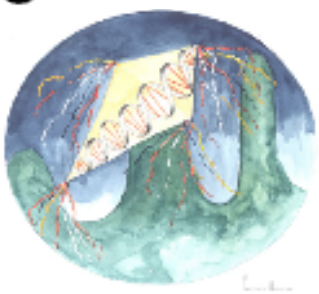
geneSkin



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GENESKIN

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Rare genetic skin diseases: advancing diagnosis, management and awareness through a European network

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1. Project execution

Project objectives

Genetic skin diseases or genodermatoses comprise almost 300 rare conditions mainly affecting the skin and its appendages. These disorders usually manifest at birth or early in life and are in most cases severely disabling or even life-threatening, thus bearing major medical and social implications. Over the last decade a burst of research in this field has resulted not only in the elucidation of the aetiology of a number of genodermatoses, but also in the development of more precise diagnostic procedures. However, the molecular bases of some genodermatoses are still unidentified and the function of many identified gene-products remains unknown. Furthermore, diagnosis, management and genetic counselling of these rare conditions remain a challenge because of the lack of wide-spread in-depth clinical knowledge, difficulties in defining how and where to investigate affected patients and lack of diagnostic tools. Indeed, experts' cognition is restrained to a few centres in Europe, each centre dealing with a limited number of specific diseases. In addition, no curative therapy is at present available for any form of genetic skin disease and, because of disease rarity, most centres do not see a sufficient number of patients to initiate clinical trials with existing or new treatments. This situation can only be overcome by a Europe-wide mobilisation of activities and resources to facilitate the development of substantially improved and cost-effective health care services.

The GENESKIN project has worked in this direction, actively supporting the cooperation between European expert groups in the field of genetic skin diseases and promoting the dissemination of knowledge relevant to the improvement of the diagnosis, treatment and care of genodermatoses. The project has involved 32 clinical and research centres and patients' organisations (27 legal entities) from 12 European countries (see contractor list at the end of the text) (Figure 1). The number of participants has insured a wide representation of European competencies and expertise on genetic skin diseases.

Focused on five major groups of genetic skin diseases, namely epithelial adhesion disorders (EAD), keratinization disorders (KD), ectodermal dysplasias (ED), connective tissue disorders (CTD), and DNA-repair disorders (DNAr), the GENESKIN project has been aimed:

- To create a European clinical and diagnostic network for the five groups of genetic skin diseases above mentioned (main objective 1);
- To integrate, test, and validate diagnostic and research tools for these diseases (main objective 2);
- To promote training of clinicians, healthcare and laboratory personnel on clinical, diagnostic and management aspects of specific disease group and organise meetings on critical emerging topics (main objective 3);
- To promote pan-European communication pathways between patients' organizations, ethics committees, physicians and scientists, as well as close accompanied alliance of patients' organisations and ethics committees with physicians and scientists (main objective 4).

Work performed

At first, an assessment of project participants' resources and competencies was carried out and centres with specific expertise in the various disease/disease groups were identified. By this mean, the strengthening of pre-existing links and starting-up of new collaborations among project participants was boosted. Thereafter, in light of the GENESKIN objective of creating a widespread European task force dealing with genetic skin diseases, a network enlargement beyond GENESKIN participants has been propelled. To this purpose, **a list of the diseases** comprised in each of the five disease groups was at first generated, and then specifically designed GENESKIN registration forms (for **clinical, diagnostic and research centres**) have been produced and submitted to the attention of European centres, either identified by project participants or through a

home-based web search. The centres which sent back the completed registration forms were recorded in the lists. In parallel, a similar activity has been carried out to generate a list of **patients' associations** and **websites** related to the diseases object of the GENESKIN project. The lists have been published in the GENESKIN website and updated during project course. Concurrently, the generation and updating of disease-related documents (e.g. **disease cards with clinical and laboratory disease description, diagnostic questionnaires, tests and procedures, tools for laboratory diagnosis, clinical trial lists**, etc), as well as of documents illustrating the GENESKIN project activities and related issues was worked out.

Several activities concerning the integration, testing and validation of diagnostic and research tools have been performed. In particular the focus has been on (i) the development and standardization of immunohistochemical/biochemical screening tests, preliminary to molecular diagnosis, in selected diseases characterized by similar clinical presentation and genetic heterogeneity; (ii) the standardization of pre- and post-natal molecular diagnostic tests; (iii) the organization of "virtual" biological sample databanks for selected diseases; (iv) the collection and exchange of samples, in particular from patients affected with still uncharacterised diseases, as a prerequisite to the identification of the causative gene.

In order to facilitate information exchange among professionals on critical topics and the training of junior colleagues on clinical, diagnostic and management aspects of specific skin disease groups, a substantial part of project activities has been devoted to the organisation of workshops/meetings and training courses.

Within the frame of the Geneskin project, a network of ethical experts has been built up. This group of experts with different scientific backgrounds, ranging from genetics to philosophy and theology, was involved in the identification and discussion of ethical issues, in particular in relation to genetic skin diseases. In parallel, patients' associations were contacted with the aim to strengthen the links between physicians/scientists and patients' support groups and to enquire about the possibility to establish a European GENESKIN-patients' association. Overall, a close accompanied alliance among ethicists, physicians and scientists and patients' organizations has been promoted.

Finally, in order to disseminate the above mentioned activities and, in a more general way, knowledge about genetic skin diseases a dedicated website (http://geneskin.idi.it) has been designed and generated.

Main achievements

Main objective 1 - To create a network of clinical, diagnostic and research centres dedicated to the five major groups of inherited skin disorders.

Cards with a general description and classification of each of the five disease groups have at first been generated for the general public and published in the GENESKIN website. The information provided in these cards includes a list of European centres offering clinical, diagnostic and research services, and of patients' associations. These lists have been generated by project participants as above described.

As to the production of **diagnostic questionnaires/protocols/check lists** specifically addressed to professionals, the following documents have been generated:

- For EAD, a multi-step diagnostic algorithm was established and a model questionnaire has been prepared by participant 4 in concert with other centres involved in EAD management.

This questionnaire comprises parts related to the clinical diagnosis and patients' follow-up, and also parts relevant to the laboratory diagnosis of EAD.

- For KD, six clinical questionnaires/checklists specifically referred to: i) ichthyosis, ii) Netherton syndrome, iii) Darier disease, iv) Hailey-Hailey disease, v) keratitis-ichthyosis-deafness (KID) syndrome, and vi) palmo-plantar keratodermas have been generated by participants 14B, 5 and 11.
- For ED syndromes, a diagnostic questionnaire has been prepared by participant 8.
- For CTD, a combined checklist for the Ehlers-Danlos syndromes (EDS), cutis laxa syndromes and pseudoxanthoma elasticum (PXE) has been generated. Additionally, diagnostic flowcharts have been prepared for PXE and for the major forms of cutis laxa and Ehlers-Danlos syndromes. These documents have been prepared by participant 20 with the help of participant 3 and distributed to clinical centres involved in management of CTD all over Europe. Positive feedback was obtained and the clinical data obtained by the use of these checklists proved to be very helpful in the characterization of the phenotype in CTD.
- Finally, three specifically designed clinical questionnaires for DNAr diseases have been generated by participant 2A with the collaboration of participant 10, 16 and 21. These questionnaires are currently used by referring clinicians all over Europe.

The above mentioned documents have been uploaded in the GENESKIN website (disease submenu, professional-restricted area) and are therefore available to interested professionals.

As to the preparation of **gene cards** (more appropriately named disease cards in the website):

- For the EAD group, 13 gene cards with clinical and laboratory disease descriptions and causative genes lists have been generated by participant 4 in collaboration with GENESKIN centres involved in the management of EAD.
- For the KD group, the 18 disease cards with description of disease pathogenesis, presentation and course, the main diagnostic criteria and key clinical features, patients' management and follow-up issues have been generated by participants 14B, 5 and 11 with the support of participant centres with specific clinical / diagnostic expertise.
- For the ED group, 17 gene/disease cards referred to the more common ectodermal dysplasia syndromes have been prepared by participant 8.
- As to CTD, 9 disease cards, with detailed clinical and laboratory disease descriptions (clinics, pathogenesis, diagnosis, management, genetic counselling, etc) and causative gene lists have been generated by participant 20 in collaboration with participant 3.
- For DNA, gene/disease cards reporting for each disorder (xeroderma pigmentosum, trichothiodistrophy and Cockayne syndrome) the key clinical features, a detailed description of disease pathogenesis, presentation and course, the main diagnostic criteria, patients' management and follow-up issues have been prepared for all DNAr disorders. These cards also contain recent information on clinical and molecular aspects of new informative cases and the mutational pattern of each gene has been included. Information on disease incidence generated during the project (**Kleijer WJ et al, DNA Repair 2008; 7:744–50,** collaboration between participants 2A, 10, 16 and 21) has also been published in the disease cards.

The above mentioned documents have been uploaded in the GENESKIN website (disease submenu, professional-restricted area) and are therefore available to interested professionals.

As to the **diagnostic reagent lists**:

- For EAD, a list of antibodies useful for antigen mapping diagnosis in EAD patients has been prepared and will be published shortly in the GENESKIN website.
- For KD, the histological/(immuno)histochemical/ultrastructural analyses useful for the diagnosis of each disease have been indicated in the disease cards. The final version of the

protocol for a diagnostic test (transglutaminase assay) for screening of patients suffering from lamellar ichthyosis/non-bullous congenital ichthyosiform erythroderma is published in the GENESKIN website.

- For ED, the relevant information has been introduced in the disease cards.
- For CTD, an outline of the diagnostic procedures currently used for this group of disorders had been made available on the GENESKIN website.
- Finally, for the DNAr, 4 detailed laboratory protocols, including cellular tests, genetic analysis by somatic complementation with tables summarising for each disorder the typical results of cellular functional assays, the responsible genes and the type of mutations have been generated and updated during the project and are downloadable from the website.

For what concerns the **mutation data bases,** in addition to the creation of a link in all disease cards contained in the GENESKIN website to the "Human Gene Mutation Database" (www.hgmd.org):

- For specific KD, links to the "The Human Intermediate Filament Database" have been created in the GENESKIN website.
- For ED, the reference for publications reporting the pattern of p63 gene mutations has been provided by participant 8. In addition, participant 2B has generated a database of NEMO mutations in incontinentia pigmenti (**Fusco F et al, Hum Mutat 2008; 29:595-604**, collaboration between participant 2B and 17).
- For CTD, a list of relevant publications from the GENESKIN project participants and others has been made available on the GENESKIN website.
- Finally for DNAr, mutational data of all the patients analysed so far have been assembled by Participant 2A with the support of participants 10, 16 and 21. The information collected has been inserted in a dedicated database by participant 2A and made available on the GENESKIN website. This adds to the existing links in the disease cards to the Human Gene Mutation Data Base and to the database of the Xeroderma Pigmentosum Society (http://xpmutations.org/).

As to the listing of **clinical trials**, the number of reports remains very limited and concerns only KD and CTD. The available information has been inserted in the GENESKIN website.

In the field of **identifying needs for meetings on specific topics and provide information on recent developments:**

- For EAD, the patients' association DebRA Europe (participant 12) has organized, under the patronage of GENESKIN, an international conference on epidermolysis bullosa (EB), which was held on October 10-12, 2006 in Dublin and involved a number of GENESKIN participants. This expert conference was focused on how the improved knowledge of the fundamental molecular biology of EB can be transformed into potential clinical applications. Following the EB Dublin conference, task forces have been established into specific areas of the quest for treatment for EB ("EB clinical trials" and "Skin cancer in EB") and two meetings, again involving several GENESKIN participants, have been organised by DebRA. In parallel, an update consensus meeting on the diagnosis and classification of EAD took place on May 19, 2007, in Vienna (at the occasion of the Annual Meeting of the European Academy of Dermatology and Venereology) with the presence of 18 leading authorities from all over the world, including 11 GENESKIN members. The result of this meeting (and of post-meeting work and discussions) was the publication of a proposal for a revised classification system and of recommendations on the use of specific diagnostic tests (**Fine JD et al, J Am Acad Dermatol 2008; 58:931-50**, collaboration between participants 1A, 4, 8, 14B, 22, 25, 27 and 28).

- With reference to KD, a major event has been the "First world conference on ichthyosis" organised by participant 5 from August 31 – September 2, 2007 in Münster. In addition to 11 GENESKIN partners, invited speakers included well-known experts from all over Europe and also from other continents (Asia, America, Africa). The meeting, which also represented a GENESKIN common training event, offered a complete overview on most recent advances in keratinisation disorders. During the congress, the need for an updating of ichthyoses classification emerged and a group of experts began to work on this topic.
- Related to CTD, a European meeting, named EuroPXE, was organised by participant 20 in Budapest (September 13, 2007). The aim of the meeting was to discuss the organization of an international collaboration with several European groups all involved in the study of PXE of which several belong to the GENESKIN project. Furthermore, participant 20 has reviewed the main clinical characteristics, management principles, and the current insights in the molecular pathogenesis of EDS (**Callewaert B et al, Best Pract Res Clin Rheumatol 2008; 22:165-89**).

A major instrument for the **dissemination of data** produced by project participants both to general public and health care providers has been the **GENESKIN website**. The website had been generated, published on the web in September 2006 and then updated by participant 1B in collaboration with participant 1A and with the support of participants 2A, 4, 5, 8, 12, 14A and B. (http://geneskin.idi.it) (Figure 2). As to the website architecture, the platform that hosts the shared GENESKIN project data is a dynamic Web site realized in PHP. A principal Unit, which is a HP Proliant DL38, has been connected to a Web server Apache 2.2, to an Interpreter PHP (hypertext pre-processor) and to an SQL database. The GENESKIN website includes sections dedicated to specific project activities and to information on the five major disease groups comprised in the project. The disease area is a collection of clinical, laboratory and research data organized in a free-access subsection addressed to general public and a subsection for professional use (accessible following registration). The information provided to general public includes: a general description of each group of diseases and a list of European centres offering clinical, diagnostic and research services, and of patients' associations. In the professional area, each disease has a dedicated page with more detailed clinical and laboratory descriptions, including key clinical features, diagnostic tests and procedures, tools for laboratory diagnosis and genetic counselling. A "community" section is dedicated to social and ethical issues related to genodermatoses and reports the activities carried up by the group of GENESKIN ethics experts (see below, main objective 4). This section also contains a press release in five European languages (English, French, Italian, German and Swedish) about GENESKIN and its objectives. From the website "home page" users can also obtain additional information about rare diseases and GENESKIN project as well as about training courses/meetings organized by project participants on selected disease groups/topics (see below, main objective 3). News in the field of genodermatoses, a list of project participants' pertinent publications and documents of particular interest are also provided. The website also contains a "link" page organized in 4 major subgroups of related websites. The **launch and dissemination of the GENESKIN website** started in September 2006, has continued during all the GENESKIN project lifetime and is still ongoing. The GENESKIN website has been presented through invited talks at various national and international meetings. The GENESKIN website has also been announced through mailing to members of dermatological societies, and on national and international journals. Finally, reference to the GENESKIN website has been inserted in the text of scientific publications in international journals (**Kleijer WJ et al, DNA Repair 2008; 7:744–50; Fine JD et al, J Am Acad Dermatol 2008; 58:931-50**).

Overall the accomplished work is in line with project planning. Major achievements comprised: (i) the generation and publication on the GENESKIN website of disease cards, with detailed clinical and laboratory description and with reference centre lists, for all major genetic skin diseases object of the project; (ii) the generation and publication on the project website of model

questionnaires/checklists/flowcharts for clinical and laboratory diagnosis of a number of genetic skin diseases; (iii) the generation by project participants of mutation database for incontinentia pigmenti and DNAr diseases; (iv) the publication in an international journal, by 11 GENESKIN participants together with experts from all over the world, of a proposal for a revised classification system of EB and of recommendations on the use of specific diagnostic tests for EB; (v) the organisation of a major meeting on KD, during which the preparation to an updated classification of ichthyoses has been started.

Main objective 2 - To integrate, test and validate diagnostic and research tools

For the **validation of immunohistochemical/biochemical screening tests** preliminary to molecular diagnosis, the activities performed have concerned congenital ichthyoses/erythrodermas and a genetically heterogeneous form of EB. Specifically:

- Participant 5 has carried out a systematic validation of a protocol for a diagnostic test, namely the transglutaminase assay, which can be used on skin sections and allows a histochemical screening of patients suffering from lamellar ichthyosis/non-bullous congenital ichthyosiform erythroderma (collectively also known as autosomal recessive congenital ichthyoses, ARCI). The standardized and validated transglutaminase assay protocol has been made available to all professionals through the GENESKIN website. Participant 6, in collaboration with participant 5, has validated the use of previously developed functional tests for ALOX12B and ALOXE3 in ARCI patients (**manuscript submitted)**. The antibodies to the epidermal proteins ALOXE3, ALOX12B, ichthyin, ABCA12, LEKTI and SLURP-1 developed in the context of project participants' 1A, 6, 9B, 14B, and 19 research activities and commercially available antibodies to ALOX12B and ABCA12 have been tested within the GENESKIN network. The results obtained so far show that activity assays are suitable to test for transglutaminase, ALOX12B, ALOXE3 in ARCI patients, while antibodies are not recommended for routine use. However, work on this topic will continue also after the end of the GENESKIN project. On the other hand, a protocol for LEKTI immunostaining on skin section to be used in the diagnostics of a severe form of congenital ichthyosis, Netherton syndrome, has been standardized in participants 1A, 5, 14B, and 19 laboratories.
- A new prenatal test based on first trimester chorionic villi $\alpha 6\beta 4$ and plectin immunodetection in families at risk for a lethal, genetically heterogeneous autosomal recessive form of EB, EB with pyloric atresia (EB-PA), has been developed and validated. Up to now, 26 prenatal tests in kindred at risk for EB-PA have been performed by immunofluorescence analysis for $\alpha 6\beta 4$ and plectin proteins on chorionic villi samples (Figure 3). In all cases, the prediction of the fetus phenotype by villi immunolabelling was confirmed at probands' birth. These results validate chorionic villi immunofluorescence examination as a novel tool for early prenatal diagnosis of EB-PA in kindred carrying unidentified genetic mutations (**D'Alessio M et al, J Invest Dermatol 2008, e-pub Jun 19**, collaboration between participants 1A, 14A and 15).

For what concerns the **standardization of prenatal and postnatal molecular diagnostic tests** and the design of new assays for genetic studies of selected diseases based on microchip technology, the activities performed have led to the following results:

- In the field of EAD, participant 1A laboratory has standardised a protocol for COL7A1 mutation screening using DHPLC methodology. Analysis of a large collection of DNA samples from patients affected with dystrophic epidermolysis bullosa (DEB) with previously identified mutations has demonstrated that DHPLC mutation detection rate is

significantly higher than the mutation scanning rate reached with a combination of conventional techniques (97% vs 86%) (**Posteraro P et al, Biochem Biophys Res Commun 2005; 338:1391-1401**). Analysis of additional uncharacterised patients has confirmed the superior performance of DHPLC technique and indicated it as the method of choice for COL7A1 molecular analysis in DEB. In parallel, COL7A1 gene was also chosen for testing new approaches for mutational screening exploiting microchip technology. Specifically, the development of a single dedicated Affimetrix GeneChip® re-sequencing array, which can be tiled with up to 30,000 base pairs of overlapping oligonucleotide information, was evaluated by participant 1A and B. However, *in silico* analysis of COL7A1 disclosed several sequence blocks sharing high degree of homology which makes automated re-sequencing of the entire COL7A1 gene with customized Affymetrix GeneChip® at present less convenient than the DHPLC method. Participant 1A has then standardised DHPLC protocols also for the screening of the LAMB3, LAMC2, LAMA3 and the ITGB4 genes, which are frequently mutated in patients with Herlitz junctional EB (HJEB) (LAMB3, LAMC2, LAMA3) and EB-PA (ITGB4) (**Castori M et al, Br J Dermatol 2008; 158:38-44**). In parallel, participant 26 has made available and standardized a new method for molecular diagnosis of EB simplex due to KRT14 mutations. It consists of a single-step, allele-specific PCR designed to avoid sequence contamination from KRT14 pseudogenes (**Glasz-Bona A et al, J Invest Dermatol 2008, e-pub Aug 14**). Furthermore, a collaborative effort among participants 1A, 4 and 8 has allowed to define the molecular basis of Kindler syndrome (KS) in a large group of Italian patients. This study has implication for optimal KIND1 mutational screening in KS individuals (**Has C et al, J. Invest Dermatol 2006; 126:1776-1783**). Subsequent work has been performed by participants 1A and 4 for molecular diagnosis of KS patients collected through the GENESKIN network in their countries and in other European countries, such as Spain, Switzerland and Turkey (**Kaçar N et al, Br J Dermatol 2008; 158:1375-7**, participant 1A; and manuscript in preparation**)**. This activity has led to the identification of recurrent mutations in specific geographic areas with implication for optimal KIND1 molecular screening. Finally, the activity of several laboratories involved in EAD molecular diagnosis has contributed to generate novel notions on population carrier risk for a given disorder within specific populations, unusual phenomena of mutation inheritance in recessive diseases, causative gene function, revertant mosaicism molecular mechanisms, and genotype-phenotype correlations [**Franzke CW at al, J Biol Chem 2006; 281:30260-30268** (participant 4); **Drera B et al, Clin Genet 2006; 70:339-347** (participant 1A); **Has C et al J Invest Dermatol 2006; 126:1776-1783** (participants 1A, 4, 8); **Castori M et al, J Dermatol Sci 2008; 51:58-61** (participant 1A); **Castori M et al, Br J Dermatol 2008; 158:38-44** (participant 1A); **Zimina EP at al, J Biol Chem 2007; 282:22737–46** (participant 4); **Pasmooij AM et al, Br J Dermatol 2007; 156:861–70** (participant 22); **Pasmooij AM et al, J Clin Invest 2007; 117:1240–8** (participant 22); **Murrell DF et al, J Invest Dermatol 2007; 127:1772-5** (participant 22)].

- In the field of KD, participant 5 developed a standardized protocol for SPINK5 sequencing, thus increasing the number of European laboratories performing molecular diagnostics of this severe and even lethal syndrome. In parallel, participant 7 has established molecular diagnostic protocols for two rare forms of KD: CHILD syndrome and X-linked dominant chondrodysplasia punctata (CDPX2). He has also collected biological samples from patients with the clinical diagnosis of CHILD syndrome and CDPX2, in whom no mutations have been found in the NSDHL or EBP genes. These samples are available for search of genetic heterogeneity in these syndromes. The groups of participants 5, 18, 14B and 9B have been involved in the mutation analysis of ABCA12 in patients with Harlequin ichthyosis (**Akiyama M et al, J Invest Dermatol 2007; 12:568-573**) and participant 9B has developed gene specific oligo arrays for ABCA12 in ARCI and Harlequin ichthyosis

patients. In addition, participant 14B was involved in: (i) mutation analysis of GJB2 gene in a cohort of families affected with KID (keratitis-ichthyosis-deafness) syndrome (**Mazereeuw-Hautier J et al, Br J Dermatol 2007; 156:1015-9**, collaboration between participants 14B, 9B and 17), and (ii) molecular analysis and prenatal diagnosis of KRT1/KRT10 genes in children affected with bullous congenital ichthyosiform erythroderma (**Chassaing N et al, J Invest Dermatol 2006; 126:2715-7**, participant 14B). These activities contributed to generate novel data on particular mechanisms of mutation inheritance and genotype-phenotype correlations.

- In the field of ED, participant 7, in collaboration with participant 5 and 1A, collected samples from patients affected with focal dermal hypoplasia (Goltz syndrome). This activity has been instrumental to the identification of the gene responsible (PORCN) for this ED syndrome (**Grzeschik KH et al, Nat Genet 2007; 39:833-5**, collaboration between participants 7, 5 and 1A). Thus, standardised molecular diagnosis of focal dermal hypoplasia is at present possible and performed by participant 7 and participant 8 laboratories (**Clements SE et al, J Dermatol Sci 2008; 49:39-42**, participant 8). In addition, the activity of participant 2B in incontinentia pigmenti (IP) molecular diagnostics has led to the implementation of a database of NEMO mutations (see main objective 1) (**Fusco F et al, Hum Mutat 2008; 29:595-604**, collaboration between participants 2B and 17). This work also showed the presence a secondary mutational hot spot in NEMO gene, located in exon 10 which contains only 11% of the protein. More interesting, familial inheritance analysis revealed an unexpectedly high incidence of sporadic cases (>65%). The sum of the observations can aid both in determining the molecular basis of IP and hypohidrotic ectodermal dysplasia with immune deficiency allelic diseases, and in genetic counselling of affected families.
- As to DNAr disorders, participant 2A, in collaboration with participants 10, 16 and 1A, has carried out the molecular characterization of a large group of nonphotosensitive trichothiodystrophy patients of different geographic origin and with different disease severity, showing that (i) genetic heterogeneity also underlies this subtype of TTD and (ii) the involvement of other factors besides TTDN1 mutations in determining the severity of the disorder (**Botta E et al, Hum Mutat 2007280: 92-96**).
- For what concerns CTD, a standard protocol for molecular testing of the recently characterized GGCX gene, which is responsible of a pseudoxanthoma-elasticum-like syndrome, has also been developed.

  Specific to prenatal molecular diagnosis, participant 2B has focused on the development of a test for non invasive prenatal diagnosis of incontinentia pigmenti, based on genetic analysis of foetal cells circulating in the maternal blood. The approach used has been the enrichment of circulating foetal cells on the basis of their size following the ISET (isolation by size of epithelial tumor/trophoblastic cells) protocol. Short tandem repeat-specific markers (DXS1187, DXS1254 e DXS8094 for X chromosome and Y1.7 e Y1.8 for Y chromosome) were used to discriminate foetal profile from maternal profile. Using peripheral blood samples from unaffected 11-12 week gestation women, the foetus specific amplification for each microsatellite under analysis could be achieved, showing that adequate amounts of DNA for microsatellite amplification can be achieved with this technique. However, the analysis for maternal allele using the above markers did not allow exclusion of a maternal contamination. Therefore, the non-invasive method developed is at present not suitable for prenatal diagnosis of IP pregnant women.

As to the **organization of a "virtual" biological sample Databank** for selected diseases:

- A patients' database representative of the European population affected by EAD was established. At present the bank contains data from 1058 families including 422 patients suffering from dystrophic EB, 294 affected with junctional EB, 220 with EB simplex, 30 with Kindler syndrome and 92 presenting still undetermined forms. Analysis of the collected diagnostic data has led to the identification of novel hot spot mutation sites in different populations. The collected information is also useful in the prospect of selecting potential candidates for clinical trials.
- A database comprising of the European population affected by DNA repair deficiency disorders has been compiled by combining the data from the diagnostic centres in France, Germany, Italy, and United Kingdom. The database comprises 343 xeroderma pigmentosum cases, 154 patients suffering from Cockayne syndrome and 59 from trichothiodystrophy. Retrospective analysis of these cohorts of DNA-repair defective patients has provided an accurate evaluation of the incidence for the three conditions in the western European population. This information has been included in the GENESKIN website disease cards. The establishment of the databank and the scientific publications relating the accurate assessment of these conditions are expected to improve the general awareness of the diagnostic options among the medical community, to promote early diagnoses and facilitate the identification of causative genetic mutations in the patients (**Kleijer WJ et al, DNA Repair 2008; 7:744–50,** collaboration between participants 2A, 10, 16, and 21).
- In parallel, participant 18/29 has organised a sample databank of patients/families affected by ARCI. This sample databank comprises 504 ARCI families: 357 from CEA/Généthon (participant 18/29) and 147 from other GENESKIN participants (participants 27, 19 and 1A). A high throughput sequencing system has been standardised for the simultaneous screening of 7 ARCI genes in 394-well plates under fully automated conditions. The sequencing activity has been carried out on 481 ARCI patients (from 481 independent families) and has provided information for the mutation database, and a more precise estimate of the percentage of implication of each gene in ARCI. This sequencing activity has also provided a large amount of data for genotype-phenotype correlation studies, which are currently ongoing. In parallel, the patients in whom no mutation in known ARCI genes could be found have been selected for mapping studies aimed at the identification of novel genes responsible for ARCI. Thus, this initiative also fulfils the objective of **collecting samples of still uncharacterized diseases as a prerequisite for identification of the causative gene**.

In the field of **collecting/distributing biological samples**, the following initiatives have been developed (in addition to those already mentioned in previous paragraphs):

- Participants 7, 27 and 18/29 have collected DNA samples from families affected by a rare KD, ichthyosis follicularis with alopecia and photophobia (IFAP), which are available for studies aimed at the identification of the causative gene.
- Participants 18/29 and 27 shared the clinical information and samples of a particular form of congenital ichthyosis, called ichthyosis prematurity syndrome (IPS).
- Participant 1A has characterized clinically two families affected with a rare ED syndrome, Schöpf-Schulz-Passarge syndrome (**Castori M et al, Acta Derm Venereol, in press**) and collected DNA samples which have been shared with participant 18/29 for future gene mapping studies.
- Participants 20 and 3 have collected clinical data and biological samples from patients' affected with various CTD. In particular, participant 20 has received samples from families with 'unclassified' variants of EDS, which will be used for investigation at the molecular level. Participant 3 has collected biological samples (skin biopsies, DNA, serum, plasma) from PXE patients. The collected samples have been used for molecular diagnosis,

ultrastructural analysis and also for studies aimed to shed light on PXE pathogenesis (**Gheduzzi D et al, Lab Invest 2007; 87:998-1008**, **Garcia-Fernandez MI et al, Biochim Biophys Acta 2008; 1782:474-81**, participant 3).

Overall, important results have been obtained within the framework of this project major objective and numerous novel collaboration links have been set up among project participants. The activity "Testing and validation of novel antibodies/enzymatic assays" has allowed to validate a novel immunofluorescence procedure for early prenatal diagnosis of a lethal form of EB in kindred carrying unidentified genetic mutations. Activity assays for enzymes defective in congenital ichthyoses have been standardised, while it has not been possible to validate immunohistochemical tests for routine diagnostic screening of congenital/neonatal erythrodermas/ ichthyoses. However, work on this topic will continue also after the end of GENESKIN. Within the framework of the activity "Setting up of standardized molecular diagnostic protocols for genetic skin diseases" diagnostic protocols have been set-up/standardized/refined for several diseases (various forms of EB, ARCI, Netherton and CHILD syndromes, X-linked dominant chondrodysplasia punctata, focal dermal hypoplasia, etc). The activity of "Setting up of a cell, DNA, RNA virtual Databank for selected genetic skin diseases" has led to generation of database on congenital ichthyoses, EAD, and DNAr. Finally, the activity of collection and exchange of biological samples among project participants has been highly fruitful, representing the prerequisite for the development of research projects on causative gene identification, disease pathogenesis, etc.

Main objective 3 - To promote training of clinicians, healthcare and laboratory personnel on clinical, diagnostic and management aspects of specific disease groups and to organize meetings on critical emerging topics.

During the reporting period nine **individual training courses** have been organised. For each course the program is downloadable in the "Training" section of the GENESKIN website. Here, the list of courses:

- A course entitled: "Course in dermatological genetics" focused on EAD and on ethical aspects of genodermatoses has been organized by participant 25 in Salzburg (Austria) on 16–20 October, 2006 (14 participants).
- A course on "Epidermolysis bullosa" has been organized by project participant 22 on clinical and diagnostic aspects of EAD, with a theoretical and a practical part. The course, which took place in Gröningen, Netherlands on 27–28 June, 2006, was attended by 16 participants.
- A course entitled "Workshop on connective tissue disorders", focused on clinical and laboratory aspects of CTD, has been organized by participant 20. The course took place in Ghent, Belgium, on December 5–8 2006 (5 participants).
- A course entitled "Management of Epidermolysis Bullosa" has been organized by project participant 22. It took place in Gröningen (Netherlands) on 17 and 18 March, 2008 (18 participants). This course has been entirely devoted to clinical management of children and adults with EB.
- A second course entitled "Course in dermatological genetics" which has been organised by participant 25 and took place in Salzburg (Austria) on 10-12 October, 2007 (15 participants). The course was focused on EB and ichthyoses and comprised a theoretical part, and a practical part on molecular diagnosis.
- A course entitled "Summer School for Ichthyosis" has been organized by participant 5. It took place in Münster (Germany) on 28-30 August, 2007 (7 participants). The programme included clinical case demonstrations of ichthyosis patients and practical exercises.
- An introductory training course entitled "Genodermatoses: basic concepts on clinical approach, diagnostic procedures and genetic counselling" organised by participant 1A and

held on 21-22 November 2007 in Rome, Italy (17 participants). The course aimed to illustrate the clinical and laboratory procedures for the diagnosis of the most relevant groups of genodermatoses, in particular Ehlers-Danlos syndromes, EAD, KD, and ED.

- The second training course organized by participant 1A, entitled "Practical course on clinical and laboratory diagnostic procedures and management of selected genodermatoses" was held in Rome, on 9-11 April, 2008 (10 participants). It was focused on EAD, Netherton syndrome, DNAr disorders and the Ehlers-Danlos syndromes.
- A "Practical Training Course on Epidermolysis Bullosa: From Bedside to Bench – Clinical, Diagnostic and Research Aspects" which has been organised by participant 4 and was held in Freiburg (Germany) from 4 to 6 June, 2008 (7 participants). The program of this specialized course on EAD included epidemiologic, clinical, diagnostic and research aspects of EB and Kindler syndrome.

In parallel, the **common training activity** was structured in the form of short symposia/meetings/workshops focused on specific topics. Nine events were organised and their program is downloadable in the "Training" section of the GENESKIN website. Here, the list of meetings:

- A workshop entitled "Understanding genodermatoses: a clinical and scientific update" has been organized by participant 9A in London (United Kingdom) on 15-16 September 2005 (12 participants).
- An international symposium on "Recent advances in ichthyoses and related keratinization disorders" has been organized by project participant 5. The symposium took place in Münster, Germany on October 7-8, 2005 (49 participants).
- A symposium entitled "Heritable Connective tissue Disorders" has been organized by participant 20. The symposium took place in Ghent, Belgium, the 16th of March 2006 (23 participants).
- A meeting entitled "UK gap junction: meeting" has been organized by project participant 11. It took place in Glasgow, United Kingdom, the 7th of September 2006 (49 participants).
- A half-a-day common training course on dermato-genetics has been organized by participant 19 on September 14th 2006 in Lausanne (Switzerland).
- A major updating meeting in the field of KD, namely the "First world conference on ichthyosis" was organized by participant 5 from August 31 – September 2, 2007 in Münster (93 participants).
- One half-a-day training course in dermato-genetics with the following topics: EAD, KD and cancer-prone syndromes, was organized by participant 19. The course was held at the occasion of the Swiss annual "Training course in paediatric dermatology", on 30-31 August 2007 (Lausanne) (252 participants).
- A "GENESKIN day" organized by participant 14C at the occasion of the "29th Course of Paediatric Dermatology" on 28 March 2008 (Bordeaux, France) (81 participants). Topics included pathogenetic, diagnostic and therapeutic aspects of selected diseases belonging to KD, ED, CTD, and also of pigmentation disorders and porphyrias.
- An international workshop entitled "GENESKIN: towards a better understanding and care of genetic skin diseases" (June 20, 2008, Rome) was organised by participant 1A at the occasion of the final GENESKIN meeting. Talks were given: (i) by project partners who presented either final data related to project achievements (GENESKIN website, diagnostic tools, ethical experts report, etc) (participants 1, 12, 14A, 18/29, and 24) or updating on specific research and clinical topics related to GENESKIN disease groups (participants 2A, 4, 5, 7, 8, 10, 14B, 16, 20, 21), and (ii) by invited speakers, external to GENESKIN (T. Magin, Bonn; U. Kornak, Berlin; E. Sprecher, Haifa) who reported about their most recent achievements in the field of genodermatoses.

Training initiatives represented an important part of GENESKIN activities, and their organization and delivery run quite smoothly. Some modifications to the planned structure of both individual and common training have been performed in order to better adhere to GENESKIN aims of knowledge dissemination on genodermatoses. Specifically, the total number of individual training courses organized during GENESKIN has been less than originally foreseen (9 instead of 12), but the range of topics treated was wider (also including ED and DNAr). On the other hand, the total number of common training events organized during the project has been significantly higher than originally foreseen (8 instead of 2). Overall, it can be considered that the project objectives in the field of training have been successfully achieved.

Main objective 4 - To promote pan-European communication pathways among patients' organizations, ethic committees, physicians and scientists, as well as close accompanied alliance of patients' organizations and ethic committees with physicians and scientists.

Towards this aim, at first a **network of ethical experts** has been built up by participant 24 group. The network consists of a European interdisciplinary group of seven experts, with different scientific backgrounds, ranging from genetics to philosophy/theology. A first meeting of ethical experts was organised by participant 24 Salzburg on October 2005, where they also met GENESKIN-scientists (participants 1A, 7 and 25) and the representative of DebRA Europe (participant 12) and exchanged their knowledge. A second challenge faced during this meeting has consisted in bringing together common ethical issues with the specific GENESKIN-problems. In a dialogue between ethical experts and GENESKIN-scientists the following issues were identified:

- Prenatal/genetic counselling within the field of genodermatoses
- Genodermatoses as an example for a trial to make a distinction between mild and severe forms of diseases
- The skin - interdisciplinary aspects
- Genodermatoses: gene therapy and genetic research
- Genodermatoses and preimplantation genetic diagnosis (PGD)

On 24th of September 2007 the ethical experts met for a second time in Vienna under the coordination of participant 24 and presented short genodermatosis-related statements to their colleagues. These statements were discussed and used as basis for a consensus draft on genetic counselling. In parallel, two questionnaires with specific questions were prepared and distributed to patients and GENESKIN-scientists, respectively. Responses were incorporated in the consensus draft on genetic counselling. Following further discussions, the text was presented to the GENESKIN-clinicians and -scientists during the final GENESKIN meeting and concomitant workshop. The final version of the **consensus statement**, entitled **"Points for consideration in the genetic counselling of people affected by genodermatoses"**, has then been published on GENESKIN website.

In parallel, an official letter to the patients' associations was prepared. Following this letter, in a permanent contact, patients' associations were invited to take an active part within GENESKIN project. Feedback was good and patients' associations used the chance to present themselves on the GENESKIN website. However, as many of them are already running out of time, energy and money, they rejected unanimously the idea of founding a European GENESKIN-Patients' Association. This deviation from the original work-plan has been compensated by regular and fruitful contacts and ongoing collaboration and initiatives between many GENESKIN participants and existing genodermatosis patients' associations. In parallel, the most well-structured and oldest patients' association for a genodermatosis group, DebRA (participant 12), played a leading role in encouraging other patients' support groups to participate in GENESKIN and in promoting the perspective of people affected by genetic skin conditions. Finally, two of the ethical experts, belonging to participant 24 group, had regular contacts with the patients' associations.

As to the preparation of **a GENESKIN information-kit for non-specialists**, it deals with the ethical- and patients' associations-related aspects of GENESKIN and its contents are presented on the GENESKIN website. Specifically, the following documents have been produced:

- a press release in five European languages (English, French, Italian, German and Swedish) about GENESKIN and its objectives. It does not only provide patients' associations but the whole public and press with material for websites/newspapers/newsletters, broadcasting, television etc.
- Two questionnaires addressed to patients' associations and GENESKIN-scientists, respectively.
- A Consensus-statement of the ethical experts about "Points for consideration in the genetic counseling of people affected by genodermatoses".

Finally, the following **"Task lists for combined activities between public groups, physicians, scientists, ethical experts"** has been delineated. It has been partly implemented during the project and, at the same time, represents a challenge for the future:

1. Continuing and intensifying the linkage between ethical experts and GENESKIN-participants: the document "Points for consideration in the genetic counselling of people affected by genodermatoses" can be used as general frame or as anchor for all further ethical discussions, but it cannot solve specific problems. Therefore, the GENESKIN-scientists were invited to contact the group of ethical experts in the future whenever they want to discuss specific cases. However, many of GENESKIN-participants discuss problems already with local ethical experts – a cooperation which is certainly going to continue.

2. Intensifying the contacts between the national hospitals/institutions specialized in rare genetic skin diseases, the patients' physicians and patients' associations. The communication between these institutions and persons is still mainly based on personal contacts. An institutionalized permanent exchange of knowledge in the whole of Europe (not only within national boundaries) between patients and scientists/physicians cannot be reached by the effort of single persons who are already overloaded with work. The GENESKIN website and "community" activities have represented an attempt to respond to these requirements. However, additional specific initiatives are needed.

3. As genetic skin diseases are very rare they are still often unknown to the public. The world wide web has improved the situation but information from this source, including the GENESKIN website, cannot replace professional promotion actions raising the awareness of journalists in TV, print media and broadcasting stations. In this perspective, during the GENESKIN project some GENESKIN participants (e.g. 8, 9B, 10 and 16 team leaders) have been involved in television documentaries/shows on harlequin ichthyosis, ectodermal dysplasias, and DNAr disorders aimed at the lay public. Also in this field additional specific initiatives should be pursued.

Overall the activities of this WP have proceeded as planned. The work and results produced by the ethical group of experts in collaboration with physicians, scientists and patients' support groups is perfectly in line with project objectives. Although a European GENESKIN Patient Association has not been established (and therefore deliverables 36 and 39 have not been achieved), this deviation from the original work-plan has been compensated by regular and fruitful contacts and ongoing collaboration with patients' associations.

2. Dissemination and use

Thirty-eight papers, ten of which resulting from a collaboration between two or more project participants, acknowledging the GENESKIN project have been published (or are in press) in peer-reviewed international scientific journals (for details, see list below).

1. **Akiyama M, Titeux M, Sakai K, McMillan J.R, Tonasso L, Calvas P, Jossic F, Hovnanian A, Shimizu H.** DNA-based prenatal diagnosis of Harlequin ichthyosis and characterization of ABCA12 mutation consequences. *J Invest Dermatol 2007; 12: 568-573.* (Participant 14B).

2. **Botta E, Nardo T, Orioli D, Guglielmino R, Ricotti R, Bondanza S, Benedicenti F, Zambruno G, Stefanini M**. Genotype-phenotype relationships in trichothiodystrophy patients with novel splicing mutations in the *XPD* gene. *Hum Mutat, in press* (Collaboration between participants 1A and 2A).

3. **Botta E, Offman J, Nardo T, Ricotti R, Zambruno G, Sansone D, Balestri P, Raams A, Kleijer WJ, Jaspers NG, Sarasin A, Lehmann AR, Stefanini M.** Mutations in the C7orf11 (TTDN1) gene in six nonphotosensitive trichothiodystrophy patients: no obvious genotype-phenotype relationships. *Hum Mutat 2006; 28: 92-96.* (Collaboration between participants 1A, 2A , 10, 16 and 21).

4. **Callewaert B, Malfait F, Loeys B, De Paepe A.** Ehlers-Danlos syndromes and Marfan syndrome. *Best Pract Res Clin Rheumatol 2008; 22: 165-89*. (Participant 20).

5. **Castori M, Covaciu C, Paradisi M, Zambruno G**. Clinical and genetic heterogeneity in keratosis follicularis spinulosa decalvans. *Eur J Med Genet, in press.* (Participant 1A).

6. **Castori M, Floriddia G, De Luca N, Pascucci M, Ghirri P, Boccaletti V, El Hachem M, Zambruno G, Castiglia D.** Herlitz junctional epidermolysis bullosa: laminin-5 mutational profile and carrier frequency in the Italian population. *Br J Dermatol 2008; 158: 38-44*. (Participant 1A).

7. **Castori M, Floriddia G, Pisaneschi E, Covaciu C, Paradisi M, Torrente I, Castiglia D.** Complete maternal isodisomy causing reduction to homozygosity for a novel LAMB3 mutation in Herlitz junctional epidermolysis bullosa. *J Dermatol Sci 2008; 51: 58-61*. (Participant 1A).

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10. **Chassaing N, Kanitakis J, Sportich S, Cordier-Alex MP, Titeux M, Calvas P, Claudy A, Berbis P, Hovnanian A.** Generalized epidermolytic hyperkeratosis in two unrelated children from parents with localized linear form, and prenatal diagnosis. *J Invest Dermatol 2006; 126: 2715-7*. (Participant 14B).

11. **Clements SE, Wessagowit V, Lai-Cheong JE, Arita K, McGrath JA**. Focal dermal hypoplasia resulting from a new nonsense mutation, p.E300X, in the PORCN gene. *J Dermatol Sci 2008; 49: 39-42*. (Participant 8).

12. **D'Alessio M, Zambruno G, Charlesworth A, Lacour JP, Meneguzzi G.** Immunofluorescence analysis of villous trophoblasts: a tool for prenatal diagnosis of inherited epidermolysis bullosa with pyloric atresia. *J Invest Dermatol 2008, e-pub Jun 19*. (Collaboration between participants 1A, 14A and 15).

13. **de Zwart-Storm EA, Hamm H, Stoevesandt J, Steijlen PM, Martin PE, van Geel M, van Steensel MA.** A novel missense mutation in GJB2 disturbs gap junction protein transport and causes focal palmoplantar keratoderma with deafness. *J Med Genet 2008; 45: 161-6*. (Participant 11).

14. **de Zwart-Storm EA, van Geel M, van Neer PA, Steijlen PM, Martin PE, van Steensel MA.** A novel missense mutation in the second extracellular domain of GJB2, p.Ser183Phe, causes a syndrome of focal palmoplantar keratoderma with deafness. *Am J Pathol 2008; 173: 1113-9.* (Participant 11).

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20. **Fusco F, Mercadante V, Miano MG, Ursini MV.** Multiple regulatory regions and tissue-specific transcription initiation mediate the expression of NEMO/IKKgamma gene. *Gene 2006; 383: 99-107.* (Participant 2B).

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23. **Gheduzzi D, Boraldi F, Annovi G, DeVincenzi CP, Schurgers LJ, Vermeer C, Quaglino D, Ronchetti IP.** Matrix Gla protein is involved in elastic fiber calcification in the dermis of pseudoxanthoma elasticum patients. *Lab Invest 2007; 87: 998-1008*. (Participant 3).

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Project website address:

http://geneskin.idi.it

Figures

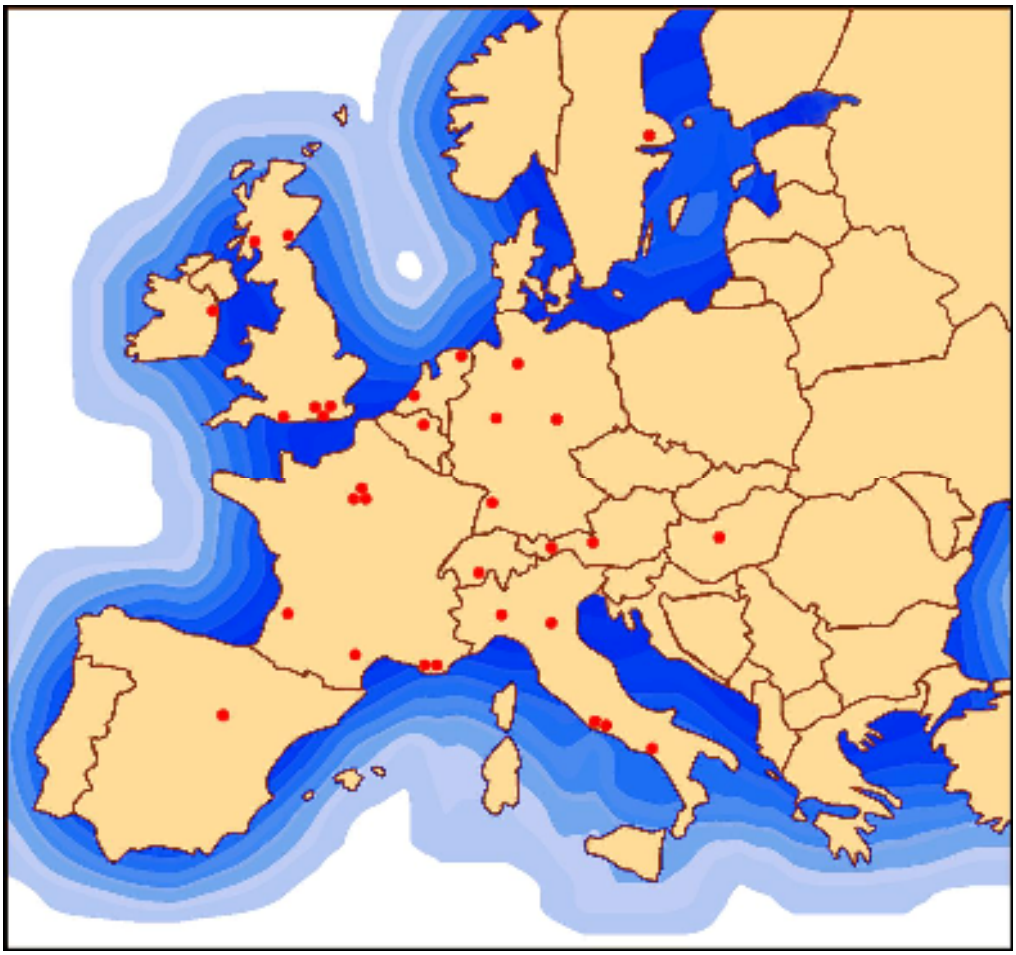

Figure 1. The GENESKIN network with participating centers (●)

Figure 2. GENESKIN website home page with project logo.

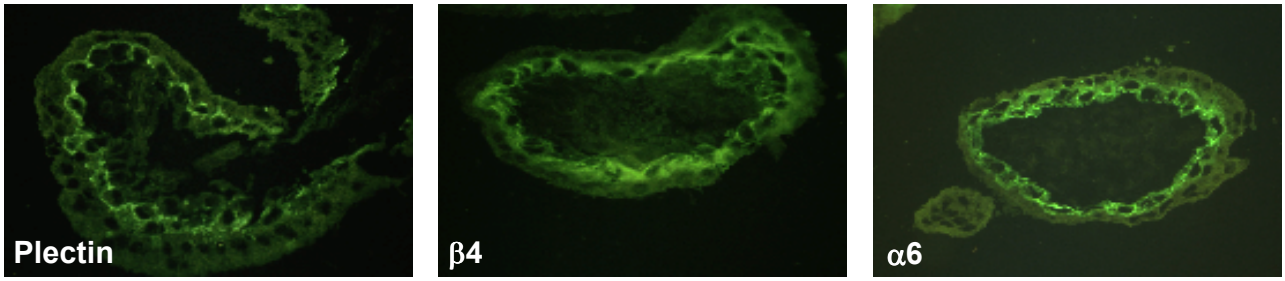

Figure 3. Immunofluorescence labelling of frozen chorionic villi obtained from a pregnancy at risk for recurrence of junctional epidermolysis bullosa with pyloric atresia due to compound heterozygous mutations in the ITGB4 gene. The positive staining for beta 4 and alpha6 integrin subunits predicts a healthy foetus. As expected, chorionic villi also express plectin. Parallel molecular diagnosis showed the foetus to be healthy carrier of the paternal ITGB4 mutation. Immunofluorescence and molecular findings were confirmed by the birth of an unaffected healthy baby.