Adding DNA-test for screening of HLA-DQ2 and DQ8 to improve the early diagnosis of celiac disease at the Dutch Preventive Youth Healthcare Centres

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Primary Objective:1. To validate the HLA-DQ typing in blood taken by a fingerprick; to make it feasible in the regular Preventive YHCCs organization.2. To establish the feasibility of HLA-DQ2/8 typing in the active case-finding at the Preventive...

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Malabsorption conditions
Study type	Observational invasive

Summary

ID

NL-OMON53370

Source
ToetsingOnline

Brief title
GLUTEN-GEN

Condition

- Malabsorption conditions

Synonym
autoimmune disease, Glutenintolerance

Research involving
Human

Sponsors and support

Primary sponsor: Leids Universitair Medisch Centrum

Source(s) of monetary or material Support: ZonMW

Intervention

Keyword: Coeliac disease, Preventive Youth Health Care Center

Outcome measures

Primary outcome

Main study parameter/endpoint

Phase 1:

• Accuracy of the test for HLA-DQ typing. The results of the HLA-DQ2 and DQ8-typing using the dried blood spots will be compared with the results of the traditional HLA-typing.

DNA extraction for HLA-DQ typing from dry blood spots will be performed using the QIAamp method [11-14]. A protocol to isolate DNA has been developed and the isolation is executable by all the well skilled laboratory analysts. DNA extraction per sample takes about 15 minutes and it is possible to determine several samples at the same time. The equipment and optical technology are already present at the LUMC.

The usage of RT-PCR on DNA acquired from dried blood samples was successful in another setting and can easily be applied to HLA-DQ2/DQ8 typing [10]. The fast, accurate and simple RT-PCR-based assay for the genotyping and homozygosity analysis of the CD-related HLA alleles has been validated [15, 16]. The assay overcomes the major limitations of protocols currently in use, allowing

HLA-DQ2/DQ8 genotyping by using only three real-time PCR reactions. For the appraisal of DQ2 homozygosity, only one more reaction is needed. These reactions are easily automated, and the running time is exactly short which make it suitable for large screening or case-finding studies in diagnostic procedures, as it is demonstrated by their successful application in our HLA diagnostic laboratory. This approach has proven its effectiveness to diagnostic HLA-typing in CD suspicion in a North-Eastern Spanish population, confirming that the HLA-DQ genotyping is a powerful tool for stratifying CD risk. This has been confirmed in other cohorts [15-18]. The validation will be done at the LUMC, department of Pediatrics.

Since no previous studies have been done on the validation of the HLA-DQ2/8-typing in dried blood spots, the aim of this part of the study is to validate the test in dried spots collected in the hospital setting. From 50 children attending the LUMC dept. of Pediatrics because of suspected CD in whom traditional HLA-typing is part of their standard of care or from children with diagnosed CD in whom their HLA typing is already known. (The parents of) these children will be asked to participate in the study. Prior to the regular consultation, (parents of) children will receive study information letter along with an informed consent form. After blood collection for medical care and upon informed consent, a blood droplet will be collected by an additional fingerprick. DNA will be isolated using the QIAamp method designated for this purpose from dried blood spots (Qiagen). The results of the HLA-DQ2 and DQ8-typing using the dried blood spots will be compared with the results of the

traditional HLA-typing. Factors that may influence the quality of the material will be taken into account in the validation process.

Phase 2:

• Children with positive HLA-DQ2/8 typing (n/%)

• Children with negative HLA-DQ2/8 typing (n/%)

• Children in whom the test failed (including reason)

• Evaluation of the acceptance and impact of the HLA-typing for CD by the Dutch population (parents) and the health care professionals.

For the HLA-DQ typing approximately 15 µL is necessary which corresponds with 1-2 droplets of blood. After informed consent, an extra droplet of blood will be collected for the novel HLA-DQ2/8 typing by the finger prick when performing the POCT for TGA determination as done in GLUTENSCREEN. The filter-papers will be stored, at room temperature, safely at the YHCCs. Once a week, all the collected filter papers with dried blood spots will be sent to the department of the Immunology in specially marked envelops. After the DNA isolation, HLA-DQ typing and interpretation, the results will be communicated via an existing, secured mailbox to the Preventive YHCCs. The Youth health care professionals will inform the parents and the general practitioner in order to avoid unnecessary diagnostics.

Secondary outcome

Secondary study parameters/endpoints

Cost-effectiveness of the investigational strategy

To evaluate the cost-effectiveness of case finding with and without HLA-DQ2/8 typing. This part of the study will be done by the health economist. Factors that will be included are cost for: the novel genetic test, health care professionals at the YHCC and laboratorial, storage and transport of the material for the genetic test. These costs will compared to the costs in the situation without HLA-DQ2/8 testing as done in GLUTENSCREEN.

Time investment by medical and nursing staff at the YHCCs (sec)

Costs of the investigational strategy (time, materials) (¤)

Study description

Background summary

Celiac Disease (CD) is an immune-mediated systemic disorder elicited by the ingestion of gluten containing cereals from the normal diet, among others wheat, rye and barley. The disease is characterized by a variable combination of gluten-dependent clinical manifestations, CD specific antibodies, human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 haplotypes and chronic inflammation of the small bowel[1,2]. T-cells in the lamina propria of the small bowel recognize the gluten peptides when they are bound to the HLA class II specificities DQ2 and/or DQ8 on antigen-presenting cells. CD is one of the most common lifelong food- related disorders; it has a frequency of 1% in the general population: this corresponds to 170.000 persons in the Netherlands, and of them at least 30.000 children[3-7]. However, CD is frequently unrecognized, partially because of its variable clinical presentations and symptoms, ranging from malabsorption with chronic diarrhoea, poor growth in children and weight loss, to nonspecific signs and symptoms like chronic fatigue, osteoporosis/reduced bone mineral density, gastrointestinal symptoms or elevated liver enzymes [5,7,8]. Unrecognized and thereby undiagnosed and untreated disease is associated with short- and long-term complications such as delayed puberty, neuropsychiatric disturbances, associated autoimmune disease, miscarriages, small-for-date-births, osteoporosis, and, rarely, malignancy. CD has a considerable health burden for society and yields extensive negative

economic consequences, thereby presenting a resource challenge for current and future health systems [9]. Once diagnosed, the patient*s health status improves after treatment with a gluten free diet (GFD). That timely diagnosis and treatment of CD could be achieved by active case-finding, show the preliminary results of the ongoing ZonMw sponsored project GLUTENSCREEN (531002001; www.glutenscreen.nl). In this active case finding project, started at February 2019, all children aged 1-4 years who attend the Preventive Youth Health Care Centres (YHCCs) in the region of Kennemerland are yearly assessed for 10 CD-related symptoms during their regular consultation. If a child has one or more symptoms, the parents of the child are invited to participate. After informed consent, a point of care test (POCT) assessing CD-specific antibodies against tissue-transglutaminase (TGA) from a droplet of blood, is performed onsite at the YHCCs. If the POCT is positive (TGA present), CD is highly suspected and the child is referred to the Leiden University Medical Centre (LUMC) for diagnosis according to the standard of care[1,2]. The preliminary results of GLUTENSCREEN are beyond expectations: From the 14.917 children attending the YHCCs, 5.512 (37.0%) of them had one or more CD-related symptoms. The parents of 3.203 (58.1%) children gave informed consent for a POC-test. In 61 (1.9%) children the POC-test was positive. After additional investigations at our hospital, CD was confirmed in 55 children (1.7% of the tested children) (serum IgA TGA >10xULN and IgA against endomysium (EMA) positive) and ruled out in 5 children with dubious/positive POC test: in two children HLA-DQ2/8 was negative with negative TGA in serum (ELISA-test), in 3 children with TGA <10 x ULN (ELISA-test) in whom small bowel biopsies showed Marsh 1 lesions. One child still needs to be seen in the hospital (parents refused till now) (www.glutenscreen.nl). From the parents who were invited for GLUTENSCREEN, almost 80% is willing to participate if the POCT could be performed during the regular visit at the YHCC. All health care professionals reported that early CD detection by case-finding adds value to the preventive care they offer at the YHCCs. These preliminary results of our active CD case-finding project at the Preventive YHCCs in the Netherlands illustrate that early detection of CD at the Preventive YHC is feasible and well-accepted by parents and health care professionals. In GLUTENSCREEN all symptomatic children will be annually tested for CD at the Preventive YHCCs till the age of 4 years. In the region Kennemerland, GLUTENSCREEN has already been implemented in the regular care and POCT will be performed during consultation. However, the development of CD requires genetic susceptibility, present in 40% of the general population. Since the disease almost exclusively occurs in individuals with the human leukocyte antigen (HLA)-DQ2 and/or HLA- DQ8 haplotypes, codified by chromosome 6. Because of the high negative predictive value of HLA-typing for development of CD, repeated CD testing will be unnecessary in HLA-DQ2/DQ8 negative individuals (60% of the general population). Currently, HLA-typing is not a part of GLUTENSCREEN because current technique presents important drawbacks in settings without the availability of a laboratory, like the Preventive Care setting as the YHCCs, since it requires DNA extraction. Material for DNA extraction is usually obtained from whole blood (minimum quantity 4-5 ml) or from other cells, such as buccal mucosa. However, venipunctures are not

feasible at the YHCCs and buccal mucosa DNA extraction in children is time-consuming. We here propose to develop a novel test for DNA isolation for HLA typing extracted from the dried blood spots obtained from the POCT at the Preventive YHCCs for early detection of CD. Other projects have shown that the usage of real-time polymerase chain reaction (RT-PCR) on DNA acquired from dried blood samples is successful and can easily be applied to HLA-DQ2/DQ8 typing in this setting[10]. Adding HLA-DQ2/8 typing to the case finding strategy for CD at the Preventive YHCC is innovative since HLA-typing for CD has not previously been done in dried blood spots in the proposed setting. Furthermore, embedding this technique represents a novel approach to active case-finding of CD and consequently will improve secondary prevention of CD by early diagnosis at the Preventive YHCCs. The outcome of the proposed study will have impact on the active case finding procedure of CD at the YHCCs. Repeated testing for CD could be omitted in children tested HLA-DQ2/8 negative, this reflects to 60% of the targeted population. This study will result in: 1. More efficient and decrease of the burden of the HLA-DQ2/8 negative children: less children have to be tested. 2. Clarity of the acceptability of HLA typing for CD in parents from young children. 3. Costs saving by reducing unnecessary follow up of children. To embed this technique in the case finding setting at the YHCCs, the test will be offered to a significant part of the general Dutch population between 0-4 years old, since more than 95% of the general population visit the YHCC.

Study objective

Primary Objective:
1. To validate the HLA-DQ typing in blood taken by a fingerprick; to make it feasible in the regular Preventive YHCCs organization.
2. To establish the feasibility of HLA-DQ2/8 typing in the active case-finding at the Preventive YHCCs in the region Kennemerland.
3. To establish the acceptability and impact of genetic testing (of HLA-DQ2/8) as part of active case-finding of CD by the parents of the children attending the Preventive YHCCs and by the health care providers at these centers

Secondary Objective(s):
To evaluate the cost-effectiveness of genetic testing of HLA-DQ2/8 as part of the active case-finding approach

Study design

Phase 1: Validation will be done on the outpatient clinic in 50 consecutive children (1-18years) who attend the LUMC dept. of Pediatrics because of suspected CD in whom traditional HLA-typing is part of their standard of care.

Phase 2 (implement the HLA-test): Prospective intervention cohort study.
All parents of) symptomatic children, 1-4 years of age, who visit the

Preventive YHCC in the region of Kennemerland for a regular visit will be invited for this study.

Acceptability and cost-effectiveness of adding the novel test to the standard of care will also be assessed by standardized questionnaires.
As no validated/published questionnaires are available for the impact of HLA-testing in the general population, questionnaires will be developed together with the medical ethicist.

The duration of this study is 18 months:
The first 6 months will be used to arrange the organizational and administrative procedures.

LUMC:
The project will be submit for approval to the Medical Ethical Committee Leiden- Den Haag-Delft (METC-LDD)

Validation of the HLA-DQ2/8 typing in DNA isolated from dried blood spots obtained from one droplet of blood from 50 children attending the LUMC dept. of Pediatrics. Every month at least 15 children with (suspicion) of CD visit our outpatient clinic at the LUMC. Assuming a conservative participation of 60% (54 children) will be sufficient to complete the work. Sensitivity and specificity of the innovative method for HLA-DQ2/8 typing win dried blood spots in children will be calculated.

Preventive YHCCs:
• Preparation of the work at the Preventive YHCCs, including:
o Information to the health care workers about the study;
o Sending information letters to the parents of the children involved;
o Provision of the material to the YHCC locations (filter paper with identification number, shipping material);
o Arrangement of secure mail (*zorgmail*) to communicate the results between laboratory and YHCCs;
o Adaptation of the YHCC medical file to allow entering the HLA-DQ2/8 results and to make a notice that repeated CD testing is not needed;
o Building a CASTOR database for storing data and analysis of the results

In month 6-12 of the study dried blood spots will be obtained at the YHCCs Kennemerland for HLA-DQ typing as part of early detection of CD. Collection of HLA results into the YHCCs files and collection of the information concerning acceptability by parents and health care providers.

During the last 6 months (month 12-18): Analysis and interpretation of the results will be done. Preparation of a scientific paper to be submitted for publication to an (international) peer-reviewed scientific journal.

Intervention

Phase 1 (Development and Validation of HLA- DQ2/8 typing using RT-PCR in DNA isolated from dried blood spots obtained from one droplet of blood) From 50 children attending the LUMC dept. of Pediatrics because of suspected CD in whom traditional HLA-typing is part of their standard of care or from children with diagnosed CD in whom their HLA typing is already known. The parents of) these children will be asked to participate in the study. Prior to the regular consultation, (parents of) children will receive study information letter along with an informed consent form. After informed consent a blood droplet will be obtained from a fingerprick which will be done after taken blood for usual medical care (blood withdrawal by venipuncture) and deposited on filter paper S&S 903* (Schleicher and Schuell). The dried blood spots require an additional fingerprick to compare the collection method with that performed at the Preventive YHCCs. DNA will be isolated using the QIAamp method designated for this purpose from dried blood spots (Qiagen). Phase 2 (Implementation of HLA-DQ2/8 typing on the Preventive YHCCs) All parents of symptomatic children, 1-4 years of age, who visit the Preventive YHCC in the region of Kennemerland, will be asked to participate in this study. Prior to the visit for POCT, (parents of) children will receive study information letter along with an informed consent form. After informed consent, an extra droplet of blood will be collected for the novel HLA-DQ2/8 typing by the finger prick when performing the POCT for TGA determination as done in GLUTENSCREEN.

Study burden and risks

Benefits and risks assessment, group relatedness

The overall burden and risk for participation in this study can be considered minimal.
Phase 1: no extra risk for participants regarding the venipuncture since it is part of the standard care children receive when CD is suspected. Risks of venipuncture are: limited pain from puncture and/or hematoma. An additional finger prick will be performed to obtain a blood droplet
Phase 2: No extra finger prick is necessary for HLA-DQ typing since the children already participate in GLUTENSCREEN and get a finger prick for POC-test for TGA determination. Only an extra droplet of blood will be obtained for HLA-DQ-typing. So the risks are negligible. Related to a finger prick: limited pain from puncture, risk of skin infection negligible, risk of hematoma negligible.

HLA-DQ2/8-positive results may cause temporary negative feelings among parents, but our group has shown that genetic testing for CD did not expected to affect perceived health and health-related quality of life of children[19]. Evaluating the acceptance of genetic testing of HLA-DQ2/8 in children by questionnaires which will be developed in cooperation with the medical ethicist, is one of the

study objectives. These results will give more insights in the usefulness to risk ratio of genetic testing for CD predisposition in children.

All things considered, the results of the study will increase the knowledge on the impact on the parents, children and Preventive YHCCs of the results of genetic testing for CD-predisposition in symptomatic children. The implementation of the results will prevent unnecessary repeated testing for CD in approx. 60% of the children tested for early case-finding at the Preventive YHCCs. In addition, it will provide relief to their parents that their children will never develop CD, not at the moment of the test or during the rest of their lives.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adolescents (12-15 years)
Adolescents (16-17 years)
Children (2-11 years)
Babies and toddlers (28 days-23 months)

Inclusion criteria

Phase 1:
- age 1-18 years,
- suspicion of gluten-related disease,
- parents have a sufficient knowledge of Dutch language,
- written informed consent from child and/or parent
Phase 2:
- age 12 months to 4 years,
- not diagnosed with CD,
- not on a GFD,
- parents have a sufficient knowledge of Dutch language,
- written informed consent from the parent(s)

Exclusion criteria

- no informed consent,
- insufficient knowledge of Dutch language and/or inability to understand the information provided,
- bleeding disorders.

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Health services research

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	05-12-2023
Enrollment:	508
Type:	Actual

Ethics review

Approved WMO Date:	11-09-2023
Application type:	First submission
Review commission:	METC Leiden-Den Haag-Delft (Leiden)
	metc-ldd@lumc.nl

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL83759.058.23