Institute of Hereditary Pathology of
Academy of Medical Sciences of Ukraine,
Lviv Regional Specialized Children's Hospital
Lviv, Ukraine

*H.Makukh[1] , L.Bober[2], M.Tyrkus[1], O.Luga [2], O.Hnateyko[1], O.Munduk[2]*

# Diagnostic challenges in Ukraine

# **Ukraine** (Total population: 48 million.)

## **Western Ukraine** ( ~10 million inhabitants)


- Every 29-th – CFTR mutations heterozygous carrier
- **35 – 40** Cystic Fibrosis children are expected to be born every year in Western Ukraine **143** in the country
- 1700 до 4000 CF patients are expected to be exist in Ukraine
- CF patients from Western region of Ukraine pass sweat test and molecular-genetic studies of CFTR mutations at the Institute of Hereditary Pathology and as a rule receive treatment in Lviv Regional Specialised Children's Hospital.
- There are differences between the CF patients' management possibilities in different regions of Ukraine.
- CFTR gene mutations analysis is performed in two centres only: in Kyiv – the capital of Ukraine and in Lviv (Western region of the country).

- Molecular genetic studies at the Institute of Hereditary Pathology were started in the early 1990's.
- In 1998 diagnostic analysis of the major CFTR mutation F508del was introduced.
- The molecular genetic testing of CFTR gene mutations was performed among 1150 persons with CF suspicion, 1200 their relatives, 126 adult men with idiopathic spermatogenesis failure and 360 healthy donors of ovocytes.

**185** CF probands were detected (182 in Kyiv).

19 different CFTR gene mutations were found and about 86% of *CFTR* alleles have been identified

# CFTR gene mutations testing

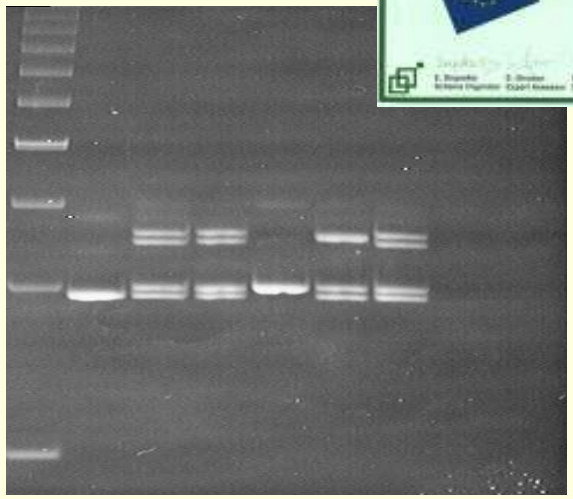

"European Quality Assessment Scheme for Cystic Fibrosis"

***Methods***

1. DNA extraction and purification *(own modification method, Makukh et al.2008)*
2. PCR.
3. Heteroduplex analysis, RFLP, deletion analysis, ARMS.

***CFTR mutations:***

*F508del, ΔI507, F508C, 1677delTA, G542X, N1303K, 2184insA, CFTRdele2,3(21kb), W1282X, G551D, R553X, 1717–1G>A, 621+1G>A, R347P, R347H, R347L, 347C, I336K, R334W, S549I, S549N, R560T, G551S, Q552X, Y122X, D1270N*



**Analysis of F508del and I507del mutations,** *10% - PAA gel.*

Від кого: **CF Network <cf.network@med.kuleuven.be>**
Кому: **Makukh Halyna <makukh_halyna@ukr.net>**
Дата: 10 квітня, 11:57:20 *(за київським часом)*
Тема: **good genetic testing reports**

Dear Halyna Makukh,

Within the CF Network we feel that there is a **need for some guidance to write good genetics reports**, especially for new laboratories that participate in the scheme.

In order to meet this need we are working on a guidance document which highlights the elements that should be covered in a report, based on what the international ISO 15189 standard asks.
To make it more visual we want to add **example reports which implemented one or more ISO elements in an excellent way**.

We found out that your report covers nice issues and therefore **we ask you if you are agree that we include one of the reports you sent us for the EQA scheme as an example in this guidance document**.
Of course we make the report anonymous.

We hope to harmonize and improve quality within the genetic testing services, by developing this document and your collaboration.

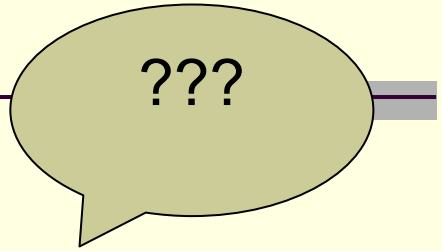
Thank you in advance.

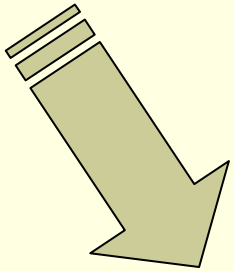
Sarah

**Sarah Berwouts**
Scheme co-organizer

**Prof. dr. Els Dequeker**
Scheme organizer
University of Leuven - Department of Human Genetics
Herestraat 49, box 602, B-3000 Leuven, Belgium
Tel:+32 (0)16 33 01 43
Fax: +32 (0)16 34 59 97
E-mail:cf.network@med.kuleuven.be
Website: CF Network

- Using our protocol in combination with the TM Bioscience protocol, 68.55% of CF mutations were detected (at 2008)
- The spectrum of mutations in our study group of CF patients is similar to that of Central and Eastern Europe.
- A significant contributor to these mutant alleles is the “Slavic” mutation CFTRdele2,3(21kb).
- 32% unidentified alleles

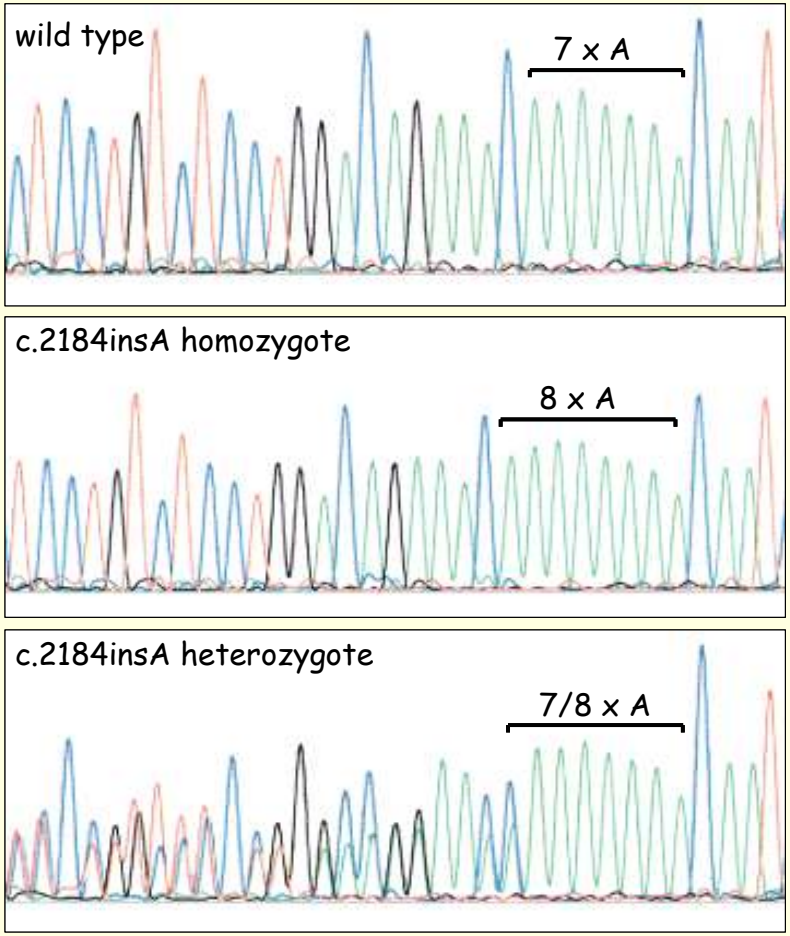


**Search for “special” mutations**

# We found it!

- After the sequencing of exon 13 we discovered the high incidence of 2184insA mutation among patients from Ukraine *(supported by Eurogentest and Prof. M.Macek ).*
- This region of CFTR gene contains poly A track (eight in normal range) and deletions of one or two nucleotides are common. 2184delA are included in commercial kits for CFTR gene mutations testing, but the insertion 2184insA is not detected by those methods.
- After inclusion of the c.2184insA mutation in the testing panel this allele was identified among 25 CF patients.
- As 2184insA mutation is rare, the phenotype which it could cause is not described sufficiently.



| No | c.2184insA | YOB | diagnosiis, *month* | Sweet test (Cl, *mmol/l)* | CF Liver Disease | Z-score | FE1,ug/g of stool | Glucose intolerance |
|---|---|---|---|---|---|---|---|---|
| 1. | c.2184insA | 2000 | 39 | 87 | No | - 1.78 | 3,3 | no |
| 2. | c.2184insA | 1992 | 42 | 105 | fibrosis | Died at the age of 7 years | | |
| 3. | p.F508del | 2000 | 3 | 98 | no | - 1.58 | 23 | no |
| 4. | p.F508del | 1997 | 11 | 104 | fibrosis | - 2.12 | 11,3 | yes |
| 5. | p.F508del | 1996 | 60 | 94 | No | - 1.36 | 15,6 | yes |
| 6. | p.F508del | 1997 | 48 | 105 | X | - 2.78 | 2,7 | no |
| 7. | c.CFTRdele2,3 | 2003 | 8 | 100 | fibrosis, hypoplasia of biliary ducts | - 2.22 | 1, 9 | no |
| 8. | p.F508del | 2004 | 4 | 86 | fibrosis | - 1.66 | x | x |
| 9. | p.F508del | 2002 | 5 | 106 | fibrosis | - 1.75 | 3,2 | no |
| 10. | p.F508del | 2002 | 44 | 105 | No | - 0.94 | 17,4 | no |
| 11. | p.F508del | 1995 | 21 | 118 | No | - 5.27 | 44 | yes |
| 12. | p.F508del | 2006 | 1 | 105 | hypoplasia of biliary ducts | - 2.21 | 8,7 | no |
| 13. | p.F508del | 2007 | 6 | 105 | No | - 3.32 | x | no |
| 14. | p.F508del | 2003 | 66 | 97 | hepatomegaly | - 0.29 | x | yes |
| 15. | p.F508del | 2004 | 2 | 73 | No | Discharged of CF centre patient | | |
| 16. | p.F508del | 2002 | 60 | 100 | cirrhosis | - 0.78 | 432,1 | No |
| 17. | p.R334W | 1982 | 5 | 95 | No | - 0.85 | 514 | no |
| 18. | p.F508del | 1992. | 12 | 97 | No | - 0.68 | x | no |
| 19. | c.3849+10kbC>T | 2004 | 60 | 102 | No | - 1.94 | x | yes |
| 20. | p.F508del | 2007 | 42 | 103 | X | - 2.85 | x | x |
| 21. | p.F508del | 2010 | 6 | 68 | X | 3.23x | x | x |
| 22. | c. 2143del | 2007 | 30 | 71 | X | - 2.32 | x | x |
| 23. | unidentified | 2005 | 6 | 82 | No | - 0.37 | x | yes |
| 24. | p.F508del | 1997 | 28 | 95 | No | - 0.44 | x | yes |
| 25. | p.F508del | 2005 | 7 | 102 | No | - 2.51 | 105 | no |

- The c.2184insA mutation is the second most common mutation in CF patients from Ukraine is ~~associated with early manifestation of CF with~~ severe disorders. *(A high frequency of the Cystic Fibrosis 2184insA mutation in Western Ukraine: Genotype–phenotype correlations, relevance for newborn screening and genetic testing. H.Makukh, P.Křenková, M.Tyrkus et al. // Journal of Cystic Fibrosis, –2010, №9, –P. 371–375)*
- The clinical manifestation of CF among patients with c.2184insA do not differ from patients with other genotypes except the colonization of bronchus-pulmonary tree.
- These observed differences could be the subject of investigation in future studies addressing the molecular defect of CFTR protein expressed from c.2184insA mutant alleles in bronchus and lung and developing mutation depending CF treatment.

# 2184insA - Galicia's (West Ukrainian) mutation

- The frequency of 7.2% of all mutated CF chromosomes makes possible to predict the origin of c.2184insA as West Ukrainian (Galicia's), which were distributed from there across Europe and has to be included at the testing panel for patients of Ukrainian origin.

| Mutations of CFTR gene | Number of mutant alleles | | Frequency % | |
|---|---|---|---|---|
| | Lviv | Kyiv | Lviv | Kyiv |
| F508del (c.1520_1522delTCT ) | 190 | 157 | 55,9 | 43,37 |
| 2184insA (c.2052_2053insA) | 26 | 13 | 7,6 | 3,87 |
| N1303K (c.3909C>G ) | 20 | 8 | 5,9 | 2,21 |
| c.CFTRdele2,3(21kb) | 14 | 14 | 4,1 | 3,59 |
| G542X (c.1624G>T ) | 10 | 3 | 2,9 | 0,83 |
| 3849+10kbC>T (c.3717+12191C>T) | 6 | 0 | 1,8 | - |
| W1282X (c.3846G>A) | 6 | 4 | 1,8 | 1,1 |
| 1898+1G>A (c.1766+1G>A) | 4 | 0 | 1,2 | - |
| R553X (c.1657C>T) | 3 | 2 | 0,9 | 0,55 |
| 2143delT (c.2012delT) | 3 | 5 | 0,9 | 1,38 |
| 621+1G>T (c.489+1G>T) | 3 | 2 | 0,9 | 0,55 |
| R334W (c.1000C>T) | 2 | 1 | 0,6 | 0,28 |
| 3272-11 A>G (c.3140-11A>G) | 2 | 0 | 0,6 | - |
| 185+1G>T (c.53+1G>T) | 2 | 0 | 0,6 | - |
| E92K (c.274G>A) | 1 | 0 | 0,3 | - |
| R347H (c.1040G>A) | 1 | 0 | 0,3 | - |
| Cystic Fibrosis Mutation Database Y362X (c.1086T>A) | 1 | 0 | 0,3 | - |
| 1717-1G>A (c.1585-1G>A) | 1 | 0 | 0,3 | - |
| 2183AA>G (c.2051_2052delAA) | 1 | 0 | 0,3 | - |
| L257G | 1 | 0 | 0,3 | - |
| R1186X (ex19) | 1 | 0 | 0,3 | - |
| W79X(ex3 | 1 | 0 | 0,3 | - |
| 2721del11 (c.2589_2599delAATTTGGTGCT) | 1 | 0 | 0,3 | - |
| Total | 300 | 210 | 88,3 | 58,1 |
| Inidentified | 40 | 152 | 11,7 | 41,9 |

- All chromosomes with 2184insA (one exeption) have the same haplotype 16 – 7.
- Next studies – patients with 2184insA from other countries.

*P.S. Patient (S., mother Ukrainian ancestry) from CF Trust (UK) was observed in Lviv CF center. No CFTR mutation identified… After analysis we detected 2184insA*

# CFTR mutations testing of infertile men with idiopathic spermatogenesis failure

- Idiopatic azoospermia

  low semen volume and acidic pH.
- Mutations associated with CBAVD: R334W, R117H, G551S, D1270N, R347P, R347H, R347L, R347C and IVS8-5/7/9T
- The molecular-genetic analysis of CFTR gene mutations and IVS8polyT polymorphic locus revealed F508del mutation in 4,02%, 5T polymorphic allele – in 10% males with spermatogenesis failure.

# Sweat testing and CFTR mutations analysis of infertile men without CF clinical symptoms

- Among adult men with idiopathic spermatogenesis failure in 60% cases sweat test was normal (<40mmol/l), in 33% borderline (40 – 60 mmol/l) and in 6,1% (>60mmol/l) positive. CFTR gene mutations analysis revealed F508del mutation and 5T allele in 5% of patients with normal and in 18% of patients with borderline sweat test.
- 2 man with high level of chloride concentration (79,5 and 90,1mmol/l) have no CF symptoms except aspermia. One of them is heterozygous for F508del, other one is negative for all analyzed CFTR mutations.
- Sweat test is important for Cystic Fibrosis diagnosis establishing as well for CFTR related diseases identification.

# Cystic Fibrosis prenatal diagnostics

- 32 prenatal diagnostics have been provided.
- 8 cases of Cystic Fibrosis were confirmed.
- 24 healthy children in CF families
- >500 heterozygous carriers of CFTR mutations among the patient's relatives have been identified.


- The results of genetic and clinical features of patients with cystic fibrosis from Ukraine are added into the European registry of cystic fibrosis **(EuroCareCF - European registry**) and will be considered for developing treatment programs and prevention of this disease in Europe.

# CONCLUSIONS and FUTURE WORK

Cystic Fibrosis in Ukraine

## "we have done"

- Good genetic diagnostics
- The care of CF patients is performed according to the European standard schemes.
- The majority of diagnosed CF patients are physically and socially adapted.
- We progress in decrease of mortality rate and improved survival of CF patients.
- Pancreatic enzymes are free of charge for CF patients.

## "we must do"

- The results have shown insufficiency of the CF cases revealing(~13 per year to 40 expected) and need of alertness and knowledge about CF
- In many cases the most serious treatment problem is impossibility of the undertaking required medicine because of financial insolvency of patients, who must pay the drug costs themselves.
- No strong patient organisation
- In Ukraine adequate CF patients' healthcare requires creation of the CF National Program and regional CF centers.
- Neonatal screening! Do we ready?

# Thanks for attention

Halyna Makukh
makukh.h@ihp.lviv.ua
makukh_halyna@ukr.net