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## Revertant mosaicism in genodermatoses

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### Abstract

Inherited monogenic skin disorders include blistering disorders, inflammatory disorders, and disorders of differentiation or development. In most cases, the skin is broadly involved throughout the affected individual's lifetime, but rarely, appearance of normal skin clones has been described. In these cases of revertant mosaicism, cells undergo spontaneous correction to ameliorate the effects of genetic mutation. While targeted reversion of genetic mutation would have tremendous therapeutic value, the mechanisms of reversion in the skin are poorly understood. In this review, we provide an overview of genodermatoses that demonstrate widespread reversion and their corrective mechanisms, as well as the current research aimed to understand this “natural gene therapy”.

### INTRODUCTION

The epidermis undergoes a regular pattern of self-renewal, and of the 85 billion cells in an average individual, 1 to 2 billion are replaced every day [1]. With age, primarily benign neoplasms appear on the skin, with most resulting from genetic mutation induced by ultraviolet radiation-induced DNA damage or via mutation arising due to the basal mutation rate of  $\sim 1 \times 10^{-10}$  inherent to DNA replication [2]. Other genetic alterations including gene conversion, mitotic recombination, and break induced repair arise after a DNA double strand break [3]. While most somatic mutations fall in noncoding regions, when they arise within coding elements, there is the potential to cause or revert disease.

Central to the evolution of new clones within the skin is fitness. Just as traits enhancing survival and reproduction undergo positive selection, at the cellular level genetic mutations conferring higher rates of proliferation, drug resistance, or growth factor independence give rise to pathogenic clones that can outcompete neighboring cells, and is a basis of tumorigenesis [4,5]. Conversely, genetic reversion, a rare process by which inherited mutations are fully or partially corrected, can produce wild-type clones with improved fitness versus affected neighbors [6]. When somatic mutations arise in affected tissue which abrogate expression of a mutant allele or give rise to the expression of a wild-type allele,

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such events generate healthy wild-type cells that proliferate and coexist alongside mutant cells, leading to revertant mosaicism (RM) [6].

RM was first observed in studying the lymphoblasts of a patient with Lesch-Nyhan Syndrome (LNS), an X-linked recessive disorder caused by mutation in the *HPRT1* gene leading to defects of the encoded hypoxanthine-guanine phosphoribosyltransferase (HGPRT) enzyme, which recycles purines during DNA turnover [7]. In LNS, accumulated substrates are instead converted into excess uric acid by xanthine oxidase, leading to hyperuricemia, neurological dysfunction, and gout [8]. In one case with a mild phenotype, revertant lymphoblasts were found with deletion of a pathologic duplication of 20 kilobases, regenerating a functional copy of *HPRT* [7]. RM has since been reported in a variety of heritable disorders, including Fanconi anemia, Wiskott-Aldrich syndrome, and epidermolysis bullosa (EB) [9–11].

Several genetic causes of revertant mosaicism have been identified, including back mutation or second-site mutation, intragenic recombination and gene conversion, or errors in DNA duplication like polymerase slippage [12,13]. Back mutation, or reverse mutation, is the correction of a pathogenic mutation via site-specific mutagenesis back to wild-type, while second-site mutations are compensatory mutations that remove a dominant negative allele, and include frameshift mutations which correct insertions or deletions, intronic splice-enhancers, mutation of enhancers and suppressors, or early termination mutations [6,14]. DNA double-strand breaks (DSBs) can initiate homology-dependent repair, leading to reversion via either intragenic recombination or gene conversion [15,16]. Intragenic recombination is the reciprocal exchange of genetic information, often between two sister chromatids during mitosis; conversely, gene conversion is unidirectional—or nonreciprocal—exchange of genetic information, as the intact “donor” (e.g. DNA of the sister chromatid) is used as template to repair the DSB at the homologous “acceptor” site [15]. Slippage of DNA polymerase occurs within tandem repeats, leading to small insertions or deletions, which can correct multiple base pair duplications [17]. However, such correction is incredibly rare and has only so far been observed in a single case of Wiskott-Aldrich syndrome, in which revertant T cells were found with deletion of a 6bp tandem repeat as result of slippage [11]. With the exception of true back mutation, these mechanisms may also lead to partial—rather than complete—reversion due to an incomplete correction, as pieces of remaining aberrant genetic information can encode for semifunctional proteins [13,18]. For example, frame-restoring revertant mutations can occur distal to the pathogenic insertion or deletion, leading to proteins with residual missense amino acids between the site of original mutation and reversion [18].

While generally a rare phenomenon, reversion can occur with strikingly high incidence in the disorders in which it occurs: up to 36% of epidermolysis bullosa (EB) resulting from *COL17A1* and 33% resulting from *LAMB3* mutations demonstrate RM [19,20]. Further, multiple independent events of reversion within one patient is not uncommon, and each independent event may be the result of one [21–23] or more [19,24] distinct corrective mechanisms.

The direct causes of genetic reversion are unknown, and it is unclear why some genetic skin disorders revert while others do not. Genodermatoses that demonstrate RM present a unique opportunity to study this phenomenon, especially in cases where revertant skin has distinguishing clinical characteristics that contrast with neighboring affected epidermis.

## THE EPIDERMIS

Human skin is comprised of the dermis and epidermis, which are separated by the basement membrane. Comprised of nearly 90% keratinocytes, the stratified squamous epithelium epidermis is organized into four cellular layers, from the innermost basal layer (stratum basale) to the spinous (stratum spinosum), granular (spinous granulosum), and lastly, the cornified layer (stratum corneum) [25]. New, differentiated keratinocytes are continuously generated from stem cells within the basal layer matching the rate of ongoing desquamation at the skin surface [26]. Basal keratinocytes are anchored to the basement membrane via cytosolic keratins 5 and 14 (*KRT5* and *KRT14*), which attach to hemidesmosome plaques comprised of bullous pemphigoid antigen 1 (*BPAG1*), type XVII collagen (*COL17A1*), plectin (*PLEC1*), and the  $\alpha6\beta4$  integrin heterodimer [27]. Type XVII collagen and  $\alpha6\beta4$  integrin extend into the lamina lucida, situated just beneath the hemidesmosome, where  $\alpha6\beta4$  associates with heterotrimeric laminin-332 filaments in the lamina densa (*LAMA3*, *LAMB3*, and *LAMC2*) [27,28]. Laminin-332 links the  $\alpha6\beta4$  integrin with type VII collagen (*COL7A1*), the major component of anchoring fibrils that extend into the papillary dermis [28,29]. Following asymmetric basal cellular division, keratinocytes rise upward into the suprabasal layer and start their differentiation program, expressing terminal differentiation markers including keratins 1 and 10 (*KRT1* and *KRT10*), loricrin (*LOR*), transglutaminase-1 (*TGMI*), and filaggrin (*FLG*) [30,31]. The majority of genetic skin diseases that demonstrate reversion involve mutation in one of the aforementioned genes.

DNA replication errors during daughter cell generation within the basal layer can produce genetic variation, even in the absence of environmental mutagens. These genetic changes can persist over time if they provide any survival advantage over neighboring cells, and their relative fitness determines the extent to which mutant—or revertant—progeny can populate the epidermis. Assessment of RM in the skin can be readily accomplished using sequence analysis of genomic DNA isolated using laser capture microdissection (LCM) of biopsies from suspected revertant areas, as well as immunohistochemistry to detect changes in protein expression [13].

## REVERSION IN BLISTERING DISORDERS

Cutaneous RM was first described in a case of generalized atrophic benign epidermolysis bullosa (GABEB), now known as generalized intermediate junctional EB (JEB), caused by mutations in *COL17A1* or *LAMB3* [10]. The 28-year-old proband displayed typical features of generalized intermediate JEB, including generalized trauma-induced blistering, universal alopecia, pigmentary changes, nail dystrophy, and dental anomalies.

Unlike the affected areas of the skin where blisters were easily evoked and *COL17A1* was absent in the basal keratinocytes, clinically unaffected patches with normal pigmentation

occurred in a symmetrical phylloid pattern over the extensor surfaces of the hands and upper arms, were resistant to rubbing, and were found to have normal COL17A1 expression. The subject was known to have compound heterozygous COL17A1 mutations, and while the paternal nonsense c.3781C>T, p.R1226X mutation was still present in the revertant patches alongside the affected skin and blood, the maternal c.1706delA frameshift leading to premature termination was absent. The concurrent absence of an intronic polymorphism 381bp downstream from c.1706delA indicated allelic loss rather than back mutation, and retention of heterozygosity in the chromosomal DNA flanking COL17A1 suggested mitotic gene conversion using the paternal allele. As the paternal mutation was not found to be duplicated, the conversion tract likely began downstream of the paternal p.R1226X mutation [10].

Second-site mutations leading to RM have also found in generalized intermediate JEB [18]. A skin biopsy of nonblistered skin from a 56-year-old female homozygous for a 2-bp, 4003delTC deletion in COL17A1, which leads to premature termination and nonsense-mediated decay, showed mosaic expression of COL17A1 at the basement membrane, indicative of reversion. LCM of the COL17A1 positive keratinocytes and sequencing of isolated genomic DNA revealed a 2-bp, 4080insGG insertion upstream of the deletion, restoring the reading frame to yield mRNA and functional protein [18].

Multiple corrective mechanisms occurring within the same individual were described in two compound heterozygous, unrelated probands, with generalized intermediate JEB arising from mutations in COL17A1 [32]. A 75-year-old male with compound heterozygous nonsense c.3781C>T, p.R1226X mutation and a frameshift c.4424insC mutation leading to premature termination, demonstrated a 2cm<sup>2</sup> circular, clinically normal patch on his right middle finger which tolerated the wearing of a wedding ring with no history of blistering. Biopsy from this site demonstrated mosaic expression of COL17A1, whereas lesional skin was completely absent of immunoreactivity. Genomic DNA extracted from the COL17A1 positive keratinocytes identified a second c.4463-1G>A mutation leading to reading frame correction. Another biopsy of clinically affected skin from the right upper shoulder also displayed mosaic COL17A1 expression; however, the COL17A1 positive keratinocytes from this site were found to have kept the c.4424insC frameshift mutation, instead correcting the c.3781C>T, p.R1226X mutation of the alternate allele. Flanking SNPs showed no evidence of loss of heterozygosity suggesting back mutation as the likely mechanism. A second compound heterozygous patient, a 45-year-old female harboring a paternal nonsense c.3781C>T, p.R1226X mutation and a maternal c.1706delA frameshift mutation leading to premature termination, was previously reported with revertant patches on the dorsum of her left hand arising via mitotic gene conversion which corrected the maternal mutation. On follow-up, LCM and sequencing of COL17A1 positive keratinocytes from the revertant patches on her ankle revealed a different corrective mechanism, a somatic c.3782G>C change which alters the paternal p.R1226X nonsense mutation to a functional p.R1226X>S missense mutation. In contrast, biopsy and microsatellite SNP analysis of revertant patches on the forearm demonstrated mitotic gene conversion leading to loss of the maternal frameshift mutation [32].

Generalized intermediate JEB arising from mutations in *LAMB3* also undergoes RM, with patchy restoration of normal histology and laminin-332 immunoreactivity [33]. Distinct, independent second-site mutations correcting the loss of function p.E210K mutation have been found in different revertant areas within the same individual, suggesting the lack of one preferred corrective mechanism. Studies of larger cohorts of this disorder following these reports have revealed that more than a third of generalized intermediate JEB cases demonstrate somatic reversion.

While less common than in JEB, somatic reversion in both recessive and dominant subtypes of EB simplex, as well as recessive dystrophic EB (RDEB) has also been reported, which arise from mutations in *KRT14* and *COL7A1*, respectively. Consequently, while the epidermal-dermal separation occurs at the lamina lucida in JEB, EB simplex suffers cleavage within the epidermis and beneath the lamina densa in RDEB. Subpopulations of basal keratinocytes cultured from lesional skin of a 67-year-old female with recessive EB (REBS), who was homozygous for a c.1842-2A>C splice site mutation in *KRT14* leading to premature termination and complete lack of keratin 14 synthesis, were serendipitously found expressing keratin 14 [34]. Immunofluorescence and electron microscopy of skin from nearby areas demonstrated mosaic expression of keratin 14 in the basal layer implicating reversion, and immunoblotting further demonstrated presence of full-length keratin 14. However, sequencing DNA from keratin 14 positive keratinocytes did not demonstrate genetic reversion, as the original splice mutation was maintained with no evidence of additional mutations or change in flanking intron borders. Instead, analysis of the *KRT14* transcripts in these cells identified a unique mRNA splice variant harboring a 6-bp deletion in combination with a U to G change, generating an intact reading frame. This reversion of cellular phenotype without corresponding changes in the clinical phenotype or genotype was attributed to changes in a modulating factor in the genome, which potentially affects processing of the *KRT14* pre-mRNA [34,35].

Somatic reversion of autosomal dominant generalized severe EBS (formerly EBS, Dowling-Meara), which features widespread herpetiform (clustered) blisters, was found in one female proband in her twenties with dominant-negative heterozygous c.373C>T, p.R125C mutation in *KRT14*, the most common missense mutation in generalized severe EBS [36]. Culturing basal keratinocytes from lesional skin revealed a subset of cells with normal keratin 14 expression due to a second c.242insG mutation upstream of the p.R125C mutation, creating a premature termination codon inactivating the dominant negative allele.

Mutations resulting in loss of normal *COL7A1* expression cause recessive dystrophic EB (RDEB), the most severe form of EB in adults which features pseudosyndactyly or “mitten deformities” of the extremities due to extensive blistering and scarring of the hands and feet, as well as an increased risk of squamous cell carcinoma. RM has been identified in two cases of RDEB to date [20,37]. A 41-year-old male compound heterozygous for two loss-of-function mutations in *COL7A1*, c.1732C>T, p.R578X and c.7786delG, p.G2593fsX4, exhibited severe blistering, mutilating scars, bilateral mitten deformities and recurrent squamous cell carcinomas, and had two clinically normal patches on his left wrist and right shin, both of which had never blistered despite repeated trauma. Positive *COL7A1* immunostaining in these revertant patches, alongside isolation of wild-type cDNA via RT-

PCR implicated genotypic reversion, and SNP genotyping revealed intragenic crossover between exons 21 and 104 leading to loss of the paternal allele. In the second case, a 42-year-old female homozygous for the c.6527insC, p.2176FsX337 frameshift mutation in *COL7A1* with classic features of RDEB demonstrated a revertant patch on her left forearm, and the revertant keratinocytes were found to have a second-site mutation c.6528delT resulting in correction of the reading frame [20].

Kindler syndrome (KS) is a recessive syndromic blistering skin disorder featuring congenital poikiloderma, mucosal inflammation, and photosensitivity, caused by mutations in kindlin-1 (*FERMT1*) [38]. Now classified as a subtype of EB, Kindler syndrome also demonstrates RM in very rare cases [39,40]. A study of 6 KS patients with germline duplication mutations in *FERMT1*, c.456dupA or c.676dupC which lead to premature termination codons p.N153RfsX4 and p.Q226PfsX17, respectively, all with a variable number of revertant patches throughout the body, found restored kindlin-1 expression along with normalized histopathology in the revertant areas [39]. Closer analysis of two patients not only demonstrated revertant keratinocytes in which the mutation was in a heterozygous state due to slipped mispairing and single nucleotide deletion but also identified keratinocytes that were homozygous wild-type, due to additional mitotic recombination. Keratinocytes with loss of kindlin-1 expression were found to have severely impaired proliferation, suggesting revertant patches clonally expand and become clinically evident by out-competing adjacent affected cells [39]. In addition, a case of KS with no genotypic evidence of reversion was also reported with RM [40], demonstrating intact *FERMT1* mRNA expression and restored  $\beta$ 1-integrin activation from functional kindlin-1 despite sustained c.676dupC, p.Q226PfsX17 mutation, suggesting the potential for KS to also revert via transcriptional modification [40].

## REVERSION IN ICHTHYOSIS WITH CONFETTI

Ichthyosis with confetti (IWC) is a rare, severe disorder of keratinization, first described in 1984 as a unique type of ichthyosis featuring congenital generalized erythema and development of pale patches of normal-appearing skin during childhood [41]. Unlike the few, scattered patches found in the EB, revertant macules and patches in IWC often number in the hundreds to thousands, representing a remarkable frequency of reversion; further, these patches expand in both size and number with age, demonstrating enhanced competitive potential against neighboring lesional cells [42]. In addition to confetti macules, patients also demonstrate dorsal acral hypertrichosis, mammillae hypoplasia, and malformation of the ears [43,44].

All mutations identified in IWC to date are *de novo* frameshift mutations with dominant inheritance affecting the carboxyl tail domain of keratin-10 (*KRT10*) or keratin-1 (*KRT1*), causing Type I IWC (IWC-I) and Type II IWC (IWC-II), respectively [21–23,45,46]. *KRT10* and *KRT1* interact via their rod domains to form obligate heteropolymers that serve as the major intermediate filament protein of the suprabasal layer of the epidermis. Mutations in *KRT1* or *KRT10* affecting the conserved rod domains necessary for heteropolymer formation lead to a distinct ichthyosis phenotype known as epidermolytic ichthyosis (EI). Notably, genetic reversion has never been reported in any case of EI, implicating a role of keratin 10 and 1 tail domains in RM [21,47].

Histologically, lesional epidermis in IWC-I displays retention of nuclei in the stratum corneum (parakeratosis), perinuclear vacuolization, and absent keratohyalin granules, with intermediate filament network collapse and KRT10 mislocalized from the cytosol to form nucleolar aggregates, whereas IWC-II displays prominent keratohyalin granules without perinuclear vacuolization or parakeratosis, with KRT1 accumulating as peri-nuclear aggregates that reflect collapse of the filament network around the nuclear envelope [21,22]. And while both IWC-I and IWC-II feature widespread development of revertant macules after birth, the ones found in IWC-II are smaller in size and more commonly in flexural areas [21,22]. The revertant macules in both subtypes show normalized differentiation, histology, and localization of KRT10 and KRT1.

The first genetic study of 7 independent kindreds with IWC-I identified distinct frameshift mutations, all of which replace the endogenous glycine-rich tail with a polyarginine sequence [21]. As polyarginated peptides are known to bind ribosomal proteins, the arginine repeats in the mutant tail were postulated to be critical for nucleolar mislocalization as well as genetic reversion; however, subsequent identification of alternate frameshift mutations leading to a polyalanine tail in *KRT10*, also featuring nucleolar aggregates and reversion, suggests that loss of the endogenous glycine-rich tail may underlie the pathobiology of IWC-I [23,45]. Likewise, in IWC-II, both a mutation leading to KRT1 with a polyarginine tail [46], as well as a mutation replacing the final 22 amino acids with a new 30 amino acid sequence [22], have been reported.

In all cases of IWC, each revertant macule arises via a single corrective mechanism: mitotic recombination. Long stretches of copy-neutral loss of heterozygosity (CN-LOH) extending from proximal 17q or 12q to the telomere, encompassing the *KRT10* or *KRT1* locus, respectively, have been identified in IWC-I and IWC-II, respectively, resulting in loss of the mutant haplotype [21].

Why does IWC demonstrate a predilection of mitotic recombination in lieu of the other corrective mechanisms? As DNA double-strand breaks (DSB) precede mitotic crossover, the LOH found in all revertant macules underscores a possible function of keratins in maintenance of DNA integrity [48], with mutation of the tail domain leading to upregulation of DNA damage and subsequent damage response.

The expansion in both size and number of revertant spots further implicates a role of enhanced cellular competition in the unprecedented frequency of RM in IWC. Focal induction of KRT14 mutation via topical Cre activation in a mouse model of EBS have demonstrated reduced fitness of cells harboring intermediate filament mutations, failing to generate stable blistering skin due to migration of surrounding normal stem cells into the wound bed [49]. Recently, modification in the adult stem cell population was demonstrated to occur via cellular competition in a JAK-STAT dependent manner within the drosophila gut, whereby wild-type epithelial cells exhibited increased fitness specifically in the company of unhealthy mutant cells, which were generated by targeted mutagenesis of the *Minute* gene to decrease ribosome biogenesis [50]. The accelerated growth of healthy cells abutting unhealthy cells was found to occur following paracrine Unpaired-3 (Upd-3) secretion from unhealthy cells exhibiting chronic JNK signaling. Binding of Upd-3 produced

from the affected cell population increased JAK-STAT signaling in nearby healthy cells, leading to increased proliferation. Interestingly, as nucleolar mislocalization of the keratins may also impair ribosome biogenesis, a similar JAK-STAT dependent modification of the basal stem cell layer may occur in IWC epidermis, promoting the expansion of revertant clones.

## PARADOX OF REVERSION IN KERATIN 10 MUTANTS

While IWC mutations remain restricted to the tail domain of KRT1 and 10, mutations affecting the conserved rod domains (1A, 1B, 2A and 2B) necessary for heteropolymer formation lead to a distinct ichthyosis phenotype known as epidermolytic ichthyosis (EI) [51]. A conditional knock-in mouse model of the most common *KRT10* mutation underlying EI, p.Arg156Cys (p.Arg154Cys in *M. musculus*), faithfully regenerates the clinical phenotype of EI, including widespread hyperkeratosis, blistering and erythroderma, as well as histological features of perinuclear vacuolar degeneration, and suprabasal cytolysis [52]. Notably, genetic reversion has never been reported in any case of EI, implicating a unique role of KRT1 and 10 tail domains in the mechanism of cutaneous RM [21,47]. Interestingly, mutations affecting the tail domain of KRT1 have been reported to cause alternate ichthyoses without evidence of reversion, including a c.1628delG generating an alanine-rich peptide causing ichthyosis hystrix Curth-Macklin (IHCM) [53], and a c.1752insG leading to an atypical case of EI with late onset of disease and absence of skin blistering [54]. The KRT1 c.1752insG mutation in the atypical EI case generates the same arginine-rich aberrant tail as the KRT1 c.1758insT mutation recently reported in IWC-II [46]. As late onset of revertant spots is a feature of IWC-II, it is possible that Sprecher et al.'s report on their 17-year-old patient preceded his development of visible revertant macules. Indeed, the first case of IWC-II reported visible white spots at age 22 [22], while the proband in Suzuki et al. developed visible spots at age 56 [46].

The tail domains of KRT1 and 10 are unique for their glycine-rich motif, arranged in X(Y)<sub>n</sub> where X is usually an aromatic or long-chain aliphatic residue, and Y is most often a glycine [55]. Furthermore, size polymorphisms exist in the tail domain, with as up to 114 base pairs deleted in frame in normal individuals (38 amino acids) [56]. These glycines are proposed to form highly flexible loop structures that have been proposed to play a role in the cornification process via “Velcro-like” interaction with a similar glycine-rich motif found in loricrin, a major constituent of the terminally differentiate corneocyte [55,57]. However, the specific function of the tail domain remains unknown.

## GENOTYPIC TO PHENOTYPIC REVERSION

Correction at the DNA level is not sufficient for development of clinically evident revertant cell clones. First, genetic correction must occur in a cell with replicative potential, such that the corrected genotype is propagated onto a clinically detectable population of cells, and leads to a durable—rather than transient—reversion. Second, reversion should confer a selective advantage which permits genetically “corrected” cells to expand and become clinically apparent. We propose that these parameters largely determine the extent of phenotypic revertant mosaicism in cutaneous disorders.



The requirement for replicative potential of the corrected cell contributes to the accumulation of a cell population capable of being clinically recognizable. Mature keratinocytes are derived from less-differentiated precursors whose self-renewal capabilities, combined with ongoing production of differentiating daughter cells, produce a population of corrected cell progeny if genetic reversion is present in the precursor cells. An analogous case occurs in blood in the bone marrow failure syndrome Fanconi Anemia, in which reversion within hematopoietic stem cells leads to repopulation of the bone marrow with healthy differentiated clones [9]. The clinical manifestation of reversion in a Mendelian disorder may, therefore, depend on which level in the cell progenitor hierarchy the reversion event occurs. Recent studies employing long-term lineage tracing have supported the stochastic model of epidermal homeostasis, in which uncommitted stem cells within the basal layer possess an equal chance to proliferate or to differentiate, with the latter generating a population of committed cells that align into vertical columns to form epidermal differentiation units (EDU), and with roughly 10% of cells within an EDU escaping their preexistent columns to form new EDUs [58,59]. Reversion within members of the EDU may propagate reversion at least over the span of its column, and the revertant phenotype will remain transient and therefore bounded, unless reversion also extends to a proliferating basal stem cell.

Bone marrow cells have been linked to the skin in other studies. It has been shown that after skin wounding in mice, bone marrow cells contribute to repopulation of the skin and can even differentiate into keratinocytes [60,61]. Notably, mouse models of RDEB following allogeneic bone marrow transplantation (BMT) demonstrated patches of healthy skin, leading to prolonged survival and partial recovery of COL7A1 expression in the dermal-epidermal junction, suggesting the applicability of BMT to treat inherited skin disorders [62]. Indeed, early clinical trials that followed soon thereafter infusing RDEB patients with allogeneic bone marrow were successful in improving skin and mucosal integrity, as well as partial correction of COL7A1 expression [63,64]. Interestingly, a substantial population of CD45-donor cells was identified in the skin, which were suggested to secrete COL7A1 into the lamina densa [63,64]. It is thus possible that clinically evident reversion in diseases featuring RM results from events occurring in the bone marrow: e.g. for diseases in the blood, these clones differentiate into corrected blood cells, and in the skin, bone marrow-derived cells could differentiate into keratinocytes.

Central to reversion appearing in a subset of genodermatoses may be a fertile cellular environment (e.g. stress, wounding, or inflammation), which promotes the selective expansion of the genetically revertant clones. Precedent for this comes from p53-mutant epidermal progenitor cells in which, upon UVB exposure, the wounded/stressed state confers mutant cells a particular advantage to promote their proliferation [65]. Competing aberrant clonal expansions (ACEs) have even been observed in macroscopically normal, sun-exposed skin, presumably following mutation of cancer-associated genes including bi-allelic inactivation of *NOTCH1* that provide mutant cells with pervasive positive selection [66,67]. Similarly, the influence of the cellular milieu on the fitness of revertant clones likely determines the rate and extent of improvement of clinical phenotype [65,68].

Some studies utilizing *in vitro* culture and *ex vivo* skin equivalents comprised of a mixture of revertant and mutant cells in EB subtypes did not demonstrate selection favoring revertant over neighboring mutant cells. Basal keratinocytes harvested from a revertant patch of a patient with JEB who was compound heterozygous for maternal c.1601delA and paternal c.3676C>T mutations in *COL17A1* contained a minor population of revertant, COL17A1-positive cells, which continued to decline down to <1% with subsequent passages [69]. Organotypic cultures generating a 3D skin equivalent found only 20% of the stratified epidermis expressing COL17A1, explaining the high failure rate previously experienced with autologous cell grafts employing naturally corrected keratinocytes for functional treatment of JEB [69,70]. It remains unclear, however, why revertant skin successfully matures and expands in some patients with RM, while mutant cells can exhibit a growth advantage *in vitro*. The distinct clinical and experimental observations raise two important questions: first, whether the presence and degree of positive selection as result of genetic reversion may be disease-dependent, whereby revertant cells in disorders like IWC, which feature the expansion of healthy macules, acquire a more significant advantage over mutant cells than the revertant cells in JEB [42]. This may serve to explain the variability of RM among distinct disorders that arise from mutations of the same gene (IWC vs. EI), as well as the absence of visible RM in the majority of cutaneous disorder despite the innate mechanisms governing genetic reversion. Second, a physiological cellular milieu may facilitate the effects of positive selection via the interplay of immune cells and cytokines which are absent *in vitro*, like the aforementioned JAK-STAT signaling pathway that requires a concerted processes of secreted molecules and cell-cell interactions [50].

## THERAPEUTIC APPROACHES HARNESSING GENETIC REVERSION

Poor survival of primary revertant EB cells in culture has motivated the generation of induced pluripotent stem cells (iPSCs) from revertant keratinocytes for subsequent autologous grafting in blistering disorders [69–71]. Subsequent differentiation of revertant iPSCs into keratinocytes have allowed generation of functional 3D skin equivalents with COL17A1 in the basement membrane at levels comparable to normal human keratinocytes *in vitro*, and these cells were successfully transplanted using a chamber assay onto immunocompromised mice [72]. Further, adenovirus-associated genome editing of *COL7A1* mutant iPSCs from an RDEB patient and successful formation of keratinocyte sheets and organotypic cultures for autologous grafting has demonstrated the potential to use any patient-derived cell for therapeutic use without the provision of having undergone natural genetic reversion [73].

## CONCLUSION

Understanding mechanisms of revertant mosaicism holds potential for therapeutic reversion of inherited and acquired disorders. Autologous transplantation of revertant skin from a patient with generalized intermediate JEB has successfully re-epithelized healthy skin in both the donor and grafted areas, suggesting expansion of revertant skin for clinical use as potential strategy against genetic blistering disorders [69]. Revertant keratinocytes from an RDEB patient has also been successfully reprogrammed into induced pluripotent stem cells, suggesting the potential to generate an inexhaustible supply of functional, patient-specific

cells for therapeutic transplantation in various end-organ systems [74]. Finally, the efficacy of BMT to induce revertant patches in RDEB implicates a yet-unknown link between the hematopoietic system and cutaneous reversion [63]. The frequent, widespread spontaneous self-correction we observe in blistering disorders and IWC raises a promising possibility that any dominant mutation, whether inherited or acquired, including oncogenic mutations in cancer, is capable of genetic reversion to wild type. Elucidating the genetic mechanisms of spontaneous genetic reversion will not only broaden our understanding of recombination and DNA repair, but also provide an opportunity to develop clinical methods to induce or increase the frequency of therapeutic genetic reversion for the treatment of human disease.

## Abbreviations used

|              |  |
|--------------|--|
| <b>RM</b>    | revertant mosaicism                        |
| <b>KRT10</b> | keratin 10                                 |
| <b>KRT1</b>  | keratin 1                                  |
| <b>KRT14</b> | keratin 14                                 |
| <b>JEB</b>   | junctional epidermolysis bullosa           |
| <b>IWC</b>   | ichthyosis with confetti                   |
| <b>RDEB</b>  | recessive dystrophic epidermolysis bullosa |
| <b>EBS</b>   | epidermolysis bullosa simplex              |

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**Table 1**

Genodermatoses demonstrating revertant mosaicism

| Disorder               | Affected gene  | Mutation(s)                                     | Mechanism of Reversion  | Reference |
|------------------------|----------------|---|---|-----------|
| JEB                    | <i>COL17A1</i> | Compound heterozygous c.3781C>T and c.1706delA  | Gene conversion correcting c.1706delA   | [10]      |
| JEB                    | <i>COL17A1</i> | Homozygous c.4003delTC                          | Framecorrecting c.4080insGG   | [18]      |
| EBS                    | <i>KRT14</i>   | Homozygous c.1842-2A>C splice-site              | Second-site mutation c.1844T>G, c.1845del6  | [34]      |
| EBS                    | <i>KRT14</i>   | Heterozygous c.373C>T                           | Second-site mutation c.242insG  | [36]      |
| JEB                    | <i>COL17A1</i> | Compound heterozygous c.3781C>T, and c.4424insC | Second-site mutation c.4463-1G>A (middle finger), and back mutation c.3781T>C (arm) | [32]      |
| JEB                    | <i>COL17A1</i> | Compound heterozygous c.3781C>T, and c.1706delA | Second-site mutation c.3782G>C (ankle), and gene conversion of c.3781C>T (arm)      | [32]      |
| JEB                    | <i>LAMB3</i>   | Compound heterozygous c.628G>A, and c.1903C>T   | Second-site mutations c.596G>C (lower leg), c.628+42G>A (lower leg)                 | [33]      |
| JEB                    | <i>LAMB3</i>   | Homozygous c.628G>A                             | c.565-3T>C (arm), c.619A>C (shoulder), c.629-1G>A (arm)                             | [33]      |
| RDEB                   | <i>COL7A1</i>  | Compound heterozygous c.1732C>T, and c.7786delG | Intragenic crossover  | [37]      |
| RDEB                   | <i>COL7A1</i>  | Homozygous c.6527insC                           | Second-site c.6528delT  | [20]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1374-2delA                       | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1450insC                         | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1369G>T                          | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1560delCG                        | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1373+1G>A                        | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1374-1G>A                        | Mitotic recombination   | [21]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1374-1G>A                        | Mitotic recombination   | [21]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.54_57del                         | Mitotic recombination   | [75]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.54_57del                         | Mitotic recombination   | [75]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.54_57del                         | Mitotic recombination   | [75]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.54_57del                         | Mitotic recombination   | [75]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.110_113del                       | Mitotic recombination   | [75]      |
| Dyskeratosis congenita | <i>TERC</i>    | Heterozygous c.95_96del                         | Mitotic recombination   | [75]      |
| RDEB                   | <i>COL7A1</i>  | Homozygous c.6508C>T                            | Second-site mutation c.6510G>T  | [76]      |
| IWC                    | <i>KRT10</i>   | Heterozygous c.1546_1551delinsT                 | Mitotic recombination   | [77]      |
| Kindler Syndrome       | <i>FERMT1</i>  | Homozygous c.456dupA                            | Mitotic recombination and slipped mispairing  | [39]      |
| Kindler Syndrome       | <i>FERMT1</i>  | Homozygous c.676dupC                            | Mitotic recombination and slipped mispairing  | [39]      |
| Kindler Syndrome       | <i>FERMT1</i>  | Homozygous c.676dupC                            | Mitotic recombination and slipped mispairing  | [39]      |
| Kindler Syndrome       | <i>FERMT1</i>  | Homozygous c.676dupC                            | Mitotic recombination and slipped mispairing  | [39]      |



| Disorder         | Affected gene | Mutation(s)                                   | Mechanism of Reversion                       | Reference |
|------------------|---------------|---|--|-----------|
| Kindler Syndrome | <i>FERMT1</i> | Homozygous c.676dupC                          | Mitotic recombination and slipped mispairing | [39]      |
| Kindler Syndrome | <i>FERMT1</i> | Compound heterozygous c.676dupC, c.1677G>A    | Mitotic recombination and slipped mispairing | [39]      |
| Kindler Syndrome | <i>FERMT1</i> | Homozygous c.676dupC                          | RNA editing                                  | [40]      |
| RDEB             | <i>COL7A1</i> | Compound heterozygous c.3840delC, c.6751-2A>G | Exon skipping                                | [74]      |
| IWC              | <i>KRT1</i>   | Heterozygous c.1865_1866insG                  | Mitotic recombination                        | [22]      |
| IWC              | <i>KRT10</i>  | Heterozygous c.1373delG                       | Mitotic recombination                        | [23]      |
| IWC              | <i>KRT1</i>   | Heterozygous c.1758_1759insT                  | Mitotic recombination                        | [46]      |

JEB, junctional epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa; IWC, ichthyosis with confetti