Using next generation sequencing to find causative genes in patients with epidermolysis bullosa

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To explore the use of whole exome sequencing in the diagnostic evaluation of patients with epidermolysis bullosa

Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Epidermal and dermal conditions
Study type	Observational invasive

Summary

ID

NL-OMON40473

Source
ToetsingOnline

Brief title
NextGen4EB

Condition

- Epidermal and dermal conditions

Synonym
butterfly child disease, inherited blistering disease

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Groningen
Source(s) of monetary or material Support: Vlinderkind: EB patient charity.

Intervention

Keyword: epidermolysis bullosa, exome sequencing, genodermatosis, mutations

Outcome measures

Primary outcome

•To explore the use of exome sequencing in the diagnostic evaluation of patients with epidermolysis bullosa, without mutations in one of the known EB genes.

•To identify new genes and genotypes causing epidermolysis bullosa.

Secondary outcome

•To correlate newly identified genes with their associated phenotypes.

•To expand the Skin Panel list of EB genes.

Study description

Background summary

Epidermolysis bullosa (EB) is a heterogeneous group of inherited skin diseases characterized by trauma induced blistering and erosions of the skin and mucous membranes presenting directly after birth. There are over 25 different subtypes of highly varied clinical severity caused by mutations in at least 18 different genes.
Inherited mutations in 1 of 18 genes cause absence or reduction in proteins stabilizing the dermal-epidermal junction. Depending on the protein affected by the genetic mutation, skin cleavage occurs at different levels ranging from the epidermis (EB Simplex) to underneath the basement membrane (Dystrophic EB).

Aside from the clinical assessment, diagnostic evaluation of a child born with EB consists of molecular analysis of skin biopsies used for immune-antigen mapping and electron microscopy followed by genetic analysis. With these methods one can assess where the level of skin cleavage occurs, which proteins are affected and eventually which subtype of EB a patient is most likely to be afflicted with.Within the Netherlands, diagnostic DNA-testing of EB is offered for almost all of the known genes. For example, in 17% of the cases with

clinically suspected EBS, no mutation is found. In most cases, genes are tested one by one, which leads to long evaluation times and high costs.

Establishing a specific (genetic) diagnosis in a child with epidermolysis bullosa is relevant because it provides valuable information regarding aetiology, prognosis, potential associated disorders and recurrence risk. The identification of a specific (genetic) diagnosis is also important for family planning and reproductive choices, and may allow prenatal diagnostic testing or pre-implantation genetic diagnosis (PGD).

The occurrence of EB in the Netherlands is approximately 20 newborns per year. There is a need for a rapid and broad approach to quicken and simplify the diagnostic process, increase the diagnostic yield and to identify the other, yet unknown, genes involved. For these reasons we want to explore the use of exome sequencing in the diagnostic evaluation of patients with epidermolysis bullosa.

Exome sequencing allows the analysis of all exons of all known genes of one individual in a single test. This technique thus allows the parallel analysis of all genes known to be related to epidermolysis bullosa. This can be achieved by either capturing all known genes related to epidermolysis bullosa and subsequent sequencing of only these genes (= targeted exome sequencing). Alternatively, all genes can be captured and sequenced and a filter for epidermolysis bullosa genes can be applied during the data-analysis process (= whole exome sequencing with targeted analysis). The latter approach also allows the identification of new genes involved in epidermolysis bullosa, because the sequence data of all other genes remains available. Moreover, the data-analysis process can easily be updated when new information on a gene causing EB becomes available.
Currently, the departments of Genetics & Dermatology are working together to set up a Skin Panel using next generation sequencing. This panel will include all of the EB genes known in order to screen each new patient for mutations in all known genes. Whole exome sequencing will further add to this Skin Panel if new disease causing genes are discovered.

Exome sequencing has a significantly higher rate of false negative or false positive findings when compared to classic Sanger sequencing. The coverage varies over exons, and some exons may not be covered at all, especially if whole exome sequencing is applied. The greatest advantage, however, is that exome sequencing allows the analysis of all EB genes in parallel and in a single test, therefore the expected diagnostic yield is much higher than with the currently used strategy of sequential and targeted genetic analysis. By using this method as a standard genetic diagnostic tool for EB, we expect a shorter evaluation time and a reduction in costs. A comparable study, using non-targeted exome sequencing and performed in our centre has shown that exome sequencing can identify pathogenic mutations in already known and novel genes in patients with microcephaly, a condition known to be extremely heterogeneous.

In a substantial number of patients, most of whom had already been waiting for a diagnosis for years, a genetic diagnosis could be achieved.

Recently, McGrath et al used genome wide whole exome sequencing in a family clinically diagnosed with EBS. They identified a homozygous frameshift mutation in 3 individuals with an atypical clinical phenotype bringing to light a new role for the Slab2b effector protein in skin fragility. They were able to confirm their exome data findings by using the immune-antigen mapping and electron microscopy. McGrath et al. not only identified a new gene, i.e. exophilin 5, involved in EB thereby furthering diagnostics, they also were able to counsel this large family properly on risk of recurrence. This study confirms that exome sequencing is a valuable tool in finding new EB genes.

A great concern to both the dermatology clinicians as well as the clinical geneticists is that there exists a chance of finding non EB relevant mutations during whole exome sequencing.
The primary focus of this study is to find new disease causing genes and to correlate their phenotypic significance (see primary and secondary endpoints). The chances of finding variants in genes not related to EB exists but is very small. Many techniques and strategies are carried out in order to decrease the chance of this happening:

• Linkage analysis either prior to or as first step after whole exome sequencing in families with multiple affected individuals may direct towards one or more genetic regions of interest. The variants in the genes in these region(s) found by exome sequencing will be prioritized for further analysis, i.e. assessment of relevance to the disease based on their function and localization of protein expression and then confirmation by Sanger sequencing.

• Trio-analysis of the exome sequencing data will be applied in families with only one affected individual. In trio analysis the variants found in the proband are filtered against the variants in the healthy parents. Effectively, the variants inherited from the parents which are present in the affected child will be filtered out, resulting in a selection of de novo variants in the child. The remaining variants, usually 1 to 6 variants will remain by using this strategy, will be further selected based in the respective gene function. Genes related to the skin or genes leading to protein expression in the skin and mucous membranes will be further studied.

Both linkage analysis and Trio analysis directs our focus to the most probable disease causing locus within each investigated EB family and decreases the chance of finding mutations in non-EB related disease causing genes.

Study objective

To explore the use of whole exome sequencing in the diagnostic evaluation of

patients with epidermolysis bullosa

Study design

Observation research with invasive measuring.

Study burden and risks

The burden and risks associated with participation are negligible. Our research is an extension of the regular diagnostic care patients have been receiving. A blood sample is taken only when insufficient DNA is available.
If a causative mutation is found, the patient and family are counselled by the dermatologist and an experienced clinical geneticist. In the unlikely, but as yet not excluded chance that an unsolicited finding occurs, the family will be counselled by a clinical geneticist with relevant experience and after inter-collegial consultation, which is part of routine procedures within the department of Genetics.

The identification of the genetic mutation within an individual benefits the patient and family in many ways. If an affected individual can be informed about his or her disease causing mutation this is helpful in many respects:
-it may improve coping with this devastating disease
-it results in better information on the prognosis.
-it may however reduce the number of other diagnostic evaluations in the future
-It may benefit the family by improving genetic counselling, aid in decision making related to reproduction, enable carrier testing in family members and offer possibilities for prenatal diagnosis.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adolescents (12-15 years)
Adolescents (16-17 years)
Adults (18-64 years)
Children (2-11 years)
Elderly (65 years and older)

Inclusion criteria

-Clinically diagnosed EB or fmaily member of EB patient
-Antigen mapping and/or electron microscopy of a skin biopsy confirming the diagnosis of EB.
-No known mutation in one of the EB genes.
-Signed Informed consent

Exclusion criteria

Lack of informed consent

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Diagnostic

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	10-09-2014
Enrollment:	20
Type:	Actual

Ethics review

Approved WMO

Date:	10-09-2014
Application type:	First submission
Review commission:	METC Universitair Medisch Centrum Groningen (Groningen)

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	NL45728.042.13