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Genetic Testing in Hungary

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Genetic testing – children

1. **acute / recurrent acute / chronic pancreatitis**

2. testing is **independent from etiology**

3. **pancreatitis under 19** years of age
   at the time of diagnosis
   independent from actual age

Analyses of Pediatric Pancreatitis
APPLE-P

Analyses of Pediatric Pancreatitis
APPLE-R

Genetic testing – Adults

Adult patients with pancreatitis

1. **recurrent acute pancreatitis**
   at least 2 acute episodes

2. **chronic pancreatitis**

3. **pancreatitis** under 35 years of age
   at the time of diagnosis
   independent from actual age

4. **Etiology is unknown (IDIOPATHIC)**

Genetic testing – Documents

1. **Informed consent** form of **genetic testing**

2. **Informed consent** form of **APPLE study / other register**

3. Completed electronic **APPLE / other registry form** on www.pancreas.hu

4. **Patient's referral for Genetic Counseling** by the therapist doctor (Pediatritian / GI). Referral have to be addressed to the Department of Medical Genetics, University of Szeged

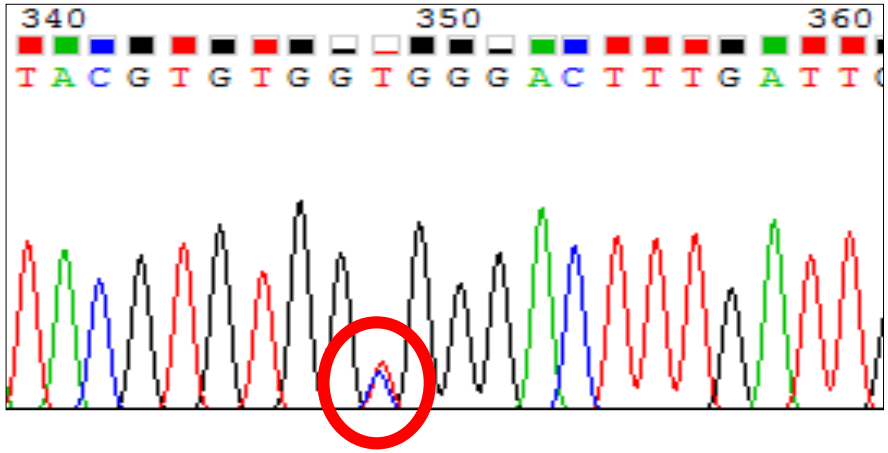
5. 2 x 6 mL **EDTA anticoagulated blood collected** from the patient and sent to First Department of Medicine, University of **Szeged**

Genetic testing – Applied methods

1. Isolation of **genomic DNA** from whole blood

2. Specific conventional **PCR** amplification of certain exons and their harbouring intronic regions

3. Sanger **sequencing** (conventional)



4. **Mutation analysis** of sequenced PCR products
   manual detection of mutations
   sequence alignment program

5. **Documented genetic counseling**
   explanation of laboratory results and clinical significance of detected mutation(s)
   *clinical genetician AND scientist togehter (*2 professionals with the patient*)*

6. **Informing therapist doctor about the results**

Genetic testing – Sanger sequencing

ADVANTAGES:

1. **GOLD STANDARD** method

2. Suitable for detection of **missense, nonsense, silent, splice-site mutations, and other intronic and promoter variants**

3. Detection of **microdeletions / microinsertions**

4. Detection of **novel (yet unknown) mutations**

DISADVANTAGES:

1.EXPENSIVE (needs a lot of **time** and **money**)
2.Unable to detect large deletions / insertions, copy number variations, haplotypes

Genetic testing – www.pancreasgenetics.org

PRSS1 *76 mutations (68 in 2015) – 26 pathogenic (26 in 2015)*
exon 2 – 10 pathogenic (p.A16V, p.N29I, p.N29T)
exon 3 – 12 pathogenic (p.R122H, p.R122C)

SPINK1 *91 mutations (79 in 2015)– 29 pathogenic (26 in 2015)*
exon 3 – 10 pathogenic (p.N34S) (9 in 2015)

CTRC *86 mutations (82 in 2015) – 33 pathogenic (33 in 2015)*
exon 2 – 4 pathogenic
exon 3 – 7 pathogenic (p.G60G)
exon 7 – 14 pathogenic (p.R254W)

CPA1 *141 mutations*
exon 7, exon 8, exon 10

CFTR ***exon 4 (p.R117H), exon 11 (p.F508del)***

Genetic testing

Genetic test results

Registry code of blood sample: **P5392**
Registry code of patient: **APPLE-R 10101010**

Summary of results

Indication:

Purpose of genetic testing:

Results:

Comment:

A clear and independent causative factor of hereditary pancreatitis is the p.R122H mutation in the *PRSS1* gene, that was NOT/- identified in the examined patient. Other mutations in the investigated genes are not independent risk factors, however may increase the risk of pancreatitis. Predisposing mutations and /or environmental factors are simultaneously susceptible for developing pancreatitis.

Genetic testing – Negative test results – example

Indication: One episode of acute pancreatitis occured in childhood (13 years of age). Idiopathic etiology / abdominal trauma.

Purpose of genetic testing: Genetic testing of genes are known to be associated with pancreatitis (*PRSS1, CTRC*, *SPINK1*, *CPA1* and *CFTR)*.

Results: We did not identified a mutation that is known to be associated with pancreatitis in the genetic sample of the investigated patient.

Comment: Genetic testing could not confirm hereditary pancreatitis. Genetic risk factors were not identified.

Genetic testing – Positive test results – example 1.

Indication: Recurrent acute pancreatitis, first episode was diagnosed under 19 years of age. Negative family history.

Purpose of genetic testing: Genetic testing of genes are known to be associated with pancreatitis (*PRSS1, CTRC*, *SPINK1*, *CPA1* and *CFTR)*.

Results: We identified homozygous *CTRC* p.G60G mutation in the genetic sample of the investigated patient.

Comment: Genetic testing could not confirm hereditary pancreatitis, however *CTRC* p.G60G mutation in homozygous form elevates the risk of pancreatitis by 10-fold.

Genetic testing – Positive test results – example 2.

Indication: Recurrent acute / Chronic pancreatitis developed in childhood.
Positive family history.

Purpose of genetic testing: Genetic testing of genes are known to be associated with pancreatitis (*PRSS1, CTRC*, *SPINK1*, *CPA1* and *CFTR)*.

Results: We identified heterozygous *PRSS1* p.R122H mutation in the genetic sample of the investigated pateint.

Comment: Genetic testing confirmed hereditary pancreatitis.

Genetic testing – Detailed description

Genetic sample: peripheral blood of the patient was sent to our laboratory by his/her physician <u>with the purpose of research</u>. Samples were identified only by registry code.

Method of genetic testing: …. Next slides

Detailed results: all of the variants we found in the investigated genes of the examined patient.

CPA1 c.697-367A>G heterozygous, c.697-260C>G heterozygous, c.697-63_-62insCC heterozygous, c.787+234G>C heterozygous, c.1073-32A>G heterozygous,

CFTR c.1408A>G p.M470V heterozygous.

Citation

Date and signature

Genetic testing – what should we tell to the patients?

1. What kind of **mutation(s)** did we find?
   causative: *PRSS1* (p.R122H)
   predisposing: *SPINK1, CTRC, CPA1, CFTR*
   harmless
2. What is the chance to get pancreatitis?
   understanding Odds ratio
3. **Almost all of the pathogenic mutations predispose to develop pancreatitis**
   BUT usually they DO NOT cause the disease by themselves!

4. **!!! DO NOT SMOKE and DO NOT DRINK ALCOHOL !!!**

5. Children should **EAT NORMALLY / NO STRICT RESTRICTION OF FAT INTAKE**, if they are otherwise healthy
   (but be careful with high fat diet in adulthood)

Genetic testing – what should we tell to the patients?

It is **NOT recommended** to test patient's family members with **NO EPISODE** of acute pancreatitis.

If we **DO NOT FIND** any causative or predisposing mutation, **IT DOES NOT MEAN** that the patient **DOES NOT HAVE** genetic risk at all.

Cancer risk in case of hereditary pancreatitis (*PRSS1* p.R122H) by the age of 75 is **~50%***

!!!!**Smoking** and **diabetes mellitus** are the main associated risk factors!!!!

*Rebours V, Boutron-Ruault MC, Schnee M, Férec C, Maire F, Hammel P, Ruszniewski P, Lévy P. (2008) **Risk of pancreatic adenocarcinoma in patients with hereditary pancreatitis: a national exhaustive series.** Am J Gastroenterol.;103(1):111-9.

Thank you for your attention!

The Hungarian Pancreatic Study Group is committed to improving the lives of patients suffering from pancreatic diseases.

www.pancreas.hu

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