Final report

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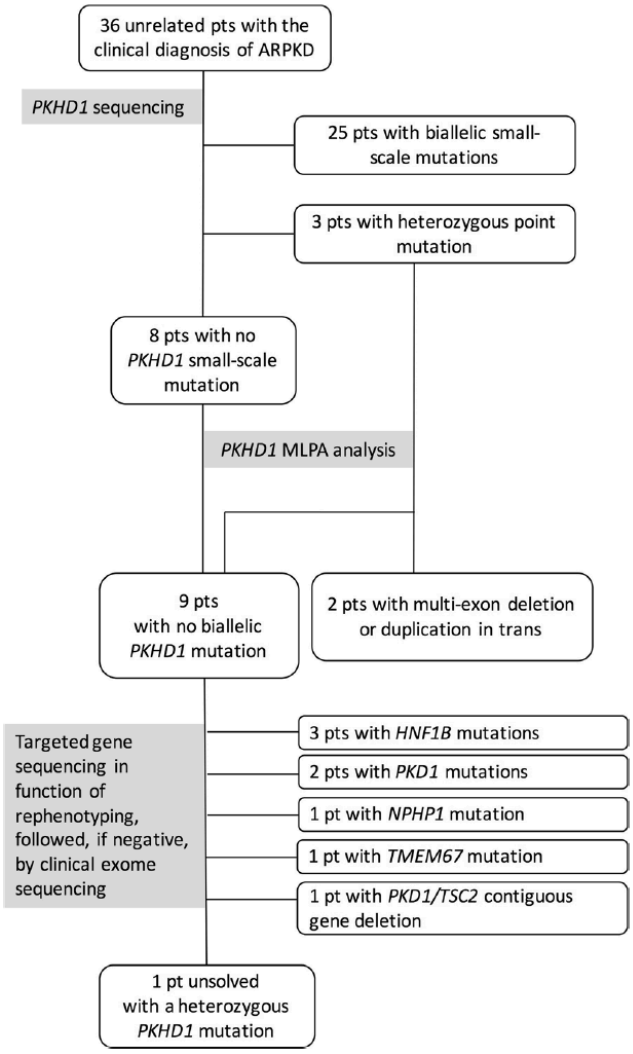
Rare disease model I (hereditary kidney disease).

In these studies we tested a samples from a devastating monogenic disease patients, autosomal recessive polycystic kidney disease (ARPKD). The responsible gene is *PKHD1*, however, phenocopies might exists (see below).

Altogether 36 unrelated patients were tested in a nationwide collaboration. First line testing included *PKHD1* sequencing which resulted in complete genetic diagnosis in 25 patients. In 11 patients, no mutation or one mutation was detected and these samples were analyzed further. MLPA analysis resulted in the detection of large heterozygous deletion or duplication in 2 patients. The remaining 9 patients were tested for the detection of possible phenocopies and this analysis showed the presence of *HNF1B* mutations (MODY5), autosomal dominant polycystic kidney disease in 3 and 2 patients, respectively. Single patients had *TMEM37*, *NPHP1* and *PKD1/TSC2* mutations. These analysis were performed either targeted way after rephenotyping of the patients, or by exome sequencing. Finally, one patient remained unsolved. Summary of this workflow is shown on the right (source: Szabo et al Pediatric Nephrology (2018) 33:1713-21).



The paper that describes the abovementioned results: **Szabó T, Orosz P, Balogh E, Jávorszky E, Máttyus I, Bereczki C, Maróti Z, Kalmár T, Szabó AJ, Reusz G, Várkonyi I, Marián E, Gombos E, Orosz O, Madar L, Balla G, Kappelmayer J, Tory K, Balogh I. Comprehensive genetic testing in children with a clinical diagnosis of ARPKD identifies phenocopies. Pediatric Nephrology (2018) 33:1713–1721. https://doi.org/10.1007/s00467-018-3992-5.**

Rare disease model II (cystic fibrosis).

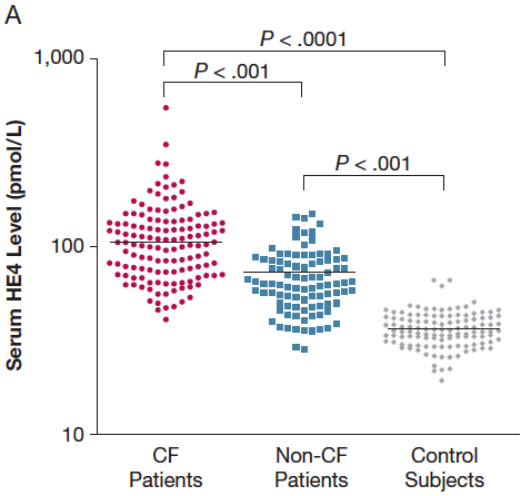
This part of the research work included
a) mutation analysis in a large Hungarian cystic fibrosis cohort,
b) identification and characterization of a novel biomarker, HE4 in cystic fibrosis
c) development a next generation DNA sequencing method for the analysis of cystic fibrosis transmembrane regulator gene

In details, we collected samples from 45 CF patients (22 males and 23 females, 10.1±8.1 years). Patient selection was based on clinical picture of classical CF, where both disease causing mutations were identified. All patient samples were sent by Hungarian care centers (mainly from the regions of Budapest, Szeged and Debrecen). The first line molecular test
was a multiplex allele specific PCR assay (detecting 29 mutations). This first tier assay was complemented by an allele specific PCR to detect the common »Slavic« deletion,
CFTRdele2,3(21kb). Sanger sequencing of the entire coding region was also done where it was needed. Last, multiplex ligation dependent probe amplification assay (MLPA) was used to detect large-scale rearrangements.
Altogether 27 different mutations were found. F508del was detected in 53.3%, four mutations (W1282X, N1303K, CFTRdele2,3(21 kb), and 2184insA) were detected in 4.4% of alleles, while another four mutations (G542X, Y1092X, 621+1G>T, and 2143delT) were found in more than one allele. The allele-specific 29-mutation assay could detect 75.9% of the mutations in our combined mutational database. Testing for CFTRdele2,3(21kb) revealed 4.7% of disease-causing alleles. MLPA analysis revealed one deletion affecting exon 2 in one patient in a heterozygous form. Allele specific PCR and sequencing analysis showed that the single exon deletion detected by MLPA was the same as previously described by others. Two novel mutations were identified. These newly described sequence alterations are most likely pathogenic. One of them changes the reading frame, generating a premature stop codon 17 amino acids downstream (c.1037_1038insA, p.Leu346Hisfs*17). Pathogenicity of the detected novel missense mutation c.1394C>T, p.Thr465Ile is supported by the following:
1) the affected residue is located at a phylogenetically highly conserved position according to the orthologs of Bos taurus, Equus caballus, Felis catus, Mus musculus etc.; 2) another pathogenic mutation (T465N) affecting the same amino acid residue has previously been described; 3) SIFT analysis predicts a damaging effect on the protein function. The paper that describes the abovementioned results: **Ivady G, Koczok K, Madar L, Gombos E, Toth I, Gyori K, Balogh I. Molecular analysis of cystic fibrosis patients in Hungary - un update to the mutational spectrum. J Med Biochem 2015; 34 (1) DOI: 10.2478/jomb-2014-0055.**

One major problem in the case of the clinical work with CF is that the lack of a reliable biomarker that can be used to follow the disease course and possibly, the efficiency of the treatment. Therefore, we put great effort to identify novel biomarker. HE4 is a tumor marker in epithelial ovarian, lung cancers and endometrial carcinomas. It was shown before that HE4 is present in CF lung biopsy samples but we were the first to test its level in blood in CF. Our research strategy was to collect samples from different CF populations with different age groups and to test HE4 levels in a case-control setting. Then, to test the possible source of HE4 in CF and also its expression regulation. Clinical data and samples from 77 CF patients were obtained and 57 adult CF patients with CF were recruited from Prague.
Sweat chloride values were analyzed by using a Sweat-Chek Conductivity Analyzer (Wescor Inc). Genetic testing was performed as previously described. The Czech adult patient cohort was genotyped by using the Elucigene EU v3 assay (Tepnel-Diagnostics) and the MLPA kit

(MRC-Holland). Serum HE4 levels were determined by using chemiluminescent microparticle immunoassay. mRNA and miRNA measurements were also performed.

As shown in the figure (source: Nagy B Jr et al Chest 2016; 150(3):661-672, DOI: http://dx.doi.org/10.1016/j.chest.2016.04.006) serum HE4 was highly elevated in CF patients. Age and sex did not affected HE4 levels. Clinical severity of the disease, however, showed a significant positive correlation with HE4 and HE4 was highly correlated with the degree of lung dysfunction. We also investigated the possible origin of elevated circulating HE4 levels in CF to understand the mechanism (destruction vs upregulation). We tested HE4 mRNA level in CF and control bronchial epithelium biopsy specimens.



We found that HE4mRNA levels in the patients with CF were significantly upregulated compared with those of the non-CF control subjects suggesting that highly elevated serum HE4 concentrations in CF may result from its increased secretion by the airway epithelium. We also found that miR-140-5p expression in CF was decreased compared to non-CF control subjects suggesting a role of miR-140-5p in HE4 expression. In conclusion, we found that serum HE4 is elevated in CF and correlates with the severity of the disease therefore it might be used as a prognostic biomarker. The paper that describes the abovementioned results: **Nagy B Jr, Nagy B, Fila L, Clarke LA, Gönczy F, Bede O, Nagy D, Újhelyi R, Szabó A; Anghelyi A, Major M, Bene Z, Fejes Z, Antal-Szalmás P, Bhattoa HP, Balla G, Kappelmayer J, Amaral MD, Macek M Jr, Balogh I. Human Epididymis Protein 4: A novel serum inflammatory biomarker in cystic fibrosis. Chest 2016; 150(3):661-672 DOI: http://dx.doi.org/10.1016/j.chest.2016.04.006.**

Next, we tried to assess the usefulness of HE4 in the course of the treatment in CF. In this study, we replicated our initial observations in other CF populations measured HE4 with the hypothesis that HE4 serve as a plasma-based biomarker in subjects receiving CFTR modulating therapy. We thus selected and tested three independent cohorts of CF patients with the Class III CF-causing mutation p.Gly551Asp, which is successfully potentiated by ivacaftor, the recently developed drug. 29 samples were requested from the Cystic Fibrosis Foundation Therapeutics (CFFT) Biorepository (Bethesda, MD, USA). HE4 plasma measurements were performed before treatment and then at 1, 3 and 6 months after the initiation of ivacaftor treatment. Samples from 12 patients from Australia were also tested at baseline, 1 and 2 months after initiation of ivacaftor treatment. 19 samples from CF patients from Ireland (baseline and after 3 months) were also analyzed. Main observations of this study were that HE4 plasma levels strongly correlate with FEV1 values and CRP concentrations, the degree of plasma HE4 level alteration is related to the improvement of CF lung disease under ivacaftor therapy and plasma HE4 is altered already in the early period of ivacaftor medication. In conclusion, we believe that HE4 can be used in the follow-up of CF patients, which may offer more sensitivity to assess drug efficacy. The paper that describes the abovementioned results: **Nagy B Jr, Bene Z, Fejes Z, Heltshe SL, Reid D, Ronan NJ, McCarthy Y, Smith D, Nagy A, Joseloff E, Balla G, Kappelmayer J, Macek M Jr, Bell SC, Plant BJ, Amaral MD, Balogh I. Human epididymis protein 4 (HE4) levels inversely correlate with lung function improvement**

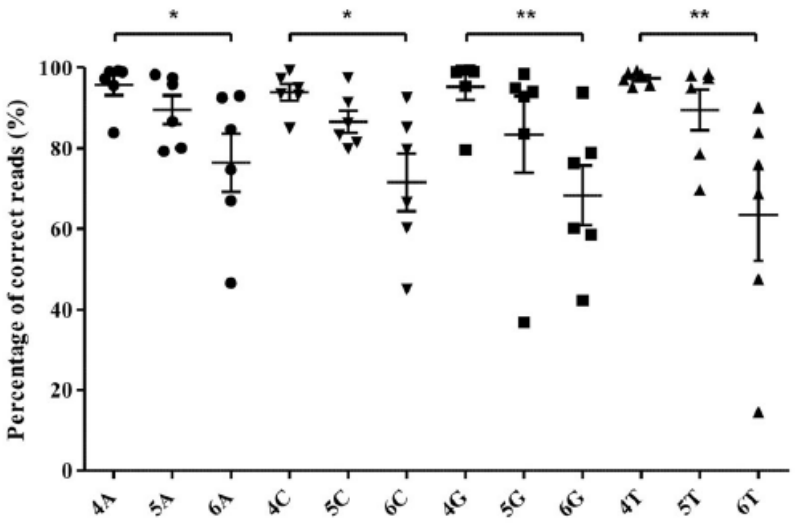
(delta FEV1) in cystic fibrosis patients receiving ivacaftor treatment. Journal of Cystic Fibrosis in press, https://doi.org/10.1016/j.jcf.2018.08.013.

Methodological developments - clinical laboratory genetic studies to detect small scale mutations

As the major part of our studies was to detect mutations in genes responsible for severe monogenic disorders, the analytical parameters of the methodologies used in these processess are of great importance. Therefore, we decided to study two methodological questions,
a) testing the analytical parameters of a next generation DNS sequencing system in detecting homopolymers
b) analyzing the effect of maternal contamination in diferent methodologies used in prenatal genetic testing.

A homopolymer (HP) is a sequence of consecutive identical bases. Approximately 1.43 million HPs exist in the human exome, with the size of 4-mer and up. The vast majority (96.7%) of them are in the range of 4-mer to 6-mer. Some sequencing-by-synthesis based next generation sequencing (NGS) techniques (pyrosequencing and ion semiconductor sequencing) have a relatively high error rate in determining homopolymer regions because of their principles used for detection. Though many bioinformatic tools exist to correct these errors, no experimental data were available about their extent. We developed two experimental test systems. In the first, homopolymer sessions were introduced into plasmid vectors and these plasmids were used as templates for sequencing experiments. Next we analyzed the *CFTR* gene to also assess the analytical performance of a newly developed pyrosequencing-based assay on patient samples. In this second experiment, all homopolymer-containing and non-HP *CFTR* exons were tested. These samples originated from cystic fibrosis (CF) patients with known *CFTR* mutation status.

When we sequenced homopolymer-containing plasmids, a negative correlation was observed between homopolymer length and read accuracy (see figure in the right). The average correct genotyping rate of all four nucleotides combined was 95.8, 87.4 and 72.1% in 4-mers, 5-mers, and 6-mers, respectively. These data suggest major limitations.



Primer localization failed to show any association with genotyping accuracy. Next, we developed an assay system for *CFTR* gene mutation analysis to see whether the currently used Sanger sequencing method can be replaced by this NGS-based one. The pyrosequencing method could identify all non homopolymer small scale mutations with 100% sensitivity, but failed in HP sequences, especially the 7A-containing c.2184insA mutation. 2184insA, a frequent CF-causing mutation in Hungary and is an eight-adenine long HP region. This was further analyzed by using 11 DNA samples from CF patients. Other mutations are also known

(2184delA and 2183delAAinsG) to affect the same poly-A tract, creating five or six adenine long HPs. We found that mutations that lead to the formation of 5-mers and 6-mers, can be detected with high specificity. While genotyping of the 8-mer c.2052_2053insA is reproducible, the detection rate failed to reach 75%, therefore this region of the gene still needs to be Sanger sequenced when testing patient samples in routine diagnostic procedures in order to avoid false positives and false negatives. The paper that describes the abovementioned results: **Ivády G, Madar L, Dzsudzsák E, Koczok K, Kappelmayer J, Krulisova V, Macek M Jr, Horváth A, Balogh I. Analytical parameters and validation of homopolymer detection in a pyrosequencing-based next generation sequencing system. BMC Genomics (2018) 19:158. https://doi.org/10.1186/s12864-018-4544-x.**

Maternal cell contamination (MCC) poses a significant risk of false genotyping in prenatal diagnostic procedures. Degree of MCC in chorionic villus sample depends on the operator's proficiency, gestational age, villus preparation, sample quality and size. Prenatal molecular diagnostics can be challenging as laboratories have to work with practically irreplaceable, often suboptimal specimens, and a very short turnaround time. Genotype of the fetus may be falsely determined in the presence of MCC because of the high sensitivity of PCR-based methods to DNA contamination. To address this issue, we decided to perform a mixing study where maternal DNA was added to fetal DNA and the degree where this contamination leads to false genotype determination was tested using different widely used genetic methods. We tested analytical sensitivity of 3 molecular genetic methods to MCC: (1) Sanger DNA sequencing; (2) multiplex ligation-dependent probe amplification (MLPA), a method of choice for copy number variation (CNV) testing; and a NGS method, (3) pyrosequencing. In all experiments, genomic DNA mixtures were created by contaminating a wild type (used as “fetal”) DNA sample with a heterozygous (used as “maternal”) DNA sample simulating 1%, 5%, 10%, 20%, 30%, and 40% MCC levels corresponding to 0.5%, 2.5%, 5%, 10%, 15%, and 20% mutant allele per DNA mixture, respectively. In the Sanger sequencing, depending on the mutation, limit of detection varied between 5% and 30%. MLPA was very insensitive for MCC, with a limit of detection of 40% in case of deletions and with even better performance in case of duplications. In line with the expected, NGS was very accurate in the determination of the MCC. Based on these data we concluded that careful interpretation of results is critical in the prenatal molecular diagnostic setting because of possible presence of MCC in fetal samples. Therefore, it is important to perform MCC analysis in parallel with the diagnostic testing. However, based on recent knowledge, even if prenatal samples are not purely of fetal origin, correct prenatal molecular diagnosis and reporting are possible and repeated sampling can be avoided in many of the cases. The paper that describes the abovementioned results: **Koczok K, Gombos E, Madar, Török O, Balogh I. Interfering effect of maternal cell contamination on invasive prenatal molecular genetic testing. Prenatal Diagnosis 2018;38:713–719. DOI: 10.1002/pd.5319.**

Rare disease model IIIa (ophthalmology/monogenic diabetes).

The fact that we published the detailed ophthalmologic phenotypic observations of a monogenic diabetes patient highlights the absolutely necessity of a multidisciplinary approach in the diagnosis and characterization of the rare genetic diseases. Heterozygous loss-of-function mutations in the *NEUROD1* gene have been described in maturity-onset diabetes of the young (MODY). However, there are only two patients with homozygous loss-of-function *NEUROD1* mutations known. Both single base pair duplication (c.364dupG) and two base pair CT deletion (c.427_428del) result in a frameshift and a premature truncation of the C-terminus of the expressed protein (p.Asp122Glyfs*12 and p.Leu143Alafs*55, respectively). These two probands were diagnosed with permanent neonatal diabetes (PNDM) and had similar neurologic abnormalities, including cerebellar hypoplasia, developmental delay, and visual and hearing impairment. In our work we provide detailed ophthalmological characterization of the patient with homozygous c.427_428delCT *NEUROD1* mutation which is the first description of the ophthalmological phenotype caused by a homozygous *NEUROD1* loss of function mutation. Given that these analyses were performed by an ophthalmologist present in the research group but these studies were not supervised by me I would rather not give a detailed data about the methods and findings just highlight some important points: severe rod–cone dystrophy which resembles retinitis pigmentosa in many aspects, it could be clearly distinguished from it. Hallmarks of the diseases were the relatively spared pigmented epithelial layer, the normal appearance of the optic disc and retinal vessel, and the disc-shaped remnant of cone photoreceptors in the fovea together with the total lack of rod photoreceptors, sharply demarcated from relatively spared foveal photoreceptors. The paper that describes the abovementioned results: **Orosz O, Czeglédi M, Kántor I, Balogh I, Vajas A, Takács L, Berta A, Losonczy G. Ophthalmological phenotype associated with homozygous null mutation in the NEUROD1 gene. Molecular Vision 2015; 21:124-130.**

Rare disease model IIIb (ophthalmology).

In this study, we have investigated members of a three generation family with X-linked high-grade myopia. A very detailed genetic investigation was performed that included exome sequencing which analysis resulted in the detection of many small scale alterations.

Gene	Coverage, -Fold IV:3	Coverage, -Fold V:1	SNP ID	Nucleotide Change	Amino Acid Change	MAF
VCX	61	6	rs75562767	c.209C>G	p.Ala70Gly	0.67
	104	15	rs80291436	c.311T>C	p.Leu104Pro	0.46
	117	29	rs78342118	c.581C>T	p.Pro194Leu	0.42
*CACNA1F**	31	12	rs150417702	c.1843G>T	p.Ala615Ser	<0.01
SLC16A2	45	15	rs6647476	c.97T>C	p.Ser33Pro	0.66
KIAA2022†	143	57	rs41306133	c.2801A>G	p.Asn934Ser	0.01
MAGT1	90	18	rs145245774	c.1028T>G	p.Val343Gly	0.13
DIAPH2‡	150	55	rs20361	c.1275C>A	p.Phe425Leu	<0.01
RBMXL3	29	13	rs6643947	c.3145A>G	p.Arg1049Gly	0.11
LOC644717	25	17	rs6528273	c.1714G>T	p.Val572Phe	0.66
SAGE1	66	23	rs4829799	c.2414T>C	p.Leu805Ser	0.22
CSAG1	91	45	rs2515848	c.185A>G	p.Lys62Arg	0.42
TMEM187	106	34	rs2266890	c.422C>T	p.Ser141Leu	0.31
	111	34	rs7350355	c.445A>G	p.Met149Val	0.37
OPN1LW§	135	55	rs145009674	c.532A>G	p.Ile178Val	0.02
	132	55	rs949431	c.538T>G	p.Ser180Ala	0.2
OPN1MW§	418	96	rs375538821	c.532A>G	p.Ile178Val	0.02

Table from Orosz et al Invest Ophthalmol Vis Sci. 2017;58:1834–1842.

From the family, affected male patients had infantile onset myopia with normal visual acuity and color vision until their forties, then their visual acuity decreased, in paralel with the development of severe protan and deutan color vision defects. A mild decrease in electroretinography response of cone photoreceptors was detected in childhood, which further

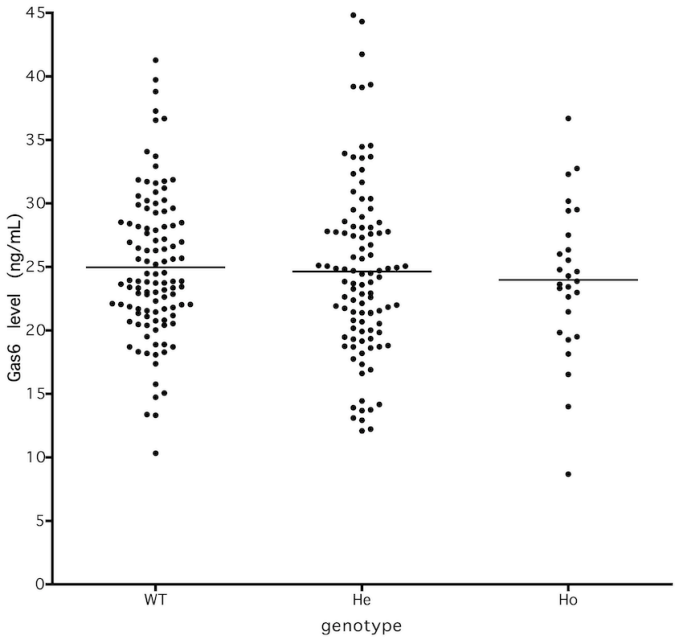
deteriorated in middle-aged patients. Rods were also affected, however, to a lesser extent than cones. Clinical exome sequencing identified the LVAVA and MVAVA haplotypes in the OPN1LW and OPN1MW opsin genes, respectively.
In conclusion, LVAVA haplotype of the OPN1LW gene and MVAVA haplotype of the OPN1MW gene cause apparently nonsyndromic high myopia in young patients but lead to progressive cone–rod dystrophy with deuteranopia and protanopia in middle-aged patients corresponding to a previously unknown disease course. This is the first report on the joint effect of these toxic haplotypes in the two opsin genes on chromosome X. We believe that these data add substantial more information about the genetic makeup and phenotype in a rare ophthalmological disease. The paper that describes the abovementioned results: **Orosz O, Rajta I, Vajas A, Takacs L, Csutak A, Fodor M, Kolozsvari B, Resch M, Senyi K, Lesch B, Szabo V, Berta A, Balogh I, Losonczy G. Myopia and late-onset progressive cone dystrophy associate to LVAVA/MVAVA exon 3 interchange haplotypes of opsin genes on chromosome X. Invest Ophthalmol Vis Sci 2017;58:1834–1842. DOI:10.1167/iovs.16-21405.**

Rare disease model IV (muscular dystrophy).

We aimed to investigate the molecular background of a muscular dystrophy using genetic, immunohistchemistry and *in silico* methods. A six-month-old male patient had mild generalized muscle weakness, hypotonia, and delayed motor development. Electromyography showed myopathic features. Laboratory analysis at 1-year-old age showed markedly elevated serum creatine kinase (1497 U/L, reference range: 24–195 U/L). *DMD* gene analysis was requested at age of 14 months. There was no family history of DMD/BMD. CNV analysis by MLPA showed a single-exon deletion which was later shown to be false postive as patient had a point mutation (c.227A>T, p.Asn76Ile) in hemizygous form that interfered with the MLPA assay. This mutation was detected by *dystrophin* gene sequencing using NGS. Immunohistochemistry showed few necrotic and basophilic regenerative fibers and reduced labeling with antibody recognizing the Dystrophin-1 epitope with segmental loss in numerous fibers while Dystrophin-2 epitope showed normal linear sarcolemmal expression and Dystrophin-3 epitope expression was seen only in occasional fibers with segmental distribution. These data together suggested a dystrophinopathy therefore *in silico* we tried to assess the functional consequence of the p.Asn76Ile missense mutation. The mutation lies in an important domain, the actin binding domain of the dystrophin protein. The analysis revealed that the presence of the mutation is highly destabilizing on N-ABD structure possibly leading to protein malfunction. We believe that this is also a good example for the comprehensive approach that is needed where a novel, previously undescribed variant of unknown significance is tested using different methods and these results can be easily translated into direct clinical care. The paper that describes the abovementioned results: **Koczok K, Mero G, P. Szabó G, Madar L, Gombos E, Ajzner E, Mótyán JA, Hortobágyi T, Balogh I. A novel point mutation affecting Asn76 of dystrophin protein leads to dystrophinopathy. Neuromuscular Disorders 28 (2018) 129–136. https://doi.org/10.1016/j.nmd.2017.12.003.**

Gas6 genetics.

This part of the study included Gas6 measurements both at the DNA and the protein level, in relation with an intronic polymorphism (c.834+7G>A) which was found to be protective against age-related macular degeneration by us previously (Losonczy G, Vajas A, Takacs L, Dzsudzsak E, Fekete A , et al. (2012) Effect of the Gas6 c.834+7G>A Polymorphism and the Interaction of Known Risk Factors on AMD Pathogenesis in Hungarian Patients. PLoS ONE 7(11): e50181. doi:10.1371/journal.pone.0050181).



For the analysis, Gas6 c.834+7G>A mutation was genotyped on 300 samples and Gas6 levels were measured by ELISA. Minigene construct was also generated. We showed by these studies that neither the protein level (see above figure), nor the splicing (data not shown) was affected by the polymorphism, therefore we concluded this as a negative result.

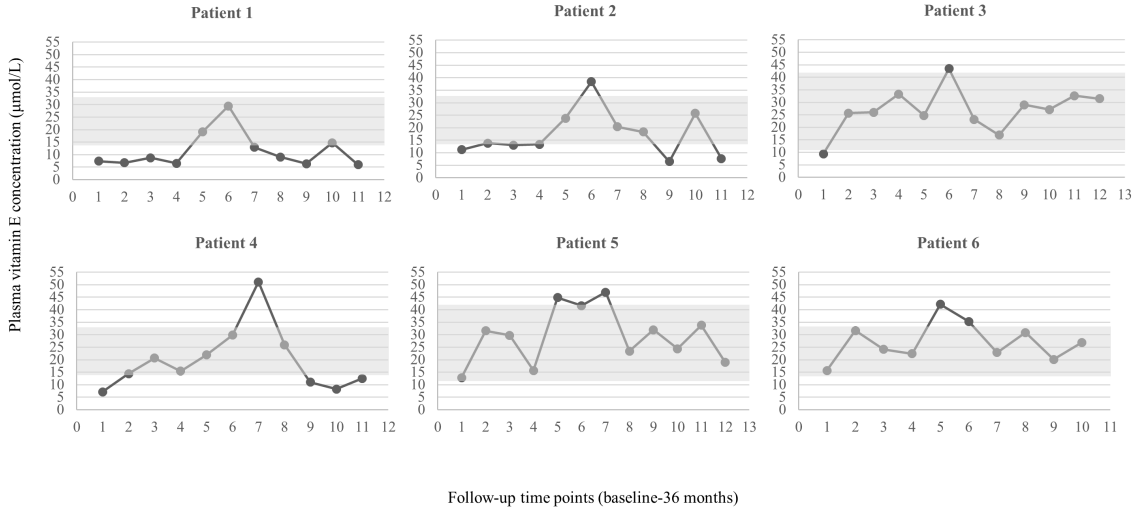
Rare disease model V (Smith-Lemli-Opitz (SLO) syndrome).

This part of the work contained different aspects of Smith-Lemli-Opitz syndrome, a severe rare disease affecting cholesterol synthesis. These were the followings:
a) the possible role of SLO syndrome in habitual abortion
b) functional consequence of a mutation detected in a patient sample
c) to study the possible beneficial effect of vitamin E on the patients' clinical status
d) to increase clinician's awareness of SLO

In order to study these questions, many different methods and procedures were introduced. These included NGS method to quickly and cost-effectively study mutations, plasmid generation, vitamin E preparation and the organization of the clinical study, international collaboration for the study of the effect of the possibly pathogenic mutations in SLO. In the habitual abortion substudy altogether 23 couples were recruited. Due to the avaliable genomic data from other sources, we decided to exclude control group analysis as these data can easily be used for that purpose. SLO gene sequencing and CNV analyses were performed and surprisingly no pathogenic alterion was found in any of the selected samples. As the clinical sample recruitment had difficulties we consider this part of the proposal negative with caution. The functional consequence of the previously detected probably pathogenic mutation was done in two ways. Experiments on the mutagenized plasmids are ongoing in an international collaboration and the possibly pathogenic nature of a mutation has been studied using fibrobasts also with the same collaborating partner. This study indentified small molecule compounds, statins, SERMs, antifungals, and several antipsychotic medications reduced levels of 7-DHC. The results of this collaboration has been published (**Korade Z, Kim HY, Tallman KA, Liu**

W, Koczok K, Balogh I, Xu L, Mirnics K, Porter NA. The Effect of Small Molecules on Sterol Homeostasis: Measuring 7-Dehydrocholesterol in Dhcr7-Deficient Neuro2a Cells and Human Fibroblasts. J. Med. Chem. 2016, 59, 1102−1115, DOI: 10.1021/acs.jmedchem.5b01696).

The possible beneficial effect of vitamin E treatment in SLO patients has been studied in a clinical study. In a 3-year prospective study we investigated the effects of vitamin E supplementation in six SLOS patients. Plasma vitamin A and E concentrations were determined by a high-performance liquid chromatography (HPLC) method. The clinical effect of the supplementation was assessed by performing parental interviews. At baseline, patients were characterized by low or low-normal plasma vitamin E concentrations (7.19-15.68 μmol/L), while vitamin A concentrations were found to be normal or high (1.26-2.68 μmol/L). Vitamin E (*all-rac*-α-tocopheryl acetate) was administered in daily doses of 230mg (age 4-10 years) and 2x230mg (age over 10 years). Supplementation resulted in correction or significant elevation of plasma vitamin E concentration in all patients. We observed reduced aggression, self-injury, irritability, hyperactivity, attention deficit, repetitive behavior, sleep disturbance, skin photosensitivity and/or eczema in 3/6 patients, with notable individual variability. Based on these data, we suggest that determination of vitamin E status is important in SLOS patients. Supplementation of vitamin E should be considered and might be beneficial. The manuscript is under preparation.



To increase clinician's awareness of SLO syndrome, we published a review paper in the Hungarian Medical Journal (**Koczok K, V Olah A, P Szabo G, Olah E, Török O, Balogh, I. [Inborn error of cholesterol biosynthesis: Smith–Lemli–Opitz syndrome]. Orv. Hetil., 2015, 156(42), 1695–1702.)**). Although it is not a merely scientific outcome, but I think it is important to note that due to these methodological, organizational and scientific developments, our laboratory performed prenatal genetic testing in dozens of pregnancies and 10 healthy baby have been born in the last years in the affected families. I think that nothing shows better the translation of genetic work into the healthcare practice than this outcome, especially that these families would not have had undertaken another child in the absence of such a prenatal genetic diagnostic possibility.

Rare disease model VI (Maturity-onset diabetes of the young (MODY)).

The following previously either undescribed or uncharacterized mutations were detected in MODY patients and introduced in pGEX-5x.2-hβGK(GST-GK) plasmid vector using site directed mutagenesis: p.del39K, p.G72A, p.D124V, p.A173D, p.K291M, p.S340N, p.V33A, p.G318R, p.S383L, p.C220*, p.R275P, p.L165P, p.D409H, p.F330S, p.M251R, p.R303Q, p.A208S, p.V89G, p.G44S, p.P153S, p.D217N, p.C461Y, p.del236E. Functional analysis parameters have been optimized and in some of the mutations also measurements were performed. Our preliminary data suggest that the kinetic parameters are mostly affected by the mutations, however these experiments have to be repeated in order to draw the final conclusion. For the first time we described a HNF4A mutation in Hungarian patient (**Jermendy G, Balogh I, Gaál Z. [Monogenic form of diabetes mellitus due to HNF4α mutation (MODY-1) – the first case in Hungary]. Orv. Hetil., 2016, 157(12), 469–473**). The MODY database has been developed and at the moment it contains 290 entries, both suspected patients and family members. The table below shows the distribution of the detected mutations. It is to be noted that in Hungary, GCK-MODY is far the most prevalent subtype.

	all mutations	novel	known
HNF1A	16	3	13
HNF4A	3	0	3
GCK	52	21	31
KCNJ11	3	1	2
HNF1B	2	0	2
INS	0	0	0
ABCC8	5	1	4

Due to the recent methodological developments, now we are able to test the following MODY genes in one NGS gene panel: *HNF4A, GCK, HNF1A, HNF1B, NEUROD1, KLF11, PAX4, APPL1, BLK, INS, PDX1, ABCC8, KCNJ11*. We think that this development will increase the diagnostic yield substantially.

The following table shows the importance of genetic testing in MODY, where data from GCK-MODY patients are shown. The correct diagnosis might result in change in the treatment as these patients usually do not require either metformin or insulin (red label), good glycemic control can be achieved by diet only. We believe that this is another good example how the mutation recognition translates into direct clinical care.

Age at the time of clinical diagnosis	Age at the time of molecular diagnosis	Treatment
32	47	metformin
17	27	diet
10	14	diet
NA	20	metformin
10	11	diet
26	27	diet
8	11	metformin
8	11	diet
8	8	diet
4	6	diet
20	33	diet
14	16	insulin
18	44	insulin
3	3	diet
11	22	insulin
16	17	diet
24	24	diet
10	15	diet

Summary of the project

In summary, using state-of-the art methodology, we could identify the genetic background of different rare diseases and could characterize the detected alterations by classical genetic, in silico, prediction, and functional ways in order to prove their pathogenicity. Our work in some cases resulted in prenatal diagnostic possibilities and clinical decisions about the change of the current treatment of the genotyped patient, therefore provide examples for the translation into direct health benefit.

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