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REVIEW

In vivo reversion to normal of inherited mutations in humans

R Hirschhorn

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There are increasing reports of multiple different types of somatic mosaicism detected in patients with inherited and non-inherited disorders. The characteristics of several of the major types of mosaicism will be outlined, and contrasted with somatic mosaicism, which is the focus of this article. This review examines examples of somatic mosaicism due to differences in DNA sequence arising from in vivo site specific reversion to normal of inherited mutations in humans. While several known mechanisms of reversion are evident in a number of these examples, they are not in some others. The possible significance of the role of selection, particularly in view of recent results of gene therapy, is discussed.

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DEFINITION OF MOSAICISM

The word ''mosaic'' is defined in standard dictionaries as ''derived from the presence of many different pieces to form a single whole''. This definition evokes the flat designs and pictures that were constructed in antiquity using many small pieces of differently coloured and shaped tiles or similar objects to form a single whole design. In this sense, the normal body can be considered to be a three-dimensional mosaic of cells that differ developmentally and structurally but yield an overall ''picture'' of a single human body and its various organs. Although this interpretation is listed in table 1, this is not the current sense or usage of the word ''mosaicism'' in biology.

The word ''mosaicism'' is relatively new, and first appeared as a scientific term with reference to the mosaic rod and cone vision of arthropods. More recently, ''mosaicism'' has been used in biology to imply genetic patterning. Genetic mosaicism can currently be defined as the presence in a single individual of two genetically distinct populations of cells that differ from each other at the level of DNA sequence but that derive from a single zygote. One of these populations is considered to be ''normal'' while the second is ''mutant'' with respect to a particular inherited deleterious alteration in the DNA. While there are several different types of genetic mosaicism, this review will focus on the more recently documented examples in humans of mosaicism resulting from reversion of inherited deleterious mutations to normal, and the possible implications of these observations. However, a discussion of the various types of genetic mosaicism, many of which are listed in table 1, serves to clarify the differences between these, the parameters that underlie their definition, and the possible mechanisms involved.[1–5]

DEFINITION OF TYPES OF GENETIC MOSAICISM

Genetic mosaicism in humans was first recognised and documented in disorders involving abnormalities of chromosomes, and intially because of unusual phenotypes in cases of intersex that suggested mosaicism. The ability to visualize the X and Y sex chromosomes enabled identification of mosaicism for sex chromosomes as the basis for the unusual observations of ambiguous or intersex phenotypes.[6 7] Subsequently, routine chromosome studies of the autosomes revealed that many infants born with syndromes due to chromosomal aneuploidy are also mosaic, with some cells containing a normal chromosome complement. Although some cases, especially involving the sex chromosomes, are due to mitotic non-disjunction, many cases with disomy/trisomy mosaicism involving chromosomes 13, 18, 21, (as well as X) are believed to be due to somatic loss of chromosomes from a trisomic fertilisation by anaphase lag, resulting in some cells with a normal diploid chromosome number.[8] While the latter type of chromosomal mosaicism could be considered as a type of reversion to normal, such gross changes (involving special mechanisms of chromosomal replication and sorting) do not fall into the type of mosaicism due to reversion that is the scope of this review.

However, the initial steps in the discovery and further studies of chromosomal mosaicism have served as a framework for the study of the additional types of mosaicism currently identified (table 1). In addition to chromosomal and mitochondrial mosaicism, these different types of mosaicism include germ line mosaicism, somatic mosaicism due to de novo mutation, and somatic mosaicism due to reversion to normal of inherited mutations. Genetic counselling is now beginning to consider such mosaicism.[9] Two of the additional forms of mosaicism will not be considered here because they are either not directly heritable or apparently involve mechanisms of embryonic lethality. These are the case of neoplasia where DNA changes occur in the tumour cells but not in other cells of the body, and the somatic mosaicism observed in disorders for a condition where the presence of a mutation in all cells is embryonically lethal, such as incontinentia pigmenti in males.[10 11] X inactivation can be considered as a form of ''mosaicism'' but not with respect to DNA sequence, and will only be discussed with reference to the significance of skewed X inactivation.[12]

Several different observations have served to raise initially the possible presence of mosaicism in individuals with inherited disorders. In vivo, apparently unexpected differences in phenotype

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Table 1 Types of mosaicism*

Condition	DNA	Resultant phenotype
Normal developmental differences in different tissues and cell types	Nuclear DNA sequence the same in all cells (not true mosaicism)	Normal
Mitochondrial heteroplasmy	Nuclear DNA sequence the same in all cells, mitochondrial DNA can differ from cell to cell due to mutations in the mitochondrial DNA and differential distribution (heteroplasmy)	Patient normal or abnormal; may lead to abnormal offspring
Chromosomal mosaicism	Nuclear DNA differs; usually two different classes due to varying chromosomal complements	Patient normal or abnormal; may lead to abnormal offspring
Germ line mosaicism (resulting from a de novo mutation in a germ cell)	Nuclear DNA differs; only in germ line cells	Patient normal; may lead to multiple abnormal offspring
Somatic mosaicism due to de novo mutation (+/− germ line) (resulting from a de novo mutation at a stage during embryogenesis)	Nuclear DNA differs in different tissues and number of cells, depending on stage and site of the de novo mutation	Patient usually abnormal; disease may be milder than expected
Somatic mosaicism due to reversion (resulting from a mutation that corrects (reverts) a deleterious inherited mutation in some cells)	Nuclear DNA differs in different tissues and cells, depending on stage and site of the reversion	Patient may be abnormal, possibly milder than expected or occasionally normal

*Does not consider other types such as those found in neoplasms.
Chimeric states such as persistence of fetal cells derived from prior pregnancies are not considered as mosaicism as they do not derive from the same zygote.

have served as signals suggesting the presence of mosaicism. These signals have included milder than expected disease phenotypes based upon comparison with the clinical course of individuals with the same genotype, improvement rather than the expected worsening of disease over time, or a parent with mild form of a dominant disorder who has offspring who are more severely affected. In vitro, the presence of the functional gene product in some cells and its absence in others, or the appearance of both functionally resistant or ''normal'' and abnormal cells in tissue culture have also been reported as indicating possible mosaicism. With the advent of molecular biology, rapid DNA sequencing and cloning of single copies of DNA, it has been possible to document the molecular or ''DNA'' basis for these in vivo and in vitro observations, suggesting the possible presence of both normal and mutant cells in a single individual. Additionally, the now routine determination of DNA sequence has allowed for identification of ''subclinical'' mosaicism that is without discernible effect either on gross clinical features or on easily observable in vitro function. The potential significance of these more subtle findings remains to be determined.

GERM LINE MOSAICISM

The presence of germ line (gonadal) mosaicism has been inferred most frequently in X linked disorders, but is most easily detected in autosomal dominant disorders in situations where an unaffected parent has more than one affected child. This has now been shown in numerous instances to result from a de novo mutation in germ line cells. More commonly, the apparently unaffected parent is also mosaic in somatic cells, but with involvement of insufficient cells/tissues to result in easily identifiable phenotypic alterations. The mosaicism may occur within the germ line itself, with the de novo mutation present in only some gametes. In some instances, the degree of mosaicism within the germline of male parents has been determined by DNA analysis of individual sperm for presence or absence of the mutation found in the affected offspring. In some cases, some of the unaffected children have been shown to lack the mutation on the same chromosome that carries the de novo mutation in the affected children. Empirical risk figures for germ line mosaicism have been used to counsel parents who apparently are not carriers as to recurrence risk after the birth of a single affected child.

SOMATIC MOSAICISM DUE TO DE NOVO MUTATIONS

Somatic mosaicism is most commonly due to a de novo deleterious mutation during embryogenesis. The effect upon phenotype is dependent upon cellular site of the mutation and the point during embryogenesis at which it occurs. The phenotype can range from full expression to presence of only very mild features and/or lack of progression of disease. The critical finding indicating that the somatic mosaicism is due to a de novo mutation is the failure to find the mutation(s) in the relevant parents (mother for X linked disorders, one of the parents for autosomal dominant disorders and in both parents for autosomal recessive disorders). Somatic mosaics have been identified most frequently in autosomal dominant and X linked disorders, both because the pattern of inheritance allows for easier ascertainment of de novo mutational events and because only a single mutation in an embryo can result in disease. For autosomal recessive disorders, disease results only when the de novo mutation occurs on the normal allele in a heterozygous carrier embryo. Therefore, the frequency of observed somatic mosaicism for recessive disorders varies with the carrier frequency for the disease. Somatic mosaicism due to a de novo mutation during embryogenesis for an autosomal recessive disorder probably is often unsuspected unless the parents have also been studied and one parent does not carry a mutation. The mosaicism may still be missed and the findings mistakenly attributed to non-paternity if the carrier is not the father.

SOMATIC MOSAICISM DUE TO REVERSION TO NORMAL OF INHERITED MUTATIONS

Similar to somatic mosaicism due to de novo mutations during embryogenesis, mosaicism due to reversions to normal of an inherited mutation have been discovered because of milder than expected clinical course and/ or presence of both phenotypically normal and abnormal cells *in vivo* and *in vitro*. The critical difference is that it can be shown that the mutation that has reverted to normal in some cells has been inherited from a parent. Although reversions have commonly been described in bacteria and other organisms such as *Drosophila* and mice, many of these instances surprisingly have not been fully documented at the molecular level. This review will focus on the examples in humans

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Table 2 Some different approaches to classifying reversions to normal of inherited mutations

Mechanisms (known and unknown)
- Intragenic recombination (Bloom syndrome)
- Mitotic gene conversion (epidermolysis bullosa *COL17A1*)
- Second site suppressor mutations (in same gene or different gene)
- Site specific insertion→deletion or vice versa of repeat
- Site specific insertion/deletion of single nucleotide in nucleotide run (slippage)
- Expansion/contraction of repeat
- Site specific single nucleotide reversion (homozygous or hemizygous originally)

Gross effect on phenotype in vivo (modifies ascertainnment)
- Milder manifestations
- Slows/abolishes progression of disease
- Normal areas in vivo (*e.g.* skin)
- Normalization of types of cells in blood (+/−disease amelioration)
- No effect observed in vivo

Effect on cells/tisssue; histology, cell protein cell function: (with or without out culturing in vitro)
- Reversion to normal function
- Reversion to presence of protein without function
- Tissue involved (modifies ascertainment)

Specific genetic disorders

where the precise molecular changes in DNA have been identified. These reversions can be classified in multiple different and overlapping ways, as outlined in table 2, including by mechanism, by gross effect on phenotype in vivo, by various effects on cells and tissues and by disease. I have chosen to classify by disease because in most cases the mechanism involved, which would be the most intellectually satisfying basis for classification, has not been definitively proved or disproved. Additionally, classification by disease allows for consideration of other factors, ranging from variation in ease of ascertainment (see table 4) to the structure of the gene involved. This review will cover eight disorders in relative detail. These comprise: (*a*) the metabolic disease tyrosinaemia type I; three immunodeficiency disorders, including severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID), severe combined X linked immunodeficiency due to deficiency of the constant gamma chain shared by several cytokine receptors, and the X linked Wiskott-Aldrich syndrome, due to deficiency of the WAS gene; the skin disorders of epidermolysis bullosa due to either deficiency of collagen 17A1 or of keratin 14; Bloom syndrome, which has high rates of ''mutation'' (or genome instability) and immunodeficiency; and the related group of disorders under the name of Fanconi anaemia. While the mosaicism in patients belonging to several of the complementation groups of Fanconi anaemia syndromes have recently been reviewed (5), newer findings reported since then will be discussed. Alterations in CMT1, and Duchenne muscular dystrophy,[13 14] which would appear to be dependent upon genomic structure, will be discussed briefly.

Tyrosinaemia type I

The initial examples of site specific reversion to normal of single nucleotide mutations were reported in studies of patients with tyrosinaemia type I (hepatorenal tyrosinaemia; OMIM 276700; reviewed in Russell *et al*[15]). Tyrosinaemia type I is an autosomal recessive disorder resulting from mutations in the fumarylacetoacetate hydroxylase (FAH) gene. The disease presents with a variable degree of clinical manifestations, even between siblings, and can range from acute to chronic with the same apparent genotype.[15] Kvittingen initially reported the appearance of immunoreactive and enzymatically active protein in regenerating liver nodules from four patients.[16] This type of mosaicism as to protein had been sporadically reported in different disorders, and primarily in studies of cells in culture. The Kvittingen group took these studies a step further to actually demonstrate reversion of mutant DNA to normal as the basis for the mosaicism.[17] They firstly demonstrated mosaicism for immunohistochemical demonstration of enzyme protein in the majority of a larger group of patients with tyrosinaemia type I. In four of these patients they analysed mutations present in different tissues. They reported the remarkable finding that in DNA from regenerating nodules of the liver that were positive for enzyme protein expression, one of the mutant alleles had apparently reverted to normal (table 3). The group either specifically demonstrated or could infer either by studies of both parents, by the finding of the mutation in non-regenerating liver, or the observation that the mutation was an extremely common inherited mutation, that the mutations involved in the reversion mosaicism were inherited and had not occurred de novo. An additional family with two siblings and a patient with extremely mild disease and extended life were demonstrated to have reverted mutations that were inherited by independent groups.[18 19] Three of the six patients were homozygous for mutations (table 3), thereby excluding the possibility of intragenic recombination or mitotic gene conversion events as the basis for reversion to normal of one allele. There are also no known pseudogenes that could serve as a template for correction.

Several aspects of the studies reported in tyrosinaemia are of particular interest with regard to potential mechanisms. Firstly, there may be a higher mutation rate in tyrosinaemia because the accumulated metabolites are mutagenic.[15] Secondly, experiments in a murine model for tyrosinaemia indicate that normal cells have a selective advantage.[20] Finally, the mechanisms often invoked for ''reversions'', such as gene conversion and mitotic recombination cannot explain the site specific reversion in the cases with inherited homozygosity listed in table 3.

Adenosine deaminase deficient severe combined immunodeficiency

ADA-SCID (OMIM 102700; reviewed in[21]) is an autosomal recessive immunodeficiency disorder characterized by multiple viral, fungal and bacterial infections early in life with marked failure to thrive, and in the absence of therapy, death in the first year of life. There are cases with somewhat later onset and initial severity, but the disease progressively worsens. At the time that Kvittingen and her group were examining immunohistochemical mosaicism in tyrosinaemia, my group was once again trying to solve the puzzle of two unrelated, but atypical patients with ADA-SCID. These investigations serve to highlight issues in ascertaining and confirming the presence of reversion to normal of inherited mutations. Both patients had presented early in life with apparent life threatening disease, but instead of dying as expected during infancy or early childhood, they had improved over time and very surprisingly were alive 12 and 18 years later, respectively. The older child, because of lack of a matched sibling donor, had not received a bone marrow transplant, at the time the only known effective therapy. He had intermittently received partial exchange transfusions, the only available alternative therapy at the time, and one that was of variable and incomplete efficacy (now replaced by enzyme replacement therapy with polyethylene glycol conjugated (PEG) calf ADA), but had not received any therapy for several years. The younger of the two patients had had a sibling who died of the disease before 2 years of age, the usual outcome in untreated cases. He had not received any therapy for religious reasons. In both patients we were eventually able to identify somatic mosaicism as the probable basis for the unusual clinical course but in only one could we prove that reversion rather than a de novo mutation had occurred.

In the older child, a missense mutation was identified in a B-lymphoid cell line and in fibroblasts. However, repeated,

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Table 3 DNA alterations in patients with reversion of genotype +/− phenotype

Disease	Nucleotide number normal→mutant→reverted aminoacid	Revertant cells	In vitro/in vivo	Type of reversion	Genotype	Reference
Tyrosinaemia	**192 CAG→CAT→CAG 64 Gln→His→Gln**	Liver nodule	Yes	Single nt	Homozygous	17
	Abnormal splice 1009GGC→AGC→GGC 337 Gly→Ser→Gly	Liver nodule	Yes	Single nt	Homozygous	17
	IVS12+5 G→A→G	Liver nodule	Yes	Single nt	Heterozygous	17
	IVS12+5 G→A→G	Liver nodule	Yes	Single nt	Heterozygous	17
	IVS12+5 G→A→G	Liver nodule	NA/yes	Single nt	Homozygous	18
	836 CAG→CGG→CAG 279 Gln→Arg→Gln	Liver nodule	Yes/yes	Single nt ?CpG hotspot	Heterozygous	19
ADA-SCID	IVS1 +1g→c→g*	B cells/PBL	Yes/yes	Single nt	Heterozygous	22*
(severe combined	467 CGC→CAC→CGC 156 Arg→His→Arg	B cells/ PBL	Yes/yes	Single nt	Heterozygous	23
immuno-deficiency)	355 CAG→TAG→CAG 119 Gln→Stop→Gln	T cells	Yes/yes	Single nt	Heterozygous	24
	704 CGG→CAG→CGG 235 Arg→Gln→Arg	T cells	Yes/no†	Single nt	Heterozygous	24
	IVS11-15t→a‡ (cryptic splice)→ del −4 to −14 IVS11‡	B cells/PBL	Yes/yes	Second site mutation	Homozygous	25
X linked SCID	**343 TGT→CGT→TGT 115 Cys→Arg→Cys**	T cells (sorted)	Yes/yes	Single nt ?hot spot CpG	Hemizygous	32
Wiskott-Aldrich (X linked)	**354 TAC→TGC→TAC 107 Tyr→Cys→Tyr**	T cells	Yes/yes	Single nt	Hemizygous	40
	[ACGAGG]→434ins6 bp/del 6 bp	T cells	Yes/yes	Del repeat	Hemizygous	41
	1131G→del 1131G→[del G+ins1100A]	Lymphocytes	NT/yes	Second site mutation	Hemizygous	42
Bloom	1544 9As→10As (insA)→9As (delA)	Fibroblast, PBL	Yes/yes	?Slippage	Homozygous	44
Syndrome§	**2702 TGT→TAT→TGT 901 Cys→Tyr→Cys**	LCL	Yes/yes	Single nt (father NT)	Homozygous	44
Epidermolysis bullosa						
COL 17A1	1706A→del 1706A→ins1706A	Skin cells only	Yes/yes	Mitotic gene conversion	Heterozygous	45
COL 17A1	4003TC→delTC→delTC+ins4080GG	Skin (laser dissected)	?Yes/yes	Second site del/ins	Homozygous	46
Keratin 14	IVS1 −2a→c	Skin	Yes/yes	**Pseudogene	Homozygous	47
Fanconi anaemia						
FANC A	1615G→1615delG→ 1615delg1637delA1641delT	Blood cells, LCLs	Yes/?	Second site deletions	Homozygous	50
	3559→3559insG→ 3559insG3580insCGCTG	Blood cells, LCL	Yes/?	Second site insertions	Homozygous	50
	2815 Ins19 bp→del ins 19BP	Haematopoietic cells (?stem cell)	Yes/?yes	Ins/del repeat hot spot	Heterozygous	53
	856 CAG→TAG→CAG 256 Gln→Stop→Gln	Blood cells, LCL	Yes/?yes	Single nt	Heterozygous	51
	862 GAG→TAG→GAG 288 Glu→Stop→Glu	Blood cells, LCL	Yes/?yes	Single nt	Heterozygous	51
	971 CTG→CGG→CTG 324 Leu→Arg→Leu	Blood cells, LCL	Yes/yes	Single nt	Heterozygous	51
	2852 CGG→CAG→CGG 951 Arg→Gln→Arg	Blood cells, LCL	Yes/?yes	Single nt & repeat ?slippage	Heterozygous	51
FANC C	322delG¶/1806insA→ wild-type with absence of both mutations in a clone	Blood cells LCL	—	Intragenic recombination	Heterozygous	50
	322G→→322delG¶→322G Sib of above: same changes	Blood cells LCL	Yes/yes	Not intragenic recombination	Heterozygous	50
	1749 CTT→CGT→TGT 496 Leu→Arg→Cys	Blood cells LCL	Yes	Second site ?CpG hot spot	—	52
	67delG ¶	—	—	Intragenic recombination	Heterozygous	51

Changes in **bold** cannot be explained by currently accepted non-random mechanisms for site specific reversion.
Single nt, site specific reversion to normal of a single nucleotide; LCL, lymphoid cell lines.
*Parents not available for study and therefore mosaicism could be somatic mosaicism due to de novo mutation during a relatively late stage of embryogenesis with a selective advantage for original heterozygous normal cells. Conversely, reversion cannot be excluded.
†Note possible effect of PEG ADA in observed loss of revertant cells: reduction of toxic metabolites and eliminatiion of selective advantage for normal cells.
‡The original t→a mutation results in a cryptic splice site and inclusion of 13 bp of intron into mRNA); a ''second site'' deletion of 11 bp that includes the cryptic splice site results in reversion to normal splicing
§Most reversions in Bloom syndrome are due to intragenic recombination, probably as a result of the basic pathology of the disease gene and are not included in this table.
Bp of intron results in use of normal splice site.
¶In FANC C, 332delG is now called 67delG.
**6bp deletion associated with nucleotide change, resulting in use of original splice site.
In vivo is also used to designate that cells and or tissues were tested without culturing *in vitro*.

albeit intermittent, efforts using cDNA cloning over more than a decade failed to identify the mutation on the second allele. Serendipitously, when the patient was 16 years of age we were able to obtain peripheral blood cells and establish a fresh B lymphoid cell line (but unfortunately could obtain neither fibroblasts from the child nor any material from the parents). The availability of PCR allowed us to compare genomic DNA from the different cell lines easily, and to demonstrate the presence of a splice site mutation (IVS 1+1G→C) in both the original B cell and fibroblast cell lines. The mutation apparently resulted in an unstable mRNA, explaining the inability to identify the mutation by mRNA studie. In striking contrast, the B cell line established 14 years later lacked the splice site mutation, and expressed 50% of

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Table 4 Possible factors in occurrence/detection of reversion of inherited mutations to normal

High mutation rate
Selective advantage
Ease of ascertainment
- Blood cells
- Skin
- Frequently biopsied tissue

ADA mRNA with normal sequence (in addition to 50% with the previously described missense mutation). Analysis of DNA from peripheral blood cells allowed us to demonstrate somatic mosaicism in vivo, with presence of splice site and missense mutant DNA and of normal revertant DNA. Consistent with mosaicism, concentrations of the toxic metabolite deoxy ATP, usually over 500-fold elevated in these patients, was only minimally elevated at 16 years of age. In the absence of parental DNA we noted that we could not differentiate between somatic mosaicism due to de novo mutation *v* site specific reversion to normal of an inherited mutation. In retrospect, several early studies, including a study in this patient, had reported isolation of cell lines expressing ADA that probably represented reversion but at a time when it was difficult to prove reversion molecularly.[26 27] Other instances of ADA positive cell lines isolated from patients, but without recognition of their possible significance, can be found in the literature.

The studies demonstrating mosaicism in this older patient simultaneously led us to pursue mosaicism as the possible basis for the clinical course in the second, unrelated younger patient. He was at this point 12 years of age and had shown progressive clinical improvement and unexpectedly mild biochemical and immunological abnormalities. We could obtain additional B cell lines and most importantly, blood samples from this patient and from his parents. In the peripheral blood of the patient and in DNA from the parents we identified a maternally transmitted missense mutation and a paternally transmitted splice site mutation in the flanking intron. Mosaicism due to reversion was evidenced by absence of the maternally transmitted deleterious mutation in 13/15 authenticated B cell lines and in 17% of single alleles cloned from blood DNA, despite retention of a maternal ''private'' ADA RFLP linked to the mutation. The maternal mutation was only 11 bp upstream of the position of the paternal splice site mutation. Although these results suggested site specific reversion, we could not definitively rule out intragenic recombination *v* either gene conversion of a short tract or some undefined site specific reversion to wild-type, because of the heterozygosity for mutations and the absence of polymorphic markers close to the 5′ end of the mutation. However, the placement of the retained and reverted mutations suggested that gene conversion was unlikely. These results also suggested that establishment of significant somatic mosaicism following reversion to normal could modify disorders in which revertant cells have a selective advantage in vivo. Reversions have now been reported in three additional ADA-SCID patients. Two compound heterozygous patients exhibited site specific single nucleotide reversions.[24] The presence of heterozygosity in this patient again leaves open the possibility of conversion or intragenic recombination as the mechanism for reversion. A selective advantage for revertant cells was suggested by inability to isolate additional revertant T cell lines following institution of PEG ADA (see below). Most recently a second site mutation (see table 3) that resulted in rescue of ADA activity was reported in a member of a family with several affected children.[25] The patient carrying the reversion had greater residual immune function and lower concentrations of toxic metabolites compared with the other family members carrying the same inherited mutations. Additionally he had presence of substantial ADA activity and protein in both T and B cell lines and was relatively healthy. The interesting features are the clinical response and the observation that enzyme replacement (with furthering lowering of toxic metabolites) was accompanied by diminution in the number of revertant cells, suggesting that normal cells have a selective advantage in the face of toxic metabolites.

Several pieces of evidence further support the presence of a selective advantage of ''normal'' cells in ADA deficiency.[26–30] Perhaps the most compelling is the recent apparent success of gene therapy for ADA deficiency in two ADA-SCID patients who did not receive enzyme replacement therapy simultaneously with gene therapy. Attempts at gene therapy for ADA deficiency were among the very first for inherited disorders. However, all patients in the United States who received gene therapy had to receive PEG ADA enzyme replacement therapy simultaneously. Such therapy, which dramatically lowers concentration of toxic metabolites, improves immune function, and extends life span, could conceivably abolish any selective advantage for ADA transduced cells. The two patients reported were apparently not candidates for bone marrow transplantation, and for various reasons could not receive ADA enzyme replacement therapy. Both of these patients, who were given retroviral ADA gene therapy in the absence of receiving PEG ADA enzyme replacement therapy, have been reported to have successfully integrated normal ADA cDNA into their genomes and to have had an initial therapeutic success. The kinetics are such as to invoke a selective advantage as a major factor in the current ''success''.[29 30]

X linked SCID

The X linked form of severe combined immunodeficiency or SCIDX1 (OMIM #300400; reviewed in [31]) is the most common form of SCID, accounting for 50% of all cases and essentially all X linked cases. The disorder is due to deficiency of the constant gamma chain of a group of lymphocyte cytokine receptors, most notably the receptor for the cytokine interleukin (IL)-2. To date, only a single example of reversion of an inherited mutation has been reported in this disorder.[32 33] The patient presented with an attenuated form of immunodeficiency, and investigations focused on diagnosis of the X linked form of SCID only because of a family history suggestive of X linked inheritance. The child was found to have inherited a mutation in gamma C from his mother and to carry the mutation in various cell lineages. However, the mutation had reverted to normal in his T cells (table 3). The immunological response was not fully restored, either because insufficient precursors had reverted to allow for sufficient immunological diversity or because of absence of reversion in other cells such as monocytes that are also important for the immune response, but studies suggested a reasonable generation of T cell diversity. The concept that cells not bearing the mutation would have a selective advantage in X linked SCID was initially based on observations of skewed X inactivation in female carriers in whom the active X was the normal chromosome allowing for expression of normal protein.[31] Studies in a murine animal model also supported the concept of a selective advantage for cells not bearing the mutation.[34] Recently, preceding the success in gene therapy reported for ADA-SCID, the group in Paris led by Alain Fischer reported successful sustained correction of X linked SCID by gene therapy using bone marrow stem cells transduced ex vivo with a retroviral vector containing the cDNA for the constant gamma chain. That initial report showed success in 4/5 patients. Given the low incidence of integration of the vector into stem cells, a selective advantage

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was thought to have played a role in the success. Unfortunately the success of gene therapy in these and additional patients[35–37] was marred by the adverse event of insertion at apparently the same oncogenic site in two of the patients, and development of what appeared to be leukaemia.[38] A risk of malignancy following random integration of retroviral vectors at an oncogenic site had been hypothesised from the earliest *in vitro* studies. Nevertheless, the results of the gene therapy trials for X linked SCID suggest that the phenotypic ascertainment of reversion to normal, and of other indicators of a selective advantage for corrected cells, can indicate that the disorder is a good candidate for the current methods for gene therapy, which can only provide for a low frequency of integration.

Wiskott-Aldrich syndrome

Wiskott-Aldrich syndrome (OMIM #301000; 300392 WAS; reviewed in[39]) is an X linked disorder, usually fatal in infancy, which is due to mutations in the WAS protein, resulting in a variable immunodeficiency with profound thrombocytopenia. Female carriers for this disorder exhibit skewed X inactivation in blood cells with expression of WAS protein from the normal X chromosome, consistent with a selective advantage of the normal allele in lymphoid cells. As for most X linked disorders, there are multiple different mutations with over 150 mutations described to date. The large number of mutations and the variable aspects of the disease manifestations make it difficult to ascertain reversions on the basis of a milder than expected clinical phenotype. Nonetheless, three examples of reversion have been published. One of these reversions is a site specific single nucleotide G→A reversion to normal (table 3; 354TAC→TGC→TAC; 107Tyr→Cys→Tyr).[40] Of the two additional reversions, one is a compensatory single nucleotide insertion that corrects the frame of the original inherited single nucleotide deletion.[42] For the final example, the original mutation was insertion of a 6 bp repeat sequence with reversion caused by deletion of the repeat sequence. In this case the original mutation and the deletion of the mutation could have occurred by DNA slippage.[41]

Bloom syndrome

Bloom syndrome (OMIM #210900 BLM; reviewed in [43]) is an autosomal recessive disorder characterized by instability of DNA as manifested in vitro by increased sister chromatid exchange (high SCE). However approximately one fifth of patients have mosaicism in lymphoid cells with a small percentage of cells exhibiting low sister chromatid exchange. Virtually all such individuals with mosaicism in the rate of sister chromatid exchange have been heterozygous for two different mutations, and the mosaicism for low exchange of sister chromatids can be shown to be caused by intragenic recombination. As a result, following segregation of the products of intragenic recombination, some cells carry one chromosome that does not bear either of the two different inherited mutations, while the second chromosome is either doubly mutant for the two inherited mutations or carries only one of the inherited mutations. We have not included these examples of mosaicism by virtue of intragenic recombination in table 3 because they may represent a mechanism secondary to the disease itself. However 2/7 patients mosaic for low SCE were genetically homozygous for mutations and therefore could not have low SCE cells because of intragenic recombination.[44] One patient had an insertion of a tenth A in a run of nine As as the inherited mutation, resulting in a frame shift, with a reversion in low SCE cells by deletion of the inserted A, restoring the frame. This suggests slippage as the mechanism for both the original mutation and for the site specific reversion to normal in this patient. The second patient had a constitutional homozygous G→A change that predicts a Cys901Tyr missense mutation as the inherited mutation. Low SCE cells were heterozygous, with reversion of the A to the normal G, predicting reversion to the normal Cys901. Although the father was not available for DNA analysis, homozygosity in the high SCE cells of the proband for polymorphic markers in a linked region of $>10^6$ bp was consistent with inheritance of homozygosity for the mutation from a common ancestor. The possible effect on in vivo phenotype with respect to amelioration of disease is currently not known.

Epidermolysis bullosa

Epidermolysis bullosa (EB) (junctional, collagen, type XVII, alpha-1; COL17A1, OMIM 113811; keratin 14 (KRT14), OMIM148066) is a syndrome encompassing a large group of inherited bullous skin disorders of varying severity. EB due to mutations in *Col17A1* is an autosomal recessive, usually relatively mild, disorder with blistering of the skin. One of the most dramatic examples of phenotypic signs indicating in vivo reversion of mutations to normal has been seen in patients with epidermolysis bullosa due to mutations in *Col17A1*. The first such patient exhibited patches of clinically unaffected skin on the extensor surfaces of both hands and upper arms, with some new patches appearing and expanding over the years. Cells from these areas showed positive immunofluorescence for type 17 collagen, absent in affected areas.[46] The patient was heterozygous for a paternally transmitted mutation (Arg1226X) that was present in both normal and abnormal cells, and for a maternally transmitted deletion of A at cDNA 1706. This maternal mutation was absent from the cells positive for type 17A1 collagen. These reverted cells had also lost a maternal intronic polymorphism that was 381 bp downstream from the maternal deleterious mutation. The authors suggested that a mitotic gene conversion event had occurred, with non-reciprocal exchange of sequence over a relatively small region. This was based upon several findings, including the simultaneous loss of the maternally derived deleterious mutation and the nearby polymorphism, the site of the retained paternal mutation relative to the maternal mutation, and retention of heterozygosity of flanking markers. Although not specifically commented upon, it is of note that the reversions occurred in the areas of the skin (extensor surfaces of arms and hands) exposed to UV irradiation. A second patient homozygous for a 2 bp deletion "reverted" with respect to appearance of the protein. This "reversion" resulted from an insertion of two different base pairs at a second site, which restored the frame. Lastly, in the autosomal recessive form of EB caused by mutations in keratin 14, reversion causing mosaic appearance of protein has been reported. However, this can be interpreted as a milder form of the disease resulting from leakiness of a splicing mutation. The unusual aspect is that the missense mutation present (in homozygosity) acts to create aberrant splicing. There are now several examples of differences in splicing between individuals carrying the same mutation, which result in phenotypic differences, probably reflecting genetic differences in unlinked loci that affect splicing and are currently being defined.

Fanconi anaemia

Fanconi anaemia (FA OMIM 227650, FANCA OMIM 607139, FANCB OMIM 227660, FANCC OMIM 227645, FANCD2 OMIM 227646, FANCE OMIM 600901, FANCF OMIM 603467, (FANCD1 OMIM 605724 is really *BRCA2*), reviewed in [48]) is a group of autosomal recessive disorders that were initially distinguished from each other on the basis of studies of in vitro complementation. They share defective haematopoiesis leading to aplastic anaemia, a wide range of congenital abnormalities, frequent AML and chromosomal instability.[48] This instability is usually identified by sensitivity

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to chromosomal breakage when challenged by mitomycin C. Separate genes responsible for the different complementation groups in this disorder have now been cloned. Recent results indicate that Bloom syndrome and Fanconi anaemia (as well as *BRCA2* and ATM) are all part of the same DNA repair pathway, and that a multiprotein nuclear complex connects Fanconi anaemia and Bloom syndrome.[49] It has been known for some time that in a number of patients with Fanconi anaemia there are cells in lymphoid cell lines (LCL) and in uncultured peripheral blood cells (PBLs) that exhibit resistance to chromosome breakage induced by mitomycin C. In some patients, increasing phenotypic cellular mosaicism has been observed over time.[50] With the recent cloning of the genes involved in Fanconi anaemia, the molecular basis of this mosaicism with respect to resistance to chromosome breakage (the result of reversion to normal DNA repair in these cells) has been identified in patients with FANC A and FANC C (table 3). In FANC C, it is of interest that four of five patients with reversion all involved the same mutation (322 delG, renamed as 67delG, an apparent founder mutation),[51 52] but at least two different mechanisms were involved in generating reversion. In one case of the delG mutation (heterozygous with 1806insA, see table 3) a hybrid cell clone was isolated that bore neither mutation but had an apparent ''crossing over'' of haplotype markers, consistent with an intragenic recombination that generated a wild-type allele.[51] Intragenic recombination appeared to have occurred in an additional patient carrying the 67(322)del G (heterozygous with a splice site mutation IVS 11–2a→g). This patient showed phenotypic cellular reversion but retained both mutations in revertant cells. However, cloning of PCR amplified cDNA revealed the presence of colonies carrying wild-type sequence and neither mutation, and a colony containing both mutations, consistent with the products of an intragenic recombination event.[52] One caveat is that ''crossing over'' can occur artefactually in vitro using PCR. In an additional instance, two siblings bearing the 322delg (heterozygous with Leu554Pro) lost the deletion mutation from the phenotypically revertant cells, while retaining the second mutation. However, polymorphic markers remained heterozygous distal to the ''reverted'' mutation, ruling out intragenic recombination.[51] A gene conversion event is possible (but not proven because markers flanking both sides were not apparently available).[51] In the last FANC C patient described, the original mutation (CTT→CGT; Leu→Arg) generates a CpG mutation hotspot, which reverts, not site specifically but via the C→T change commonly found at CpG hotspots, to become TGT (Arg→Cys). The authors demonstrated that the Leu→Cys change was compatible with normal function.[50] For FANC A, the most common form of Fanconi anaemia and the first of the genes to be cloned, the molecular basis for reversion has been identified in seven patients. Four of these patients all carried missense or nonsense mutations that reverted to wild-type by single base pair changes in phenotypically revertant cells. All four were heterozygous for other mutations. Because of the heterozygosity, gene conversion or intragenic recombination cannot be excluded as the mechanism in these patients. However, the authors could identify multiple different mutation motifs and/or direct repeats and palindromes within 20 bp of the reversions.[52] The first two patients with FANC A to have reversions defined molecularly did not have site specific reversions but rather initially had single basepair deletions or insertions that were compensated for by either additional deletions at nearby sites or additional insertions some distance from the original insertion, which restored the original frame. The return to functionality was experimentally demonstrated for these two mutation/reversions.[50] In the seventh patient the deleterious mutation was an insertion 0f 19 bp with site specific deletion of these 19 bp resulting in reversion to wild-type. This apparently occurred at a mutational repeat hotspot, resulting in reversion.[53] This study was consistent with reversion having occurred in a haematopoietic stem cell. However, in all of the cases with reversion in haematopoietic cells, fibroblasts did not show reversion when studied. A selective advantage for wild-type cells has been demonstrated in a murine model of FANC C.[54] While selection probably accounts for the difference in observable reversion between fibroblasts and blood cells, the role of the normally occurring recombination and somatic mutation present in immune cells is not clear.

Based upon additional reports of phenotypic cellular mosaicism, there are undoubtedly many more instances of reversion of inherited mutations. However, for most examples either the molecular basis has not been elucidated, the alteration has not been demonstrated in uncultured cells, or the inherited nature of the deleterious mutation could not be determined. However, based upon prior reports, such as for CMT1 and DMD,[13 14] areas of genome duplication or pseudoduplication may be a fruitful area for further identification of reversion of inherited mutations.

SUMMARY

This review has examined several examples of in vivo somatic reversion of inherited disorders in humans and evidence for several different mechanisms responsible for these events. The mechanisms include intragenic recombination, mitotic gene conversion, second site compensating mutations, DNA slippage, and site specific reversion of a mutated nucleotide to normal by an unknown mechanism. In cases of site specific reversion, the possible role of gene conversion or intragenic recombination cannot be dismissed in cases with compound heterozygosity. In most such cases (listed in table 3), the studies that would be required to rule out mechanisms such as intragenic recombination and gene conversion in compound heterozygotes either have not or cannot be performed. However, there remains a core of examples (in bold in the table), where intragenic recombination and gene conversion can be excluded because the patients are either homozygous or hemizygous and there is no known pseudogene or highly homologous gene to provide template for conversion-like events. We have listed the DNA changes in these examples in some detail to allow others to determine if there is a unifying principle that we have not been able to detect that defines specific type(s) of ''repair''.

Alternatively, this ''back mutation'' to function is random and/or reflects an increased mutation rate in some disorders or occurs at mutational hotspots (such as CpG dinucleotides or repeats) that are dependent upon DNA sequence. This return of function becomes evident when the mutation corrected cells have a selective advantage. For several of the diseases discussed there is evidence for either increased genomic instability (Bloom syndrome, Fanconi anaemia), or accumulation of mutagenic metabolites (ADA-SCID and tyrosinaemia), or reversion in areas of exposure to UV (epidermolysis bullosa). There is evidence of selective advantage from animal models for tyrosinaemia, X linked SCID, ADA-SCID and Fanconi anaemia group C, and from the finding of skewed X inactivation in both of the X linked disorders of Wiskott-Aldrich syndrome and SCID. The results of the gene therapy trials for X linked SCID and ADA-SCID suggest that the phenotypic ascertainment of reversion to normal and of other indicators of a selective advantage for corrected cells can indicate whether the disorder is a good candidate for the several methods for gene therapy that are in both preclinical and clinical trials and that can, at this time, only provide initially for a low frequency of corrected cells.

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