

THE ROLE OF INHERITANCE AND THE CARE OF CHILDREN

Dry skin – the sort that itches, peels, flakes or cracks in cold weather – is extremely common. As many as one in ten of the population – five to ten million people – suffer from it at some stage. The cosmetics industry, with its emollient creams and lotions, is founded on it.

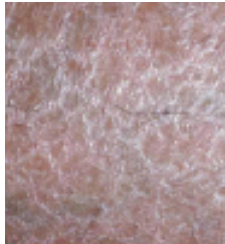
Some people have a more serious condition known as *ichthyosis* in which the skin is dry, thickened and scaly. Ichthyosis is derived from the Greek, meaning “fish scales”, a reference to the characteristic appearance of the skin. The commonest type, accounting for 95 per cent of cases, is *ichthyosis vulgaris* which affects an estimated 800,000 people in Britain. It is mostly mild and in many cases mistaken for normal dry skin but rare types of ichthyosis can be severe and even life threatening.

Ichthyosis is a genetic skin disease, passed down the generations. There are an estimated 5,000 genetic diseases in all, of which around one third involve the skin. The best hope for patients suffering from these diseases is to identify the gene (or genes) that is the culprit and to develop a therapy based on that.

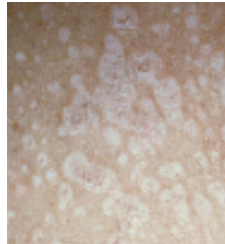
That is the task of the Paediatrics and Genetics department headed by Professor **John McGrath**. He is a gene hunter with a collection of scalps on his belt. To take one example, he and his team investigated *lipoid proteinosis*, a rare condition affecting one in 300,000 people which causes scarring and infiltration (thickening and hardening of the skin) and found the gene that caused it, *ECM1* (*extracellular matrix protein 1*). Working on this protein, he then found a link to a much more common inflammatory skin condition, *lichen*



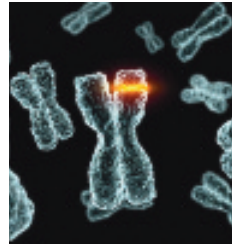
Ichthyosis



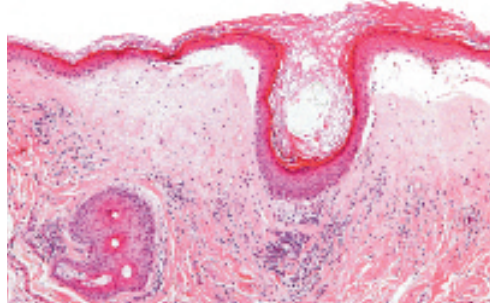
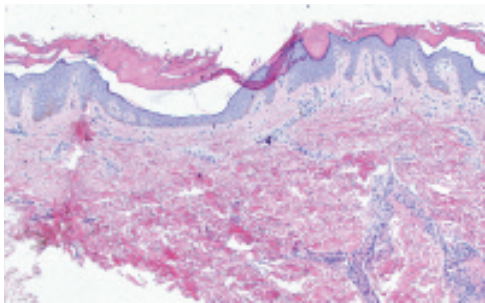
Ichthyosis Vulgaris



Lichen Sclerosus



Chromosome Mutation

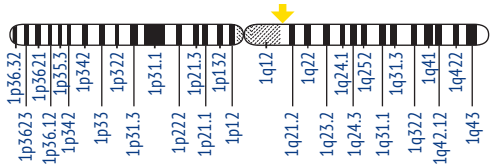


One in ten people – five to ten million – suffer from dry skin but some have a more serious conditions. Ichthyosis Vulgaris (left) affects around 800,000 people in Britain and Lichen Sclerosus (right) affects 200,000

sclerosus, which affects one in 300 people. The breakthrough in understanding led to new approaches to treating both diseases.

Professor McGrath and colleagues have since discovered several single gene mutations that are the cause of other skin diseases and opened the way for the development of new and more effective therapies.

At the same time the clinical service of paediatric dermatology offered at St John’s has expanded rapidly since the mid-2000s. Prior to 2007, there was one paediatric clinic a month for the severest cases run by a consultant from Great Ormond Street Hospital for Sick Children.



The gene that causes lipoid proteinosis: ECM1

Today, the St John’s clinic is designated as a national centre for the treatment of highly specialised paediatric skin disease. Last year, under consultant dermatologist **Jemima Mellerio**, it saw 2,000 patients.

“Until 2007, we used to get the children, babies with eczema and all the rest and put them in with the adults. Now that seems extraordinary,” she said.

History



John McGrath



Geoffrey Dowling



Bob Meara



George Wells



Robin Eady at work in the 1960s

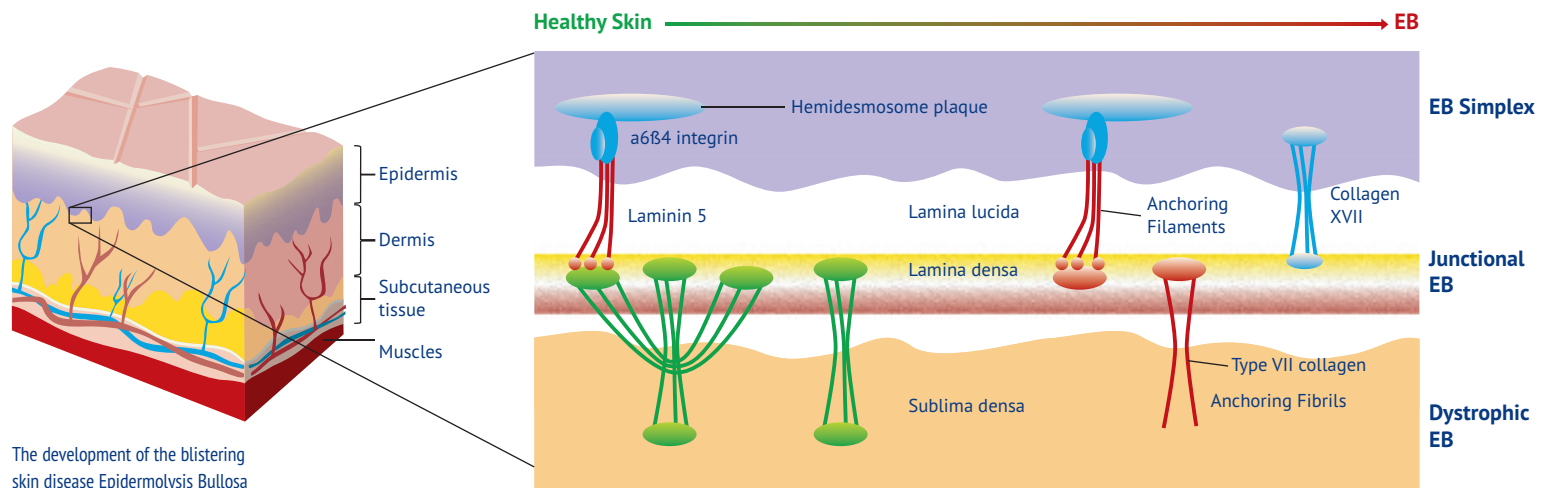
The origins of St John's expertise in genetics can be traced back to the 1950s when dermatologists from around London used to meet at the hospital, then in Lisle Street, to discuss unusual cases. "It was the collection of these cases and their careful description that laid the groundwork for future advance," said Professor McGrath.

Geoffrey Dowling, consultant and director of St Johns from 1951-56, described with registrar **Bob Meara** a form of the blistering skin disease *Epidermolysis Bullosa* (EB, see below) since called *Dowling-Meara EB simplex*.

R S Wells – known as Charlie – developed prototype classifications for the different types of

EB and ichthyosis. He was also an inspirational teacher, raised money to upgrade the dermatology section of Guy's medical museum to become a "teaching laboratory" and by the early 1980s St John's was producing one quarter of all the dermatologists in the country.

By then **Robin Eady**, another pioneer, was using



electron microscopy to see inside cells and inspect their structure. It was a long laborious process, but it led ultimately to quicker diagnosis through the introduction of *immunohistochemistry* (staining techniques), reducing the wait for results from three weeks to three days.

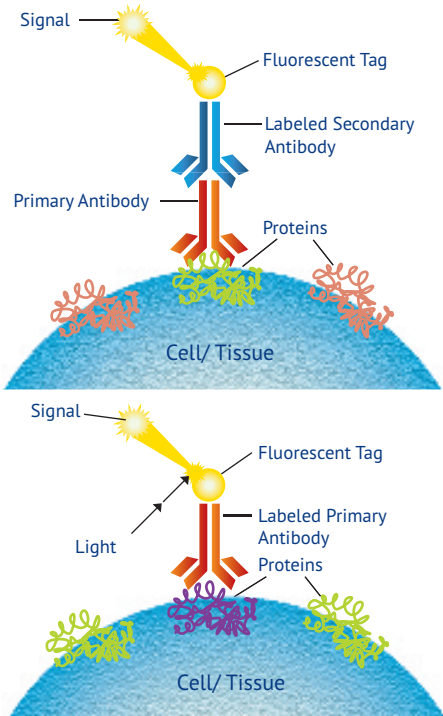
Eady, working with obstetricians, also pioneered the development of foetal skin biopsy – taking tiny skin samples from babies in the womb to examine for EB. The test was offered to parents who already had an affected child to give them the option of a termination. But the biopsy could not be done until 16 weeks, very late in the pregnancy. Foetal skin biopsy has now largely been superseded by *chorionic villus sampling*, taking a tiny sample from the placenta, which is carried out at 10-11 weeks and has already been applied to over 400 couples at St John's.

Nevertheless, this still left parents carrying an affected baby facing the heartrending decision of

whether to seek a termination. It would have been far better to make the diagnosis before the mother became pregnant.

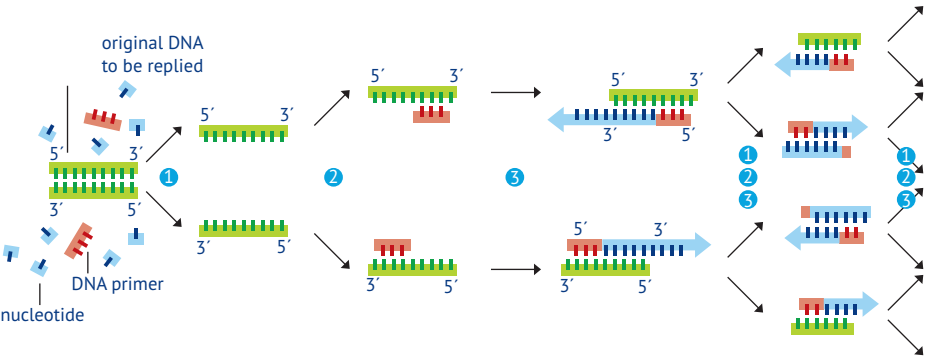
With the development of single cell *polymerase chain reaction (PCR)* technology, which could amplify a single piece of DNA by several orders of magnitude, pre-natal diagnosis became possible in the late 1990s. Parents could opt for in-vitro fertilisation (IVF) with pre-implantation diagnosis to select unaffected embryos for replacement in the womb. It was the natural extension of Eady's work

Eady, who was awarded an MBE in the New Year Honours 2014, was also a pioneer in a different way. He is the world's longest surviving kidney failure patient, at the time of writing, who as a medical student in 1963 flew to North America for treatment. He was so weak he had to be carried off the Boeing 707. He spent 24 years on dialysis and the last 26 with a kidney transplant.



Indirect immunohistochemistry and immunofluorescence methods

Pre-natal diagnosis of certain skin diseases became possible with the development of polymerase chain reaction (PCR) technology



Genetic breakthrough



"It was going through the analyser and I could see there was an exciting mutation. It was a Eureka moment. We had discovered a new disease" – Professor John McGrath

In 1995, McGrath, who returned to St John's from a sojourn in the US, began his gene hunting in earnest. He established a molecular diagnostics laboratory and raised money for an automated gene sequencer, then regarded as a "new-fangled" piece of kit.

As it was being installed, McGrath pulled a tissue sample from his "special bottom draw" of mystery patients, to use as a test.

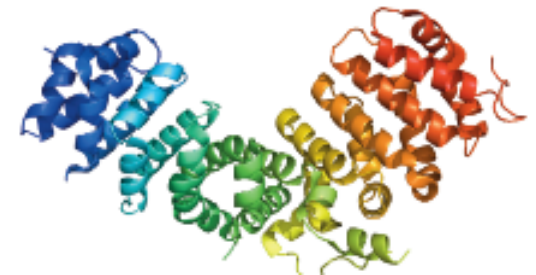
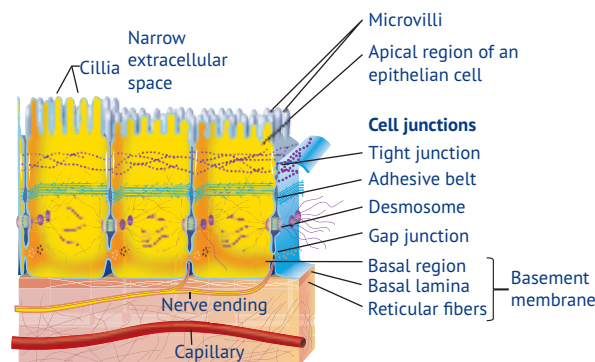
"It was going through the analyser and I could see the company technician frowning. He thought there was a malfunction. But I could see something exciting was unfolding - there was an exciting frame-shift mutation. It was a Eureka moment – we had found a new disease."

The sample in the sequencer was from a patient with *ectodermal dysplasia-skin fragility syndrome* and it had revealed the cause to be a desmosomal gene disorder resulting from a mutation in plakophilin 1. It was the world's first genetic disorder of cell attachment complexes called *desmosomes*. After McGrath published his findings

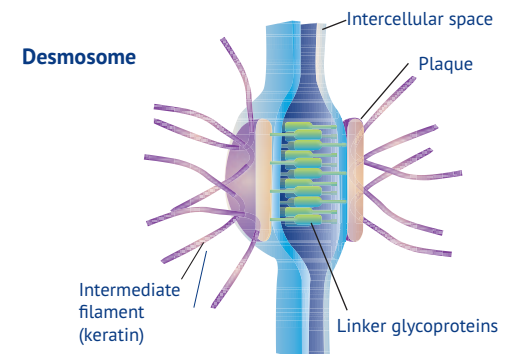
in 1997 the condition subsequently became recognised as a form of *EB*.

Over the next 15 years, McGrath examined the structure, function and protein composition of

Special Characteristics of Epithelia-Cell Junction



Plakophilin 1 Protein



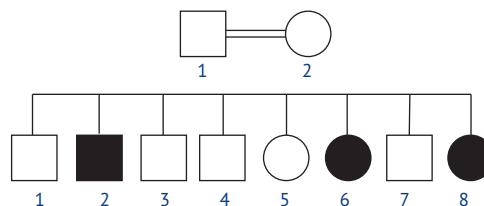
Desmosomes: Anchoring junctions bind adjacent cells together and help form an internal tension-reducing network of fibers.

tissue samples from a range of patients to gain some idea of what was going wrong in order to target the search for the genetic culprit with what is known as the “candidate gene” approach. With larger families where several members were affected, genetic linkage studies could reveal bits of the genome that were shared among all those with the disease.

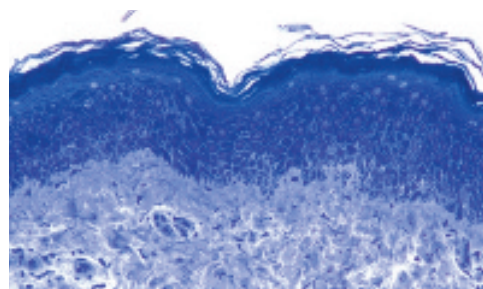
In the last four years, the search for disease genes has advanced again with the introduction of “next generation sequencing” which has enabled all 20,000 genes in the human genome to be sequenced for the same price as a single gene a decade ago. The new approach involves “dredging up everything in the sea” and using computer modelling to filter out what might be significant.

The technique yielded success in 2012 with the discovery of a new form of autosomal recessive skin **EB** caused – unexpectedly – by a mutation in the gene *exophilin 5* that controls the microtubul transport system in cells.

“The cause was so different from other forms of **EB**. Without next generation sequencing, this disease would have remained a mystery,” said McGrath.



The family pedigree. Squares denote male family members, and circles female family members; filled-in symbols indicate clinically affected individuals.



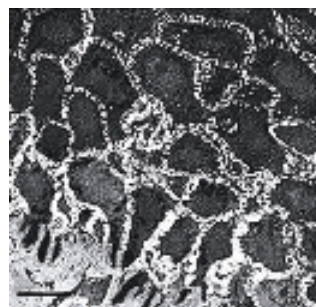
Light microscopy of skin reveals mild acanthosis and hyperkeratosis as well as a ruffled appearance to the dermal-epidermal junction (Richardson's stain; scale bar represents 50 μ m).



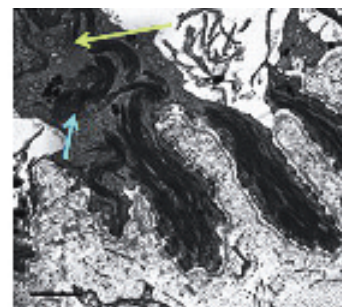
Affected individual II-2 with skin crusting at the site of a recent trauma-induced erosion.



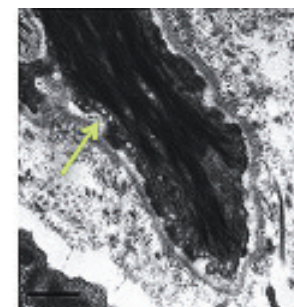
Higher magnification of the crusted erosion.



Low-magnification transmission electron micrograph shows widening of spaces between keratinocytes in the lower epidermis with some aggregation of keratin filaments (scale bar represents 3 μ m).



Higher-magnification transmission electron micrograph reveals keratin filament disruption (blue arrow) as well as perinuclear accumulation of vesicles (green arrow) (scale bar represents 0.5 μ m).



There is also focal accumulation of vesicles close to the plasma membrane (green arrow) (scale bar represents 0.25 μ m).

Epidermolysis Bullosa

Imagine having skin that blistered at the slightest touch, that was so fragile a light knock could open a wound or a gentle rub leave it sore and bleeding. There are many different types and degrees of severity of the rare inherited skin disorder that affects an estimated 8,000 people in the UK and 500,000 worldwide. Around 420 of the most distressing cases are under the care of St John's.

Jemima Mellerio said: "It is a devastating disease. I can't think of any other condition where you are in pain every day of your life. It is not like something dreadful happens to you at age 10, say. It starts from birth, it is continuous every day, and it is your parents who are inflicting the pain, as they change your dressings. It affects the whole family."

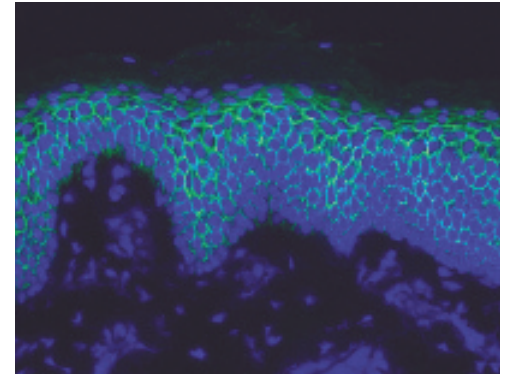
It is not only the skin that is affected. Corneal blisters can appear on the eyes, the teeth may be affected, the oesophagus narrowed causing difficulty swallowing and other internal organs and membranes including the anus may be sore. Anaemia and constipation are the consequences.

The symptoms are caused by mutations in any of 18 different genes which make proteins that "stick" the top and bottom layer of skin together. There is no cure – doctors can only strive to minimise the effects. But that can mean the difference between a full life and half a one.

"Most of our patients are in mainstream school. We have young adults who are learning to drive, attending university, some have relationships, some have children. When you visit patients in other countries you realise: we do a lot," said Mellerio.

Until a decade ago the care delivered to **EB**

patients was haphazard, relying on the goodwill of the many specialties involved to give of their time and expertise. In 2002 the National Specialised Services Commissioning Group established St John's as one of four national centres for patients with **EB** (the others are at Great Ormond Street in London and Birmingham Children's Hospital and Heartlands Hospital in Birmingham). This meant, for the first time, there was a ring fenced pot of money to pay for the specialists required, ensuring a multidisciplinary approach. The National **EB** Diagnostic Laboratory is based at St John's providing analysis of skin biopsies, genetic testing



Immunofluorescence in Epidermolysis bullosa acquisita skin



A patient with EB meets the Countess of Wessex. The condition causes blistering of the skin at the slightest touch



The National EB Diagnostic Laboratory is based at St John's providing analysis of skin biopsies, genetic testing and pre-natal diagnosis

and pre-natal diagnosis. A significant number of the St John's EB patients have severe disease and need services ranging from plastic surgery to palliative care.

The big threat to patients with severe EB is *squamous cell skin cancer*. Most start to develop it in their 20s, 30s and 40s but the youngest case was a child aged six, and there have been several in their early teens. It is a leading cause of death – Mellerio recalls a teenager who died aged 19 of the cancer.

"Patients tend to get one cancer, you cut it out and then another appears a year later. Then the disease accelerates. They face a lot of work cutting it out and having plastic surgery," she said.

For young people, on the cusp of adulthood this is hard news to absorb. They must be warned of the risk because they need to watch for changes in their skin and alert doctors when they notice one, so it can be biopsied. But information is shared on social networking sites – and some are terrified of what may happen.

"It is a sword hanging over them. It can be very difficult to manage. You jolly them along as kids but there is a real shift in their teens. I enjoy the transition – they can be infantilised in the children's department. But it is hard. Eventually they realise what they are facing – they vary enormously in their resilience. I quite like the stropky teenagers – those with a certain strand of belligerence tend to do well."

"One patient said he had always thought having EB was like climbing a mountain. When he got his first cancer he realised that all the time he had just been in the foothills. He died as a young adult."

New Treatments

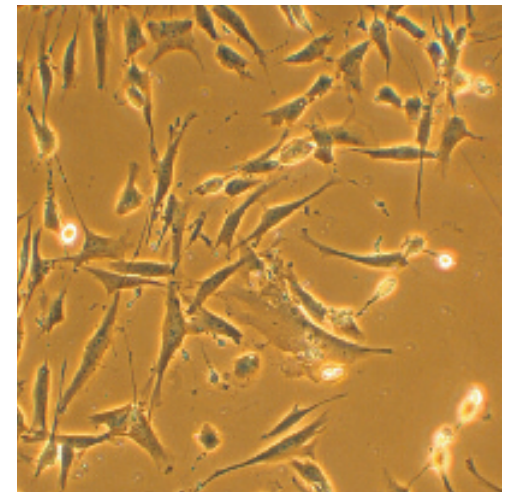
St John's is pioneering efforts to find new treatments for EB and other skin diseases. At least four trials were underway at the time of writing.

1 Stem cell therapy

One of the most exciting is an attempt to ease the impact of the disease by infusing bone marrow-derived stem cells from unmatched donors to create a generic anti-inflammatory effect. Ten children have been included in the trial which began in the autumn of 2013 and each has had three infusions of the mesenchymal stromal cells over 28 days into a vein. It is a small, Phase 1 trial to test the safety of the procedure. However, early results suggest the children have less pain, less itching, more appetite, more energy and their wounds heal faster. "We expect the effects to wear off but we may be able to do it again," said McGrath. The technique is also being tried in the Netherlands. The group at St John's also works closely with colleagues in the USA who are developing bone marrow transplantation for EB (the external advisory committee is chaired by Professor McGrath). Much work still needs to be done, however, before bone marrow transplantation can ever be considered a routine treatment for the clinic.

2 Fibroblasts

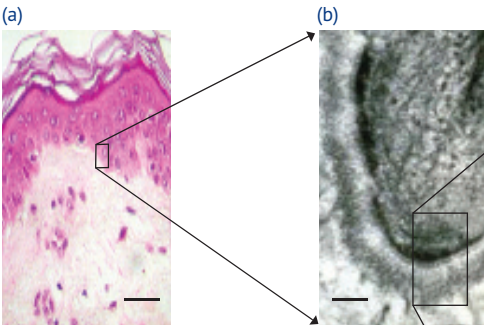
In this research, adults with EB have been injected with fibroblasts, the cells that produce collagen, to speed healing. The cells were injected around the edges of wounds and the results showed a statistically significant improvement in rates of healing for the first 28 days.. The study was published in the British Journal of Dermatology (October 2013). It is likely that more injections will be needed to heal most wounds. Injecting fibroblasts can be painful, and new devices are being developed to make the injections hurt less.



Human fibroblast cells – injected into EB patients to speed healing

3 Gene therapy

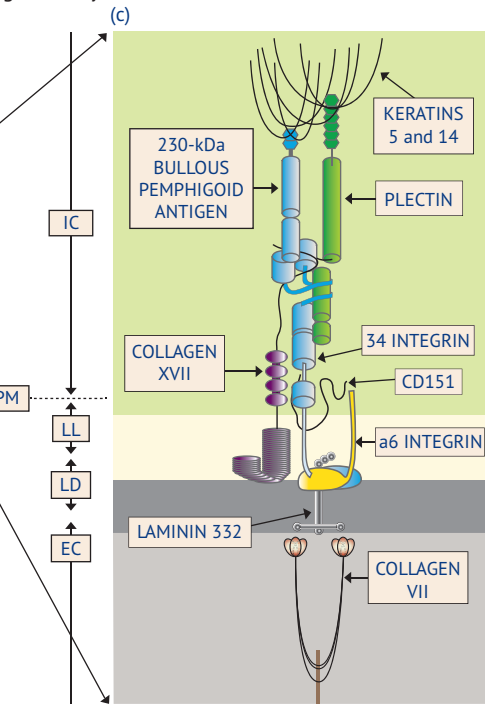
Clinical trials of correcting EB skin cells by replacing the responsible gene outside the body and then grafting them back is in progress in the USA and Europe, but the group at St John's is focusing on gene correction of the patient's own fibroblasts. "We have a lot of experience with injecting fibroblasts. Now we want to go to the next step of putting back the patient's own cells after we have corrected the gene. Hopefully this will lead to stronger skin that remains so for a long time," said McGrath.



The molecular basis of inherited skin blistering involving hemidesmosome-associated proteins. (a) Light microscopy image of the skin; the boxed area indicates a dermal-epidermal junction (b) Transmission electron microscopy image of a dermal-epidermal junction; hemidesmosome attachment complexes are boxed (scale bar=0.1 μ m); (c) A schematic representation of the protein organization of dermal-epidermal attachment complexes, the intrinsic proteins and the genes encoding them, and the associated genetic diseases. Revertant mosaicism has been reported for keratin 14 (KRT14), laminin-332 (LAMB3), type XVII collagen (COL17A1) and type VII collagen (COL7A1).

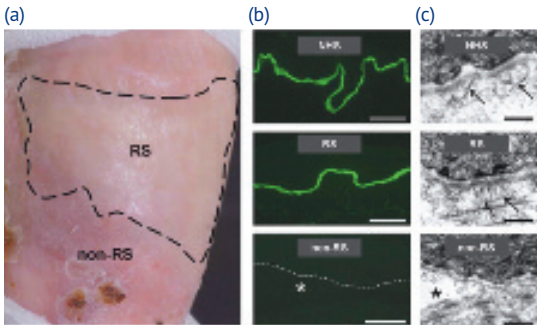
4 Natural gene therapy

One of the curiosities of EB is that some patients have areas of skin that never get blisters. The genes in those areas appear able to reboot themselves providing doctors with an opportunity to use the "healthy skin" to treat the "unhealthy" areas. By culturing keratinocytes – the outer skin cells – from the healthy tissue it should be possible to create skin grafts to repair the wounds. In the Netherlands, researchers have used punch-grafting in one patient – taking small pieces of the healthy skin and seeding them in areas of unhealthy skin – with good early results.



5 Inducible pluripotent stem cell therapy

One of the most significant advances of the last seven years has been the discovery that an ordinary skin cell can be re-programmed to behave like an embryonic stem cell and develop into any tissue in the body. The technique has so far been used on keratinocytes taken from healthy patches of skin on EB patients, which have been programmed to form skin progenitor cells which could be used for treating EB. Because they are the patient's own cells rather than provided by a donor there is no risk of rejection. In patients who lack healthy patches of



Revertant mosaicism in RDEB. (a) Clinical evidence of reverted (RS) and unreverted (nonRS) skin; note the severe blistering phenotype in the nonRS area. (b) Immunofluorescence image showing Type VII collagen expression shows bright linear labeling at the dermal-epidermal junction in normal human skin (NHS) and in the RS but no signal is detected in the nonRS sample (dashed line indicates dermal-epidermal junction; asterisk depicts subepidermal blistering, scale bar=50 μ m). (c) Transmission electron microscopy images showing anchoring fibrils beneath the lamina densa in NHS and also in RS (arrows) but not in nonRS samples. There is also blistering beneath the lamina densa in nonRS sample (asterisk). Scale bar=0.2 μ m

skin, the researchers plan to use gene editing techniques to correct the genetic fault. Writing in the *Journal of Investigative Dermatology* (January 2014) the authors, including McGrath from St John's, say the approach should be "the starting point for autologous [from the same body] cellular therapies using natural gene therapy" in **EB**.

Other research is assessing the potential of protein and drug therapy. As many of the severely affected **EB** patients lack collagen VII, essential for maintaining the structure of the skin, one approach is to replace it directly. Micro-needles, no bigger

than the mouth parts of a mosquito, are coated with collagen VII and stuck through the skin in the hope that the collagen will dissolve off directly into the cells, helping the inner and outer layers of the skin to stick together. If the technique proves effective the collagen could be delivered by a dressing covered with fine needles that have first been coated with it. Research is also underway to give replacement protein therapy by intravenous injection - in France, experiments with this approach have been carried out on dogs.

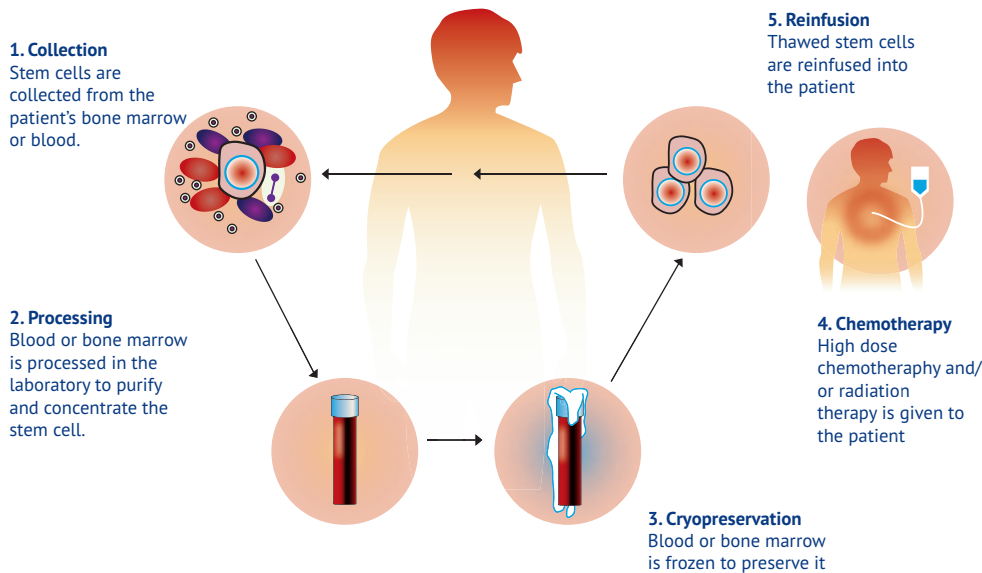
Drugs are being developed to repair some types

of genetic mutation, and have been trialled in *muscular dystrophy* and *cystic fibrosis*. If successful they may be extended to include **EB**.

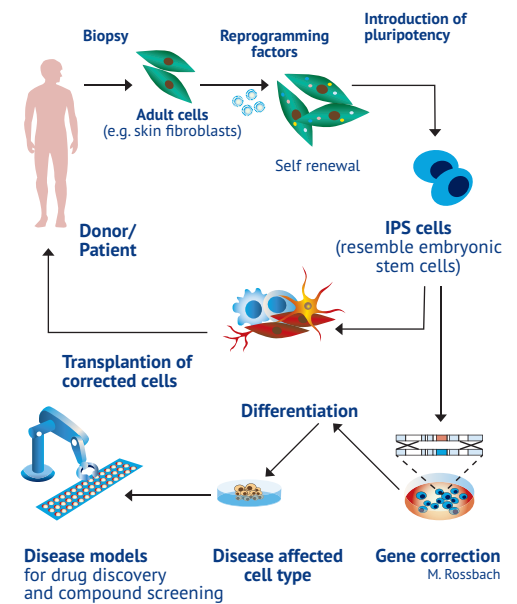
McGrath said: "St John's is not about treating conditions seen at the local district hospital. This is where the buck stops. We are here for patients who can't be treated anywhere else."

"I joined the Institute in the 1990s and have had a couple of decades making discoveries. Now I want to give something back to the patients - designing and carrying out clinical trials of new therapies. That is going to be my work for the next decade."

The Autologous Transplant Process

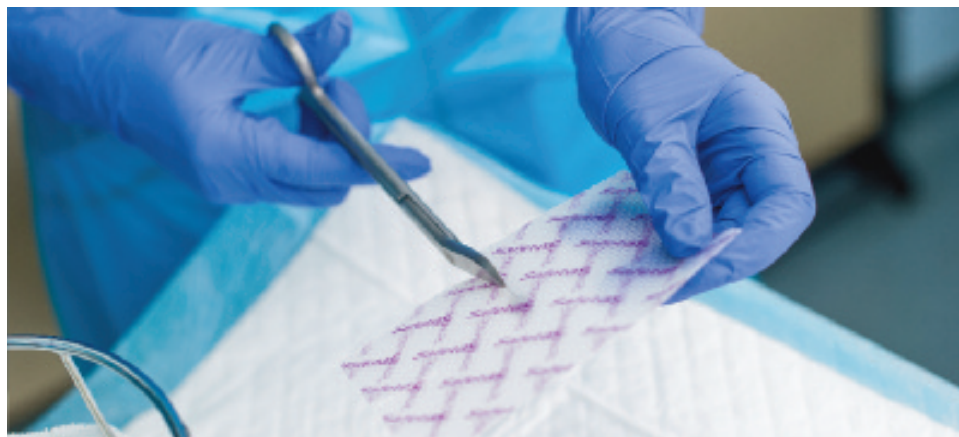


The autologous transplant process - removing stem cells from a patient before treatment with high dose chemotherapy, then replacing them afterwards



Induce pluripotent stem cell therapy - re-programming skin cells to become stem cells

Nursing



Dressings have advanced from traditional gauzes to soft silicone and there are a range of garments, including tubular vests and leggings to hold them in place.

Caring for people with EB who have fragile, blistering skin is extremely challenging. St John's has five specialist nurses who go out into the community to help adult EB patients and their families in their own homes, advising about skin care, liaising with employers or university/colleges and educating local nurses. They also provide end of life care to patients with advanced cancer related to their EB.

In the past they were funded by DEBRA, the national charity for people with EB, but are now funded 75 per cent by the NHS.

Separately, Great Ormond Street provides specially trained nurses to support families with EB. When notified of the birth of an affected baby they will give advice to the local nurses caring for it over the phone and make home visits to the family throughout childhood.

"Within 24 to 48 hours one of the Great

Ormond Street nurses will travel anywhere in the country to take skin biopsies and blood for genetic testing. They will advise nurses about putting babygros on inside out, so the seams don't rub, and avoiding plastic name tags. They will also take dressings and give advice on feeding," said Mellerio.

Dressings have advanced from traditional bandages and gauzes pre-1990 to Vaseline gauze in the mid-1990s to soft silicone dressings post-2000.

Today nurses have developed a range of garments, including tubular vests and leggings to hold the dressings in place.

Mellerio said: "If you have a baby with severe ichthyosis and the nurses on the neo-natal ward have no expertise, it is hard. I would like to see a community outreach service introduced for other skin diseases."

CASE STUDY - BETHAN THOMAS

Every eight weeks or so Bethan Thomas has to return to St Thomas' from her home in South Wales to have her oesophagus dilated – a challenging procedure that requires several days in hospital.

"They can never predict how long because nothing ever goes smoothly with me," she says with a shrug.

She is 37 and a lifelong sufferer from *recessive dystrophic EB* of an unusual type – *inversa*. She has a badly affected mouth with a narrow opening, a tied tongue, blisters on the inside of her cheeks and difficulty swallowing and eating. In addition to the regular dilatations of her oesophagus, she has a feeding tube direct into her stomach.

Despite these difficulties she has never let her illness stop her doing what she wanted. "My parents never treated me any different to my brother. I wasn't molly coddled. If they said I couldn't do it, I did it anyway."

A self confessed tomboy, she played rugby as a teenager, hung out with the lads and discovered alcohol. "I was never in. I was always out with the boys. I worked in a pub. I'm not scared of nothing."

Her truculence has served her well. Today she lives with her partner, Bady, 42, a window maker, and her teenage daughter, Georgia, who is taking her GCSEs.

"I know people who let EB rule their life. When Georgia was born they said come and have it in St Thomas'. I said no child of mine is

going to be born in England.” She had a planned Caesarean in her local district hospital instead.

She has had a number of crises, including several spells in hospital for *septicaemia*. Blue light ambulances have rushed her through the streets. “It’s my oesophagus that’s knackered. But if you can’t swallow you panic and then you can’t breathe.”

She tries to do without her feeding tube, drinking up to 16 pints of milk a week. Solid food is more challenging – it may take her two hours to eat a sandwich.

“I have managed for 37 years. I don’t know how I do it but I do. My throat hurts a lot but I can’t do nothing about it. That’s life.”

She has stopped coming for out patient appointments. “I’m not coming all the way to London for half an hour. They know me – I don’t bother them unless I need to. If I ring they know there is something wrong. I have seen enough doctors.”

She tries not to think about the future. She knows there is a risk of cancer. But anyone can get it, she says. “It is always in the back of my mind. But if you start thinking that way you might as well just curl up.”

CASE STUDY - MANDY ALDWIN

The unpredictability of her condition is the hardest part for Mandy Aldwin. She has ichthyosis of a rare type called Netherton syndrome which causes reddened scaling skin, with her face being most severely affected. It is an inherited condition that can be managed but it can’t be cured.

“Unlike psoriasis and eczema, it’s not patchy but affects all of my skin all of the time. I manage it with a daily regime of creams and lotions. My type flares up and I get infections so I have to take antibiotics. It’s on and off sore most days – but it can be extremely painful and then I have to rest. The best treatment, apart from a lot of grease and emollients, is bed rest.”

Now aged 37, she was cared for at Great Ormond Street as a child because her local hospital in Reading did not know how to cope. On reaching the age of 16 and becoming an adult she was discharged but four years later she had a crisis at the age of 20.

“My skin suddenly deteriorated. There was a major change in the way it was behaving. My skin was very bad and did not get better – it went on for months.”

Her mother was desperate for help and called St John’s for advice. Mandy was admitted to a bed in St Thomas’ and remained in hospital for over two weeks.

That was 17 years ago. She has been under the care of the Genetics department at St John’s ever since and attends the hospital every six months for a check.

“I can contact them when I need to. But under their guidance I am self managing now. I know what to do – I just refer to them for reassurance and any new information.”



“Other people’s reactions were hurtful as a child.”

Coping with the condition is not just a matter of managing the physical symptoms. Like all skin ailments, it has a psychological impact too. “My skin is very inflamed and peels. Other people’s reactions leave something to be desired. It comes down to a lack of knowledge – but it was hurtful as a child. To a certain extent I have learnt to deal with it, but if it looks particularly bad I might decide I do not want to go and do the food shop.”

Mandy and her mother, Maggie are two of the founder trustees of the Ichthyosis Support Group which now has 600 members across the country. Sharing experiences helps, she said, and the group also helps focus efforts to improve treatment and care.

“The attitude of doctors has changed. My consultant, Jemima Mellerio, is very empathetic and appreciates the person behind the condition. In earlier years, consultants were very much focused on the condition. It is so much better, psychologically, to be treated as a person than as a fascinating condition.”

