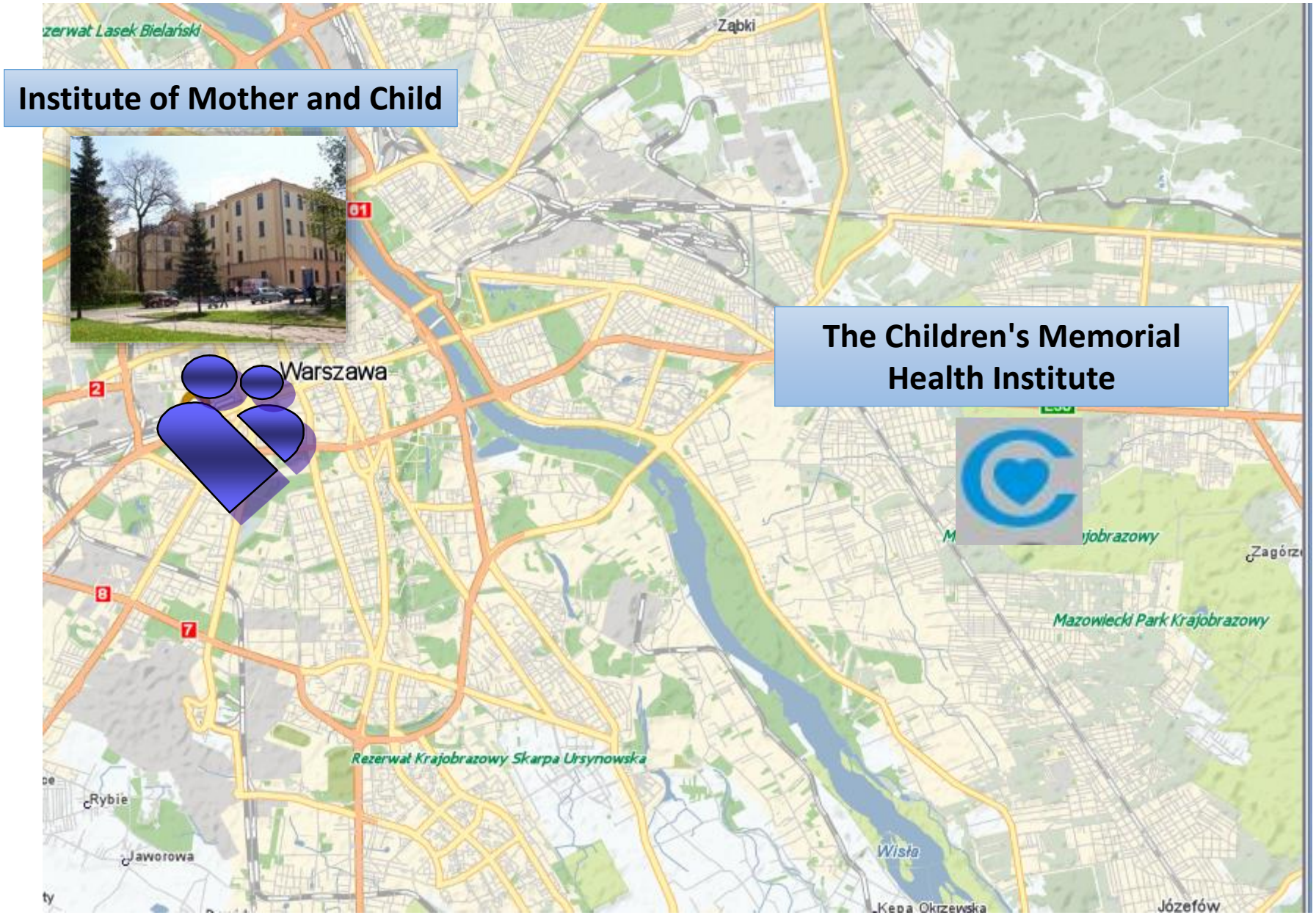
Polish pancreatitis working group and activities

Institute of Mother and Child, Warsaw

Associated Prof. Agnieszka Rygiel, PhD; Katarzyna Wertheim-Tysarowska, PhD

The Children's Memorial Health Institute, Warsaw

Associated Prof. Grzegorz Oracz, MD,PhD


Institute of Mother and Child
Warszawa
The Children's Memorial Health Institute
Ząbki
Mazowiecki Park Krajobrazowy
Rezerwat Krajobrazowy Skarpa Ursynowska
Wisła
Józefów
Rybie
Jaworowa
Zagórze
2
8
7
61

1. Institute of Mother and Child, Warsaw, Poland

Department of Medical Genetics (Molecular, Cytogenetic unit and Genetic counseling)

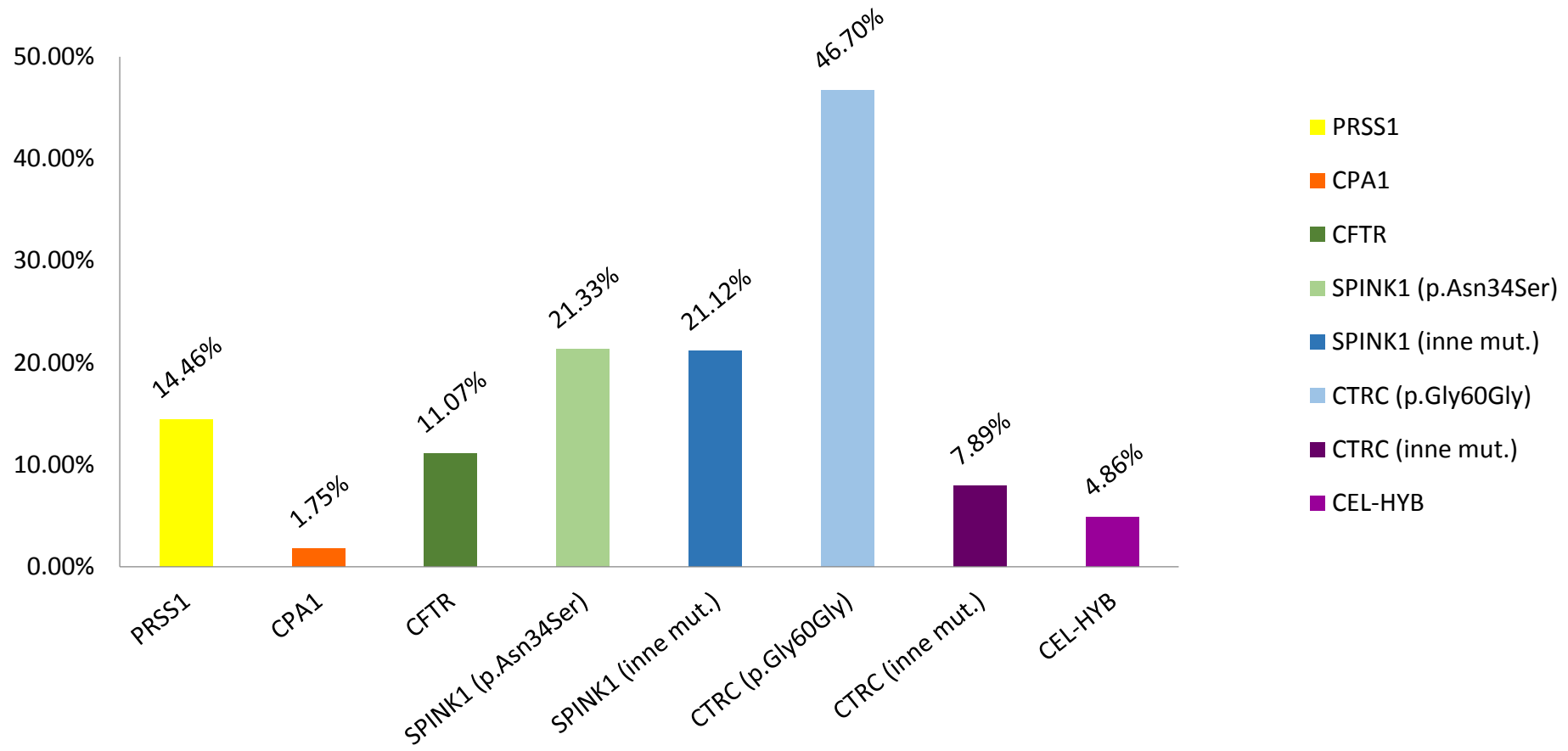
- Since 1970s molecular diagnostic unit for various inherited disorders
- DNA bank of 6000 DNA samples including around 500 cases of RAP/CP from various hospitals
- **30 and 18 years of experience in molecular diagnostic of cystic fibrosis (CF) and chronic pancreatitis (CP), respectively**

2. The Children's Memorial Health Institute, Warsaw, Poland

Department of Gastroenterology, Hepatology, Feeding Disorders and Pediatrics

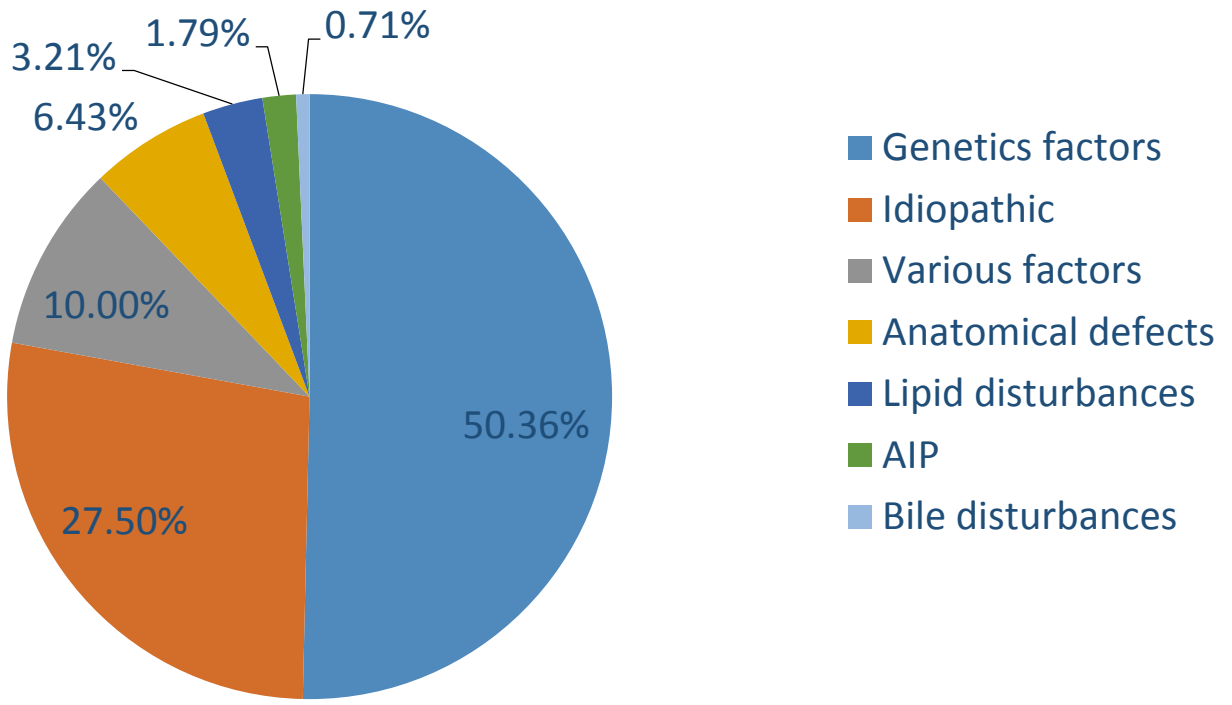
- **Polish reference center for children with CP**
- **Single center cohort of around 400 cases: Grzegorz Oracz**

Genetic variants in CP children cohort (mean age at diagnosis 10 years)



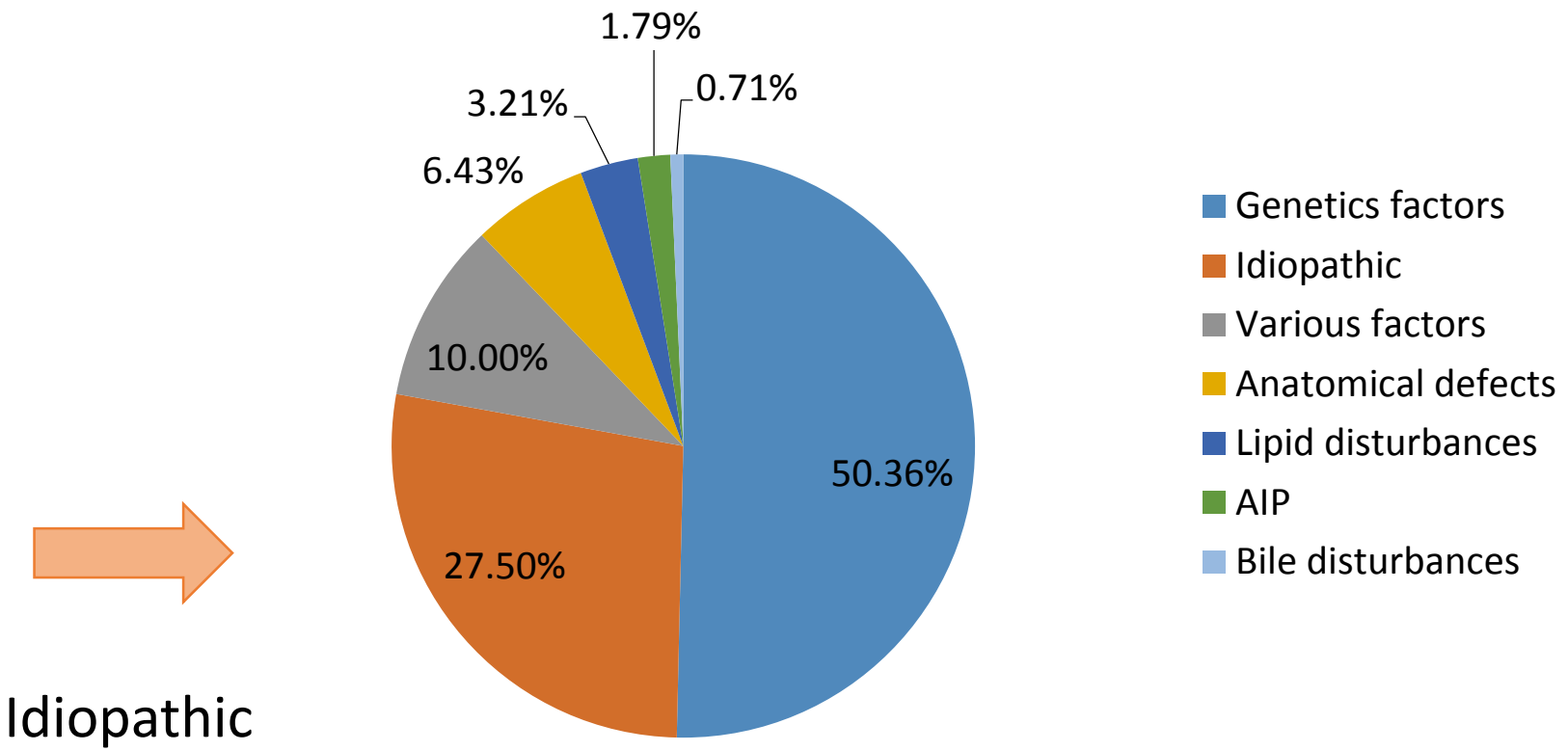
CP children cohort of the Children's Memorial Health Institute

Etiological factors in CP children (mean age at diagnosis 10 years)



CP children cohort of the Children's Memorial Health Institute

Etiological factors in CP children (mean age at diagnosis 10 years)



Idiopathic

CP children cohort of the Children's Memorial Health Institute

Ongoing Project

Identification of novel genetic variants associated with risk of chronic pancreatitis by whole exome sequencing.

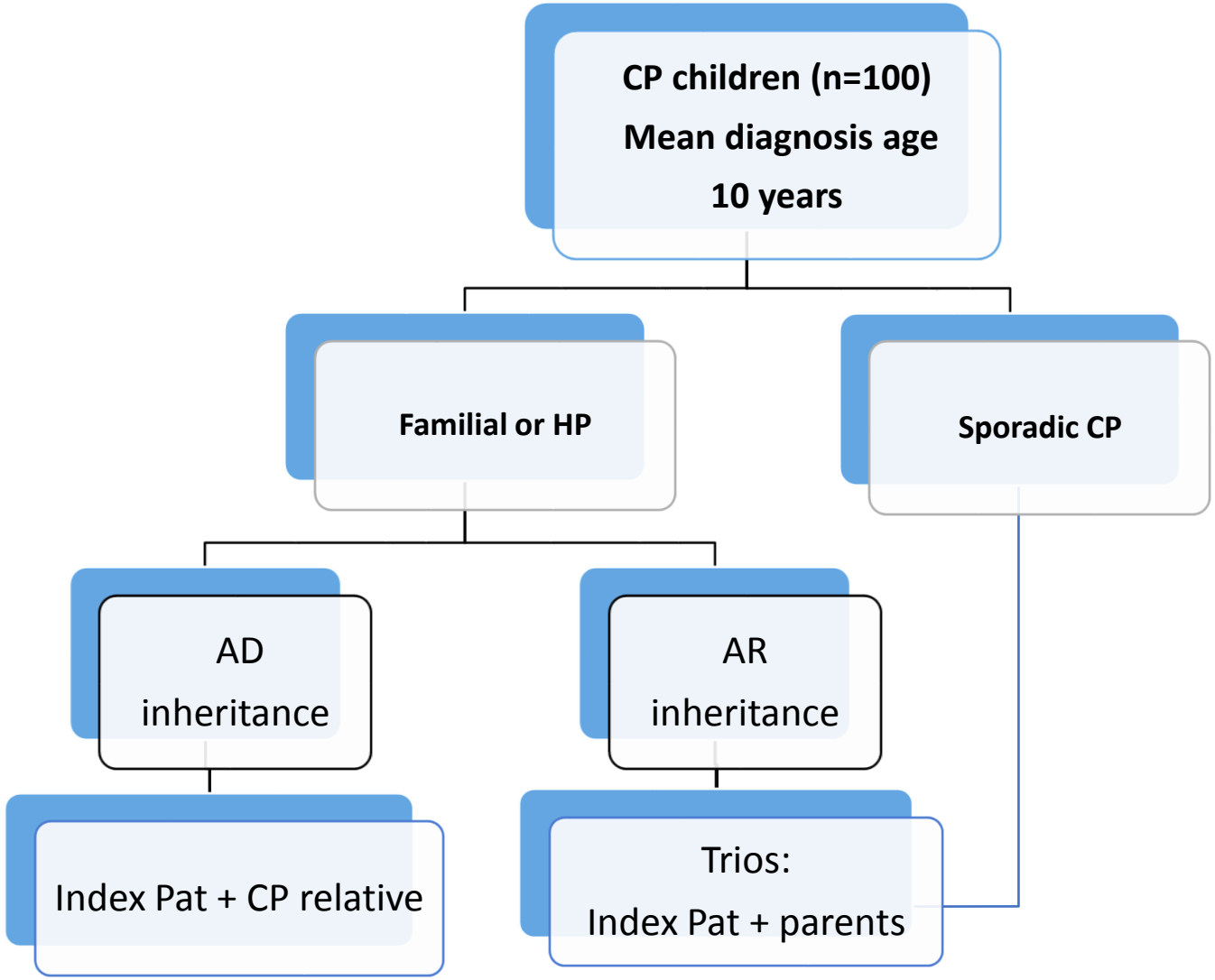
Lider: Institute of Mother and Child, **Agnieszka Rygiel**

Collaboration:

The Children's Memorial Health Institute, **Grzegorz Oracz**
Medical Warsaw University: Prof. **Rafał Płoski**

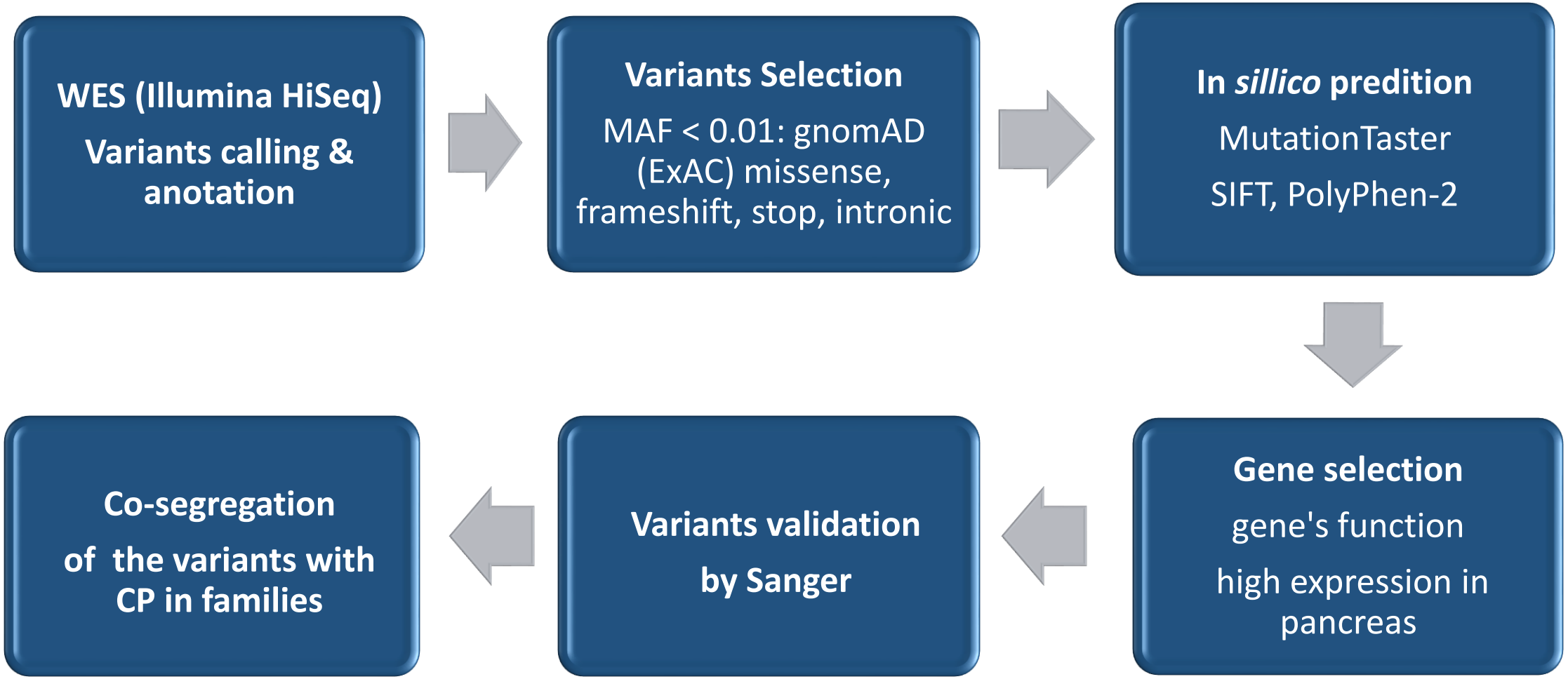
Finansed by National Science Center Poland:2015/19/B/NZ5/02224

Patients enrolled for WES



AD - autosmal dominat
AR - autosomal recessive

Pipeline of data analysis



Identification of novel and rare recurrent genetic variants in early onset chronic pancreatitis by whole exome sequencing

Agnieszka Magdalena Rygiel[1], Grzegorz Oracz[3], Tomasz Gambin[1], Elwira Kołodziejczyk[3], Joanna Kosińska[2], Piotr Stawiński[2], Katarzyna Wertheim-Tysarowska[1], Rafał Płoski[2], Jerzy Bal[1]

[1]Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland
[2]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland
[3]Department of Gastroenterology, Hepatology and Feeding Disorders and Pediatrics, The Children's Memorial Health Institute, Warsaw, Poland

Background

The early onset non-alcoholic chronic pancreatitis (CP) is often associated with genetic mutations driving uncontrolled trypsin activity or inducing endoplasmic reticulum stress. Despite of a progress in a discovery of new CP genes, the genetic basis of the disease in considerable number of patients remains unknown.

Aim

To identify novel and rare genetic variants associated with early onset CP patients by using whole exome sequencing (WES).

Patients and Methods

Before WES, Sanger sequencing was performed to exclude known genetic causes of CP. In total, 27 CP children (mean age at diagnosis 8 years) with familial or sporadic CP and their relatives (n=22) were included for WES. WES data (HiSeq 2500, Illumina) were compared between index patient and affected or/and unaffected relatives. The variants were selected taking into account: *in silico* prediction (SIFT, MutationTaster, PolyPhen-2), MAF <0.01 in genomic databases, gene function and expression in the pancreas (Figure 1). All selected variants were confirmed by Sanger sequencing.

Results

Upon qualification for WES, we detected novel, pathogenic variant Ser282Pro in *CPA1* and Glu190Lys in *PRSS1*. In 7 out of 27 cases analyzed by WES, 2 novel variants (Leu100VfsX21 *CTRC* and c.56-1G>A *SPINK1*) and 5 recurrent variants were detected in other know CP genes (Table 1).
Besides, we selected novel or rare variants in several susceptibility genes candidates as depicted in Table 2. In 9/27 of the index patients, the trans-heterozygous variants in two different genes (known CP gene and susceptibility gene candidate) were observed.

Conclusion

We identified novel and rare recurrent variants in fragments of known CP genes routinely not examined by Sanger sequencing (*PRSS1:* exon 4; *SPINK1:* intron 1, exon 1*; CTRC:* exon 4; *CFTR:* 3, 14a, 17a, 18). We have selected several potentially pathogenic variants in susceptibility genes candidates encoding proteins highly expressed in the pancreas. Their clinical significance needs to be elucidated by the functional and the case-control studies.

Financed: National Science Center Poland:2015/19/B/NZ5/02224
The authors declare no competing financial interests.
Informed consent from all patients was obtained.

Figure 1: Strategy to select the potentially pathogenic variants

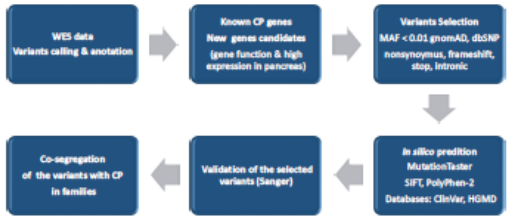


Table 1: Novel and recurrent variants in CP genes

CP gene	Exon/intron	Variant	CP carriers Nr	Novel/recuurent
*CPA1**	8	Ser282Pro	2	novel
	10	Arg382Trp	1	recurrent
	10	Trp318X	1	recurrent
*PRSS1**	4	Glu190Lys	1	novel
CTRC	4	Leu100VfsX21	1	novel
SPINK1	intron 1	c.56-1G>A	1	novel
	1	Ser10VfsX5	1	recurrent
CFTR	3	Arg75Gln	1	recurrent
	14a	Arg851X	1	recurrent
	18	Asp1152His	1	recurrent
	17a	Leu997Phe	1	recurrent

**CPA1* and *PRSS1* variants were detected by Sanger during qualification for WES. The variants pathogenic status was confirmed by functional studies: Kujko AA et al., *Gut* 2018 ; Jancsó Z et al., *Frontiers in Genet.*2019.

Table 2: New candidates for CP susceptibility genes

Gene candidate	Protein function	Variant	CP carriers Nr	GnomAD MAF %
CUZD1	zymogen granule protein	Cys229Ser	1	novel
		Cys207Thr	1	novel
PNLIP	triglyceryde lipase	Gln323Leu	1	novel
GCK	glucokinase	Val101Met	1	0.009
RFX6	transcription factor, pancreatic islet cell differentiation	Ala648Thr	1	novel
		Ser883Phe	1	novel
		Thr680Lys	2	0.001
CPB1	carboxypepeitase	Aso172Glu	2	0.01
SERPINA12	serine-type endopeptidase inhibitor	Gly327Asp	1	0.003

Contents lists available at ScienceDirect

Pancreatology

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Original article

The clinical course of hereditary pancreatitis in children – A comprehensive analysis of 41 cases

Grzegorz Oracz [a, *], Elwira Kolodziejczyk [a], Agnieszka Sobczynska-Tomaszewska [b, c], Karolina Weinarska [a], Maciej Dadalski [a], Alicja Monika Grabarczyk [b], Jarosław Kierkus [a]

RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Gene Conversion Between Cationic Trypsinogen (*PRSS1*) and the Pseudogene Trypsinogen 6 (*PRSS3P2*) in Patients with Chronic Pancreatitis

Agnieszka Magdalena Rygiel,[1†] Sebastian Beer,[2†] Peter Simon,[3] Katarzyna Wertheim-Tysarowska,[1] Grzegorz Oracz,[4] Torsten Kucharzik,[5] Andrzej Tysarowski,[6] Katarzyna Niepokój,[1] Jarosław Kierkus,[4] Marta Jurek,[1] Paweł Gawliński,[1] Jarosław Poznański,[7] Jerzy Bal,[1] Markus M. Lerch,[3] Miklós Sahin-Tóth,[2‡] and Frank Ulrich Weiss[3*‡]

Published in final edited form as:
Gut. 2017 September ; 66(9): 1728–1730. doi:10.1136/gutjnl-2017-313816.

A novel p.
dominant

Aleksandra Ku
Antoniuk[1], **Ka**
Sahin-Tóth[2,#],

frontiers in Genetics

Novel
p.Glu1
Pancre

Zsanett Jancsó
Evette S. Radisk

ORIGINAL ARTICLE: PANCREATOLOGY

Chymotrypsinogen C Genetic Variants, Including c.180TT, Are Strongly Associated With Chronic Pancreatitis in Pediatric Patients

*Alicja Monika Grabarczyk, †Grzegorz Oracz, *Katarzyna Wertheim-Tysarowska,
*Aleksandra Anna Kujko, †Karolina Wejnarska, †Elwira Kolodziejczyk, *Jerzy Bal,

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

The hybrid allele 1 of carboxyl-ester lipase (*CEL-HYB1*) in Polish pediatric patients with chronic pancreatitis

Grzegorz Oracz [a], Aleksandra Anna Kujko [b], Karianne Fjeld [c, d], Katarzyna Wertheim-Tysarowska [b], Wioletta Adamus-Białek [e], Solrun Johanne Steine [f], Dorota Koziel [e], Stanislaw Gluszek [e], Anders Molven [f, g], Agnieszka Magdalena Rygiel [b, *]

Instytut Pomnik Centrum Zdrowia Dziecka,
dr hab. G.Oracz

Uniwersytet Jana Kochanowskiego w Kielcach,
Prof. S.Głuszek

Uniwersytet w Bergen
Prof. A. Molven

Instytut Biochemii i Biofizyki, PAN,
Prof. J.Poznański

Technische Universität München

Uniwersytet w Monachium, Niemcy
Prof. H. Witt

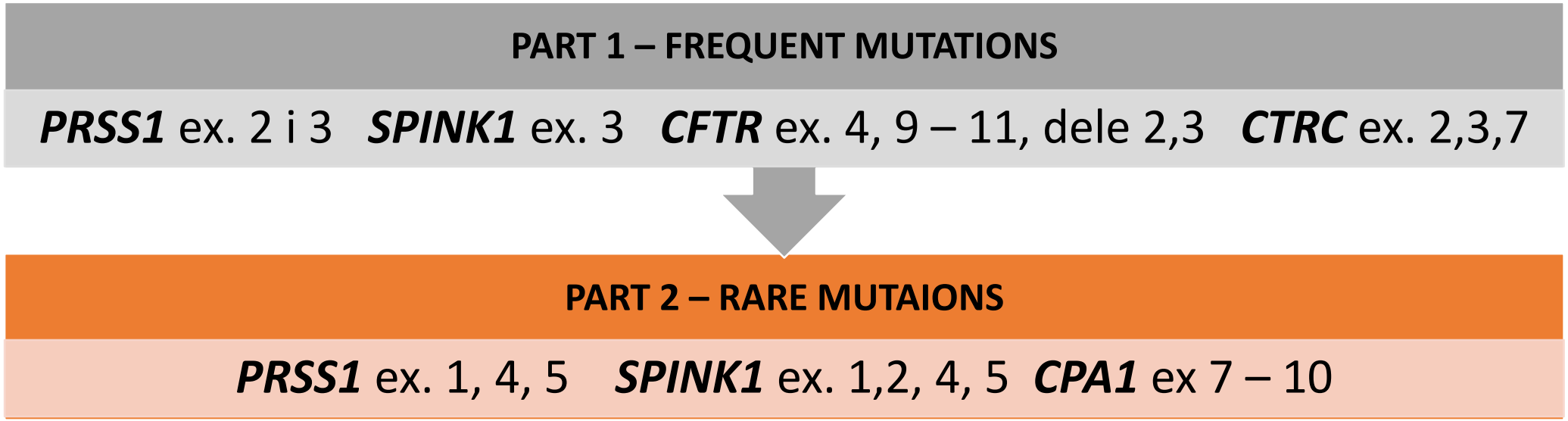
Uniwersytet w Bostonie,
Prof. M. Sahin-Thoth

Uniwersytet w Greifswald, Niemcy
Prof. U.Weiss

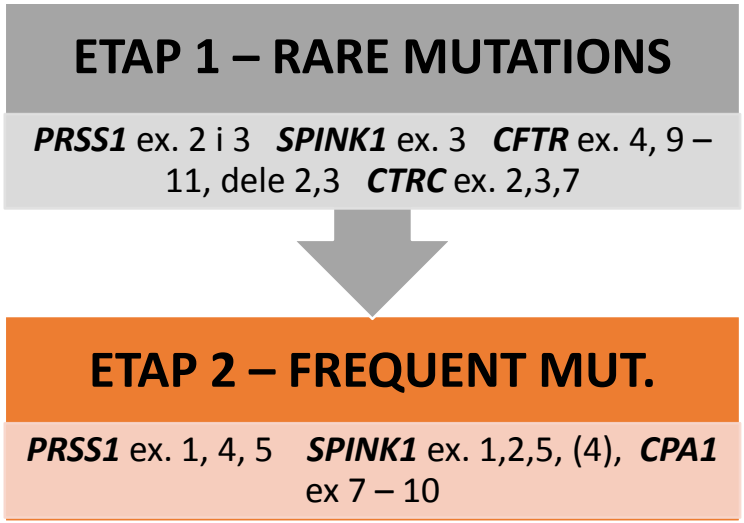
Uniwersytet Warszawski,
Prof. Płoski;Dr Drożak

Molecular diagnostics at Department of Medical Genetics, IMCh

Diagram of routine molecular diagnostics



Implementation of Panel-based CP genetic analysis using NGS sequencing



NGS GENE PANEL for CP:

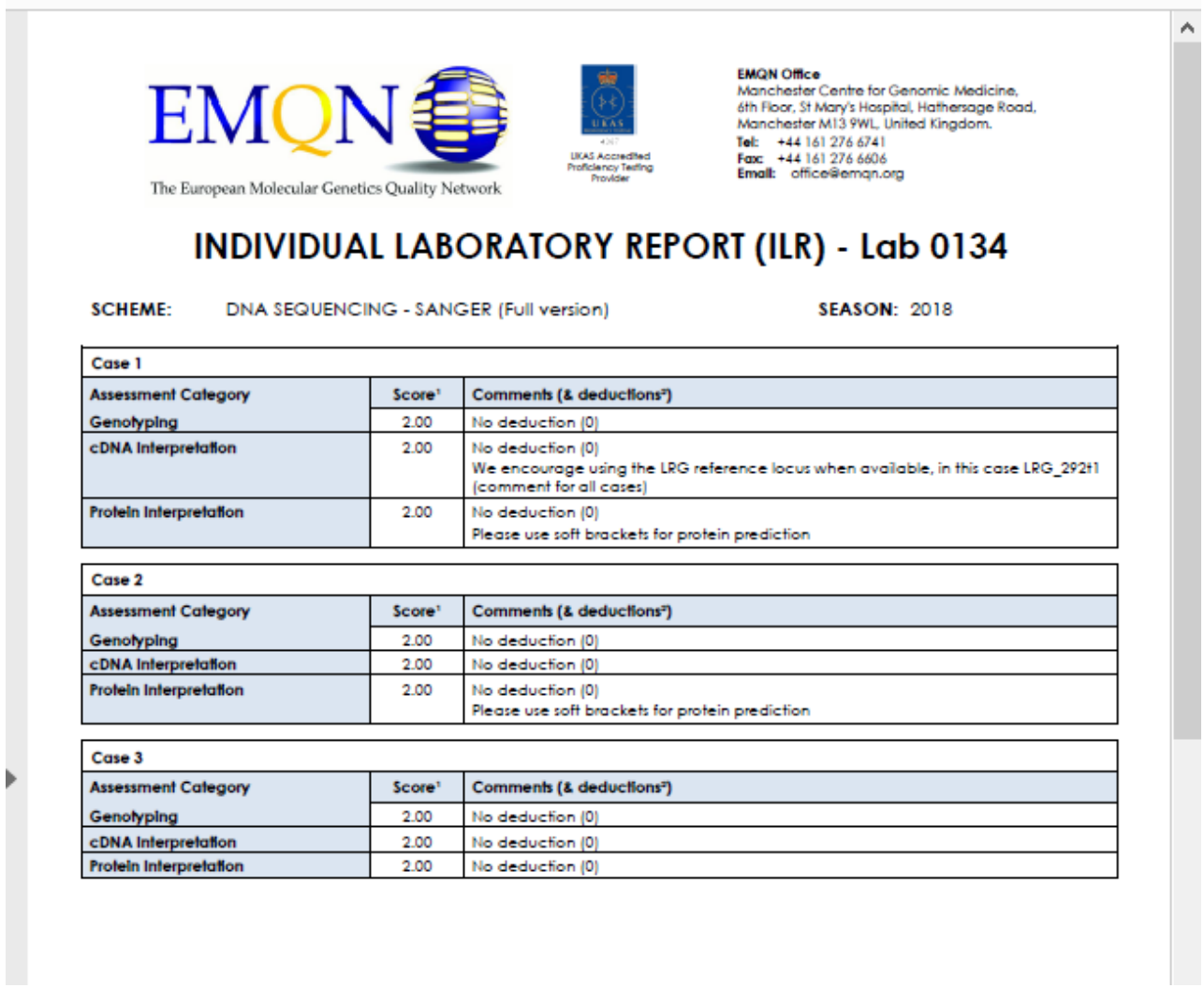
One step analysis for all CP genes

Shorter procedure and lower cost

POWER „Choroby genetycznie uwarunkowane - edukacja i diagnostyka”

Fundusze Europejskie Wiedza Edukacja Rozwój
Rzeczpospolita Polska
Instytut Matki i Dziecka
Unia Europejska Europejski Fundusz Społeczny
edu-metgen.imid.med.pl

The European Molecular Genetic Quality Network (EMQN) for Sanger and NGS sequencing



EMQN
The European Molecular Genetics Quality Network

UKAS Accredited Proficiency Testing Provider

EMQN Office
Manchester Centre for Genomic Medicine,
6th Floor, St Mary's Hospital, Hathersage Road,
Manchester M13 9WL, United Kingdom.
Tel: +44 161 276 6741
Fax: +44 161 276 6606
Email: office@emqn.org

INDIVIDUAL LABORATORY REPORT (ILR) - Lab 0134

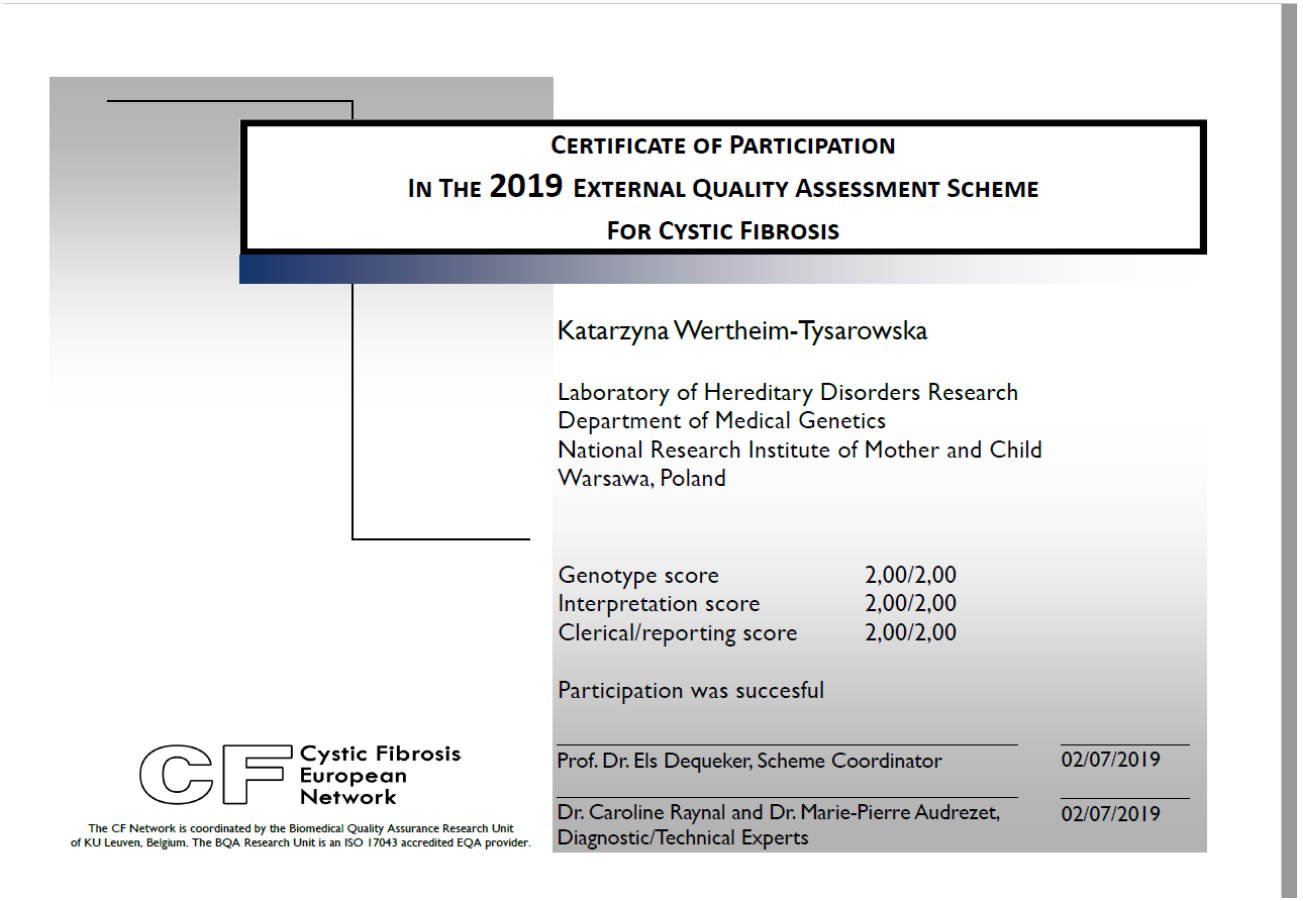
SCHEME: DNA SEQUENCING - SANGER (Full version) **SEASON:** 2018

Case 1		
Assessment Category	**Score[1]**	**Comments (& deductions[2])**
Genotyping	2.00	No deduction (0)
cDNA Interpretation	2.00	No deduction (0) We encourage using the LRG reference locus when available, in this case LRG_292t1 (comment for all cases)
Protein Interpretation	2.00	No deduction (0) Please use soft brackets for protein prediction

Case 2		
Assessment Category	**Score[1]**	**Comments (& deductions[2])**
Genotyping	2.00	No deduction (0)
cDNA Interpretation	2.00	No deduction (0)
Protein Interpretation	2.00	No deduction (0) Please use soft brackets for protein prediction

Case 3		
Assessment Category	**Score[1]**	**Comments (& deductions[2])**
Genotyping	2.00	No deduction (0)
cDNA Interpretation	2.00	No deduction (0)
Protein Interpretation	2.00	No deduction (0)

External Quality assesment scheme for Cystic fibrosis (CF European Netwok)



CERTIFICATE OF PARTICIPATION
IN THE 2019 EXTERNAL QUALITY ASSESSMENT SCHEME
FOR CYSTIC FIBROSIS

Katarzyna Wertheim-Tysarowska

Laboratory of Hereditary Disorders Research
Department of Medical Genetics
National Research Institute of Mother and Child
Warsawa, Poland

Genotype score	2,00/2,00
Interpretation score	2,00/2,00
Clerical/reporting score	2,00/2,00

Participation was succesful

Prof. Dr. Els Dequeker, Scheme Coordinator — 02/07/2019

Dr. Caroline Raynal and Dr. Marie-Pierre Audrezet, Diagnostic/Technical Experts — 02/07/2019

CF Cystic Fibrosis European Network

The CF Network is coordinated by the Biomedical Quality Assurance Research Unit of KU Leuven, Belgium. The BQA Research Unit is an ISO 17043 accredited EQA provider.

Department of Medical Genetics, IMID

Laboratory of research on inherited diseases: CP genetic group

The Children's Memorial Health Institute Warsaw and others

Thank you !