The 2nd Nordic symposium on Epidermolysis Bullosa

24–26 April, 2005

Stockholm, Sweden

Organizing committee
Malin Netz, project leader, DEBRA Sweden/Nordic
Kristina Gustafsson-Bonnier, social worker/organizer
Birgitta Schiött, DEBRA Sweden
Heidi Ellingsen Silseth, administrator International EBForum, DEBRA

Scientific committee
Anders Vahlquist, co-ordinator, Prof. MD, PhD
Gabor Koranyi, ophthalmic surgeon
Carl-Fredrik Wahlgren, MD, Assoc.Prof.
Bitte Ahlborg, DDS, Senior Consultant
Gerd Wohlin, occupational therapist
Christina Eklund, dietitian
Invited Speakers (in order of appearance)

Robin Eady, MD, Prof, St John’s Institute of Dermatology, St Thomas’s Hospital, London, UK

Leena Bruckner-Tuderman, Prof. Dr.med., Universitäts-Hautklinik, Freiburg, Germany

Marcel Jonkman, MD, Prof., University Medical Centre Groningen, The Netherlands

Janice Carrera, Social worker, DEBRA International Support, UK

Elisabeth Wallenius, Chairman, Association for Rare Disorders, Sweden

Jacqueline Denyer, Senior clinical nurse specialist, UK

Bryan Mayou, Consultant plastic surgeon, Cadogan Clinic, London, UK

Nina Skogedal, Specialist of Pediatric Dentistry, TAKO-centre, Oslo, Norway

Karin Lorenz, journalist, Swedish Television, Karlstad, Sweden

Ruth Bernssen Bø, EB Simplex, DEBRA Norway

Mikko Blomqvist, EB Simplex, DEBRA Finland

Jesper Bønning, Junctional EB, DEBRA Denmark

Malin Ekunger, Dystrophic EB, DEBRA Sweden

Kris Aaseth, Chairman, DEBRA Norway

Tobias Gedde-Dahl Jr, MD, PhD, Senior Researcher, Rikshospitalet, Oslo, Norway
Programme for the Scandinavian EB-conference 2005

Sunday 24 April
18.00 Registration
19.00 Get-together party

Monday 25 April
8.00 Registration

Plenary morning session I
(Chairs: Malin Netz, Anders Vahlquist)
8.30 Official opening
Mats Wilander/John Dart & Malin Netz
A short history of EB in Sweden
Anders Vahlquist
8.50 Classification and microscopic diagnosis of EB
Robin Eady
9.30 The pathogenesis and prognosis of EB
Leena Bruckner-Tuderman
10.10 Discussion
10.30 Coffee break + poster and exhibition viewing

Plenary morning session II
(Chairs: Heidi Ellingsen Silseth, Carl-Fredrik Wahlgren)
11.00 Multiprofessional care
Marcel Jonkman
11.30 My role as a social worker in the hospital setting
Janice Carrera
12.00 Introduction to the workshop sessions
Gabor Koranyi
12.10 Lunch
13.30 Workshops, session I; all workshops includes a multidisciplinary panel
Workshop A
Neonatal care + nutrition + parental support
Carl-Fredrik Wahlgren
Workshop B
Wound dressings + pain management + ophthalmology
Gabor Koranyi
Workshop C
Surgery + physiotherapy + occupational therapy
Gerd Wohlin
Workshop D
Psychological and social support + oral care + prophylactic + skin care
Malin Netz
15.00 Coffee break + poster and exhibition viewing
15.30 Workshops, session II
(repeated with new participants)
17.00 End of workshops
18.30 Bus leaving for the conference dinner, including a guided tour of the museum
20.00 Conference dinner at Restaurant Atrium, National Museum Art Gallery

Tuesday 26 April

Plenary morning session
(Chairs: Flemming Brandrup, Kris Aaseth)
8.30 "Focus on everyday-life" - Presentation of an inquiry study
Elisabeth Wallenius
Therapeutic summaries:
8.50 The skin, Jackie Denyer
9.10 Surgery, Bryan Mayou
9.30 Mouth and teeth, Nina Skogedal
9.50 Future therapy; gene therapy?
Leena Bruckner-Tuderman
10.10 Discussion
10.20 Introduction to EB-forum on the Internet and Professionals’ forum
Heidi Ellingsen Silseth & Anders Vahlquist
10.30 Coffee break + poster and exhibition viewing
11.00 Professionals’ forum – interactive sessions
* Physicians Anders Vahlquist
* Dental professionals Mats Jonell
* Nurses and dietitians Jackie Denyer & Lesley Hayn
* Occupational- and physiotherapists Gerd Wohlin
* Psychologists and social workers Janice Carrera
12.30 Lunch

Plenary afternoon session I
(Chair: Kristina Gustafsson-Bonnier)
13.40 "Personal experience"; panel interview of four EB-patients
Karin Lorenz, Ruth Bernssen Bo, Malin Ekunger, Mikko Blomqvist, Jesper Bonning
14.40 Coffee break + poster and exhibition viewing

Plenary afternoon session II
(Chair: Bitte Ahlborg, Christina Eklund)
15.10 Patients’ organisation/DEBRA; peer counselling, co-operation between patients, professionals and society
Kris Aaseth
15.30 "A life time story of EB"; case studies
Tobias Gedde-Dahl Jr
15.50-16.00 End of conference
Malin Netz & Anders Vahlquist
WORKSHOP A

NEONATAL CARE – NUTRITION – PARENTAL SUPPORT

Chair
Carl-Fredrik Wahlgren, MD, PhD, Department of Dermatology, Karolinska University Hospital & Karolinska Institutet, Stockholm

Setting
Two cases are presented by two clinicians and discussed with the audience and the panel.
Hans-Ulrik Stark, MD, Department of Dermatology, Central Hospital, Karlstad,
Magnus Domellöf, MD, PhD, Dept of Pediatrics, Norrland’s University Hospital, Umeå

Panel
Leena Bruckner-Tuderman, Professor Dr. med, Universitäts-Hautklinik, Freiburg, Germany
Jackie Denyer, Senior clinical nurse specialist, DEBRA UK & Great Ormond Street, London
Lesley Haynes, Specialist paediatric dietitian for EB, Great Ormond Street, London
Anders Lindfors, MD, PhD, Astrid Lindgrens Children’s Hospital, Karolinska, Stockholm
Ann Nordgren, MD, PhD, Dept of Clinical Genetics, Karolinska, Stockholm
Kerstin Rudstedt, Social worker, Astrid Lindgren Children’s Hospital, Karolinska, Stockholm

WORKSHOP B

WOUND DRESSINGS - PAIN MANAGEMENT - OPTHALMOLOGY

Chair
Gabor Koranyi. MD, Ophthalmologist, St. Eriks Eye Hospital, Stockholm, Sweden

Panel
Per Montan, MD, Ophthalmologist, St. Eriks Eye Hospital, Stockholm
José Duipmans, Nurse consultant, University Hospital Groningen
Elizabeth Pillay, Senior EB Nurse Specialist (Adults UK), London
Gunnar L. Olsson, MD, Anaesthesiologist, Astrid Lindgrens Barnsjukhus, Stockholm
Ingrid Bäckmark, RN, Dept Anaesthesiology, St. Görans Hospital, Stockholm (FC: 15)
Elisabeth Steinlein, RN, Nurse specialist, Norway

WORKSHOP C

SURGERY – PHYSIOTHERAPY – OCCUPATIONAL THERAPY – ANAESTHESIOLOGY

Chair
Sippie Formsma, Occupational therapist, Groningen University Hospital

Post-operative hand therapy in RDEB (FC: 16)

Panel
Bryan Mayou, FRCS, Consultant Plastic surgeon, Cadogan Clinic, London
Hand surgery
Sigrid Hanem, MD, PhD. Dept of Anaesthesia, National hospital, Oslo
Anaesthesia in EB-patients (FC: 11)
Jitka Vokurkova, MD, PhD & Hana Buckova, MD, PhD, University Hospital, Brno
Surgical management of the hand in Dystrophic EB (FC: 9)
WORKSHOP D

PSYCHOLOGICAL & SOCIAL SUPPORT – ORAL CARE – PROPHYLACTIC SKIN CARE

Chair
Janice Carrera, Social worker DEBRA International support
Malin Netz, Social worker, Handicap & Habilitation, Stockholm

Panel
Malin Netz, vice president DEBRA Sweden

Personal experience from everyday life with JEB
Christina Renlund, psychologist and psychotherapist, Child Guidance Clinic, Stockholm

Children know in their own way. How can we talk?
Nina Braathen, occupational therapist, Asker, Norway

Everyday life with EB from the occupational therapist’s view
Agneta Gánemo, PhD, dermatological nurse, Uppsala and Malmö, Sweden

How to take care of both blistered and non-blistered skin
Bitte Ahlborg, dentist, Mun-H-Center, Gothenburg, and
Nina Skogedal, dentist, TAKO-centre, Oslo

Presentation of a leaflet on the odontological needs for persons with EB
Janice Carrera and Malin Netz

Conclusions

PHYSICIANS’ FORUM

Chair
Anders Vahlquist, MD, PhD, Professor of Dermatology, Uppsala University

Programme

Genetic counselling
Robin Eady, Prof, FRCP, S.t Johns Hospital, London

The UK experience of prenatal diagnosis in EB
Kaisa Tasanen, MD, Dept Dermatology, Oulu University, Finland

Phenotype and molecular mechanism associated to glycine substitution mutation in the largest collagenous domain of collagen XVII (FC: 14)

Systemic disease & cancer
JM Mandema, MD, Groningen University Hospital, The Netherlands

The vicious circle of blisters, pain, anemia and malnutrition in children with EB
Marcel Jonkman, Prof., MD, Groningen University Hospital

Malignant degeneration of chronic wounds in EB: Signs, prevention and prognosis

New treatments
Carl Swartling, MD, PhD, Dept Dermatology, Uppsala University Hospital

Treatment of sweat-induced blisters in mechano-bullous diseases (FC: 13)
Gabor Koranyi, MD, St Eriks Eye Hospital, Stockholm

Amniotic membrane in ocular surgery for EB (FC: 10)

DENTAL PROFESSIONALS’ FORUM

Chair
Mats Jontell, OD, PhD, Professor, FDSRCSEd, Oral Medicine, Institute of Odontology, The Sahlgrenska Academy at Göteborg University, Sweden
Setting
Four short communications related to oral and dental problems in patients with JEB and DEB will be presented:

Karin Höökil, DDS, Specialist in Pediatric Dentistry, Eastman Dental Institute, Stockholm

**Case report: An EB patient with high caries activity**
Torstein Tryti, DDS, Specialist in Orthodontics, Asker, Norway

**Experiences of orthodontic treatment in patients with EB**
Jens O. Andreasen, DDS, Specialist in Maxillofacial Surgery, Righospitalet, Copenhagen

**How to perform minor surgery in a patient with limited mouth opening**
Birgitta Bergendal, DDS, Specialist in Prosthetic Dentistry, National Oral Disability Centre, The Institute for Postgraduate Dental Education, Jönköping, Sweden

**A review of experiences in prosthetic therapy with oral implants in patients with EB**
Carin Vahlquist, MD, Dept Dermatology, Uppsala University Hospital, Sweden

The discussion will focus on therapy planning of patients with severe DEB. Also included in the panel:

Nina Skogedal, DDS, Specialist in Pediatric Dentistry, TAKO-senteret, Oslo, Norway

**NURSES’ AND DIETICIANS’ FORUM**
Chair
Jacqueline Denyer, Senior clinical nurse specialist
Lesley Haynes, Specialist paediatric dietitian, Institute of Child Health, London

Setting
The proposed topics for discussion is that of optimal weight status and how best to achieve this, especially in relation to those with EB whose mobility is significantly compromised and partially/wholly wheelchair-dependent (FC:18)

Irene Herpertz et al, University Medical Center Groningen

Body mass index is a reliable tool to optimize length growth in patients with severe dystrophic EB (FC: 17)

**OCCUPATIONAL- AND PHYSIOTHERAPISTS’ FORUM**
Chair
Nina Braathen, Occupational therapist, Norway

Panel
Nina Braathen

**Nursery school and school (mobility and tailoring) (FC: 19)**
Louise Wright, Physiotherapist, Birmingham, UK

**Physio-assessment and treatment of EB patients in Birmingham**
Gerd Wohlin, Occupational therapist, Botkyrka Habilitarnings Center, Stockholm

**Life quality and a meaningful daily life**
Also participating:
Berit Wideroe, Occupational therapist, Rikshospitalet Oslo, Norge
Lena Thuander, Physiotherapist, Centralsjukhuset, Karlstad, Sweden
Lotta Ågaurdh, Occupational therapist, Centralsjukhuset, Karlstad, Sweden

**PSYCHOLOGISTS’ AND SOCIAL WORKERS’ FORUM**
Chair
Janice Carrera, Social worker, DEBRA International Support, UK

Setting
At this Forum we plan to introduce new members to the international EB-forum group and hear about their experiences of working with people with EB. One member to present a case they have worked on followed by a short discussion. Discussion on supporting clients with a diagnosis of cancer and terminal care.
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The following abstracts will be published in Forum for Nordic Dermato-Venereology
EB-symposium 25-26/4-05 (list of abstracts)

**Plenary lectures**

PL1: A short history of EB in Sweden. *Anders Vahlquist*

PL2: Classification and diagnosis of EB. *Robin A J Eady*

PL3: Pathogenesis and prognosis of EB. *L Bruckner-Tuderman*

PL4: Multiprofessional care. *J.C. Duipmans and M.F. Jonkman*

PL5: The role as a social worker in the hospital setting. *J. Carrera*

PL6: Focus on daily life - a member study. *E Wallenius*

PL7: Therapy: The skin. *J. Denyer*

PL8: Therapy: Surgery (no abstract). *B. Mayou*

PL9: Therapy: Mouth and teeth. *Nina Skogedahl*

PL10: Future therapy. *L Bruckner-Tuderman*

PL11: Patient organisation/DEBRA. *Kris Aaseth*

PL12: A lifetime story of EB (no abstract). *T. Gedde-Dahl*

**Workshops/Forum/Posters (= Free Communications in order of submission)**

FC1: Expression of Matrilysin-1 (MMP-7) and collagenase-3 (MMP-13) and loss of MMP-19 in Cutaneous SCC in RDEB (poster). *Atte Kivisaari et al.*

FC2: EB Centre in Czech Republic (poster). *Bucková H. et al.*

FC3: First experiences with mutation analysis of Dystrophic form of Epidermolysis bullosa in the Czech Republic (poster). *Jerábková B. et al*

FC4: Somatic repression in junctional pretibial epidermolysis bullosa (PEB) is reversible (poster). *Laimer M et al*

FC5: The Austrian Centre for Epidermolysis Bullosa (poster). *Diem A. et al*

FC6: Case Report: Junctional Epidermolysis Bullosa (EB) with Ecthyma Gangrenosum (poster). *Diem A. et al*

FC7: Esophageal Dilation and PEG - Tubes in Children with EB (poster). *Bauer J et al.*

FC8: Release of pseudosyndactyly of hands in children with EB (poster). *Ludwikowski, B.*

FC9: Surgical management of the hand in Dystrophic EB. *Vokurkova J & Buckova H.*

FC10: Amniotic membrane in ocular surgery for EB. *Koranyi, G.*

FC11: Anaesthesia in EB-patients. *Hanem, S*

FC12: Alterations of oral mucosa in hereditary EB (poster). *Sadler E et al*


FC14: Phenotype and molecular mechanism associated to glycine. *Väissänen L, Tasanen K*

FC15: The importance of information and prevention of pain to children. *Bäckmark, I*

FC16: Post-operative hand therapy in RDEB. *Formsma, SA*

FC17: Body mass index is a reliable tool to optimize length growth. *Herpertz, I*

FC18: In severe EB, what is desirable weight status. *Hayenes L, Denyer J*

FC19: Nursery school and school - tailoring for EB children. *Braathen N*
PLenary Lectures

PL:1

A SHORT HISTORY OF EPIDERMOLYSIS BULLOSA IN SWEDEN.

Anders Wahlquist
Department of Medical Sciences, Genodermatosis Centre, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden

The Swedish pediatrician Gillis Herlitz (1902–1982) will be remembered for describing 8 cases of lethal EB already in 1935 (Acta Pediatr Acta 17, 315–371). Later on, Dr. Tobias Gedde-Dahl, Norway, has helped many Swedish physicians to correctly classify new cases of EB. According to his data, collected in 1965-95 and reported at the Nordic EB conference in Oslo in 2002 (Acta Derm Ven 82, 238), the cumulative number of Swedish EB families is: EBS 14, JEB 46 and DEB 25 (9 dominant/16 recessive).

In a study from 2001, initiated by the late Dr. Sven Wittboldt of DEBRA-Sweden, we asked 200 Pediatric and Dermatology units all over the country about the number of EB-patients recorded in their files. A total of 80 patients were identified: EBS 39, JEB 5 (subtype not specified) and DEB 24 (only 4 recessive). However, the true prevalence of EB in Sweden is probably in the order of 150-200 cases (1:50 000), because EBS in particular is likely to be underreported. These figures do not include patients with bullous ichthyosis or pachyonychia congenita who frequently have foot blisters indistinguishable from EBS (about 20 patients identified in Sweden; Acta Derm Ven, Suppl. 213, 34–37, 2003).

All the abovementioned genodermatoses are at the focus of a new EU-project (GENESKIN) which officially starts in June 2005 (Nordic participant: Uppsala University). A network of scientists and health staff with a special interest in EB will hopefully help to improve the diagnostic accuracy, genetic counselling and care of EB patients.

PL:2

CLASSIFICATION AND DIAGNOSIS OF EB

Robin A J Eady
St John’s Institute of Dermatology, St Thomas’ Hospital, London SE1 7EH, UK

Various classifications have been proposed for EB, ever since the disorder was first described over a century ago. Recently, as many as 26 subtypes of EB have been suggested. With the explosion in our knowledge of the causative genes underlying all the major and most of the more minor forms of EB, it has become evident that the division of EB into so many subtypes is not always supported by elucidation of the genotype (mutant gene or type of mutation underlying the disorder). There is therefore a strong case for reverting to a simpler classification of EB, yet retaining the 3 main types of EB, namely EB simplex, junctional EB and dystrophic EB (EB dystrophica). In other words, the classification will still depend on the depth in the skin where the primary split or blister occurs. In EB simplex, this is at the level of the epidermis; in junctional EB it is within the dermo-epidermal junction (DEJ) and ultrastructurally at the level of the lamina lucida; and in dystrophic EB it is also at the DEJ, but slightly lower and beneath the lamina densa. With either simple or more complicated classifications, the placing of certain subtypes of EB may still be a challenge. For example, the form of EB associated with pyloric atresia is conventionally grouped under junctional EB, yet, in a high proportion of patients, the primary level of skin cleavage is found to be intraepidermal. Do these cases therefore have EB simplex rather than junctional EB? We also need to consider whether we should be more inclusive and incorporate certain genetic skin fragility disorders not normally recognized as EB, such as Kindler syndrome, Shabir syndrome and skin fragility with ectodermal dysplasia (plakophilin deficiency), within the EB ‘family’.

Inclusion would benefit the patients, especially infants, from the increasing levels of specialist care that are now available for those with EB. Inference is the main disadvantage for EB still relies on the provision and microscopic analysis of an appropriate skin biopsy, these methods will be briefly discussed.

PL:3

PATHOGENESIS AND PROGNOSIS OF EB

Leena Bruckner-Tuderman
Department of Dermatology, University of Freiburg, Freiburg, Germany

The term epidermolysis bullosa (EB) refers to a clinically, genetically and biologically heterogeneous group of inherited disorders characterized by blistering of the skin and certain other epithelia. The blisters are a manifestation of a separation between the epidermis and the dermis along the basement membrane and usually result from minor trauma. This characteristic is common to the whole group, although the severity of expression ranges from mild blistering to extensive bulla formation, erosions, scarring, mutilation and lethal outcome. The group contains many distinct subtypes, and defects in 10 different genes are known to lead to the clinical abnormalities. Enormous progress has been made in the last years in understanding the genetic basis of EB and, as a consequence, some old concepts about this disease have been modified. The new, simplified classification is based on categorization according to the precise level of blister formation, the molecular background and clinical presentation.

The dermo-epidermal junction zone, the site of attachment of the epidermis to the dermis, is pathologically altered in EB. Three major categories are defined: EB simplex, in which the separation occurs within basal keratinocytes, is caused by mutations in the genes for keratin 5/14, or plectin. In junctional EB, the cleavage occurs along the basement membrane, and mutations in at least 6 genes are involved, including LAMA3, LAMB3 and LAMC2 encoding laminin 5, COL7A1 for collagen XVII, and ITGA6 and ITGB4 for integrin α6β4. Dystrophic EB, with separation below the basement membrane, is caused by mutations in the collagen VII gene, COL7A1. A large number of mutations are associated with a surprisingly broad spectrum of EB phenotypes.
Not only definition of mutations but also cell biological, protein chemical and suprastructural studies of the mutated molecules have shed light on the molecular pathomechanisms in EB and provided a basis for both prognostication and development of novel therapeutic strategies. International collaborative efforts have resulted in successful gene transfer into EB keratinocytes and in stable expression of correctly folded proteins by these cells in vitro. Although the clinical application of such therapies for EB may still be years away, the rapid development of new technologies holds promise for individually designed and biologically valid curative treatments.

**PL:4**

**MULTIPROFESSIONAL CARE AND CASE MANAGEMENT**

_J.C. Duipmans, M.S., and M.F. Jonkman, M.D._

Center for Blistering Diseases, University Medical Center Groningen, the Netherlands

Epidermolysis bullosa is a complex genetic disorder affecting more than only skin. The complications associated with EB are plentiful and include pain, itch, dental and eye problems, airway obstruction, dysphagia, oesophageal strictures, gastro-oesophageal reflux, constipation, anaemia, scarring and contractures, and limitations in mobility. Furthermore, the complex problems in EB conditions more or less serious developmental and psychosocial problems (affective, social, and occupational) and disturbances in the integration of the personality, both for patients with EB and their care takers.

Research in patients with a chronic condition and/or their parents demonstrates that the most important needs of patients/parents are being informed, being acknowledged, being partners in care, and recognized by professionals.

For all the needs and problems patients with EB need specialized care from many disciplines. An interdiscipli-

ary professional team is the standard approach for complex diseases like EB. This includes multidisciplinary out-patient clinics and hospital admissions, as well multidisciplinary care when the patient is at home.

Medical specialists are experts in their field. The main problem is that experts cannot prioritise the patient's needs and wishes beyond their professional field. For that the patients need a case holder. In many patients one of the parents functions as case holder. However, the parent has no formal relation with the professionals. For patients with a chronic disease it is important to have also case manager in the hospital, who both can focus on the individual and the group of patients.

Our choice is to use a nurse practitioner as case manager working from the hospital. In addition DEBRA NL supports a social worker who works as a case manager from the home. The nurse practitioner and social worker do take a central position, with the parent/patient, in the network of professionals.

The nurse practitioner (typically female) is fitted for the job as case manager because of the generalistic and easy approachable and accessible role of her function. Besides her knowledge of options in care and cure in EB, of patients needs, and with competence in care for patients with EB, she also gives excellent advice to specialist during hospital procedures. She is able to make use of an accessible network of professionals. Furthermore, she also facilitates the collaborative relationship between medical professionals and administrators of the multidisciplinary EB-team. From the patients view, the nurse practitioner creates a relationship with the patients that emphasizes reciprocal exchange and mutual decision making.

Together with the patient/parents the nurse practitioner lists and prioritizes the problems and coordinates and communicates the needed care by referring to the relevant professionals. In this way the collaborative practice of the EB-team provides superior patient care by combining the unique expertise of all professionals; it maximizes effective and efficient care.

It results in high levelled, demand driven, coordinated and collaborative care for EB-patients with complex problems and the collaborative multiprofessional care of the EB-team, including the social worker, meets the needs of the patients: optimal treatment, information, acknowledgement, and partnership in care.

**PL:5**

**MY ROLE AS A SOCIAL WORKER IN THE HOSPITAL SETTING**

_Janice Carrera (social worker)_

DEBRA International Support (Magnolia Cottage School Rd, Saltwood Hythe, CT21 4QB, UK)

The role of the Social Worker in the hospital setting is somewhat different to colleagues who work in the Community as there is rarely the opportunity to visit clients in their own homes due to distances. In a Specialist Centre such as St Thomas’ Hospital London patients are treated from all over Europe and the world so not only is it difficult to assess living conditions but cultural and language differences become of prime importance. The Social Worker must act as an advocate on behalf of the patient. There is a need to be flexible, to advise and liaise, enable, promote and encourage disciplines to work together.

Communication with the patient is central to all care. Often we pay only 'lip service' to this concept and continue to talk ‘about’ the patient instead of ‘with’ him/her. We tell and advise with the very best of intentions, instead of listening to what the persons needs are. We often decide for him on his behalf since he/she is in ‘no fit state to decide for him/herself’. This is often, we tell ourselves to protect the person, but it is often to protect ourselves.

On contacting the patient the Social Worker must find out whether or not their help is wanted. The official offer of help must be specific (not just “let me know if there is anything I can do”):

- Become informed about the medical situation and offer to be at the clinic when the patient visits.

- Ask the patient to think about the questions that they want answered, help them by writing down their
questions and put them in order of importance as time may be limited.

- During the appointment don’t speak on behalf of the patient unless asked.
- Listen to the information the doctor gives, as the patient may want part of it repeated at a later date.

One of the greatest services a Hospital Social Worker can provide is to listen to the patient’s fears and allow them to talk, often a service that the doctors do not have the time or training to deal with:

- We need to understand the patient’s attitude to treatment:
- Expectations - desired outcome - level of motivation
- Anxieties – depression - fears of failure etc
- Quality of interaction with parents, siblings and relatives
- Parental attitudes and behaviours towards sexuality
- Ethnic and religious influences

Patients must be encouraged to visit the hospital on a regular basis for their skin to be checked for suspicious areas. This can be painful and frightening for them and this is where the Social Worker can be of great help in befriending, supporting and encouraging regular visits.

When a diagnosis of cancer is made it is often relayed to the patient or carer by a junior doctor who has not had the training in such matters. It would be preferable if the Social Worker were involved perhaps with the Specialist Nurse or Psychologist when an appropriate ‘time and place’ can be chosen to give such news.

Often the patient and/or carer want to discuss treatment, the stay in hospital and other practical arrangements in which the Social Worker can be of assistance. Patients and relatives have often been dissatisfied and confused by the information given and have felt it too difficult to seek clarification from the doctors.

Another very important role that a Hospital Social Worker undertakes is to advise and ensure that every EB patient has all the State Benefits and Allowances that they are entitled to, plus aids and adaptations to their home. This entails liaising with many multidisciplinary colleagues and official bodies within the Hospital & in the Community.

PL.6

WITH FOCUS ON DAILY LIFE – A MEMBER STUDY

Elisabeth Wallenius, President of the Swedish Association of Rare Disorders

As president of the Swedish Association of Rare Disorders, I have many times expressed that “rarity in itself” – the fact that a disorder is rare – is an additional disability. Within the association this has been the focus of our discussions rather than the problems with the various disabilities. A rare disorder makes the contacts with other people more difficult and complicates the contacts with those professionals whose duty it is to give care and support.

According to our members, they encounter the greatest problems in the contacts with care staff and the social insurance office. However, there have also been problems in the contacts with officials of the municipalities and in school. Thus, the aim of the study was to establish the extent of the problems mentioned above, and to investigate the similarities and differences that exist between our different groups of disorders.

The study has been carried out through a questionnaire with mainly quantitative questions. The questionnaire was divided into two sections. One was concerned with how the family is affected and the other dealt with the problems that the person with the rare disorder encounters. Three of the questions were open questions, where members were asked to describe their situation with their own words. Members were also encouraged to write a story telling about their daily life and the problems they have to face. These stories make up the basis of the different case descriptions. The research material has been coded in such a way that the result can be applied to the entire group in the study, as well as to each individual disorder.

The results of the study not only reveal that most persons with a rare disability have very complex problems, but that the rarity as such is a particular problem. Consequently, the need for associations for various disorders has been expressed clearly in the study. The questionnaire has been answered by 37 (27 women and 10 men) of the DEBRA association’s members. The results show that the problems in practical life are extensive and that plenty of time is needed for personal care. 50% of those who have answered also declare that they have extra expenses of SEK 200 or more per month due to DEBRA.

In comparison to others with a rare disorder, many persons with a DEBRA diagnosis experience that being diagnosed facilitates contacts with specialised care. But still more people state that they have problems with the latter. Many have also experienced problems in the contact with other care givers and more than 50% say that they have been exposed to malpractice on one or more occasions.

PL.7

THERAPEUTIC SUMMARY: THE SKIN

Jackie Denyer, EB clinical nurse
London

This short presentation will highlight the danger of clinical diagnosis in the absence of supportive immunohisto-chemistry and electron microscopy findings.

It will raise the question “are skin lesions in infants a reliable indication of disease or prognosis?”

Factors such as mode of delivery and use of inappropriate dressing materials will be used to demonstrate factors responsible for severe skin loss, often suggesting severe disease.

A short case study will relate an unusual scenario with disturbing consequences.
Epidermolysis bullosa (EB) is characterized by blistering of the skin and mucosal membranes, EB exist in different forms, and the most severe affliction is seen in the dystrophic EB.

Oral health problems include vesicular and bullous eruptions of mucous membranes, scarring, restricted mouth-opening, impaired movement of lips and tongue and pain. Hypomineralyzed and hypoplastic teeth are recognized as features in some types of EB. Oral hygiene may be painful and difficult to carry out due to affected hands and oral blisters.

Dental treatment of the EB patient is a challenge due to microstomia, fragility of oral mucosa, obliteration of oral vestibulum, reduced tongue-mobility and hypersensitive teeth. Therefore, the highest priority in the EB-group as a whole, is prevention of oral disease. Counselling in proper oral hygiene routines should start before eruption of the first primary tooth. The preventive regime includes frequent professional cleaning and fluoride treatment as well as dietary advice.

The presentation will focus on implications complicating daily oral hygiene in these patients as well as precautions which need to be taken by dentist/dental hygienist to avoid unnecessary blisters and pain for the patient. Various methods available to make an oral hygiene programme easier will be presented. Furthermore, examples of dietary advice for caries prevention will be emphasized. Cooperation with other health care personnel is essential in order to give good oral care to the EB-patient.

PL:10

FUTURE THERAPY: GENE THERAPY

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Due to the severe symptoms in some EB subtypes, a scientifically valid therapy is urgently needed. Different strategies using genetic or protein materials have been critically evaluated, with all of their positive and negative aspects. The conclusion is that the most promising therapeutic developments today are in the field of skin gene therapy, although clinical applications still may be years away. The skin, or keratinocyte, gene therapy is based on introduction of a normal gene into cells isolated from a skin biopsy of a person with EB. The “repaired” cells will be grown into a skin graft which can be transplanted onto a very fragile skin area. The graft should develop into stable adherent skin.

Many research centers work on the development of gene therapy protocols and several international networks have been formed in order to combine expertise and to optimize progress. The expertise of dermatologists, cell and molecular biologists, virologists, and geneticists is needed to develop optimal gene vectors, cell cultures, product quality controls, transplantation techniques and clinical management. Many technical problems must yet be solved, and the prevailing opinion is that the efficacy of skin gene therapy must be proven in animal models before clinical trials can be considered. In addition, significant bureaucratic and legal hurdles must be overcome before treatment of individuals with EB can be tested.
Matrix metalloproteinases (MMPs) are a family of zinc-dependent metalloendopeptidases collectively capable of degrading essentially all extracellular matrix components. Expression of MMP-7 and MMP-13 is associated with invasive phenotype of many malignant tumors including UV-induced squamous cell carcinomas of the head and neck in non EB-population. MMP-19 is induced in hyperplastic epithelium in wounds, but absent from invasive areas of squamous cell carcinoma.

Recessive dystrophic epidermolysis bullosa (RDEB) is one of the most severe hereditary mecano-bullous diseases, characterized by scarring blister formation, nail dystrophy, cutaneous contractures, mutilations of the hands and feet and oesophageal stenosis. Cutaneous squamous cell carcinoma (SCC) is a frequent complication in chronic ulcers in these patients. Although the cutaneous SCCs in RDEB are in general histologically well differentiated they are characterized by rapid invasion and development of metastases resulting in poor prognosis. Cutaneous SCC is one of the major causes of the premature death of RDEB patients.

In this study, we wanted to elucidate the role of extracellular matrix degrading enzyme matrilysin-1 (MMP-7), collagenase-3 (MMP-13) and MMP-19 in development and invasion of these carcinomas.

We have obtained formalin-fixed, paraffin embedded samples from 25 cutaneous SCCs and 3 lymph node metastases from 18 RDEB patients. Immunostaining of the sections was performed by the avidin-biotin peroxidase complex. 3-amino-9-ethylcarbazole (AEC) was used as chromogenic substrate and Mayer hematoxylin as a counterstain. We used mouse monoclonal antibodies to study localization of MMP-7 and MMP-13. For detection of MMP-19 we used rabbit polyclonal antibody. Negative control stainings were performed without primary antibody.

In tissue sections cytoplasmic staining for MMP-7 and MMP-13 was noted in tumor cells but not in stromal cells, MMP-7 expression was also detected in tumor cells in lymph node metastases examined. MMP-7 was also expressed by exocrine epithelial cells in sweat glands as a positive control where as no staining was noted in normal epidermis or in negative control stainings. Staining for MMP-19 was detected in keratinocytes in hyperplastic epithelium, but not in malignant cells.

Expression of MMP-7 and MMP-13 is specifically induced in malignantly transformed keratinocytes in this aggressive subset of cutaneous SCCs suggesting a role for these MMPs in early cutaneous SCC development, invasion and metastasis in RDEB patients. Also the loss of expression of MMP-19 may have a role in malignant transformation of the keratinocytes.

These results indentify MMP-7 and MMP-13 as a potential therapeutic targets to inhibit growth and invasion of SCCs in patients with RDEB.

RDEB patients have abundantly chronic ulcers and non-malignant wounds which may resemble histologically well differentiated SCCs. The loss of MMP-19 might help in making the differential diagnosis.
FIRST EXPERIENCES WITH MUTATION ANALYSIS OF DYSTROPHIC FORM OF EPIDERMOLYSIS BULLOSA IN THE CZECH REPUBLIC

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Dystrophic epidermolysis bullosa (DEB) is a clinically heterogenous skin disorder, characterized by abnormal anchoring fibrils at the dermal-epidermal basement membrane zone. DEB has been linked to the COL7A1 gene at chromosome 3p21 which encodes collagen VII, the major component of the anchoring fibrils. The gene consists of 118 separate exons. DEB is transmitted in autosomal recessive (RDEB) or dominant (DDEB) fashion.

We investigated 13 patients, two with DDEB and 11 with RDEB. We designed 87 primer pairs corresponding to flanking intronic sequences which allow PCR amplification of all 118 exons directly from genomic DNA. The first step of mutation analysis of each patient is sequencing of exon 73 (over 10% of all COL7A1 mutations have been found in it), and exon 74, 75. Others exons are examined by denaturing high performance liquid chromatography (DHPLC) and if the result is positive, the PCR products are sequenced. The exons with high probability of occurrence of some mutation are analysed using DHPLC in the next step (see mutation map on pages: http://archive.uwcm.ac.uk/uwcm/mg/search/128750.html).

Four mutations in exon 73 were identified: the substitution G2049E in three patients and the insertion 2027insC in exon 74. These are recurrent mutations which have been reported previously. We would like to analyse all exons and flanking intronic sequences of each patient and his relatives by this procedure. Our intention is also introduction of DNA diagnostic of EB simplex and junctional EB.

The present diagnostics is based on clinical manifestations, immunohistochemical analysis and electron microscopy. We would like to extend a spectrum of methods used in EB diagnostics in the Czech Republic by analysis of the genes linked to the individual types of EB. Identification of mutations in affected families has important implications for genetic counselling - the detection of carriers, assessment of the mode of inheritance and early prenatal diagnosis. It is also the first step towards gene therapy.

EB-HAUS*: THE AUSTRIAN CENTRE FOR EPIDERMOLYSIS BULLOSA

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The various cutaneous and extra-cutaneous problems of Epidermolysis bullosa (EB) require interdisciplinary care and extensive coordination of all who are involved. Thus, the decision was made to provide the necessary infrastructure by building a small centre of excellence for EB: the "eb-haus" in Salzburg.

To reach this objective in times of low-budgets in health care systems the Department of Dermatology at the General Hospital Salzburg has initiated a unique cooperation with the support group debra-austria (HYPERLINK "http://www.debra-austria.org" www.debra-austria.org) which raises funds for the building and supports the research in an outstanding and very successful way.

The "eb-haus" consists essentially of three parts:

- A centre for therapy on an out-patient base with an EB-physician, an EB-nurse, co-operating with a team of experts of various medical disciplines.
- A research department for basic science, genetic research and clinical trials.
- An academy which facilitates the intense interchange of knowledge and experience between scientists, physicians, nursing staff, patients and their relatives.

This centre of excellence will concentrate all strategies to face the difficulties of this at present incurable, severely disabling and sometimes life threatening disease. The resulting efficiency will be of great benefit to all EB patients with localised disease expression.

SOMATIC REPRESSION IN JUNCTIONAL PRETIBIAL

EPIDERMOLYSIS BULLOSA (PEB) IS REVERSIBLE

Department of Dermatology and *Institute of Pathology, Paracelsus Private Medical University Salzburg

Pretibial epidermolysis bullosa (PEB) is a genetic mechanobullous disease with an anatomically restricted phenotypic expression, the reason of which is unknown. We describe a patient suffering from the typical clinical symptoms of PEB that is principally a subtype of dystrophic epidermolysis bullosa (DEB). Ultrastructural, immunohistochemical and laminin-5 gene analyses, however, were consistent with junctional PEB. This notion was further substantiated by the DNA sequence analysis indicating compound heterozygosity for the missense mutation C290S and the nonsense mutation R635X in the LAMB3 gene. Skin grafting was successfully performed as a therapeutic approach with a favourable long-term out-come. Remarkably, at the graft donor sites the patient developed reproducibly prolonged blistering of up to 6 months suggesting activation of a formerly repressed blistering pheno-genotype. Therefore we conclude that the morphologic characteristics of PEB are ubiquitously inducible if provoked by mechanical and/or inflammatory stimuli (Koebner phenomenon). It is likely that this somatic repression is a mechanism operative in other patients with localised disease expression.
sufferers in Austria – and also Europe-wide – and considerably improve their quality of life.

**FC:6**

**CASE REPORT: JUNCTIONAL EPIDERMOLYSIS BULLOSA (EB) WITH ECTHYMA GANGRENSUM**

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We report on a girl with junctional EB of the Herlitz type with Ecthyma gangrenosum. A few days after hospitalisation because of fever she developed skin lesions on her neck, shoulders and back with dark red colour, a central haemoragic blister and subsequent necrotic skin lesions. Pseudomonas aeruginosa was found in tissue and blood culture. Despite intravenous antibiotic therapy she died of Pseudomonas sepsis.

Skin infection with Pseudomonas aeruginosa is very common in junctional EB of the Herlitz type, but this progressive course of the infection is rare. We will discuss possible underlying mechanisms and treatment options.

**FC:7**

**ESOPHAGEAL DILATION AND PEG-TUBES IN CHILDREN WITH EPIDERMOLYSIS BULLOSA**

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Six pediatric patients with dysphagia due to esophageal stenosis were treated by esophageal dilation in our clinic. The onset of dysphagia generally occurred between the sixth and the seventh year of age.

The repeated esophageal dilation relieved dysphagia and improved the nutritional status in all patients.

Unfortunately, esophageal strictures and developing microstomias made necessary to create PEG-Tubes in three of our patients.

**FC:8**

**RELEASE OF PSEUOSYNDACTRYLY OF HANDS IN CHILDREN WITH EB?**

Barbara Ludwikowski, Dept. of Paediatric surgery Paracelsus Private Medical University Salzburg- Austria

Children with recessive dystrophic epidermolysis bullosa and development of pseudosyndactyly of the hands are presented. Surgical intervention is commonly recommend-ed and performed. Unfortunately the need for repeated surgery is necessary. Musculoskeletal complications are described, if no intervention is performed and early interventions are recommended (J Hand Surg [Br]. 2005 Feb; 30 J: 14-22).

We want to discuss the indication for surgical interventions. A benefit of this surgery should be for the children and families in their daily life. We reinvestigated our patients (clinical, x-ray and questionnaire) to get more information about the outcome and questioned early surgery.

**FC:9**

**SURGICAL MANAGEMENT OF THE HAND IN DYSTROPHIC EPIDERMOLYSIS BULLOSA**

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Surgical correction of hand deformities and treatment of spinaliomas of DEB patients has a long history on Plastic surgery department in Brno, Czech republic. Now, all patients are registered in the Center for Epidermolysis Bullosa (EB) in Brno which is based on the multi-disciplinary team cooperation – DEBRA,CZ.

In the last ten years our center gathered 89 patients with EB, 46% of them had DEB. Eleven out of 14 patients with acral form of DEB have undertaken 56 hand surgeries. The mean age was 16 years (range 4-48 years). Extensive surgical procedure with separation of the fused web spaces and releasing flexion contractures was performed. The raw areas were covered by split skin grafts harvested on the thigh. The splitting of the hands was continuous for 3 months and then fingers were webbed daily with splitting during night.

Assessment of the results of surgery was measured by extension and flexion deficit and the adduction deformity of the thumb. The recurrence of pseudosyndactyly was observed each 3 months.

Clinically major improvement was observed in the patient’s grasp and the ability to pick up objects. The young patients without secondary joint disease achieved better results with surgery than patients with long standing uncorrected hand deformity. The better long term splittage the better functional results.

**FC:10**

**AMNIOTIC MEMBRANE IN OCULAR SURGERY FOR EB.**

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Symblepharon, adherens between the eyelid and the eyeball, is not uncommon in dystrophic EB. It is progressive, obstructs vision and eye movements. Material and methods: A 5 years old patient with a major symblepharon in the left eye was presented for surgery. The lesion affected more than half of the cornea. At the surgery a minor symblepharon was found also in the right eye. The eyes were operated with the use of amniotic membrane.
Results: After 5 years follow up, the right eye is still without symblepharon, while the surgery on the left eye had to be repeated several time with still unsatisfying result.

Conclusion: Early surgery of symblepharon could prevent further development of this sight threatening condition.

FC:11
ANAESTHESIA IN EB-PATIENTS.
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Patients with epidermolysis bullosa need careful preoperative screening regarding infection, anaemia and electrolytes in order to correct any disturbances before the planned procedure.

In the operating theatre special steps must be taken to ensure protection of the skin and airways. A general principle is to avoid all kinds of tape and adhesives. Intavenous cannulas must be fixed with gauze and bandage, and stitches might also be used. Gentle airway manipulation is of utmost importance. If tracheal intubation is necessary, great care must be taken when introducing the laryngoscope and endotracheal tube in order to avoid blistering of the mucosa or damage to the skin.

The monitoring systems anaesthetists routinely use, are electrocardiography (ECG), pulse oxymetry, blood pressure and capnography.

ECG monitoring with adhesive pads must be avoided. The pulse oxymetry probe if possible to use, need to change position so as not to harm the skin. Blood pressure monitoring with cuffs must be placed over soft gauze.

The position of the patient on the operating table is important. The operating table should be soft and comfortable and the armrests must be equipped with atraumatic padding.

All personnel taking part in the preoperative, operative and postoperative procedures should be properly informed about the skin problems of EB patients in advance, in order to avoid any additional blistering.

FC:12
ALTERATIONS OF ORAL MUCOSA IN HEREDITARY EPIDERMOLYSIS BULLOSA
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The appearance and structure of perioral and intraoral epithelia differ markedly from one anatomic site to another, reflecting unique functions of different oral tissues under the variety of stresses that they endure. Oral mucosa is variable and frequently affected in hereditary epidermolysis bullosa (EB). The most severe and most frequent alterations are found in patients with recessive dystrophic EB (RDEB) subtypes. These patients show intraoral disease activity in 92,3-100%. Mucosa of dominant dystrophic EB (DDEB) subtypes is affected in 81,1%, of junctional EB-Herlitz (JEB-Herlitz) in 83,3%, of JEB-non-Herlitz in 91,6% and of EB simplex in 34,7-58,6%. Most abnormal oral findings in EB patients are oral blisters and erosions, chronic gingivitis and oral milia (DEB 49-54%), occurring most often on the palatal mucosa. In RDEB chronic blisters and erosions can lead to perioral and intraoral scarring resulting in lack of lingual papillae, lack of palatinal rugae, ankyloglossia, obliteration of the vestibulum oris and microstomia. Additional, continuous oral blistering, which results in oral scarring, ankyloglossia, and vestibular obliteration, may produce tissue changes with malignant potential. Because squamous cell carcinomas have been reported to occur in perioral and intraoral (tongue) sites in patients with generalized RDEB, periodical oral examinations of patients are important throughout life. Therapy of oral mucosal alterations is limited and consists of prophylactic strategies as soft diet, sucralfate and dental care.

FC: 13
TREATMENT OF SWEAT-INDUCED BLISTERING OF THE FEET
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Plantar sweating at high ambient temperatures increases the blistering in patients with EB simplex (EBS), which is due to KRT 5 or14 mutations, as well as in pachyonychia congenita (PC), which is due to KRT 6, 16 or 17 mutations. The latter condition is also associated with painful, focal hyperkeratosis on the soles. Treatment of plantar sweating is notoriously difficult, but local injection of botulinum toxin (btx) can produce longstanding anhidrosis in patients with primary essential plantar hyperhidrosis without skin disease (C.Swartling, Thesis 2002). The anaesthesia required for the plantar injections is however a problem.

Here we report 5 patients with EBS and PC who had great walking problems, especially in summer time, due to painful blistering of the soles. They were treated with intracutaneous plantar injections of btx type A (Dysport, 100 units/ml, Ipsen, Slough, UK) after prior intravenous regional anaesthesia of the foot with a low tourniquet and 25 ml prilocain (5 mg/ml). Within one week all 3 PC patients experienced dryness and a remarkable relief of pain at plantar pressure sites. The effect duration was between 6 weeks and 6 months. Repeated injections over a 2 year period confirmed the good results with no side-effects or tachyphylaxis noted. Two patients with EBS were treated in the same way with unilateral btx injections. However, despite obvious anhidrosis on the treated side in both cases, the intensity of blistering and pressure-induced pain only marginally benefited from the therapy. It remains to be seen whether PC and EBS are truly different in this respect or if subsets other patients with EBS may also show an excellent response to btx therapy.
Mutations in the collagen XVII gene, COL17A1, are associated with junctional epidermolysis bullosa. Most COL17A1 mutations lead to a premature termination codon (PTC), while only a few mutations result in amino acid substitutions or deletions. We describe here a novel glycine substitution, G612R. A transition c.1834G>A causing a Gly to Arg substitution at the amino acid position 612 (G612R) was found from an 8-year-old Finnish girl, who had rather severe blistering at birth already. Generalized moderate blistering continues, and she also has nail dystrophy, dental problems, poorly healing wounds and partial alopecia of the scalp. Immunohistochemical staining with basement membrane zone antibodies showed junctional blistering with reduced collagen XVII staining. Her 22-year-old brother has had blisters since the neonatal period. His disease is milder than his sister’s, but he also has partial alopecia and tooth and nail dystrophy. Based on clinical data and electron microscopic analysis in the early 1980s, he and also his sister were thought to have epidermolysis bullosa simplex. Their case demonstrates very clearly the importance of immunofluorescence antigenic mapping and mutation analysis for correct diagnosis of epidermolysis bullosa.

In order to investigate the molecular pathomechanisms of this glycine substitution, G612R was introduced into recombinant collagen XVII. The mutated collagen was expressed by transfection in COS-7 cells and its thermal stability was assessed using trypsin digestions at incremental temperatures. G612R significantly destabilized the ectodomain of collagen XVII, which manifested as 16°C lower Tm (midpoint of the helix-to-coil transition) in trypsin assay. Thus, glycine substitution interferes with tight folding of the triple helix and renders the ectodomain of collagen XVII sensitive to non-specific degradation.

**FC: 15**

**THE IMPORTANCE OF INFORMATION AND PREVENTION OF PAIN IN CHILDREN UNDERGOING PAINFUL PROCEDURES**

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According to Swedish laws, all patients, including children and their parents, have the right to get correct information before undergoing examinations or procedures. This information should be given in a positive way on a level that the child can understand, according to the child’s age and level of development.

Prevention of pain according to the procedure or surgery which is to be done should be started in a preventative way with local anaesthetics or different type of analgesia.

**FC: 16**

**POST-OPERATIVE HAND THERAPY IN RDEB**

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The aims of postoperative treatment of the hand in children with EB are to maintain optimal range of motion of the wrist, fingers and thumb and to delay recurrence of deformity in order to enlarge the possibilities of hand function. In literature two types of postoperative treatment programs are described: a program with static splinting and a program with dynamic splinting. The splints are aimed for abduction of the thumb and extension and abduction of the fingers.

In the University Medical Centre Groningen (UMCG) in the Netherlands the postoperative treatment is done by the occupational therapist/hand therapist and the rehabilitation physician, in close co-operation with the plastic surgeon. This treatment includes dynamic splinting, followed by static splinting in combination with exercises.

The dynamic splint is fabricated prior to surgery. The patient starts wearing the dynamic splint day and night after the first wound dressing, approximately ten days following surgery. The dynamic splint allows very early mobilisation of the hand to prevent recurrence of the deformities, but does not interfere with wound healing and dressings.

Approximately 21 days after surgery, depending on the progression of the healing of the wounds, a static splint is made for wearing during the night. The dynamic splint has to be worn several times a day in order to give well-balanced stretching of the wound and soft tissue of the hand. This splint is gradually cut back. The static splint should be worn at night as long as possible. Mullett (1998) wrote; “improved survival of children with RDEB seems to be accompanied by gradually decreasing severity, and relatively long-term stability may be achieved after the mid-20’s”. This might indicate that night splints should be worn at least until the mid-20’s.

After approximately ten days following surgery, an exercise-programme is initiated in order to improve the joint mobility, prevent joint-contractures and prevent adhesions of the M. flexor digitorum profundus (fdp) and the M. flexor digitorum superficiale (fds). Initially, the wound and fragile skin should be protected against bumping and risk of wound infection. It is very important for the child to regain trust in using the hands in daily activities like playing, eating and writing. Regular exercising is phased out step by step. This depends on the function and the degree of usage of the operated hand by the child.

In addition to hand problems, the child with RDEB is at risk of a great deal of other physical problems. Surgery and the postoperative treatment of the hand are very time-consuming and have a great impact on the lives of both the child and their parents or caregivers. In the process,
if and when surgery should take place the possibilities and compliance of the child and his or her environment should be taken into consideration. The final result of the operation depends on the perseverance of both.

**FC: 17**

**BODY MASS INDEX IS A RELIABLE TOOL TO OPTIMIZE LENGTH GROWTH IN PATIENTS WITH SEVERE DYSTROPHIC EB**

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The incidence of length growth retardation in patients with severe dystrophic epidermolysis bullosa is high. It is generally accepted that an optimum height gain requires an adequate weight. Until now, the weight-for-height chart is a frequently used method to determine the ideal weight. However, weight for height can be normal in children with a growth delay. On the other hand the body mass index (BMI) is a generally accepted tool to identify overweight or obesity in patients. BMI is sex and age specific.

Therefore, we hypothesized that BMI standard deviation score (SDS) is a reliable tool to determine an ideal weight in children with severe dystrophic epidermolysis bullosa.

We performed, retrospectively, a survey on five patients (age 7-27) with severe dystrophic epidermolysis bullosa (3 RDEB-HS, 2 RDEB-inv). Weight and length of the patients and length of the parents was obtained. Standard deviation curves for BMI (weight/height²) for age (years) and height (cm) for age and the target height channel were constructed using Growth Analysor 3 (version 3.0) software.

Results: A BMI SDS below -1 leads to a progressively deviation from the previously defined growth channel.

Conclusion: In clinical practice the BMI can also be feasible to establish the ideal weight and, as such, create the ideal circumstances for an optimal length growth in patients with severe dystrophic epidermolysis bullosa.

We recommend the ideal BMI SDS 0, (minimum requirement -1/2 SDS and maximum requirement +1 SDS) to establish an optimal length growth in these patients.

**FC: 18**

**IN SEVERE EB, WHAT IS DESIRABLE WEIGHT STATUS TO PROMOTE BOTH MOBILITY AND OPTIMAL NUTRITION?**

Lesley Haynes, dietician, and Jacqueline Denyer, EB specialist nurse

London

Maximising quality of life in severe EB is paramount and maintenance of optimal nutritional status and mobility are major challenges. Many factors influence, and are influenced, by both these issues and the forum will consider some of these, for example:

- # optimal weight gain when patients' heights are often compromised (use of growth and waist circumference charts), definition/assessment of overweight/underweight
- # effect of neonatal lower limb lesions and dressings on later weight-bearing
- # impact of pain relief on weight-bearing
- # promotion of wheelchair use
- # impact of steroids on excess weight gain
- # provision of adequate nutrition to promote immunity and wound healing whilst minimising tendency to accrue fat in preference to lean tissue

**FC: 19**

**NURSERY SCHOOL AND SCHOOL - TAILORING FOR EB-CHILDREN**

Nina Braathen, occupational therapist, Norway

The lecture will exemplify: 1) how the physiotherapist uses different positions and exercises to teach and maintain body control and good balance for the EB-client while at school, and 2) how the occupational therapist can work with the EB-family and school staff to tailor the environment in order to prevent sores and blisters to occur; i.e., what to look out for outside and inside buildings.

The need and contents of information to caretakers and other persons around the client will also be discussed. The main focus is to prepare for as good life quality as possible for the whole family. This includes:

1. A meaningful “every- day life” for the client
2. Independence to the greatest possible extent
3. Preventing sores, wounds and skin blisters by tailoring the environment
4. Planning for suitable transportation with mechanical aid (car, wheel chair and bicycle)
5. Providing examples for play-ideas and leisure time
Forum for Nordic Dermato-Venereology is owned by the Society for publication of Acta Dermato-Venereology which also publishes Acta Dermato-Venereology.

The following articles about Epidermolysis Bullosa have recently been published in Acta Dermato-Venereology.
We report here on three patients suffering from recessive dystrophic epidermolysis bullosa and one suffering from generalized atrophic benign epidermolysis bullosa, all of whom developed cutaneous squamous cell carcinoma. Our observations and a review of the literature suggest that squamous cell carcinoma in generalized atrophic benign epidermolysis bullosa is very frequent and has a better outcome compared to skin cancer in recessive dystrophic epidermolysis bullosa. These differences could be explained by the distinct pathophysiology and clinical course of each of these variants of epidermolysis bullosa. In contrast to UV-induced skin cancer, the tumours in epidermolysis bullosa develop on distal extremities at sites of chronic wound healing. The cases reported here underline the exceptional importance of early histopathological assessment of suspicious skin lesions in patients with epidermolysis bullosa. Key words: epidermolysis bullosa dystrophicans Hallopeau–Siemens; generalized atrophic benign epidermolysis bullosa; skin cancer.

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Epidermolysis bullosa (EB) comprises a heterogeneous group of genetically determined disorders in which minor trauma leads to blister formation on the skin and mucous membranes (1). Recessive dystrophic EB Hallopeau–Siemens (RDEB-HS) is one of the most severe forms of EB, with blister formation below the lamina densa due to mutations in the gene of type VII collagen resulting in lack of, or abnormal, anchoring fibrils (2). It is characterized by chronic mucocutaneous erosions/ulcers and atrophic scarring, resulting in pseudosyndactyly, scarring alopecia, symblepharon, dysphagia due to oesophageal strictures, loss of nails and teeth and formation of multiple milia. Chronic wound healing and inflammation cause hypochromic anaemia and growth retardation. RDEB-HS is associated with profound morbidity requiring intensive medical care throughout life. Life expectancy is reduced in patients with RDEB-HS, with a substantial cumulative risk of death in late adolescence (3).

Generalized atrophic benign EB (GABEB), which has recently also been termed junctional EB non-Herlitz (4), is a less severe but still disabling autosomal recessive type of EB. Mucocutaneous blisters arise due to cleavage in the lamina lucida and heal without scar but often result in notable atrophy. Atrophic alopecia, dental abnormalities and nail dystrophy are characteristic clinical features (5). In addition, some patients have large melanocytic naevi at sites of prior blistering (6). There is a tendency towards spontaneous improvement in symptoms in adulthood. As most patients live to adulthood, GABEB has a more favourable prognosis than RDEB-HS.

Chronic inflammation and tissue repair are thought to be responsible for tissue alterations which allow tumour formation from pre-existing transformed cells (7). Tumours arising under such conditions are most commonly squamous cell carcinomas (SCCs) and have been described as a complication of chronic infections (e.g. lupus vulgaris, Hansen’s disease, gummatous syphilis and chronic osteomyelitis), chronic ulcerations (e.g. venous stasis, decubitus ulcers), burn scars and, of particular relevance to this article, EB (8). Under these conditions, and also in EB, these carcinomas tend to exhibit very aggressive growth and lead to high mortality rates within this patient group (1, 3).

We report herein on our experience with four patients with EB – one suffering from GABEB and three from RDEB-HS – all of whom developed SCC during the course of the disease.

CASE REPORTS

Patient suffering from junctional EB

Case 1. This male patient was born in 1939 with a chronic blistering disorder. He is the oldest son of consanguineous parents (cousins); three of eight surviving siblings are affected by the same condition [see Hintner & Wolff (9)]. In both the patient and the affected siblings, the diagnosis of GABEB was established by histopathology, electron microscopy and immunofluorescence (absence of the 180 kD bullous pemphigoid antigen, i.e. type XVII collagen). Genetic studies later revealed a 4003delTC mutation of the type XVII collagen gene (5, 10).

Blistering of the skin occurred continuously throughout his life, with predilection for the hands, feet, knees and elbows. There was a clear spontaneous decrease in disease activity after childhood. In the chronically affected areas, the skin was atrophic and reddish and hair follicles were absent. During childhood, blisters had also developed in the oral mucosa. Atrophic alopecia developed after the age of 20 years; eyelashes, eyebrows and beard were sparse, and pubic and axillary hair had never developed. Nails were partially absent and the remaining nails were dystrophic. On the right aspect of the trunk, two large shagreen-like EB naevi were present.

At the age of 58 years, a verrucous exophytic tumour was noticed on the back of the left foot. Histopathology revealed a well-differentiated SCC. The entire lesion was excised and the defect closed with a split skin graft. No regional or distant metastases were detected either then or during the follow-up period.

Patients suffering from dystrophic EB

Case 2. A 26-year-old female patient without a family history of bullous disorders exhibited persistent generalized mucocutaneous blistering with subsequent scarring since birth. Owing to severe and early dental caries, dysphagia allowing only a liquid diet, chronic
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constipation, dilatative cardiomyopathy and recurring skin and airway infections the patient showed severe growth retardation (height: 115 cm; body weight: 16 kg). Her hands and feet exhibited pseudosyndactyly with complete loss of nails and formation of multiple milia. Furthermore, the patient developed symblephara of both eyes, scarring alopecia of the scalp with massive crusting, chronic hypochromic, microcytic anaemia requiring multiple blood transfusions, thrombocytosis of up to 800,000/μl and leukocytosis of about 14,000/μl. The clinical course and histopathologic features, including immunofluorescent antigen mapping, were diagnostic for RDEB-HS. Systemic therapy with phenytoin, vitamin E and etretinate had failed to show objective improvement.

In 1998, the patient presented with a rapidly growing exophytic tumour of the left sole measuring 6 cm in diameter (Fig. 1), accompanied by large firm subcutaneous masses of the left femoro-inguinal region (Fig. 2). Histology revealed a moderately differentiated SCC with inguinal lymph node metastases. No further metastases were detected by sonographic or radiologic examinations.

As the patient and her parents refused any surgical intervention, radiotherapy (60 Gy) was performed to the left foot, left groin and left thigh. Despite an initial reduction in tumour mass, the tumour resumed growth later during therapy. Seven months later the patient died of weight loss and heart failure.

Case 3. A 33-year-old woman had developed severe persistent bullous eruptions of the skin and oral mucosa soon after birth. In her family one of her two brothers suffered from the same disorder. Owing to scarring with milia formation, pseudosyndactyly, complete loss of nails and scarring alopecia, the diagnosis of RDEB-HS was made and confirmed by electron microscopy (dermal cleavage and rarefaction of anchoring fibrils). In the course of the disease the patient suffered from dysphagia due to oesophageal strictures, scarring of genital skin and mucosa with contraction of the introitus vaginae, chronic erosive keratitis of both eyes with pannus formation, growth retardation (height: 144 cm; body weight: 32 kg) and persistent anaemia, with haemoglobin levels varying between 6 and 9 g/dl.

At the age of 33 years the patient was admitted to hospital because of a hypertrophic granulomatous lesion in scar tissue of the right prepatellar region (Fig. 3) which had developed after a trauma a few months earlier. Biopsy revealed a well-differentiated SCC and the tumour was surgically removed. After biopsy, multiple lymph nodes of the right inguinal region were found to be enlarged, suspicious for reactive lymphadenopathy or metastatic disease. In order to differentiate between an inflammatory process alone, as often occurs in patients with EB, and an additional lymph node metastasis a sentinel lymph node biopsy was performed. Histopathology of the lymph node revealed a squamous cell carcinoma.

Fig. 1. Exophytic tumour on the left foot of patient 2. Histopathology revealed a squamous cell carcinoma.

Fig. 2. Metastatic tumour tissue on the left proximal thigh of patient 2 at the time of presentation with the primary tumour.

Fig. 3. The non-healing erosive area prepatellar on the right knee of patient 3 developed hypertrophic granulations (arrow) with the histopathology of a differentiated squamous cell carcinoma.
revealed only inflammation without metastatic tumour cells. During follow-up, the enlarged lymph nodes regressed spontaneously and there were no signs of a metastatic process.

Case 4. This 28-year-old male patient is the oldest of four siblings. Since birth the patient had suffered from a chronic mucocutaneous blistering disorder which led to scarring, formation of milia within scars, pseudo syndactyly of hands and feet, scarring alopecia, complete loss of nails and teeth, microstomia, oesophageal stenosis and growth retardation. The diagnosis of RDEB-HS was made by histopathology and antigen mapping.

At the age of 26 years, the patient had developed a deep bacterial infection of the right hand. Despite vigorous antibiotic treatment and appropriate surgical measures, which finally included the amputation of the fourth and fifth fingers, wound healing was still not complete after 1 year. At this time, an exophytic verrucous tumour arose on the back of the hand within the chronic wound area which proved to be a well-differentiated SCC by histopathology. No regional or distant metastases were detected. The patient’s hand had to be amputated. Further treatment was refused by the patient.

DISCUSSION

Malignancies of the skin are a well-documented complication of EB. The most common neoplasms are SCCs and they occur most commonly in RDEB-HS; including this series, 56 patients with generalized RDEB associated with SCC have been reported, most commonly as single case reports (11–17). The National Epidermolysis Bullosa Registry (NEBR) registered 87 RDEB-HS patients with SCC and 12.6% of the RDEB-HS patients had at least one SCC at the time of first presentation to the NEBR. By the age of 25 years, 21.7% were at risk of having had at least one SCC, and the risk rose steadily to 39, 6%, 53% and 76.5% by ages 30, 35 and 60 years, respectively (1).

SCCs in RDEB-HS are typically well-differentiated, but they frequently show rapid growth and metastatic spread with an overall poor prognosis (1, 3), as is common for non-actinic variants of SCC (18). In contrast to UV-induced SCCs, they are mostly located on the distal extremities (19). Sometimes more than one primary SCC is found in these patients (17).

In contrast, junctional EB is probably very rarely complicated by skin tumours: only four cases have been reported, who developed a total of eight SCCs and three keratoacanthomas (Table I) (20–22). All these patients were males and had GABEB; all SCCs were exclusively located on the distal extremities and were well-differentiated. No lethal outcome has been reported to date.

Although it is difficult for numerous reasons to compare the biological behaviour of SCCs arising in GABEB to those in RDEB-HS, it appears that they occur less often, at a later age, predominantly in males and behave less aggressively. This may be linked to the generally milder disease severity of GABEB, with less inflammation and little scarring. Also, the disruption below the basal lamina in RDEB-HS may facilitate lymphogenic spread of tumour cell aggregates. Based on conventional histological grading schemes the prognosis of SCCs is not predictable in these patients (1). Possibly, genetic profile studies of cutaneous SCCs in the future will explain the aggressive behaviour of these tumours in RDEB-HS patients (23).

The cases reported here underscore the necessity of monitoring EB patients for the emergence of SCCs at reasonable intervals, e.g. every 3–6 months, by the same skilled examiner. Although patients with RDEB-HS are at highest risk, SCCs may also occur in other types of EB, such as GABEB. Only few cases of SCCs in GABEB are documented, but the course of the disease is likely to resemble that of non EB patients. Notably, the case presented here is, to the best of our knowledge, the first example of SCC complicating junctional EB with a documented primary defect in type XVII collagen.

Owing to the reduced longevity of patients with RDEB-HS, this severe type is less frequent in the adult population than milder types of dystrophic EB, such as dominant dystrophic EB Cockayne–Touraine, dominant dystrophic EB Pasini and localized variants of dystrophic EB. Most of these types are at increased risk of developing SCC (1) and therefore also require careful clinical surveillance.

Cutaneous ulceration and hyperkeratotic crusting are common findings in EB but should always arouse suspicion of SCC when persistent or enlarging. We therefore propose that persistent hyperkeratotic crusting, especially on distal extremities, should be detached for clinical (and later histological) assessment of the underlying skin changes.

In our three patients with RDEB-HS complicated by SCC, one patient had regional lymph node metastasis at the time of diagnosis. Another patient had reactive inflammatory lymphadenopathy without metastatic tumour cells. This case under-

### Table I. Squamous cell carcinoma (SCC) in five male patients with generalized atrophic benign epidermolysis bullosa: a review of the literature

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Location</th>
<th>Diagnosis</th>
<th>Differentiation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>43</td>
<td>Left lower leg</td>
<td>Keratoacanthoma</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left lower leg</td>
<td>Keratoacanthoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left lower leg</td>
<td>Keratoacanthoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>Dorsal hand</td>
<td>SCC</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower leg</td>
<td>SCC</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>Right calf</td>
<td>SCC. Well diff.</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left shin</td>
<td>SCC. Well diff.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCC</td>
<td>SCC. Well diff.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>Inner ankle</td>
<td>SCC</td>
<td>ND</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower leg</td>
<td>SCC</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left calf</td>
<td>SCC</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right shin</td>
<td>SCC</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>58</td>
<td>Left foot</td>
<td>SCC</td>
<td>Well diff.</td>
<td>Present work</td>
</tr>
</tbody>
</table>

SCC: squamous cell carcinoma; ND = not determined; diff. = differentiated.
In acting or believing that non-metastatic lymphadenopathy can occur in EB patients with SCC and histological assessment is necessary in order to differentiate between reactive inflammatory lymph node augmentation alone or a suspected metastatic process. As SCC in RDEB-HS is known to behave aggressively, with a rather high potential for lymphogenic metastasis, sentinel lymph node dissection could be of early diagnostic, prognostic or therapeutic value. Of course, the utility of such a technique in this group of patients is unknown at present and needs to be confirmed in a larger number of patients.

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Dystrophic epidermolysis bulosa pruriginosa, a subtype of epidermolysis bullosa dystrophica and a heterogeneous inherited disease, is characterized by pruritus, excoriated nodular prurigo-like lesions, skin fragility, altered anchoring fibrils and loss of dermal-epidermal adhesion. Mutation in type VII collagen gene (COL7A1) is thought to be implicated in the underlying change for dystrophic epidermolysis bullosa pruriginosa. We report here a large family of dominant dystrophic epidermolysis bullosa pruriginosa. Mutation analysis using polymerase chain reaction and direct sequencing demonstrated a novel nucleotide substitution of 6899A→G in exon 87 in one COL7A1 allele of the proband and 18 affected family members. This substitution was not found in 100 normal alleles. Polymerase chain reaction and sequencing of the cDNA, reverse transcribed from the proband’s peripheral lymphocyte RNA, suggest that this mutation causes aberrant COL7A1 mRNA splicing of exon 87 skipping. Clinical features and pedigree analysis suggest that 6899A→G substitution is a mutation with full penetrance and variable expressivity. Key words: COL7A1 mutation; dystrophic epidermolysis bullosa; DEB pruriginosa.

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Dystrophic epidermolysis bulosa (DEB) is an inherited skin fragility disorder, with characteristic trauma-induced blistering associated with scarring and nail dystrophy (1). The ultrastructural hallmark of DEB is the separation in sub-lamina densa level of the dermal-epidermal junction, usually associated with abnormal quality or quantity of anchoring fibrils (2). DEB pruriginosa is a rare clinical type of DEB, characterized by marked itching and the presence of nodular prurigo-like or lichenoid lesions (3, 4). Autosomal dominant, autosomal recessive, and sporadic inheritance patterns have been described in this disease (4, 5). In this study, we screened mutation in the COL7A1 gene in a Chinese family with dominant DEB pruriginosa.
phosphate buffer, postfixed in OsO4, dehydrated in ethanol and embedded in epoxy resin. An ultra-thin section was stained with uranyl acetate and lead citrate and examined using a JEOL 100 CX-II electron microscope.

**Mutation detection**

Genomic DNA was extracted from peripheral blood lymphocytes of the proband and 18 other affected family members available for study by standard method (7). Polymerase chain reaction (PCR) was used to amplify all of the 118 exons of the COL7A1 gene from the proband's genomic DNA using oligonucleotide primer pairs described elsewhere (according to GenBank accession numbers L02870 and L23982) (8). Specifically, the following primers were used to amplify a 404-bp segment spanning exons 86–87 and their flanking intron sequences: left primer, 5'-GTC AGG TGT GCT CCA GG; right primer, 5'-TGG AAA CAG GCT TGT GGG TG. The amplification condition was 94°C for 5 min, followed by 94°C for 45 s, 63°C for 45 s, and 72°C for 45 s for 33 cycles in a total volume of 50 μl containing 100–200 ng genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 10 pmol of each primer and 1.25U of Taq DNA polymerase. The PCR product was purified in a 1.5% agarose gel and then subjected to direct sequencing on an automated DNA sequencer (ABI Prism 377 Sequencer).

**PCR using allele-specific oligonucleotide primers**

We used allele-specific oligonucleotide primers to detect the nucleotide substitution of 6899A→G in 20 members of this family (including one healthy member and 19 patients) and 50 unrelated healthy individuals. Primers for wild-type allele: left, 5'-ACA GGG GTC TCC AGG TTT GC; right, 5'-GAA GTC AGG GTC AAA GAT CAC CT. Primers for mutant allele: left, 5'-ACA GGG GTC TCC AGG TTT GC; right, 5'-GAA GTC AGG GTC AAA GAT CAC CC. The expected product size is 402 bp.

**Reverse transcription polymerase chain reaction and sequencing**

To examine the consequences of the 6899A→G transition in exon 87, reverse transcription-PCR (RT-PCR) was performed using total RNA extracted from the proband's peripheral lymphocytes. cDNA was generated from the total RNA by random priming and reverse transcription. cDNA 6783–7083 was amplified in a total volume of 50 μl containing 100–200 ng genomic DNA, 1× PCR buffer, 1.5 mM MgCl2, 10 pmol of each primer and 1.25U of Taq DNA polymerase. The PCR product was purified in 2% agarose gel and cloned into pBluescript Sk+ Vector (Stratagene). Recombinant plasmids were sequenced on an ABI Prism 377 Sequencer, using T7 primer.

**RESULTS**

**Histopathologic, immunofluorescent and electron microscopic studies**

Examination of the proband's skin specimen by light microscopy showed compact hyperkeratosis, mild acanthosis, dermal-epidermal separation and dense fibrosis in the upper dermis (results not shown). Indirect immunofluorescent study, using monoclonal antibody LH 7:2 recognizing collagen VII, revealed bright and linear fluorescence located in the dermal-epidermal junction (results not shown). Electron microscopic examination of a lichenoid, papular lesion showed abnormal high density of collagen bundles beneath the dermal-epidermal junction (results not shown).

**Mutation detection, including screening of other family members**

All exons and their flanking regions of the COL7A1 gene of the proband were scanned by PCR amplification and direct sequencing. The proband showed an A-to-G transition at nucleotide 6899 (numbering from the A of the translation initiation codon ATG) in one allele (Fig. 3). This point mutation is located in exon 87 at –2 of the donor site. We performed PCR using allele-specific oligonucleotide primers to detect the nucleotide substitution of 6899A→G in 20 family members (19 patients and 1 healthy member) and 50 unrelated healthy individuals. In 19 patients, the 402 bp fragment could be amplified by PCR using primers either for wild-type allele or for mutant allele. One of the PCR products was sequenced and demonstrated a heterozygous 6899A→G mutation. In the healthy family member and 50 unrelated healthy individuals, however, this fragment could be amplified by PCR using primers only for the wild-type allele. One of these PCR products was sequenced and showed a homozygous A at nucleotide 6899.

Eighteen other DEB pruriginosa patients in this family were examined clinically. As shown in Table I, their age at onset, area and severity of the skin symptoms varied
Table I. Clinical features of 19 patients available for examination

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Age at onset</th>
<th>Location of skin lesions</th>
<th>Pruritus#</th>
<th>Nail involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-13</td>
<td>72</td>
<td>7–8</td>
<td>Legs, mostly on shins</td>
<td>+</td>
<td>Toenail atrophy</td>
</tr>
<tr>
<td>II-17</td>
<td>66</td>
<td>1</td>
<td>Limbs</td>
<td>+ +</td>
<td>Toenail absent</td>
</tr>
<tr>
<td>III-5</td>
<td>48</td>
<td>5–6</td>
<td>Limbs, and hands</td>
<td>–</td>
<td>Absent</td>
</tr>
<tr>
<td>III-7</td>
<td>41</td>
<td>4–5</td>
<td>Hands, feet, elbows and knees</td>
<td>+/+ +</td>
<td>Toenail absent</td>
</tr>
<tr>
<td>III-10</td>
<td>50</td>
<td>1</td>
<td>Legs, elbows and ankles</td>
<td>+/+ +</td>
<td>Nail thickness</td>
</tr>
<tr>
<td>III-13</td>
<td>63</td>
<td>1</td>
<td>Trunk and limbs</td>
<td>+ +</td>
<td>Nail thickness, and toenail absent</td>
</tr>
<tr>
<td>III-14</td>
<td>62</td>
<td>10</td>
<td>Legs and forearms</td>
<td>+ +</td>
<td>Absent</td>
</tr>
<tr>
<td>III-30</td>
<td>1</td>
<td>7–8</td>
<td>Limbs</td>
<td>+</td>
<td>Absent</td>
</tr>
<tr>
<td>IV-2</td>
<td>13</td>
<td>5</td>
<td>Limbs, hands and feet</td>
<td>–</td>
<td>Toenail absent</td>
</tr>
<tr>
<td>IV-8</td>
<td>13</td>
<td>0.1</td>
<td>Legs</td>
<td>+/+ +</td>
<td>Atrophy</td>
</tr>
<tr>
<td>IV-9</td>
<td>22</td>
<td>8</td>
<td>Trunk, elbows and knees</td>
<td>+</td>
<td>Toenail absent</td>
</tr>
<tr>
<td>IV-11</td>
<td>35</td>
<td>3</td>
<td>Limbs</td>
<td>+ +</td>
<td>Toenail absent and nail thickness</td>
</tr>
<tr>
<td>IV-14</td>
<td>42</td>
<td>10</td>
<td>Limbs</td>
<td>+ +</td>
<td>Nail thickness</td>
</tr>
<tr>
<td>IV-16</td>
<td>38</td>
<td>4–5</td>
<td>Limbs</td>
<td>+ +</td>
<td>Thickness</td>
</tr>
<tr>
<td>IV-36</td>
<td>29</td>
<td>4–5</td>
<td>Legs and forearms</td>
<td>+ +</td>
<td>Nail thickness and toenail atrophy</td>
</tr>
<tr>
<td>IV-41</td>
<td>17</td>
<td>2</td>
<td>Legs, hands, elbows and back</td>
<td>+</td>
<td>Toenail dysmophia</td>
</tr>
<tr>
<td>V-2</td>
<td>10</td>
<td>1</td>
<td>Limbs, hands and feet</td>
<td>+ +</td>
<td>Toenail atrophy</td>
</tr>
<tr>
<td>V-4</td>
<td>16</td>
<td>5–6</td>
<td>Trunk, limbs</td>
<td>+ +</td>
<td>Normal</td>
</tr>
<tr>
<td>V-6</td>
<td>13</td>
<td>4–5</td>
<td>Legs and feet</td>
<td>+</td>
<td>Thickness</td>
</tr>
</tbody>
</table>

For details see Fig. 2. All were found to have heterozygous 6899 A→G substitution in the COL7A1 gene.

#Severity of pruritus: – = no; + = mild; + + = severe.

considerably. However, all of them bore a substitution of 6899A→G in one of the COL7A1 alleles, as identified by PCR using allele-specific oligonucleotide primers.

**RT-PCR analysis**

RT-PCR of total RNA from the proband’s peripheral lymphocytes revealed two bands on agarose electrophoresis: a band of normal size (301 bp) and an extra band of smaller size (232 bp) (Fig. 4A). After cloning of the PCR products into plasmids and sequencing of the recombinant plasmids, two kinds of DNA sequence were obtained. One had a normal sequence and the other lost 69 bp nucleotides corresponding to those of the entire exon 87 (Figs 4B and 4C) and resulting in the deletion of 23 amino acid residues in the COL7A1 polypeptide.

**DISCUSSION**

The term “epidermolysis bullosa pruriginosa” was proposed by McGrath et al. (4). DEB pruriginosa is a rare form of DEB, characterized by intense pruritus usually accompanied by nodular prurigo-like lesions and lichenoid papulars, hypertrophic violaceous scars and nail dystrophy. Blisters and erosions may not be evident clinically. This condition must be distinguished clinically from a variety of acquired inflammatory dermatoses, such as hypertrophic lichen planus, keratosis lichenoides chronica, nodular prurigo and dermatitis artefacta (5).

Here we report on a large Chinese family with 59 patients in which indirect immunofluorescent and histological features of the proband confirmed the clinical diagnosis of DEB pruriginosa. In this family, we found a 6899A→G mutation in the COL7A1 gene, which is located at –2 of the donor site of exon 87 and was not reported previously (9–11). This mutation was closely linked to the individuals with DEB pruriginosa in this family and was not found in 50 normal, unrelated individuals. Therefore, it is likely to be a pathogenic mutation that caused DEB pruriginosa in this family.

This mutation may affect the splicing of the corresponding exon, because the A nucleotide in donor position –2 in human genes is relatively conserved in 58% of the splice junctions (12). Another possibility is that it may change the codon glutamine (CAG) at 2,300 to CCG and resulting in the deletion of 23 amino acid residues in the COL7A1 polypeptide. The deleted segment resides within the collagenous domain of 8 Gly-X-Y triple repeat sequences. In many studies the importance of the Gly-X-Y repeat sequence in type VII collagen such as hypertrophic lichen planus, keratosis lichenoides chronica, nodular prurigo and dermatitis artefacta (5).
In this pedigree, all of the offspring from normal parents were normal (see II-8, III-16, 18–21, 23, 38–44, and 46 in Fig. 2), and roughly half of the progeny from a DEB pruriginosa parent was abnormal. This suggests that the 6899 A→G substitution is a mutation with full penetrance, though we had only one normal individual in this family with the consent of mutation screening. Moreover, patients in this family showed variable clinical features in age at onset, area and severity of skin lesion. Other factors (genetic and environmental) responsible for the variable expressivity of DEB pruriginosa need to be studied further.

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Management of Generalized Pruritus in Dominant Dystrophic Epidermolysis Bullosa Using Low-dose Oral Cyclosporin

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Sir,

Dystrophic epidermolysis bullosa (DEB) comprises a group of inherited mecanobullous disorders characterized by blisters and erosions occurring after minor traumas frequently associated with milia formation, nail dystrophy and scarring. The blisters occur at sublamina densa level associated with quantitative or qualitative changes in anchoring é brils due to a congenital abnormality of collagen VII (1–3). Several subgroups and phenotypical variants of DEB have been described (4). Itching is a common symptom in many types of epidermolysis bullosa (EB), including junctional and dystrophic types (1). Here we present a patient dia- gnosed as DEB associated with intense pruritus without typical prurigo-like or lichenoid lesions of epidermolysis bullosa pruriginosa (EBP).

CASE REPORT

A 27-year-old female patient was referred with a complaint of severe itching. She had been suffering from skin blistering after minor trauma since the age of 3 months. Pruritus started 2 years ago. She had no atopic history. The father, two sisters, one brother had similar complaints, showing a probable otosomal dominant hereditary pattern. Dermatological examination revealed generalized erythematous plaques with crust formation, scattered excoriations, occasionally atrophic scars with milia formation, and some intact bullae localized on the trunk, back skin and extremities of the patient (Fig. 1a, c). Most of the toenails were dystrophic. General health was not impaired. Histopathological examination showed dermal blistering at papillary dermis level. Direct and indirect immunoreoreuence was performed without showing a co-existing acquired autoimmune bullous disorder. Electronmicroscopical investigation revealed that the blister was beneath the lamina densa of the dermal-epidermal junction. Serum iron levels, IgE, thyroid function, renal function and liver function tests were in the normal ranges. The patient received 2.5 mg/kg/day of oral cyclosporin. During the therapy, the patient was carefully monitored by renal function tests and blood pressure. The majority of the lesions had responded to the therapy after 2 months (Fig. 1b, d).

DISCUSSION

Fine et al. (4) reported the revised classification system of inherited EB, which was a consensus paper of the second international consensus meeting on diagnosis and classification EB. According to this classification system, DEB has three major subtypes, including dominant DEB (DDEB), recessive DEB-Hallapeau Siemens (RDEB-HS) and recessive DEB-non Hallapeau Siemens (RDEB-nHS). However, EBP is classified in another table as one of the rare phenotypic variants of DEB. The term EBP was first used by McGrath et al. (1) to describe a group of patients with DEB characterized by lichenoid lesions, toenail dystrophy and hypertrophic violaceous scars associated with intense pruritus. It is well known that pruritus may also occur in other EB subtypes, including junctional and dystrophic types and many cases associated with generalized pruritus cannot be included within any classical subgroup of DEB (1).

Our patient showed the clinical, histopathological and ultrastructural features of DEB, the probable dominant type. She also had intense pruritus. However, the lesions were not typical for EBP. It was recently reported that all types of DEB were due to alterations of the same gene (COL7A1) that encoded type VII collagen, the major component of the anchoring é brils (4–7). DEB may be considered as a group of diseases presenting a wide range of phenotypic features in different patients based on the same genetic pathology.

The itchy lesions of our patient were more generalized than those of the other affected family members. The pruritus may be the consequence of an abnormal reactivity of some patients against their inherited bullous disorder. This reaction may also be considered as a late reaction in the blistering site, probably due to histamine liberation (2). Our patient did not show typical prurigo-like or lichenoid features of EBP, so we made the diagnosis of DDEB associated with generalized pruritus.

The treatment of DEB is generally based on the prevention of blister formation. Until today, oral and topical agents, including pulse topical corticosteroid therapy, cyclosporin, minocyclin, phenytoin, tocopherol acetate and tacrolimus, have been reported to be successful in occasional cases of DDEB, RDEB and EBP (8–14). The lesions of our patient responded very well to oral cyclosporin after 2 months of therapy. On the other hand, the remission of blisters can be contributed to the relief of severe itching and scratching. According to the literature and our own observation, we suggest that low-dose oral cyclosporin is the best medical treatment for DEB associated with pruritus. However, we believe that this treatment must be used with care for short periods, since we know that neoplasia formation.
is one of the risks of long-term use of cyclosporin and DEB patients have an increased risk of developing squamous cell carcinoma.

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A Novel Insertion Mutation in COL7A1 Identified in Hallopeau-Siemens Recessive Dystrophic Epidermolysis Bullosa


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Sir,

Different forms of dystrophic epidermolysis bullosa (DEB) caused by mutations in the type VII collagen gene (COL7A1, GenBank L23982, L02870) are inherited either in an autosomal dominant or autosomal recessive fashion. Hallopeau-Siemens (HS) type is the most severe form of recessive DEB and is characterized by the total absence of type VII collagen in the epidermal basement membrane. The mutations in DEB vary widely and more than 170 kinds of mutations have been reported so far, most of them representing single nucleotide substitutions. We report on a child with HSDEB showing compound heterozygous mutations with a novel four base-pair insertion in one allele, and a nonsense point mutation on the other allele of COL7A1.

CASE REPORT

A male patient was born with bullae and erosions predominantly on the trunk and the inverse sites of the axillaries, hands, feet, elbows, knees and genito-anal regions starting continuously or shortly after birth (Fig. 1a, b). The mucous membranes of the mouth, nose and conjunctiva of the eye were also erosive (Fig. 1a). The fingernails and toenails dropped off 3 months after birth (Fig. 1c). The infant's skin was fragile and bullae appeared easily after minor mechanical trauma. He was fed by nasogastric tube because he could not suckle milk from his mother’s breast owing to continuous formation of bullae in his mouth. The patient was the parents’ first child and there was no family history of any signs of skin fragility or nail dystrophy.

Skin biopsy specimens obtained from a fresh blister were snap frozen with liquid nitrogen and 5 mm cryostat sections were prepared. Immunostaining for antigen mapping was performed using S1193 (BPAG1), HDD20 (BPAG2), GoH3 (α-6 integrin), 3E1 (β-4 integrin), 121 (plectin), 19DEJ1 (uncein), GB3 (laminin 5), LH7.2 (type VII collagen), and type IV collagen as described elsewhere (1). Histologic examination revealed a subepidermal blister. Immunohistochemically, type VII collagen (LH7.2) was completely negative. Other basement membrane zone antigens, including plectin, α-6 β-4 integrins, type XVII col...
lagen (BPAG2), and laminin 5, were expressed normally. Antigen mapping confirmed the occurrence of blister formation below the lamina densa. An electron microscopy examination revealed that there were no normal anchoring fibrils at the basement membrane zone. Genomic DNA extracted from peripheral blood mononuclear cells was examined for mutations in COL7A1, as described elsewhere (1, 2). In brief, the PCR product was screened by conformation sensitive gel electrophoresis. The products that showed hetero-duplex bands were directly sequenced by an automated sequencer (ABI PRISM 3100 Genetic Analyzer, PE Biosystems, Foster City, CA, USA). The mutation was confirmed by restriction enzyme digestion; the 434insGCAT was confirmed with SphI, and R2261X with TaqI. Since the present mutations were novel and had not been previously reported, we examined DNA extracted from 50 unrelated healthy Japanese volunteers as a control. All were negative for the 434insGCAT and R2261X mutations. Genetic screening of the COL7A1 gene revealed that both mutations led to premature termination codons (PTC). The paternal mutation (434insGCAT; Fig. 1d) resulted in a frame shift and lead to PTC in exon 5, at amino acid position 180. The maternal mutation (R2261X) (Fig. 1e) was caused by a C to T transition at nucleotide 6781 in exon 86.

DISCUSSION

We identified a four-base-pair insertion (434insGCAT) in the present case of HS-RDEB. According to The Human Mutation Database Cardiff (http://archive.uwcm.ac.uk/uwcm/mg/search/128750.html), multiple base insertion is a rare cause of DEB. Among 174 different kinds of mutations registered before July 1, 2002, only 10 were insertion mutations; of 7 cases of single-basepair insertions (3 – 6), 2 were two-base-pair insertions (5, 6), and only one was a four-base-pair insertion (6). The maternal mutation R2261X was also novel. In the present case, the severe clinical symptoms could be explained by a total absence of type VII collagen owing to the mutations in both alleles resulting in PTC.

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Simultaneous Occurrence of Three Squamous Cell Carcinomas in a Recessive Dystrophic Epidermolysis Bullosa Patient

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Sir,

Squamous cell carcinoma (SCC) is a life-threatening and frequent complication in recessive dystrophic epidermolysis bullosa (RDEB) (1). SCC in RDEB usually develops between the fourth and fifth decades and predominantly on the extremities (2). We have identified three independent SCCs occurring on the extremities of an RDEB patient who had failed to undergo regular dermatological consultations.

CASE REPORT

A 44-year-old Japanese woman who had no history of parental consanguinity or family history of bullous disease consulted our hospital complaining of painful nodules on both her feet (Fig. 1). Her own history revealed that within a few days of birth she developed trauma-induced blisters with scarring. She was soon diagnosed by a dermatologist as having RDEB. Detailed examination later revealed a non-Hallopeau-Siemens RDEB subtype with G1815R and 5818delC mutations in COL7A1 (3). Despite appropriate dermatologic care, she was suffering from continuous widespread erosions over her limbs and back, extensive dystrophic scarring, alopecia, pseudosyndactyl fingers and toes, loose nails, oesophageal stenosis and dental caries. She had stopped regular medical examination spontaneously 20 years previously because her condition failed to improve. Histology of the dome-shaped painful masses on her right foot, the left medial malleolus and the left heel showed all three tumours to be invasive SCCs. They were treated by wide excision. Too much emphasis on the possibility of developing carcinomas may cause patient disapproval and lead to the patients and their families losing hope for coping with this disabling disease. However, early and on-going patient education raising the awareness of developing SCC from ulcers and scars is necessary for all patients with RDEB. We should at least repeatedly advise them to keep in touch with their dermatologist and not to stop visiting completely.

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INVESTIGATIVE REPORT

Pathogenic Mechanisms in Epidermolysis Bullosa Naevi

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Epidermolysis bullosa naevi are large, eruptive melanocytic naevi which frequently arise in areas of former blisters in patients suffering from inherited epidermolysis bullosa. Morphologically, these naevi are similar to malignant melanoma, although so far no malignant transformation has been observed. To investigate the pathogenesis of these moles we documented their clinical evolution and their histopathological and immunocytological characteristics in three patients with epidermolysis bullosa. Clinically, we observed signs of malignant transformation, such as explosive growth and the occurrence of satellite lesions of epidermolysis bullosa naevi. However, malignant melanoma was excluded by histopathological evaluation. In addition, we evaluated the concentrations of various factors known to stimulate melanocyte growth in blister fluid. Human interleukin 8, basic fibroblast growth factor, human hepatocyte growth factor, GM-CSF, leukotriene B4 and prostaglandin E2 revealed concentrations comparable with the levels in inflammatory blisters. We were able to detect individual melanocytes/naevus cells in blister fluid from a blister over an epidermolysis bullosa naevus. The factors detected in the blister fluid might therefore promote the proliferation, migration and melanogenesis of disconnected melanocytes/naevus cells representing the basis of the highly dynamic appearance of epidermolysis bullosa naevi. Key words: blister fluid; cytokines; epidermolysis bullosa; growth factors; naevi.

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Epidermolysis bullosa (EB) hereditaria comprises a group of mechano-bullous diseases characterized clinically by blister formation of the skin and mucous membranes following minor trauma; they are caused by mutations in the genes of various structural proteins of the dermo-epidermal basement membrane zone (1).

We have recently described a new entity of naevi which predominantly occur in recessively inherited forms of EB, i.e. EB simplex (EBS), non-Herlitz junctional EB and dystrophic EB (DEB): “EB naevi” are eruptive, large, asymmetrical, irregularly pigmented, highly dynamic melanocytic lesions with sharply demarcated borders outlining former blisters (2, 3). These moles are highly suspect if one applies the ABCD rules, which have been elaborated for clinical recognition of early malignant melanoma (4, 5). Microscopically, they either show aspects of common naevus cell naevi or, quite frequently, reveal criteria mimicking malignant transformation similar to those seen in persisting naevi/pseudomelanoma (6). Despite the clinically and sometimes also histopathologically highly suspect features of EB naevi, we have not noticed malignant transformation of these moles in over 20 years of clinical surveillance (3). Recent studies on EB naevi have been carried out by Annicchiarico et al. (7) and Bichel et al. (8).

The pathogenesis of common acquired naevi has not yet been elucidated. Cramer (9) speculated that these and congenital melanocytic naevi derive from melanocyte precursors located in nerve sheaths, which undergo a four-step differentiation pathway (nerve sheath precursor stage, dermal migratory stage, junctional migratory stage and dendritic stage). With regard to the pathogenesis of EB naevi, it has been shown histopathologically that melanocytes probably deriving from incipient naevi or subclinical nests of naevus cells are found in the blister cavity (2). This has led to the assumption that single melanocytes/naevus cells, after disconnecting and settling down, proliferate excessively in the microenvironment of epidermal regeneration, thus constituting the malignant aspect with alarming size and satellite lesions. So far, however, there has been neither formal proof of single melanocytes in the blister fluid nor data on growth factors/cytokines promoting the proliferation of melanocytes (10, 11) in EB blisters. We therefore investigated the clinical evolution, histopathology and immunocytology of EB naevi in three children with recessive EBS, EBS with muscular dystrophy and recessive DEB, respectively.

CASE REPORTS

Patient 1
An 8-year-old boy, the only child of healthy parents of Austrian origin without apparent consanguinity and with a negative family history of blistering skin diseases. A homozygous nonsense mutation in the KRT14 gene (744delC/insAG; Y248X) leading to a
premature termination codon was shown to have caused complete loss of keratin 14 protein expression in basal keratinocytes of the patient. The clinical, histological and genetic findings in this patient have been reported elsewhere (12). The boy has been carefully examined at least once a year with regard to the status of his skin and secondary extracutaneous complications of EB. Multiple, rapidly enlarging, asymmetrical, irregularly pigmented melanocytic naevi, some of them with poorly defined borders, i.e. EB naevi, have been developing since the age of three (in 1998). A large EB naevus on the left buttock with an alarming clinical aspect has been reported previously (Patient 1 in Ref. 3). Initially, the patient presented with a newly developed approximately 4.5 x 4 cm wide, sharply demarcated naevus extending along the outlines of a former blister on the left buttock (Fig. 1a). One year later, a protrusion approximately 3 cm in length appeared at the cranial portion of the naevus (Fig. 1b) and more EB naevi elsewhere. The latero-basal border became lighter and blurred and speckled satellite lesions turned more prominent in colour. After a further year, the naevus has lost parts of the protrusion but has gained new satellite lesions, appears lighter and is more raised (Fig. 1c).

**Patient 2**

A 5-year-old boy, the son of unaffected, not consanguineous parents of Turkish origin. The diagnosis “EBS with muscle dystrophy” was established by mutation analysis, which revealed compound heterozygosity for a 3-bp insertion at position 1287 in the PLEC1 gene, that encodes for the linker protein plectin, leading to the insertion of leucine as well as the nonsense mutation Q1518X leading to a stop codon (13). The boy developed a large EB naevus at the dorsum of his right thumb/thenar at the age of 3 years. The lesion showed stippled pigmentation ranging from dark to light-brown and coalesced in a prominent centre. The irregular border outlines the shape of preceding blisters, giving this mole its bizarre configuration (Fig. 2).

![Fig. 1. Epidermolysis bullosa naevus on the left buttock of Patient 1 followed-up over 3 years: (a) In 1998 the mole showed typical criteria of an EB naevus, i.e. rapid growth, large diameter, irregular borders and satellite lesions (reprinted from Bauer JW, et al. Large melanocytic naevi in hereditary epidermolysis bullosa. J Am Acad Dermatol 2001; 44: 577–584; with the permission of Elsevier Science). (b) In 1999 a 3-cm long protrusion on the cranial aspect of the initial naevus was noticed. (c) In 2000 the naevus has lost a distal portion and new satellite lesions have developed on its lateral border.](image)

![Fig. 2. The epidermolysis bullosa naevus on the thumb/thenar of Patient 2 appears clinically highly suspect because of its size, polycyclic irregular configuration, mottled pigmentation and satellite lesions. Note the tense blister on the thenar and the older flaccid blister over the interphalangeal joint.](image)
**Patient 3**
A 9-year-old Austrian girl with a history of blister formation following minimal trauma since birth and a negative family history with regard to bullous skin diseases. Erosions healed with milia formation and atrophic scarring. Mucosal involvement, i.e. erosions in the oral cavity and oropharynx, varied in intensity. Toenails were completely lost in early childhood, whereas hair and teeth were unaffected. The girl was identified to be heterozygous for a mutation at position 425 (425A→G) in the COL7A1 gene, which encodes for type VII collagen (14). A second mutation has not yet been revealed in this girl with presumed recessively inherited DEB. A large EB naevus with satellite lesions developed at the dorsal aspect of the right heel within a few months (Fig. 3).

**Histopathology**
In Patient 1 the naevus on the right buttock was biopsied when he was 5 years of age. In Patient 2 the biopsy was taken from the naevus on the right thenar at the age of 5 years, while he underwent circumcision in general anaesthesia. Because of the highly suspect clinical aspect of Patient 3’s EB naevus we took two punch biopsies, one from the centre the other from a satellite lesion, to exclude malignant melanoma. Histological evaluation of the naevus of Patient 1 revealed nests of melanocytes localized along the dermoepidermal junction, particularly in partly confluent rete ridges. Between the nests, single melanocytes predominated, a few of which were also located in upper Malphigian layers (often described as pagetoid spreading). Melanocytes were also located in the follicular epithelium. In addition, there was a discrete lymphocytic infiltrate in the papillary dermis (Fig. 4a). The melanocytic lesion in Patient 2 consisted of slightly unequal melanocytic nests in epidermal and dermal location. Single melanocytes were rarely seen

![Fig. 3. The polycyclic margin of a large epidermolysis bullosa naevus on the heel of Patient 3 outlines preceding blisters. A flaccid, partially torn bulla causes the blurred, opaque aspect of this dark-brown naevus with satellite lesions.](image)

![Fig. 4. Histopathology of epidermolysis bullosa naevi. (a) Patient 1. Melanocytes arranged in intraepidermal nests, particularly in partly confluent rete ridges. Single melanocytes are found in upper Malphigian layers (× 200). (b) Patient 2. Uneven nests of melanocytes in epidermal and dermal location. Slight fibroplasia around rete ridges. Dermal melanophages are in vicinity to melanocytes (× 400). (c) Patient 3. The dermal part of the biopsy showed melanocytes in nests in the papillary dermis and a lymphocytic infiltrate with melanophages. There are no cytological atypia or mitoses present (× 400).](image)
in suprabasal epidermal layers. Besides slight focal fibroplasia, there were only a few scattered lymphocytes and melanophages in the dermis (Fig. 4b). In conclusion, the histological features of these two EB naevi were consistent with a melanocytic naevus of the compound type with some features of a Clark’s naevus. Because of dermoepidermal separation of the biopsy of Patient 3’s naevus during the surgical procedure, the epidermis and the dermal part were embedded separately. The epidermal part of the biopsy showed acral epidermis with single melanocytes in the basal layer, but only one melanocytic nest located directly beneath the dermoepidermal junction (subepidermally). No melanocytes were found in the upper Malphigian layers (not shown). In contrast, the dermal component of the biopsy showed melanocytes in nests in the papillary dermis surrounded by a lymphocytic infiltrate mixed with melanophages (Fig. 4c). There were neither cytological atypia nor mitoses. This melanocytic lesion was therefore an acral naevus of compound type. In contrast to other EB naevi that we have investigated previously, there were no histological changes of a persistent naevus and/or a pseudomelanoma in any of the biopsies.

**Immunohistochemistry**

Sections of specimens from all three patients were stained with antibodies against the S-100 protein (Z 0311, Dako, Glostrup, Denmark), HMB-45 (M0634, Dako) and the Ki-67 (M 7240, Dako) proliferation marker-protein. S-100 stains showed the expected reactivity of melanocytic cells (also in the upper epidermis) and of Langerhans’ cells, dermal dendritic cells and neuronal structures (not shown). HMB-45 as a marker for premelanosomes, stained positive in melanocytes along the basal layer and in dermal nests, but also in melanophages. Interestingly, Ki-67 was preferentially expressed in epidermal and adnexal keratinocytes, while expression was low in melanocytes of all three naevi (Fig. 5). In the naevus of Patient 3, staining was completely negative for Ki-67 in the dermal part of the biopsy (not shown).

**Blister fluid analysis**

Blister fluids were collected in sterile syringes by puncturing skin blisters. “Control” samples included fluids from burn blisters, blisters of a patient with Stevens-Johnson syndrome and blisters of two patients with bullous pemphigoid. Samples were aliquotted and immediately stored at –70°C after the addition of aprotinin (500 Units ml^-1) to protect proteins from degradation.

For quantification of factors known to stimulate melanocyte growth, the following commercial ELISA kits (Quantikine, R&D Systems, Minneapolis, IL, USA) were used, all in accordance with the manufacturer’s instructions: Interleukin 8 (IL-8, No. D8050); basic fibroblast growth factor (bFGF, No. DFB50); hepatocyte growth factor (HGF, No. DHG00); granulocyte monocyte colony stimulating factor (GM-CSF, No. DGM00); leukotriene B4 (LTB4, No. DE0275); and prostaglandin E2 immunoassay (PGE2, No. DE0100). For each compound, duplicate readings were averaged and concentrations (pg ml^-1) were calculated corresponding to the standard curve.

The concentrations of IL-8, GM-CSF, LTB4, PGE2 and HGF in the blister fluid of the patients with EB showed no significant differences compared to the concentrations in the blister fluid from “control” patients. Interestingly, bFGF was barely detected in any of the blister fluids (Table I).

To demonstrate any free-floating melanocytes in the blister fluid, haemorrhagic fluid was obtained from a large blister over the EB naevus on the right heel of Patient 3 and was immediately centrifuged in a
Cytospin® 3 Cytocentrifuge (Thermo Shandon, Pittsburgh, PA, USA) at 800 rpm for 5 min, fixed in acetone and immunohistochemically stained with anti-HMB-45 antibodies (M0634, Dako). Two melanocytic cells were detected in this sample, one of which is shown in Fig. 6.

DISCUSSION

EB naevi, which bear the exact outlines of the borders of preceding bullae as a morphological hallmark, i.e. “the imprint” of the underlying blistering skin disorder, undergo the same fate as most common acquired melanocytic naevi (15). They gradually appear in the first or second decade of life, begin as flat lesions that grow horizontally, and later while acquiring dermal components and losing pigment (16), develop papular areas resulting in the chagrin-like appearance of dermal naevi (see Fig. 3 in Ref. 3).

Sander et al. (17) describe 17% of 126 melanomas in Swedish persons below 20 years of age having had an associated precursor lesion. In 17 out of 36 children with malignant melanoma, Schmid-Wendtner et al. (18) documented a precursor lesion at the site of the subsequent melanoma and emphasized that prophylactic excision of suspect pigmented lesions was mandatory. According to the ABCD rules (4, 5), the EB naevi in our three children with recessive EB clinically resembled malignant melanomas, including satellite lesions. However, we have not seen any malignant transformation of EB naevi in 19 patients during a follow-up period of more than 20 years in some cases. The benign nature of EB naevi has also been emphasized by Voglino & Voglino (19). However, one has to keep in mind that chronic injury, inflammation and wound healing provide optimal conditions for tumour promotion, as is shown by the high incidence of aggressively metastasing squamous cell carcinomas and the increased risk for the development of malignant melanomas in patients with recessive, dystrophic EB (20). There is also the occurrence of multiple keratoacanthomas (21) and squamous cell carcinomas in patients with junctional EB (22, 23). Nevertheless, after histopathological evaluation of the EB naevus (occasionally multiple biopsies) a “wait-and-see” strategy with regular (at least annual) clinical follow-up can be an alternative complete excision. This approach is especially suited for patients with EB with skin fragility and potentially impaired wound healing.

Kopf et al. (24) followed up two children with hundreds of benign, eruptive naevocytic naevi after a severe bullous disease over 4 and 6 years, respectively, and recorded the condition as having reached a point of stability in terms of the number of lesions with no tendency to involute spontaneously. Interestingly, such naevi have not been reported in autoimmune blistering diseases. EB naevi differ from these eruptive naevi in size and shape (i.e. the imprinted shape of the preceding blister) and, most notably, show a continuing dynamic growth pattern over years, as seen in Patient 1, until they finally disappear or turn into a chagrin-naevus.

To gain more insight into the pathogenesis of EB naevi, we compared cytokine/growth factor levels in the blister fluid of our patients with the levels from patients with second-degree burn-blisters, Stevens-Johnson syndrome and bullous pemphigoid. We were able to detect HGF, IL-8, GM-CSF, PG-E2 and LTB4, but virtually no bFGF in the blister fluids of our three patients with recessive EB or in other patients with junctional and dystrophic EB (25). The lack of bFGF is surprising, since Arbiser et al. (26) showed elevated urinary bFGF in patients with recessive dystrophic EB and hypothesized that this could contribute to the development of squamous cell carcinomas in these patients. There are two explanations for this discrepancy: on the one hand bFGF may rapidly degrade in blisters (27, 28), or, on the other, as bFGF is a marker of chronic activation of fibroblasts it may not be involved in the acute event of blister formation.

According to Riley (29) and Valyi-Nagy et al. (30), the loss of adhesion in the dermo-epidermal basement membrane zone alone could be an initial factor promoting the proliferation and migration of free-floating melanocytes. These cells have been shown to disperse like “flocking birds” in a histological section of a skin blister of a patient with junctional EB (2). In addition, we were able to demonstrate two melanocytic cells in a cytospin specimen made of fluid drawn from a blister located on top of a large EB naevus in Patient 3 by immunohistochemical HMB-45 staining. We assume that the cytokines/growth factors detected in acute blisters of patients with EB may be ancillary to enhance

![Image](https://via.placeholder.com/150)

**Fig. 6.** HMB-45 stain of a cytospin specimen of blister fluid from Patient 3. A melanocyte was detected floating free in the blister fluid of a blister over an epidermolysis bullosa naevus (×1000).
the rapid proliferation and spreading of free floating melanocytes/naevus cells to form the typical, large, “blister-shaped” EB naevi.

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**CLINICAL REPORT**

**Life-long Course and Molecular Characterization of the Original Dutch Family with Epidermolysis Bullosa Simplex with Muscular Dystrophy due to a Homozygous Novel Plectin Point Mutation**


Plectin is one of the largest and most versatile cytolinker proteins known. Cloned and sequenced in 1991, it was later shown to have nonsense mutations in recessive epidermolysis bullosa with muscular dystrophy. A dominant mutation in the gene was found to cause epidermolysis bullosa simplex Oga without muscular dystrophy. Here we report the DNA sequencing of the plectin gene (PLEC1) in a Dutch family originally described in 1972 as having epidermolysis bullosa with muscular dystrophy. The results revealed homozygosity for a new plectin nonsense mutation at position 13187 and its specific 8q24 marker haplotype profile. Western blotting of cultured fibroblasts and immunofluorescence microscopy of skin biopsy confirm that the plectin protein expression is grossly reduced or absent. A summary of the life-long clinical course of the two affected brothers homozygous for the new E1914X mutation is given.

**Keywords:** EBS-MD; genotype-phenotype; nonsense mutation; plakins; plectin; PLEC1.

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Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) is a rare recessive disease associated with plectin deficiency (1) and caused by nonsense mutations, small deletions or insertions in the PLEC1 gene (2–14) coding for the protein plectin. Targeted inactivation of the plectin gene in mice caused a phenotype similar to that of human EBS-MD patients (15). Plectin was first cloned and sequenced from rat (18) and later from man (19) and mouse (20). The only dominant mutation described in PLEC1 has been shown to cause the rare blistering skin condition epidermolysis bullosa simplex Oga (EBS-O) (21).

The first detailed clinical report on epidermolysis bullosa with muscular dystrophy was published in 1972 in Dutch by the neurologist C. J. de Weerdt and dermatologist S. Castelein, who named it EB dystrophica because of skin sequelae and nail dystrophies (22). Electron microscopy of the skin of the two affected brothers revealed low intraepidermal basal cell blistering and hemidesmosomes with hypoplastic attachment plates and impairment of keratin filament insertion into the inner hemidesmosomal plaque (23). This blister pathogenesis differed from both dystrophic and junctional EB (23–26). The group was initially denoted “pseudojunctional” (P-JEB), but has later been renamed EBS-MD (27).

Altogether 15 patients with individual mutations suffering from EBS-MD have been described (2–14), all suffering from a generalized blistering skin condition or fragile skin with blistering due to trauma at birth or in infancy, nail dystrophy and a severe muscular dystrophy resembling the limb-girdle type with a variable onset from infancy (delayed walking) to their early 30s.

We report Western blotting of fibroblast cultures, immunofluorescence microscopy of the skin, and molecular findings in the genomic plectin (PLEC1) locus and its flanking 8q24-haplotype in the original Dutch EBS-MD family. The life-long clinical course of the two adult patients is also summarized.

**PATIENTS AND METHODS**

**Patients**

The Dutch family (NEB1) first published in 1972 (22) comprises both lethal and non-lethal cases. The parents did not report consanguinity, but both knew of ancestral lines to rural Western Germany. Later other relatives reported that
the parents were third cousins, hence the inbreeding coefficient is 0.00391 for the patient generation.

In a sibship of 14, 3 girls and 3 boys had generalized blisters from birth, and 4 of those died in infancy (see Fig. 4 for a partial pedigree). The 3 girls (nos. 3, 6, 8) died within a few weeks because of their skin condition; the boy (no. 12) died at 5 months of age due to pneumonia. He was recalled to have fewer blisters than his affected siblings. Another boy (no. 2) looked normal at birth, but was never able to move out of bed, never learned to walk or talk, had epilepsy and was severely psychomotorically retarded. He died at 10 years of age.

Two boys with EB (nos. 9, 11) grew up and both developed progressive muscular dystrophy from age 15 (no. 11) and age 17 (no. 9), respectively, but none of their 7 healthy living siblings did so. Based on the suspicion that this family did not have dystrophic EB, but rather a non-Herlitz EB junctional type of the disease, their neurologist, Dr. de Weerdt, kindly arranged for two of us (CPdG, TGD) to see the whole family in their home town in North Holland, the Netherlands, in 1982. In addition to a detailed clinical history from the parents and the two patients, blood samples for classical genetic marker testing and skin shave biopsies for fibroblast cultures were taken of all 11 living family members. The parents and the 2 affected boys had normal karyograms (analyses by Anton Brøgger, Oslo, Norway).

The patients were followed up by one of us (C. J. de Weerdt) in their home town and had frequent admissions until their deaths in 1990 (no. 9, 44 years) and 1995 (no. 11, 47 years). A skin biopsy for immunofluorescence study was taken several hours post-mortem from patient no. 11. Autopsies were not done.

### Immunofluorescence study

Skin samples were processed for immunofluorescence as previously described (28). The primary antibodies raised against human and rat antigens are listed in Table I. In combination with primary mouse IgG we used biotinylated horse anti-mouse IgG (Vector Laboratories, Inc., Burlingame, CA, USA) and FITC (fluorescein isothiocyanate) conjugated neutravidin (Southern Biotechnology Associates (SBA), Inc., Birmingham, AL, USA). For the primary mouse IgM antibody we used FITC conjugated goat anti-mouse IgM (SBA). In combination with primary rat IgG we used FITC-conjugated goat anti-rat IgG (SBA). Digital fluorescence images of tissue sections were obtained with a Leitz Orthoplan microscope equipped with a video imaging system with exposure time control board designed for the detection of low levels of fluorescence (29).

### Fibroblast cultures

Fibroblasts stored in liquid nitrogen after establishment in 1982 were recovered and used for DNA extraction by standard protocols from all 11 family members and for plectin protein expression from 2 patients (nos. 9 and 11) and one unaffected sibling (no. 10). Primary fibroblasts derived from a non-EB-MD patient (EB107-1) were used in the preparation of control lysates for Western blotting.

### Western blotting

Cultured patient and control primary fibroblasts were lysed directly in hot 2 × PAGE sample buffer and run on SDS 3.5% polyacrylamide mini gels. Separated proteins were transferred to PVDF membranes (Millipore, Bedford, MA, USA) and membranes blocked with 3% BSA over night. Immunodetection of plectin on the blots was carried out with mAbs 10F6 and 5B3, followed by goat anti-mouse antibodies conjugated to alkaline phosphatase (Promega, San Luis Oshio, CA, USA). Plectin purified from cultured rat glioma C6 cells was used as size marker.

### Genetic markers and linkage studies

After plectin was reported in situ assigned to 8q24 (19) we typed the then most 8q telomeric DNA marker, the tetranucleotide repeat D8S373 (23). Recently, we added the following 8q-assigned dinucleotide Genethon STR-markers: D8S274, D8S1717, D8S1836, AFM128xh5, D8S1925 and D8S2334. The primers were as specified from Genethon and/or the Genome Data Bank (gdbwww.gdb.org) and labelled with FAM or HEX. Fragment sizes were determined on an ABI 377 sequencer. The results were entered in Cyrillic format. Lod scores for linkage analysis were calculated where needed by standard methods.

### DNA sequencing

Plectin sequences deposited in GenBank (acc. nos. U63610; Z43676; X59601; U53204; and NM_000445) were used to design PCR primers that would yield overlapping 400–600 bp-long DNA fragments covering the sequence from the start of exon 2 to the end of exon 32 (19) (exon 33 in the McLean et al. terminology (3)), and skipping only the longest introns.

All PCR primers were designed as described earlier (21). The PCR products were purified either using the QIA quick PCR Purification Kit (Qiagen) or the PCR product pretreatment kit (USB) and subsequently sequenced using PRISM AmpliTaQ FS Dye Primer Cycle Seq. Kit (Perkin Elmer) and run on the ABI 377 automatic sequencer. Sequences were aligned and compared using Sequencher 4.1 from Gene Codes Corporation.

### Mutation detection by restriction enzyme

The restriction patterns after 4 h (at 37 °C) HindIII digestion of exon 31 DNA amplified with the PLE47F/PLE48R primers (PLE47F: AGGACGAGAGCCAGCGTAAAG; PLE48R:CCT CTGCTTGGACTTCTC) followed by nested PCR with the PLE47FR primers (PLE47F: AGGACGAGAGCCAGCGTAAAG;
PLE47R:TTCCAGCTCTGCGGTTTC, were read after a 2 h run at 70V on a 10% PAGE gel.

RESULTS

Clinical features and course of the disease

The course of the disease shared the following skin manifestations: Blisters at birth and in 2 of them specifically on the fingers, soon widespread occurrence of new blisters, buttocks invariably affected with sores. Lifting up the baby in the usual way induced blisters on lateral thorax in a pattern showing the finger grips. No blister sequelae, including no miliae, remained upon healing. In childhood, both of them had had attacks of eye irritation. No. 9 had a hoarse voice throughout life. By age 33, no. 11 had lost all his permanent teeth, while his 38-year-old brother, no. 9, had decayed teeth similar to those often seen in severe-dystrophic EB. Both patients had dystrophic nails (Fig. 1), no oral blistering but new blisters at 2–4 week intervals on the peripheral extremities, leaving many spots of pale atrophic skin often surrounded by hyperpigmentation.

No. 11 was deaf in his right ear and had reduced hearing in the left. From age 15 he had experienced slowly increasing muscular weakness. At first he had problems getting out of bed without the help of his arms, and the knees tended to sink together when he tried to get up from a lying position. Muscular biopsy of the m. quadriceps at that time revealed extensive variation of the diameter of muscle fibres – with big rounded fibres with several central nuclei and signs of regeneration.

At 33 years, the muscular atrophy was still most striking at the proximal extremities (limb-girdle-like; Fig. 1), but included facial muscles, ptosis, Bell’s phenomenon and bulbar dysarthria. At age 46 he could still walk, but had a typical waddle and drop foot. He died the same year as a result of respiratory insufficiency probably caused by his muscular disease.

The older brother (no. 9) experienced the same muscular dystrophy from age 17. He had recurrent pulmonary infections during childhood and died at 44 years of age because of respiratory insufficiency and kidney failure.

![Fig. 2. Immunofluorescence microscopy of the skin of no. 11 with recessive epidermolysis bullosa simplex with muscular dystrophy (A, C, E) and of a normal human individual (B, D, F). The nuclei are counterstained in blue with bisbenzimid. Anti-HD1/plectin antibody does not bind to the epidermal basement membrane zone in the patient (A). Anti-plectin antibodies (5B3 and 5C10) also do not bind to this region (C, E). The staining with anti-plectin 5C10 antibody at the cell periphery in the suprabasal layers is also lost, whereas the perinuclear staining in epidermal cells, fibroblasts and endothelial cells remains conserved (E). Bar is 10 μm.](image-url)
Absence of plectin in skin and fibroblast cultures

Immunofluorescence microscopy of the skin: monoclonal antibody (mAb) HD-121 to 500-kD HD-1/plectin (30) did not bind to the epidermal basement membrane zone (EBMZ) in patient no. 11 (Fig. 2A, B). Also mAbs 10F6, 5B3 (Fig. 2C, D), 6C6 and 5C10 (Fig. 2E, F) against plectin did not bind to the EBMZ, although some epitopes appear to remain in the suprabasal layers (6C6) or perinuclear region (5C10) of the epidermis.

Knowing that the patient’s skin lacks plectin, the tissue distribution of other hemidesmosome components becomes important. Basal keratins 5 and 14 were normally present in the basal epidermal cells. The 230-kD and 180-kD bullous pemphigoid antigens, as well as the integrin subunits α6 and β4 and laminin-5, were all unreduced and normally polarized in the EBMZ (data not shown).

Western blotting: When lysates from cultured fibroblasts from the patients (nos. 9 and 11) were subjected to Western blotting, no protein band at the position of plectin was seen, contrary to lysates from an unaffected sibling (no. 10) and control lysate (Fig. 3).

Linkage and haplotype studies

All family members were homozygous 1-1 in glutamate pyruvate transaminase (chromosome 8q24) (Fig. 4). When short tandem repeat markers became available, the two common limb-girdle muscular dystrophy loci (on chromosome 2 and chromosome 15), the two laminin-5 loci tested (on chromosome 1q) and the collagenase locus (on chromosome 11q) were also excluded as candidate loci.

When plectin became assigned to chromosome 8q24, we first tested D8S373, most telomeric on the tetranucleotide marker CHLC (Collaborative Human Linkage Center) map, and confirmed full co-segregation with the EBS-MD disease (23). We have elaborated the extended 8q24 haplotypes, as shown in Fig. 4. During our current plectin DNA sequencing studies we have observed several polymorphisms, including an insertion/deletion polymorphism with alleles S and F (to be published). In Fig. 4 one intragenic plectin marker on each side of the disease mutation is flanked by short tandem repeat markers located according to our ongoing 8q24 fine mapping. This reveals an identical long haplotype from both parents strongly suggesting identity in descent and therefore predicting homozygosity for an 8q24 disease-causing mutation.

Mutation detection by gene sequencing and restriction enzyme

DNAs from two unaffected siblings (nos. 7 and 13) and one patient (no. 11), representing one normal and one disease-carrying chromosome (as shown in the haplotype analysis), together with DNAs from 12 other haplotypes (21), were subjected to systematic sequencing from exon 2 to exon 32 (see Methods). A new plectin nonsense mutation was found using the Ple48 primer (PLE48F AAACGGCAGGAGCTGGGAAG: PLE48R) (Fig. 5). The substitution which was verified homozygous in both patients is a G>T transversion at genomic position 13187 (GenBank...
accession no. U63610), corresponding to cDNA position 5740 (codon 1914) in GenBank accession no. Z54367 (position 5461 in cDNA U53204) located in exon 31 (exon 32 in (3)). This mutation leads to a shift from GAG to TAG resulting in the substitution of glutamic acid codon with a stop codon (E1914X) in the plectin polypeptide. This base substitution abolishes a normal HinfI restriction site and allows a restriction-based identification of the mutation in all carriers of the NEB1 family (Fig. 6). Normal DNA has a restriction site splitting the 156 bp nested PCR product into 106 bp and 50 bp fragments (including m13 tail), whereas the EBS-MD mutation abolishes this restriction site leaving the pattern of the homozygote (no. 11) with unsplit fragments similar to undigested fragments. One hundred Norwegian random chromosomes (50 individuals) lacked this mutation. Homozygosity for the stop mutation in both affected individuals and heterozygosity in unaffected family members are in full accordance with the distribution of 8q24 haplotypes, supporting that this nonsense point mutation is in fact the cause of EBS-MD in this family.

Further downstream another mutation was found using the PLE57 primer (PLE57F: TGTGAAGGAGGTGTTGTTG; PLE57R: CTGCCGTGAAGTATCG). This is an A>G substitution in position 16455 in genomic DNA (U63610) creating a restriction site for NgomIV. It corresponds to cDNA position 8900.

Fig. 4. The 8q24 haplotypes in the NEB1 family. The given order is based on our ongoing family and radiation hybrid studies. Note the long identical haplotype shared by both parents and carrying both the new disease mutation 13187 G>T (E1914X) and the 16455 A>G substitution.

Fig. 5. DNA sequencing revealing a G>T mutation in the affected proband NEB1-9 (a), G/T heterozygosity in non-affected family members NEB1-7 (b) and NEB1-13 (c), whereas the healthy control shows homozygous G (d).
(codon 2967) in Z54367 and position 8621 (codon 2857) in U53204. This change results in alteration of the amino acid tyrosine (TAC) to cysteine (TGC). Of 140 chromosomes, 7 carried G, shown by Ngom IV typing, which reflects a polymorphism (A = 0.95, G = 0.05).

**DISCUSSION**

All EBS-MD mutations known to us up to December 2002 are detailed in Table II. Our novel genomic mutation 13187G->T or cDNA 5740G->T, changing a GAG glutamic acid codon to a UAG termination codon (E1914X), is located in exon 31 (19) between the Italian 5728C->T (Q1910X) (5) and the Japanese 5806C->T (Q1936X) (9,10) EBS-MD mutations. The skin of patient no. 11 with the homozygous PLEC1 late stop mutation makes mapping of monoclonal antibodies in the absence of plectin possible. Note- worthy is the remaining expression of plectin epitopes in the suprabasal layers (6C6) of the epidermis and Fig. 6.

**Table II.** Reported disease mutations in the plectin gene (PLEC1) as in December 2002. The total of 34 alleles depict 23 different recessive EBS-MD mutations and one dominant EBS-O mutation. Updated after Table 145.3 in ref. 34

<table>
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<tr>
<th>McLean et al. (3) exon 2- exon 33</th>
<th>PLEC1 cDNA (U53204)</th>
<th>4574aa codon (U53204)</th>
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<th>Disease alleles (no.)</th>
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<sup>a</sup>In U63610 (gDNA); 13187; in Z543067 (cDNA): 5740G->T and E1914X.

<sup>b</sup>In U63610: 13775; in Z543067: 6328C->T and R2110W.

<sup>c</sup>In U63610: 21358; in Z543067: 13805.
perinuclear (5C10) region in all cells of the skin. These antigens may represent alternative splice variants of plectin, which is distributed in most tissues. Western blotting of the huge plectin molecule often results in a ladder of bands that may not always be degradation products, but instead alternative splice variants. Similar to other cases of EBS-MD, it should be noted that plectin mRNA was significantly reduced (to ~5% of its normal level), as determined by RNase protection assays using fibroblasts derived from patient nos. 9 and 11 (data not shown).

The absence of plectin did not result in altered distribution of other hemidesmosome components such as BP230, BP180, and integrin α6β4, nor in its associated intermediate filaments keratins 5 and 14. Thus plectin is not essential for the recruitment of these hemidesmosome components (31).

Compared to extensive tissue sampling and examinations recently performed on young adult patients (14), we only had fibroblasts and 2 postmortem skin samples available for study. However, the size of the extensively sampled family 2 decades ago made it ideally suited for gene mapping by linkage. Unfortunately both parents happened to be homozygous in the GPT isozyme system, so no association was made with the GPT-linked dominant epidermolysis bullosa simplex Ogna (32) back in 1982. EBS-Ogna is now explained by an exon 31-specific base substitution and plectin amino acid exchange (R2110W) (21).

In the history of clinical delineation of Mendelian disease the coexistence of different organ signs and symptoms has led to prolonged discussions of (a) the chance co-occurrence of independent diseases, (b) the pleiotropic effects of a single disease mutation, or (c) the close genetic linkage of different Mendelian diseases. This is also the case with EBS-MD.

When the Dutch report (22) of EB dystrophica with muscular dystrophy was later complemented by a “similar” association in a Finnish family with EBS and muscular dystrophy (25), both chance coincidence and linkage of two separate loci became less likely, but classification of the EB involved was at stake. Like Anton-Lamprecht’s ultrastructural results in the Dutch family, the Finnish workers saw the low basal cell intraepidermal origin of the blistering. The intraepidermal pathogenesis led to their term EB simplex, although the clinical signs were similar to junctional EB, hence the original term pseudojunctional EB (23, 24). The term EBS-DM was recommended by Fine et al. (27). The low intrabasal cell split level categorizes this form as EBS (27). The same very low split level within the basal cell is also seen in EBS due to a ITGB4 mutation deleting part of the cytoplasmic tail of integrin beta4 (33). When plectin first came into focus, our initial D8S373-typing revealed full linkage to this at that time the most telomeric marker on the genetic chromosome 8q map (23).

The extended haplotypes (Fig. 4) define a region of 8q24 for which the EBS-MD patients of the family are homozygous and haplotype identical from both parents. The considerable length of the haplotype common to both parents is explained by the inbreeding (parents 3rd cousins), but we do not know if this inbreeding line includes the initially reported West German ancestors. When other detected EBS-MD mutations are likewise haplotyped, this will give a rapid indirect way of pinpointing previously known mutations in new patients.

In the review by Anton-Lamprecht & Gedde-Dahl (34) no specific Dutch origin mutations, but 2 previously reported exon 31 German mutations (5188C->T, Q1713X: and 7102C->T, R2351X), are described (12). To this can be added the exon 32 North-East German 16 basepair insertion mutation 13803ins16 which leads to loss of the normal 35 last amino acids in the plectin polypeptide and thereby the keratin binding motif (14).

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