gaubert M, Council



- F, Varaigne F, Bienvenu T, Bellis G, Dusser D. Non-classic cystic fibrosis associated with D1152H CFTR mutation. *Clin Genet.* 2010 Apr;77(4):355-64. doi: 10.1111/j.1399-0004.2009.01294.x. Epub 2009 Oct 15. PubMed PMID: 19843100.
- [21] Ren CL, Borowitz DS, Gonska T, Howenstine MS, Levy H, Massie J, Milla C, Munck A, Southern KW. Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis. *J Pediatr.* 2017 Feb;181S:S45-S51.e1. doi: 10.1016/j.jpeds.2016.09.066. PubMed PMID: 28129812.
- [22] Sermet-Gaudelus I, Brouard J, Audrézet MP, Couderc Kohen L, Weiss L, Wizla N, Vrielynck S, Llerena K, Le Bourgeois M, Deneuille E, Remus N, Nguyen-Khoa T, Raynal C, Roussey M, Girodon E. Guidelines for the clinical management and follow-up of infants with inconclusive cystic fibrosis diagnosis through newborn screening. *Arch Pediatr.* 2017 Dec;24(12):e1-e14. doi:10.1016/j.arcped.2017.07.015. Epub 2017 Nov 22. PubMed PMID: 29174009.
- [23] Ceyhan-Birsoy O, Murry JB, Machini K, Lebo MS, Yu TW, Fayer S, Genetti CA, Schwartz TS, Agrawal PB, Parad RB, Holm IA, McGuire AL, Green RC, Rehm HL, Beggs AH; BabySeq Project Team. Interpretation of Genomic Sequencing Results in Healthy and Ill Newborns: Results from the BabySeq Project. *Am J Hum Genet.* 2019 Jan 3;104(1):76-93. doi: 10.1016/j.ajhg.2018.11.016. PubMed PMID: 30609409; PubMed Central PMCID: PMC6323417.
- [24] Kingsmore SF. Newborn testing and screening by whole-genome sequencing. *Genet Med.* 2016 Mar;18(3):214-6. doi: 10.1038/gim.2015.172. Epub 2015 Dec 17. PubMed PMID: 26681311.

Table 1: Epidemiological and penetrance data associated to *CFTR* variants

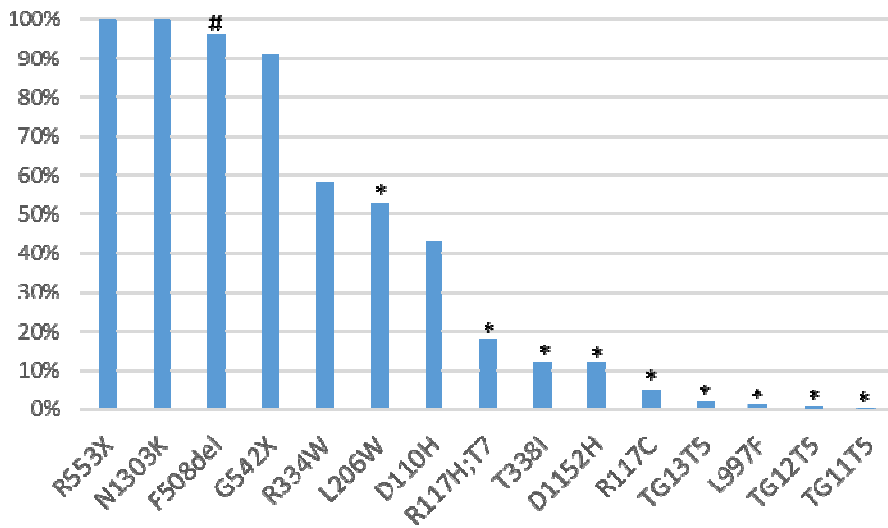
Variant name			Phenotypic classification		Allele frequency		Ratio General population/ CFTR2 frequency	Ratio CFTR2/ general population frequency	No. NN expected F508del/variant (n=12320023 NN)	No. NN detected (2002-2017 period)	Ratio detected/ expected	95% IC (%)	Likelihood of a positive NBS test	Significance (Exact binomial test)
cDNA (HGVS)	Protein (HGVS)	Legacy	CFTR2	CFTR-France	General population (gnomAD/ French*)	CF patients CFTR2 (n=142,036)								
c.[1210-34_1210-6TG[11]T[5]	p.(=)	TG11T5	VCC	CFTR-RD	0.0234*	0.00025	94.96	0.01	6111.7	2	0.0003	0.0003 to 0.0003	<b>0.03%</b>	<0.0001
c.[1210-34_1210-6TG[12]T[5]	p.(=)	TG12T5	VCC	CFTR-RD	0.0098*	0.00128	7.65	0.13	2559.6	9	0.0035	0.0035 to 0.0035	<b>0.35%</b>	<0.0001
c.[1210-34_1210-6TG[13]T[5]	p.(=)	TG13T5	VCC	VCC	0.0008*	0.00030	2.64	0.38	208.9	4	0.0191	0.0191 to 0.0191	<b>1.91%</b>	<0.0001
c.2991G>C	p.Leu997Phe	L997F	Non CF	CFTR-RD	0.002544	0.00077	3.28	0.30	664.5	4	0.0060	0.0060 to 0.0061	<b>0.60%</b>	<0.0001
c.349C>T	p.Arg117Cys	R117C	CF	VCC	0.0003724	0.00103	0.36	2.76	97.3	5	0.0514	0.0513 to 0.0515	<b>5.14%</b>	<0.0001
c.3454G>C	p.Asp1152His	D1152H	VCC	VCC	0.0003261	0.00402	0.08	12.33	85.2	10	0.1174	0.1172 to 0.1176	<b>11.74%</b>	<0.0001
c.1013C>T	p.Thr338Ile	T338I	CF	CFTR-RD	0.00006201	0.00037	0.17	5.97	16.2	2	0.1235	0.1233 to 0.1237	<b>12.35%</b>	<0.0001
<b>c.[350G&gt;A;1210-12[7]]</b>	<b>p.Arg117His</b>	<b>R117H;T7#</b>	VCC	CFTR-RD	0.0029*	0.00088	3.30	0.30	617.7	110#	0.1780	0.1779 to 0.1783	<b>17.81%</b>	<0.0001
c.328G>C	p.Asp110His	D110H	CF	VCC	0.00003522	0.00046	0.08	13.00	9.2	4	0.4348	0.4346 to 0.4351	<b>43.48%</b>	0.0969
c.617T>G	p.Leu206Trp	L206W	CF	VCC	0.00024	0.00234	0.10	9.77	62.7	33	0.52645	0.5262 to 0.5267	<b>52.64%</b>	<0.0001
<b>c.1000C&gt;T</b>	<b>p.Arg334Trp</b>	<b>R334W</b>	CF	CF	0.00004652	0.00302	0.02	64.93	12.2	7	0.5761	0.5758 to 0.5764	<b>57.61%</b>	0.1928
<b>c.1521_1523del</b>	<b>p.Phe508del</b>	<b>F508del</b>	CF	CF	0.0106*	0.69744	0.02	65.78	1384.3	987	0.7130	0.7128 to 0.7133	<b>71.30%</b>	<0.0001
<b>c.1624G&gt;T</b>	<b>p.Gly542Ter</b>	<b>G542X</b>	CF	CF	0.0003571	0.02542	0.01	71.17	93.3	85	0.9113	0.9112 to 0.9115	<b>91.13%</b>	0.437
<b>c.3909C&gt;G</b>	<b>p.Asn1303Lys</b>	<b>N1303K</b>	CF	CF	0.0001796	0.01581	0.01	88.03	46.9	47	1.0019	1.0019 to 1.0020	<b>100%‡</b>	0.9418
<b>c.1657C&gt;T</b>	<b>p.Arg553Ter</b>	<b>R553X</b>	CF	CF	0.0001243	0.00931	0.01	74.90	32.5	33	1.0164	1.0164 to 1.0165	<b>100%‡</b>	0.8608

ND: not documented; NN: neonates; \* refers to allelic frequencies in the French general population; # c.350G>A (R117H) is no longer part of the French NBS panel as of January 2015. The data were thus collected during the 2002-2014 period with 10 046 679 NN. ‡ Value set at 100% because observed was greater than expected; Variants included in the French NBS panel are indicated in bold.

Note: NBS panel Elucigene®CF30 (legacy nomenclature): F508del, I507del, 1078delT, 1717-1G>A, 2183AA>G, 3659delC, 3849+10kbC>T, 621+1G>T, A455E, E60X, G542X, G551D, N1303K, R1162X, R117H, R334W, R347P, R553X, S1251N, W1282X, 1811+1,6kbA>G, 2789+5G>A, 3120+1G>A, 3272-26A>G, 394delTT, 711+1G>T, G85E, Y1092X, Y122X, W846X. NBS panel Elucigene®CF30v2 as of January 2015: same panel as above without R117H.

Figure 1: Likelihood of a positive NBS test on a scale for genotypes combining F508del and one of the 15 variants

Likelihood of a positive NBS test



\* Statistically significant difference between detected and expected number of neonates carrying a given genotype.

# For the F508del homozygous genotype, correction with the expected number of termination of pregnancies after prenatal diagnoses was taken into account.

Likelihood of a positive NBS test for genotypes combining F508del and one of the 15 variants

