DELIVERABLE D.T3.2.10 IMPLEMENTATION OF PILOT PROJECT "EVALUATION AND FOLLOW UP OF FAMILY MEMBERS"

Version 1
05 2019

INTRODUCTION

PP10's pilot action targets further undiagnosed groups of patients with subtle or subclinical manifestations in addition to diagnosing CD patients with clinical symptoms who spontaneously visit health care facilities. Since CD has a genetic background, first degree family members have elevated risk, but they often do not seek medical advice, or children are not tested by gastroenterologists if an adult patient is diagnosed or vice versa. In this pilot action, we established an open-access family evaluation regardless of age limits. The only criterion for enrolment was an already ongoing gluten consumption in case of testing serology markers (transglutaminase and endomysial antibodies) in young children, but even without that even newborns were evaluated for the presence HLA-DQ2 and HLA-DQ8 genetic allele variants which confer susceptibility for developing CD. Workshops were organised for stakeholders (eight in total) where the index patient and his/her family were informed about risks, possible clinical signs and the screening procedure. If consent was given, venous blood (or cord blood) was collected and processed. Serum antibodies against transglutaminase 2 (TG2) were measured by a capture ELISA using human red blood cell TG2. Endomysial antibodies were detected and titrated by indirect immunofluorescent method using human umbilical cord and appendix substrates. Genomic DNA was extracted from EDTA-treated venous blood samples by the Flexigene (Qiagen) kit. HLA-DQ risk alleles were detected by SNP-based Taqman probes using PCR amplification. A simple fluorescent method was developed and optimised during WP1 (diagnostic evaluation of new diagnoses) that could be read by fluorescent ELISA already available in the hospital. This worked well for the alleles DQ2.5 and DQ8. However, allele DQ2.2 needs 3 reactions and amplification is more difficult to detect. Therefore, for larger scale testing, a LightCycler automated real-time PCR machine was needed (rented). The organized workshops had 366 participants. During the pilot project 1486 newly enrolled family members were tested and 235 positives found (15.8%). In most cases the diagnosis of CD was confirmed by small bowel biopsy.

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DELIVERABLE D.T3.1.1 REPORT ABOUT PILOT PROJECT IDEAS & ESTABLISHED STAKEHOLDER GROUPS

Pilot project Start-up description template: "Evaluation and follow up of family members"

Version 1
07 2017

1. Pilot Background

Please describe here the background of your pilot in terms of ideas, preliminary actions, plans defined earlier and methods already chosen, etc. Some of the aspects you can tell about are as follows:

- *How did the project idea surface?*
  - Celiac disease (CD) is a chronic, lifelong disorder induced in genetically predisposed subjects by gluten found in wheat, rye, barley. Given the same genetic background, the disease occurs often in several family members.
  - CD is frequently transmitted from parents to offsprings, but the exact mode of inheritance is not known and thus disease prediction is difficult. Most often mothers and their daughters are affected and the disease also in general occurs more often in females who make up two thirds of all CD patients.
  - The genes behind disease induction are multiple and currently only partially known. Intense research in this field recognizes more and more important inherited properties each day. Also our own previous research has contributed to this collection of possible markers. The most important permissive factor is a certain normal polymorphism of the class II major histocompatibility complex DQ (HLA-DQ) with DQ2 and DQ8 as the only permissive alleles.
  - Consequently, DQ2 or DQ8 are mandatory for developing CD, but as almost 25% of the normal European population are DQ2/8 carriers, this test cannot confirm the presence of the disease. Therefore, CD can be only confirmed when gluten is shown to induce disease features. However, DQ tests are often used to select subjects for follow up.
  - CD is often asymptomatic for a long time and this is particularly true in family members. However, undetected disease can lead to severe complications with time, the very same as symptomatic disease can cause. Some of them may develop insidiously, like osteopenia and osteoporosis, autoimmune disorders, diabetes mellitus and small bowel malignant tumors.
  - Subclinical CD also can cause long term or debilitating health consequences, such as chronic fatigue, infertility, hair loss, underachievement in academic performance, chronic anxiety and psychological problems. Most of these can be attributed to the low levels of nutrients, minerals and vitamins. The small bowel shows a villous damage both in symptomatic and asymptomatic CD.
  - Such health hazards increase with time, and CD diagnosed in childhood has a more favourable outcome both physically and also socially, as young children adapt better and faster to the gluten-free diet. It has been shown that celiac antibodies (transglutaminase-specific antibodies: TGA and EMA) are detectable much earlier in the blood than symptoms arise.

- Laboratory tests detecting TGA and EMA are suitable for screening purposes, but this tests need a previous gluten exposure and cannot be used in newborn age. Sometimes only repeated testing will show a seropositive conversion and the time until positivity may be variable between individuals.
- Screening tests can detect CD among family members, but the best time to perform such evaluations is not yet clarified. Too early testing can still produce false negative results while a testing in late adolescence or adulthood increases the hazard for health deterioration during the elapsed time. Further the timing of gluten introduction and the consumed amount may influence the presentation of CD.
- Large quantities of gluten may induce more severe symptoms early in life, but it is still controversial whether late start or low intake would have a protective role. Parents in affected families often reduce gluten intake on their own and this may interfere with the proper performance of TGA and EMA testing.
- Although CD is a prototype condition fulfilling medical requirements for screening (frequent, severe, treatable upon early detection and possessing easily detectable and reliable serum markers), investigation of family members is often neglected in clinical practice and therefore everyday care is compromised. This is the case especially for adult and elderly index cases or relatives. Although more attention is paid to infants and young children, adult health services usually do not provide a screening for those either.
- National pediatric guidelines on Hungary recommend the screening of celiac family members at the age of 1 year, but this screening has never been implemented in clinical practice due to its bad timing (too early) and lack of allocated financial resources. This approach is not feasible in the majority of cases, when the diagnosis of the index case occurs later. Further, children mostly start to eat gluten around this age and thus antibodies are still not detectable and such an early screening may produce false negative results.
- Some of the affected family members have a more severe disease than index cases, but undiagnosed or misdiagnosed and therefore not properly treated. CD may be diagnosed even at old age.
- Further, in Hungary laboratory CD antibody tests are ordered at the discretion of specialists and family doctors cannot make these studies in their own practice. The frequency of referrals is low and waiting time in gastroenterology services may be very long.
- It is important that the screening should be performed as soon as possible after diagnosing the index case, because gluten consumption in the whole family will decrease when preparing the dietetic items for the index case.
- A fast and open service accepting family members for testing would improve the level of care.

- *Are there preliminary works that the project is based on? What are they?*
  - During 2007-2011 we participated in the PREVENTCD FP6 project investigating the possibilities to modify development of CD by a feeding intervention in early life. During this project we established TGA and EMA testing in our laboratory for family members.
  - During analysis of patient records (WP1) we found that approx. one third of all diagnosed cases have another CD relative in the family.

- We asked the number and age of family members in all newly diagnosed CD cases
- We still follow a part of the PREVENTCD cohort

- *What is the knowledge base behind the project (studies, methods, statistical data etc.)?*

Studies and articles related to family screening in CD are numerous and serum antibody testing for TGA and EMA are proven tools in older children. In the very young age group, other antibody tests, such deamidated gliadin-specific antibodies or HLA-DQ determination is often used as well.

- 1: Chou R, Bougatsos C, Blazina I, Mackey K, Grusing S, Selph S. Screening for Celiac Disease: Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2017 Mar 28;317(12):1258-1268.
- 2: Ferretti F, Branchi F, Dell'Osso B, Conte D, Elli L. Coping with celiac disease: how heavy is the burden for caregivers? Rev Esp Enferm Dig. 2017Apr;109(4):250-255.
- 3: Balasopoulou A, Stanković B, Panagiotara A, Nikčevic G, Peters BA, John A, Mendrinou E, Stratopoulos A, Legaki AI, Stathakopoulou V, Tsolia A, Govaris N, Govari S, Zagoriti Z, Poulas K, Kanariou M, Constantinidou N, Krini M, Spanou K, Radlovic N, Ali BR, Borg J, Drmanac R, Chrousos G, Pavlovic S, Roma E, Zukic B, Patrinos GP, Katsila T. Novel genetic risk variants for pediatric celiac disease.Hum Genomics. 2016 Oct 24;10(1):34.
- 4: Roy A, Smith C, Daskalakis C, Voorhees K, Moleski S, DiMarino AJ, Kastenberg D. Physicians Caring for Celiac Patients do not Routinely Recommend Screening of First-Degree Family Members. J Gastroenterol Hepatol Res. 2015 Dec;4(12):1838-1843. doi: 10.17554/j.issn.2224-3992.2015.04.585.
- 5: Uusitalo U, Lee HS, Aronsson CA, Yang J, Virtanen SM, Norris J, Agardh D; Environmental Determinants of the Diabetes in the Young (TEDDY) study group. Gluten consumption during late pregnancy and risk of celiac disease in the offspring: the TEDDY birth cohort. Am J Clin Nutr. 2015 Nov;102(5):1216-21.
- 6: Singh P, Arora S, Lal S, Strand TA, Makharia GK. Risk of Celiac Disease in the First- and Second-Degree Relatives of Patients With Celiac Disease: A Systematic Review and Meta-Analysis. Am J Gastroenterol. 2015 Nov;110(11):1539-48.
- 7: Aronsson CA, Lee HS, Liu E, Uusitalo U, Hummel S, Yang J, Hummel M, Rewers M, She JX, Simell O, Toppari J, Ziegler AG, Krischer J, Virtanen SM, Norris JM, Agardh D; TEDDY STUDY GROUP. Age at gluten introduction and risk of celiac disease. Pediatrics. 2015 Feb;135(2):239-45. doi: 10.1542/peds.2014-1787.
- 8: Mavrinac MA, Ohannessian A, Dowling EP, Dowling PT. Why celiac disease is so easy to miss. J Fam Pract. 2014 Sep;63(9):508-13.
- 9: Tomlin J, Slater H, Muganthan T, Beattie RM, Afzal NA. Parental knowledge of coeliac disease. Inform Health Soc Care. 2015;40(3):240-53.

- 10: Barbero EM, McNally SL, Donohue MC, Kagnoff MF. Barriers impeding serologic screening for celiac disease in clinically high-prevalence populations. BMC Gastroenterol. 2014 Mar 5;14:42. doi: 10.1186/1471-230X-14-42.
- 11: Uenishi RH, Gandolfi L, Almeida LM, Fritsch PM, Almeida FC, Nóbrega YK, Pratesi R. Screening for celiac disease in 1st degree relatives: a 10-year follow-up study. BMC Gastroenterol. 2014 Feb 20;14:36.
- 12: Korponay-Szabó I, Kovács J, Lörincz M, Török E, Gorácz G. Families with multiple cases of gluten-sensitive enteropathy. Z Gastroenterol. 1998 Jul;36(7):553-8.

- *What methods will you / do you plan to use (to motivate stakeholders, to involve lead users, to develop ICT infrastructure, to communicate online etc.)?*
  - Developing an educative programme for newly diagnosed CD patients is an effective tool to communicate basic knowledge about the disease. As part of this training, situation and risks for family members will be explained and families directly approached.
  - Symptoms of CD not commonly known or not directly related to the gastrointestinal tract or beyond the disease burden currently experienced by the index case will be explained to newly diagnosed cases to draw attention to other family members who may be affected as well but may have different symptoms. Such knowledge will be communicated to doctors, other HCPs, dieticians, coeliac society members and district nurses involved in the care of pregnant women, young children or elderly people during educational programmes.
  - Screening will be offered to all first-degree family members and to other relatives with suspicious symptoms in an open-access fashion. Family doctors will be asked to provide a formal referral to the testing, but then appointments will be arranged directly with the volunteering family members.
  - Recruiting at least 200 new family members not yet tested before. Recruitment will be continued until October 2018 to allow sufficient time for the evaluation and reporting.
  - An initial interview will be conducted and data for anthropometry, dietary habits, gluten consumption, health status and possible complaints will be collected. Then serum antibodies will be measured from a blood sample. Also EDTA-blood suitable for DNA extraction will be collected at the same occasion and stored for later use. In case of newborn infants, a special approach will be used. Umbilical cord blood will be used for HLA determination. In case parents are willing to donate also the umbilical cord itself, endothelial cells may be prepared and maintained in culture to study early features of the disease process.
  - Laboratory space will be arranged for blood collection, testing and appropriate storage of collected samples. Serum and DNA should be stored at -20-30C and cells at – 80C or in liquid nitrogen tank. Laboratory test reagents and cell culture reagents will be purchased for antibody testing,

blood chemistry determinations (e.g. deficiencies, like in iron and vitamins, basic blood chemistry), DNA and cellular studies. Further, newly discovered genetic markers will be designed for the testing and used as pilot.

- In antibody positive cases, further clinical evaluation will be offered and diagnosis of CD established by performing small bowel biopsy or other confirmatory tests accepted in clinical practice. In seronegative cases a further decision for follow up will be done based on the age, gluten consumption pattern and presence of symptoms.
- Young children will be followed at several occasions. As important milestones, a check-up at age 3 years and at a later timepoint will be targeted.
- A subsample will be tested for HLA-DQ2 and DQ8. The follow up results of the DQ-tested and not tested cohorts will be compared.
- Follow-up results will be used to evaluate the best timing of the screening and derive a model for use in other regions. Results will be communicated to all involved stakeholders.

2. Pilot Objectives

Please describe here the objectives of your pilot in terms of what the pilot project plans to achieve at the project's end and by what means. Some of the aspects you can tell about are as follows:

- *What are the main outputs of the pilot project (service, process, new management approach, new knowledge…)*
  - The service will pick additional patients, decrease the burden of undetected disease in several aspects of health, e.g. bodily complaints, underachievement and low energy status, chronic dietary deficiencies, osteopenia & osteoporosis, fertility problems, co-morbidities and complications. Early health intervention by appropriate diet will contribute to healthy and productive ageing even in a high-risk population.
  - The screening service will decrease anxiety and fears in the affected families especially in cases of newly born children and help parents to sustain a healthy life and diet for young children. Negative screening results will make not justified dietary restrictions unnecessary and will promote the participation of children without special precautions in the educational services (kindergarten, schools) and social programmes.
  - Added value to the lives of patients, because it is easier to prepare dietary items in the family, if several members need to follow a diet.
  - The pilot will evaluate the new management approach to deal with whole families instead of treating only index patient individuals. Such an approach may help overcome isolation, depression and fears related to a chronic disease condition and promote socialization.
  - Authorities will be notified about the results in the hope that a more applicable and effective recommendation will replace the current pediatric recommendation.

- *What is the approach that makes the project viable and sustainable?*
  - The need for the service was expressed by future end users, patients and the Hungarian celiac disease society. Further, several other health institutions and adult gastroenterology specialists asked us to develop a family member evaluation service methodology. Importantly, evaluation should be simple and should not have high costs.
  - The management plan is easily applicable in institutions which already perform celiac antibody testing. The testing methodology does not differ whether symptomatic or asymptomatic cases are tested, but the personnel has to learn to deal with not symptomatic subjects and learn the judgment of such cases. Our hospital will provide assistance to other gastroenterology centres in this respect and will remain committed to this initiative. In some settings, only a cross-sectional evaluation can be targeted.
- What kind of problems are you anticipating and what is your "plan B"-s if something doesn't turn out as you counted in certain situations?

- Non-participation, indifference or mistrust of individual patients may occur
- Some parents may not be willing to expose young children to venous blood sampling. In this case, a minimally invasive finger prick sampling can be offered and DNA extraction from cheek swab sample can be attempted. In other cases waiting until later (school) age may be appropriate.
- Financial problems may influence sustainability of the newly developed service (model) use – consumable costs. Other funding needs to be obtained after ending this project.

- Will the pilot have cross-regional impacts? Which are they?
  - Pilot activities and achievements will be transferred to other regions and countries through partners in their own countries and through our participation at transnational events (D.C.6.2): AOECS, ESPGHAN, UEG and other events and project communication channels.

- Any other aspects you find important?
  - Family involvement
  - Infant feeding practices
  - Secondary prevention
  - Contribution to a healthier future generation
  - Policy
  - Contribution to improved health services

3. Partnership

Please describe your stakeholders and their roles in the pilot project. Insert rows according to your needs.

Name	Specialization Area	Role in Project	Motivation / Benefits
If you plan to include a certain type of stakeholder but you don't yet know the specific organization, write "[TBD]" (to be determined) in this column.	*Healthcare professional/ patient/presentative of NGO/policy maker…*	*Participating in development phase/participating in testing, communication, evaluation etc.*	*What is the main motivation of the organization to participate in the pilot project? What will be their anticipated benefits?*
1. Celiac Societies in Hungary	NGO	Recruiting participants, implementation of pilot project's main activities	Service provided to members, evaluation of policy and future recommendations
2. Medical experts (specialists gastroenterologists, family doctors	Healthcare professionals	Recruiting family members, medical evaluations, diagnosis and follow-up of family members	Monitoring and evaluation of family members
3. Nutritionist / dietitians	Healthcare professionals	Presenters at educational training and patient workshops promoting screeining. Consuelling during implementation	Monitoring and evaluation of newly diagnosed patients
4. General public	Patients and their relatives, normal population	Potential participants	End-users of the service

5. Health authorities in Hungary (public health organizations and Ministry of Health	Policy maker	The representatives will be invited to a meeting, when the pilot partnership will have evaluation of pilot results, to discuss and propose future guidelines related to patients with life-long diseases.	Gaining information about newly tested practices
7. Other Hungarian health societies and foundations that are active in support of life-long chronic diseases	NGOs	cooperation with other NGOs, exchange experience	Exchange of experience and knowledge between NGOs.

4. Business Model Canvas

Please summarize your project plan and approach model described above in this table. Write bullet points in each cell of the table

<table>
<tr>
<td rowspan="2">Key pilot Partners
Project partners (HP PP10,PP8)
Hungarian Celiac societies
Medical experts (specialists gastroenterologists, phycologists)
Nutritionist / dietitians
Public health authorities and policy makers
Other Hungarian health societies and foundations that are active in support of life-long chronic diseases</td>
<td>Key Activities
1. Preparation of training programme and information materials

2. Recruitment and selection of candidates for screening

3. Performing the screening and evaluation of clinical results

5. Follow-up of family members

6. Testing of the service

7. Evaluation of the programme (questionnaire)</td>
<td rowspan="2">Value Proposition of the pilot
(what is the benefit?)
Implementation of screening in 200 new cases and follow up of initially negative subjects (recruitment, testing, diagnosis, consuelling, follow-up)
Establish screening protocols according to age, previous diet and initial results
Assistance in daily management, social and emotional support, awareness raising, education for patient families, relatives, teachers)
Reviewing of newly diagnosed cases in every 3 months</td>
<td>End-user (patient) Relationships
• Index patients (or their parents) bring in their family members

Participation in the screening and follow-up

Feedback</td>
<td rowspan="2">End-user (patient) Segments

At least 200 new family members
Families with children
Parents
Adult sibs
Elderly</td>
</tr>
<tr>
<td>Key Resources
1. Human (PP10) medical staff, technicians
2. Financial
HP – staff cost
Consumables, reagents for testing</td>
<td>Communication channels
personal contact, email patient and HCP lists
workshops
fieldtrip
media channels. newsletter of the celiac society</td>
</tr>
</table>

	• Promotional material preparations		• newsletters to HCPs via medical societies	

Cost Structure Pilot development coordination costs: distribution of working hours Medical/laboratory evaluation – staff costs Consumables (plastic labware and laboratory reagents for the screening and medical evaluation, external services for certain tests (experts, genetic, cell studies)	**Revenue Streams** Not planned

6. Preliminary work plan

Please give a time plan of how you plan to proceed with your pilot project. Define the main stages and milestones of the workflow. Insert rows according to your needs.

Phase Title & Description	Participating Stakeholders	Milestones	Planned Date
Give the title and/or short description of the phase (identification process, focus group meeting, survey, testing… etc.).	*According to the Partnership table above. You can write "All" if all of the stakeholders participate in the Phase.*	*Describe the milestone that you plan to achieve at the end of the phase*	*Planned date of milestone*
1. Advertise screening of family members	Celiac society, medical experts, general public	continuous until August 2013	End of August 2018
2. Providing infant feeding advices to decrease future risk of celiac disease	Celiac society, medical experts, nutritionists, dieticians, general public, pregnant women e.g. those previously diagnosed with or having coeliac disease in the family	continuous until May 2013	End of May 2018
3. Selection of candidates for evaluation	Celiac families, general practitioners, other proxy's	enrolment according to birth, new celiac disease diagnoses and willingness of participation upon interview	End of September 2018
4. Entry evaluation of individuals	Medical experts, Dietician	1. Birth & developmental data in children 2. Current gluten consumption 3. Symptoms/complaints	End of October 2018

4. Immunological evaluation	Medical experts, biomedical research staff, technicians	1. Antibody status (transglutaminase, endomysium) 2. Chemical abnormalities 3. Genetic predisposition (if consented) and predisposing other factors 4. Diagnostic evaluation (histology if needed)	End of September or 1st week of October 2017
5. Decision on further follow up and its schedule	Medical experts	Follow up visits at predefined age	From October 2017 to October 2018
6. Evaluation of the programme	Project partners, celiac society, medical experts	Evaluation of medical and follow up data & costs and preparation of report	From November to December 2018

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ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT

Pilot Status Report 1

Version 1
03 2017

1. Pilot Status According to Objectives defined in D.T3.1.1

- Pilot implementation progressed as planned with good number of newly tested family members (up t0 800). Affected people were scheduled for biopsy and final evaluation of celiac disease. A Class II sterile laminar box was purchased to ensure contamination risk-free DNA isolation and further work with cellular systems. A real-time PCR platform was rented from Roche. Earlier investigated young family members' cohorts, including the PREVENTCD children followed from birth, their age-matched controls and other young family members were called for a check at age 9, and if possible, at age 12. Altogether 100 currently 12 years old children's parents were contacted and a new blood examination was offered. Up to now, about 60 of them came to a new visit to our department and one new positive case was found.
- Follow-up results were analysed and an ESPGHAN abstract for next years' annual meeting in Glasgow (5-8 June, 2019) was submitted.

2. Activities implemented so far

- We organized patient/family workshops in our department on a monthly basis where families got the opportunity to learn more on celiac disease and discuss the status of family members, also the policy to deal with newborns and young children. Staff extensively explained the mechanism of the disease, possible heredity, family risks and tolls for screening. The workshop were intended to help recruitment of family members into our screening and follow-up programme.
- Enrolment into the screening proceeded well. Almost 800 subjects have been screened for transglutaminase and endomysial antibodies so far. EDTA samples for isolation of DNA were also collected and part of this material already processed for SNP-based HLA-DQ typing.
- HLA-DQ2 and DQ8 testing was further developed. Initially, we used a simple fluorescent reader to measure the PCR products, but this was not sensitive enough for detecting DQB1*0202 alleles. Therefore, we concerted the method into real-time PCR measurement using Light-Cycler 96 platform rented from Roche as a genetic service. This solved *0202 detection but we need further adjustment and optimisation for detecting allele *03012 to assess the presence of the full trans DQ2 heterodimer.

Analysis of follow-up results yielded the following abstract (for ESPGHAN 2019). This abstract has been accepted for oral presentation.

How long children with a first-degree coeliac relative should be followed by antibody screening?

Ilma Korponay-Szabo, Judit Gyimesi, Tamás Kerekes, Luisa Mearin, Sibylle Koletzko, Jernej Dolinsek, Jasmina Dolinsek

Family members of patients with coeliac disease (CeD) have 10-40% risk of CeD during lifetime, depending on genetic risk and environmental factors, but most affected subjects develop the disease between the age of 2-6 years according to prospective cohort studies. It is, however, unknown which proportion of family members develop CeD only after the age of 6. In this study we investigated whether 9 and 12 years old children have new seroconversion and whether gluten consumption habits influence the prevalence at this later age.

Methods: First-degree family members (FDR) presenting for screening were prospectively enrolled and followed by measuring serum transglutaminase 2-specific (TGA) and endomysial (EMA) antibodies at age 3 and 6. The investigated cohorts also included children (n=134) who participated from our country at the international PREVENTCD study (www.preventcd.com) with randomised early gluten introduction at age 4 or 6 months, a wild cohort (n=302) born in the same years as PREVENTCD children starting with gluten at age preferred by the parents and other FDR persons with multiple screening occasions. Children currently at age 9 and 12 were called for new blood drawings. FDRs presenting first time with already detected TGA+ or EMA+ results elsewhere were excluded. CeD diagnosis was confirmed by jejunal biopsy showing Marsh III lesions. Results were compared with prevalence data of cross-sectional FDR screening performed first time at the specified age time-points and with population screening results in the background population at age 6 and 12. HLA-DQ testing was performed if a genetic sample was available.

Results: Altogether 1007 FDR children at risk had an evaluation by TGA testing at age 9 (n=506) or 12 (n=501), or both. The median age of the prospectively followed cohorts is currently 10.4 years and most affected children developed seropositivity before or around the age of 3 years, independently of the time of gluten introduction. No cases occurred in children who were negatuve for both HLA-DQ2 and DQ8 alleles. From the children who were still negative at age 3, 10.2% (19/185) developed CeD by age 6, and from those still negative at age 6, 12.0% (3/25) developed CeD by age 9. However, no new cases occurred between 9 and 12 years of age in the 49 children who had been found still negative at age 9. Higher proportions of positives were found at 9 years of age (66/362, 18,2%) or 12 years of age (51/326, 15.6%, p<0.01), if screening has not been implemented before these timepoints or the index patient was diagnosed only at that time.

Conclusions: Periodic screening of children at risk should be continued until the age of 9 years, but thereafter, the seroconversion rate is falling below the population average.

3. Changes in stakeholder's partnership

STAKEHOLDERS NO LONGER PARTICIPATING	
Name	Reason for leaving
-	-
NEW STAKEHOLDERS	

Name	Specialization Area	Role in Pilot Project	Motivation / Benefits
Genetic testing provider company	Genetic tests	Service	-

ACTIVITY A.T3.2 IMPLEMENTATION OF PILOT PROJECT "EVALUATION AND FOLLOW UP OF FAMILY MEMBERS"

Pilot project Final Report PP10

Version 1
03 2017

1. RESULTS ACHIEVED ACCORDINGLY TO OBJECTIVES

- **Please review the objectives you have set up in your D.T3.1.1 description, in the Status report Phase 1 and describe activities and results achieved by your pilot. Give an overview of the processes that are part of your pilot project.**

During this pilot we had the followinng objectives

A/ *Objective*: Information and education of families about the risk of first degree relatives, facilitate enrolment for screening

Action and results: Screening is done on a voluntary basis. To achive motivation to be enrolled, 8 stakeholder workshops were organized at the Heim Pál National Paediatric Institute between 24.11.2017-10.01.2018. Discussions with the families were done on the risk condition itself and possible actions to discover hidden disease. Also was discussed how CD can be diagnosed in family members if they are asymptomatic. Stakeholders were informed about the risk of family members and its components. The possibility, ways and investigations for screening were also presented. The screening was offered. We talked about Focus in CD project and how it supports the care of family members. Main topics were:

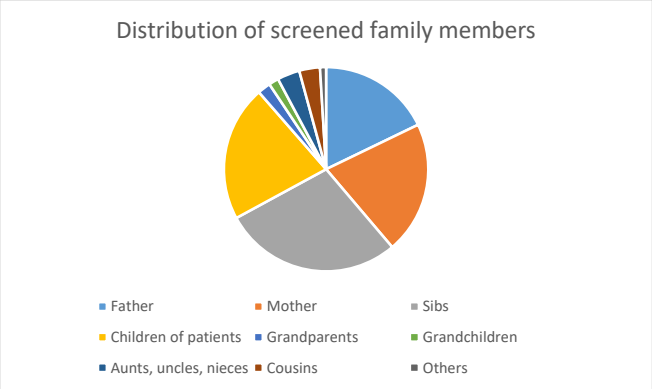
1. Family members have on average 10% risk
2. Risk factors
3. Possible symptoms (also others than in index case)
4. Possibilities for screening
5. Early childhood feeding advices (for future babies)

Date	Participants	Number
24.11.2017	General public, patients, family members	39
11.12.2017	General public, patients, family members	52
15.01.2018	General public, patients, family members	39
27.02.2018	General public, patients, family members	51
24.4.2018	General public, patients, family members	53
18.6.2018	General public, patients, family members	42
24.9.2018	General public, patients, family members	38
1.10.2018	General public, patients, family members	52
Total		366

B/ *Objective*: Screening for transglutaminase and endomysial antbodies

Action and results: Altogether 1486 new family members voluteered for the screening by blood tests. Of these 1297 (87%) were first degreee relatives and 13% were second degree relatives, but also these family members were accepted in an open-access fashion in our hospital (with or withour formal referral from the primary care doctors). Blood for serological studies and EDTA blood for genetic

studies were collected at the hospital's central laboratory from 261 fathers (17.6%) 307 mothers (20.7%, 414 sibs (27.9%), 315 offsprings of the patients (21.2%). Among the second degree relatives 30 were grandparents (2%), 23 were grandchildren (1.5%), 48 were cousins (3.2%), 52 were aunts, uncles, nephews or nieces (3.5%) and 14 were other relatives (0.9%). From the enrolled persons, 719 (48.4%) were older than 18 years (adults) and 767 (51.6%) were children. Altogether 336 children (22.6% of all subjects) were younger than 6 years at the time of the initial screening. At the time of enrolment, the subjects filled a questionnaire about gluten consumption habits, family relations with the index patients and consent or no consent to genetic studies. Family trees were prepared. Further, the family members were asked to bring in the medical report of the index patient and the CD diagnosis was reviewed and revised, if necessary from original histology slides.



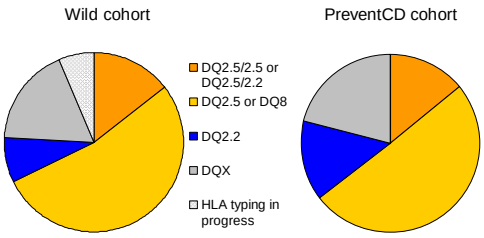
Serum antibodies against transglutaminase 2 (TG2) were measured by a capture ELISA using human red blood cell TG2 (cut-off 5 U). Endomysial antibodies were detected and titrated by indirect immunofluorescent method using human umbilical cord and appendix substrates. Results positive at 1:2.5 serum dilution were regarded as positive. Altogether 235 persons were found to be positive for both transglutaminase and endosmysial antibodies (15.8%). Additionally, 12 persons had slightly elevated tramsglutaminase-2 antibodies, but negative endomysial antibodies. Of these, 6 were found to react with the capture system antibodies only and not with transglutaminase. From the other 6 confirmed seropositive persons, one was found the have overt celiac disease, the others are still in follow up with biopsies pending.

From the 235 transglutaminase and endomysial antibody positive persons, 142 (60%) were females. Up to now 167 (71%) received a final CD diagnosis, 135 by biopsy, the rest by the no-biopsy route. 16 adults with high transglutaminase and endomyial antibodies refused biopsy and started a gluten-free diet by their own decision. Although these persons do not fit into the currently available no-biopsy policy due to their age, they can be regarded as celiac as well to to high and convincing antibody results. In the remaining family members, biopsies are scheduled or planned. Since enrolment continued till March 2019, appointments are still in course or biopsies performed but histology evaluation still pending.

C/ *Objective*: Development of a simple HLA-DQ2 DQ8 detection tool

Action and results: A fluorescent PCR kethid was developed based on the disctinctive single-nucleotide polymorphism in the different alleles. SNP probes were designed after earlier piblications by Monsuur

et al. PlosOne 2008 and adapted to Hungarian population. For the detection of alleles DQB1*0201 and *0302, a simple measurement tool by ELISA fluorescent reader was successfully developed using a single SNP for each. For the detection of alleles *0202 and *0301, more SNP-s are needed (3+1) and the amplification signal is lower. Therefore they were measured by a rented real-time PCR machine (Light cycler 96 from Roche). A sequentlial decision testing tool was developed. DNA was isolated by the Flexigene Qiagen kit, then quality checked. First the *0201 and *0302 alleles were tested. In subjects with negative results for both, two SNP test were run for *0202 and if any of these positive, two more SNP-s were runf for coinfirming *0202 and confirming/excluding *0301. When *0202 is found together with *0301, this makes a full DQ2 heterodimer with standard risk, but if only *0202 is present, only a low risk is present.



Distribution of HLA alleles in children younger than 6 years of age

D/ *Objective*: Interpretation of screening results and personalized decision making

Action and results: Persons with positive transglutaminase results were further investigated as decribed in Actibn B. For the person found negative, the results were comminucated in writing with explanations. In children younger than 6 years of age and negative serology results, a personalized discussion was atrranged and discussed whether a DNA testing to detect HLA-DQ2 or DQ9 alleles would be needed. Altogether DNA test results were available in 422 persons. Only 21% of sibs or children of patients were HLA-DQ2 and DQ8 negative and none had a positive serology result. For the DQ2 and/or DQ8 positive persons, the result was discussed. Having these risk alleles does not mean overt celiac disease and the risk is still low in the majority of them (10-15%).

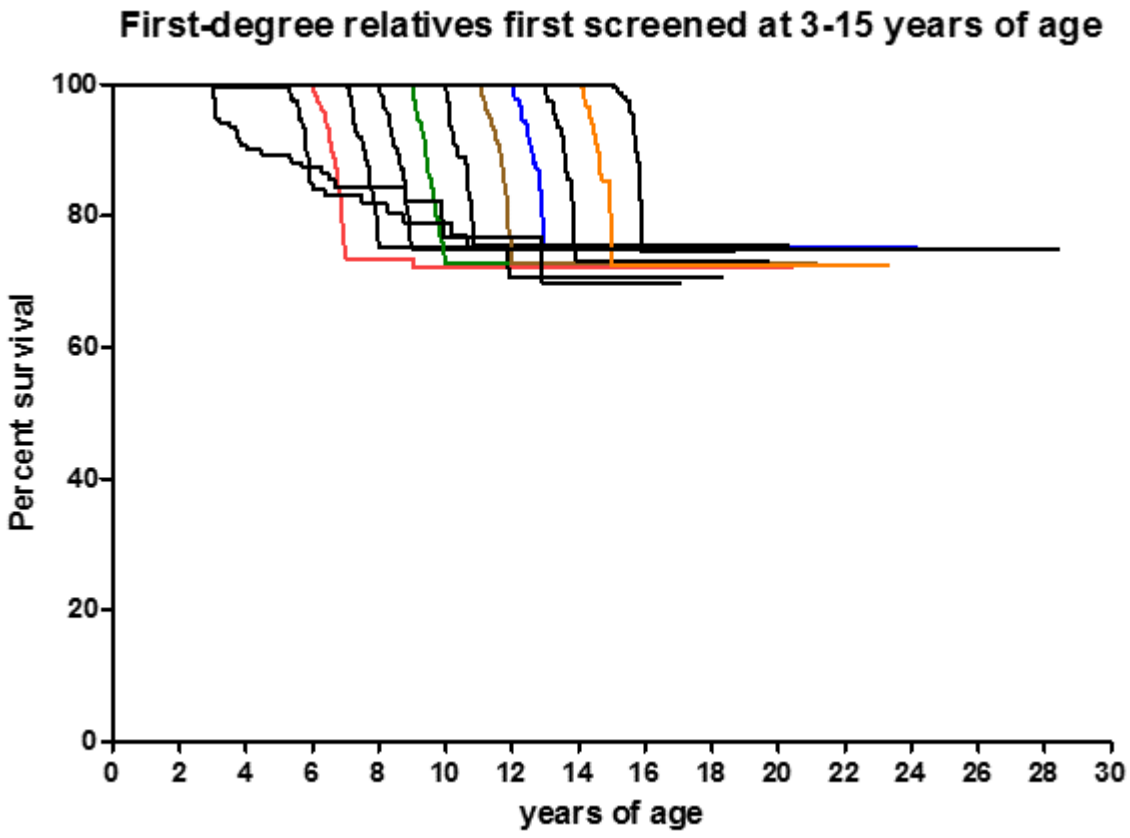
E/ *Objective*: Follow-up of young family members

Action and results: In persons with HLA-DQ2 és DQ8, celiac disease is gradually developing, so screening results in young age may be still negative. We evaluated a timing strategy for children below the age of 6 years. In guidelines, it is suggested to screen them in every 1-2 years, but this is not always possible and families often do not collaborate. We compiled results fron the recent screening and also used earlier results from the prevent CD (n=186) and age-matched control group (n=307). We found that celiac disease detection is only satisfactory if a negative results is availabel at 6 years of age. At the age of 9 years, only vetry few new positives are found. When the screening by serology was still negative at age 9, no new cases arose by the age of 12 or later (See table). However, already a

consireable number oif children were positive at age of 3 years and it is advisable to start the screened by then and repeat at 6 years (obligatory) and optionally at 9 years.

Age at first screening	Prevalence at cross-sectional first screening		Newly seroconverted / diagnosed						
	3 years	%	6 years	%	9 years	%	12 years	%	p
≤3 years			19/185	10.3	11/123	8.9	3/43	7.0	
6 years	-		4/28	14.2	3/25	12.0	1/43	2.3	
9 years	-		-		66/362	18.2	0/49	0	
12 years	-		-		-		51/326	15.6	

When the testing was first done at age 6 or later, almost complete discovery of hiden celiac disease was achieved. However, anxiety of the family and possible clinical symptoms necessitate to start at least at 3 years of age with initial testing and make one repeat test at age 6.



2. ADDED VALUE OF THE DEVELOPED & TESTED PILOT SOLUTION IN YOUR REGIONAL ENVIRONMENT

- Please describe shortly, what is the gained added value for the end-user of pilot service solution

ADDED VALUE for END-USER	
Short term effects	Long-term effects
1. Screening for a risk condition in the user or in his/her children. In case of positive result, discovery of a disease process that should be treated. In case of negative result, reassurance that the disease condition is not present.	1. Prevention of CD related health issues and complications. Enhancement of fitness and chance for healthy ageing
2. Information about the need for action for his/her minors in the family (children).	2. Planning for the future of children made easier. Coping with the gluten-free diet is better if started from young age
3. Psychological help to cope with anxiety about the possibility of potential or hidden disease	3. Long-term wellbeing and balanced mental health, improved quality of life
4. Patients are diagnosed in an early mostly asymptomatic condition	4. Less medical costs for the health system
5. It was easy to diagnose and educate newly screened out patients in our integrated CD care facility and center, because earlier provided information to the index patient on the gluten-free diet and lifestyle could be directly utilized also for the second patient in the same family	5. Less efforts and worload for the medical care and personnel
6. During the evaluation of the family members, original data for the CD of the index patients were reviewed and evaluated by contemporary standards	7. Some of the old CD diagnoses were upgraded or dropped. Subjects were freed of the necessity of the gluten-free diet if the old CD diagnosis was wrong or unjustified.

3. DEVIATION AND PROBLEMS ENCONTERED

- The planned action structure was followed and it was successfully operated to recruit and test family members for the clinical parameters. All clinical parts We only had to modify our action for performing the genetic tests. First, we had to apply modified reagents for the DNA isolation and revert back to

tradional solution/salting-out based technique instead of using DNA binding columns which had too low yield. We also had to modify DQ testing methods, primers and probes.

4. LESSON LEARNED RELATED TO CO-CREATION OF PILOT SOLUTIONS WITH ENGAGED STAKEHOLDERS

- Please describe what were the benefits and setbacks related to co-creation of pilot project with stakeholders.

LESSONS LEARNED	
Benefits	Setbacks
1. In our pilot project patients and their families were our stakeholders. It was easy and rewarding to collaborate with them in the screening process.	1. Screening was perfomred on a voluntary basis. Some of the families did not volunteer for screening, so hidden disease could not be detected in them.
2. Number of newly diagnosed CD cases increased in our center by	2. There was additional workload for the clinical diagnostics and endoscopy, but it could be adjusted by careful timimg and planning.
3. It was easy to diagnose and educate newly screened out patients, because earlier provided information to the index patient on the gluten-free diet and lifestyle could be directly utilized also for the second patient in the same family	3. Some of the adults had only borderline elevated transglutaminase antibodies and needed repeat testing

5. FURTHER ACTION PLAN (ACTIVITIES FOR THE FUTURE)

- What are your further activities of the pilot project development,
  - On the local level ?

    See also D.T3.3
- In the study period, we were able to screen almost 1500 subjects and collected blood samples from them also for a broader database and biobank. Up to now, HLA-DQ testing results were obtained from the very young (aged <3 years) children and other selected subgroups, like the 12 years old cohort and children matching in birth years the international preventcd study. Further tests can be planned with the same reagents and platforms in the others. CD is having HLA-DQ2 and 8 only as one of the predisposing factors. During the Focus in CD project, we tested Lpp genetic variants, but they did not have a predictive value. When CD research advances to uncover other genetic markers, we wish to process more samples also for these new markers.

- On transnational level ?

  See also D.T3.3

- Similar screening procedures can be performed by antibody testing also in other centers in Hungary provided local referral from the primary care physician can be obtained and costs covered either by national insurance or by research projects. Since family screening increases new CD diagnoses at least by 20%, we recommend to perform regularly this screening in each center diagnosing CD also outside our region. Our results show that screening at specific timepoints (3 and 6 years) is cost-effective in genetically predisposed children and very few new ones will be found at later ages (if still negative at 6 years).

- How did you plan to ensure sustainability to your pilot? Have you plan any action for the maintenance/follow up/development of the actions implemented, after the project ends?
- See also D.T3.3

We foresee that results will convince health authorities that family screening is beneficial and affordable to support and pay for the antibody tests from central budget for the persons at risk even if they are asymptomatic. Although current practices allow testing if justified medically, regular testing should become a practice. Recruitment efforts should be continued to provide appropriate information to families that they seek for screening. Workshops were efficient to promote motivation in our setting, we plan to continue with this practice also in the future. New e-tools will be useful also for the primary care doctors that they learn more on this possibility and provide more often referrals for the family members.