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Transgenerational genetic effects

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Abstract

Since Mendel, studies of phenotypic variation and disease risk have emphasized associations between genotype and phenotype among affected individuals in families and populations. Although this paradigm has led to important insights into the molecular basis for many traits and diseases, most of the genetic variants that control the inheritance of these conditions continue to elude detection. Recent studies suggest an alternative mode of inheritance where genetic variants that are present in one generation affect phenotypes in subsequent generations, thereby decoupling the conventional relations between genotype and phenotype, and perhaps, contributing to 'missing heritability'. Under some conditions, these transgenerational genetic effects can be as frequent and strong as conventional inheritance, and can persist for multiple generations. Growing evidence suggests that RNA mediates these heritable epigenetic changes. The primary challenge now is to identify the molecular basis for these effects, characterize mechanisms and determine whether transgenerational genetic effects occur in humans.

Keywords

epigenetics; heritability; transgenerational

Throughout history, humans have sought metaphysical and scientific explanations for the fascinating similarities between parents and offspring. Between 500 and 350 BC Anaxagoras, Hippocrates and the atomists proposed that blending of fluids or particles from parents result in new individuals that share phenotypic characteristics with their parents [1]. In the 19th century Gregor Mendel, in a series of elegant experiments with carefully selected traits in peas, demonstrated that as yet unidentified factors duplicate in each parent and segregate in random but equal parts to offspring according to remarkably precise, simple and quantitative laws [2,3]. With the rediscovery of Mendel's work, Francis Galton formalized the statistical ideas of correlation and regression as ways to evaluate genotype–phenotype relationships and characterize inheritance of quantitative traits [4]. Then, in the early 1900s, Thomas Hunt Morgan demonstrated that chromosomes carry the information controlling inheritance [5], and later Alfred Sturtevant made the first genetic linkage maps that correlated phenotypic inheritance with the linear arrangements of genetic factors on chromosomes [6,7]. Finally, this profoundly important thread of logic, experimentation and

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discovery over millennia culminated with the identification of DNA as the agent of inheritance [8,9].

With the unprecedented opportunities that the genomics revolution enables, reconciling Mendelian genetics (individual genes) with phenotypic inheritance (complex traits) in an empirical as well as theoretical manner is now one of the primary challenges in modern genetics [10,11]. Associating genotype and phenotype, which is the essence of Mendelian genetics, seeks in part to estimate the extent to which individual genetic differences contribute to individual phenotypic differences. This perspective has dominated much of our current understanding of phenotypic variation and disease risk, and has led to the identification of the molecular basis for many aspects of organismal biology in health and disease, and profoundly shaped our understanding of evolution.

Despite remarkable technological and computational advances; however, the heritable components for common diseases such as diabetes, heart disease, cancers and many other conditions remain remarkably elusive [11,12]. The combined phenotypic effects of known genetic variants often account for less than 10% of estimated heritability, suggesting that many of the determinants underlying disease risk remain undetected. Several explanations for ‘missing heritability’ have been proposed including overestimates of heritability, unexplored regions of the genome, untested classes of genetic variants, variants that are rare, that have weak phenotypic effects or that demonstrate low penetrance, and gene–gene and gene–environment interactions [12–15]. Ongoing studies seek to establish the relative importance of these various factors.

With the discovery of Mendel’s laws of inheritance and DNA as the hereditary molecule, other modes of inheritance have been largely ignored and, perhaps unintentionally, our view of heritability has narrowed dramatically to a predominant focus on associations between genotype and phenotype within individuals [1]. However, recent genetic discoveries together with new evidence from epidemiological observations and epigenetic studies suggest that the Mendelian paradigm may not fully account for heritable phenotypic variation. In particular, evidence is rapidly accumulating for phenotypic variation in the present generation that can result from the action of genetic variants in previous generations. Here, we focus on this evidence and its implications.

Kinds of transgenerational effects

Aristotle, Lamarck, Darwin and many others thought that an important way to transmit phenotypes from one generation to the next involves inheritance of characteristics that are acquired with environmental exposures and life experiences [1,16,17]. This controversial proposal fell out of favor after a series of papers successfully argued for ‘hard’ (Mendelian) inheritance rather than ‘soft’ (epigenetic) inheritance [18–20]. However, increasing evidence now shows that phenotypic variation can sometimes occur when environmental factors and genetic variants in previous generations create an epigenetic state that persists across generations in individuals who are not directly exposed to that environment or who do not inherit the original genetic variant [21–26]. In some cases, environmental exposures lead to heritable epigenetic changes, a phenomenon that we term ‘transgenerational environmental effects’. In other cases, the original genetic variant is sufficient to initiate epigenetic inheritance (transgenerational genetic effects). In addition, environmental effects can lead to heritable epigenetic changes only in genetically predisposed individuals (trans-generational gene–environment interactions). Similarly, interactions can occur between genetically determined epigenetic states in parents and conventional genetic variants in offspring (transgenerational epistasis).

Environmental influences

Many examples have been reported for trans-generational epigenetic effects in which environmental exposures lead to heritable phenotypic changes that pass through male, female and sometimes both germlines [22,24,27–30]. Some of these factors are chemical agents [31–34], others involve irradiation [35–47], and others involve in enriched (or impoverished) environments in mice [48–52] and in humans [53–55]. Whether genetic factors modulate susceptibility to trans-generational inheritance for these environmental exposures is unknown, but strain specificities in model organisms raise this possibility [56].

Genetic factors

An important example of transgenerational genetic effects involves an engineered mutation in the *Kit* receptor gene (Kit^{tm1Alf}) in mice [57]. *Kit* encodes a cell surface receptor that, upon binding with the ligand encoded by the *Kitl* gene, signals through several downstream pathways including the PI3K, JAK/STAT and MAPK cascades [58–62]. Hematopoiesis, melanogenesis and gametogenesis are among the cell lineages that KIT-KITL signaling modulates [63–66]. In a seminal study, white-spotting of the digits and tail, which is typically inherited only in mutant mice, was unexpectedly also found in genotypically wild-type offspring [55], in a mode of inheritance that is reminiscent of paramutations. Paramutation occurs when one allele at a locus epigenetically changes expression in a heritable manner for other alleles at the same gene_[67,68]. These white-spotted wild -type mice also showed reduced *Kit* gene expression despite a 'normal' *Kit* genotype. The white-spotting phenotype in wild-type mice eventually reverted to a conventional coat color and mode of inheritance after several generations, arguing against a permanent change such as a DNA sequence mutation in the wild-type allele. Abnormally high levels of heterogeneously sized KIT RNAs were identified in sperm from *Kit* mutants. In fact, injection of RNA from sperm from these *Kit* mutant males into single-cell wild-type embryos led to the white-spotting phenotype in wild-type mice [57,69]. Moreover, injection into early embryos of two miRNAs that target *Kit* mRNA produced similar results [57,69], suggesting that small regulatory RNAs packaged in sperm serve as the transgenerational 'signal' to the developing embryo. Similar evidence for miRNA-mediated paramutation-like inheritance was found in *Cdk9* and *Sox9* mutant mice [70,71]. To understand the specific mutational requirements to initiate heritable epigenetic changes, a systematic survey of the many spontaneous and engineered mutations at the *Kit* locus should be undertaken. In particular, these tests should determine whether paramutation-like effects are restricted to specific kinds of mutations such as those resulting from genetic engineered or spontaneous deletions, or whether any sequence change in the *Kit* gene is sufficient to initiate epigenetic inheritance. Similarly, whether these heritable epigenetic changes affect the other primary phenotypes in *Kit* mutants, namely hematopoiesis and gametogenesis, remains to be examined.

Interestingly, another example of a trans-generational genetic effect involves *Kitl*, the ligand for the *Kit* receptor. Mice that are heterozygous for the Sl^{gb} deletion mutation in the *Kitl* gene develop spontaneous testicular germ cell tumors (TGCTs) at an elevated rate (15% affected males) compared with the baseline rate (5% affected males) on the 129 inbred strain background [72]. As expected among progeny of $Sl^{gb/+}$ heterozygous mutant females, wild-type sons are affected at the baseline rate and $Sl^{gb/+}$ sons at the elevated rate. Surprisingly, among progeny of $Sl^{gb/+}$ heterozygous males, wild-type sons are tumor free, whereas $Sl^{gb/+}$ heterozygous sons are affected at the expected elevated rate [72]. Thus, paternal but not maternal $Sl^{gb/+}$ heterozygosity protects wild-type males but not deletion mutation heterozygotes. This is the first example of complete suppression of TGCTs in genetically susceptible mice. Whether these wild-type sons transmit resistance to TGCTs to subsequent generations is currently being tested [Leung E et al., Unpublished Data], and the molecular

basis for protection in a dosage- and genotype-dependent manner remains to be studied. It is striking that the ligand-receptor protein pair, which is involved in signaling between soma (*Kitl*) and germline (*Kit*), shows strong evidence for transgenerational genetic effects. By contrast to many other examples of transgenerational epigenetic effects [25,26,57,70,71], silencing of the wild-type allele of the *Kit* ligand gene is unlikely to suppress tumorigenesis because loss-of-function *Kitl* mutations typically increase rather than reduce TGCT susceptibility [72].

Gene–environment interactions

Environmental exposures sometimes lead to heritable phenotypic changes only when specific genetic variants are present. Two notable examples involve coat color and disease risk in A^{vy} mice [73,74] and also vertebral development in $Axin1^{Fu}$ mice [75,76] In both cases, maternal dietary supplementation with folate or other methyl donors alters DNA methylation patterns, which in turn alters offspring phenotypes. Interestingly, both mutations result from insertions of intracisternal A particle retrotransposons that are important targets of silencing through DNA methylation [77]. Whether these diet-dependent epigenetic effects are restricted to intracisternal A particle-related mutations, and whether other mutant alleles at these and other loci show similar diet-induced epigenetic changes remain to be determined. In addition, phenotypic reversibility with diet switches after birth is another important issue that does not seem to have been studied.

Gene–gene interactions (epistasis across generations)

Transgenerational epistasis differs from conventional epistasis in that the interacting genes do not occur together to modify an individual's phenotype. Instead, one of the genetic variants is present in at least one of the parents and the other is present in offspring. In these cases, the parental variant elicits a heritable epigenetic change that reveals its phenotypic consequences only when transmitted to a vulnerable genome that carries the second genetic variant. Neither variant alone is sufficient to modify phenotypes in the way that is found when they interact across generations within the same cross or family.

Two examples in *Drosophila* highlight several important properties of transgenerational epistasis. The *wimp* mutation in the *RpII140* gene (the *Drosophila* 140 RNA polymerase II subunit) in the parental generation interacts with a remarkable variety of mutant genes in progeny to compromise viability [78]. In this case, the transgenerational effect is attributed to *wimp* mRNA deposited in the oocyte before meiosis has been completed, thereby allowing its gene product to interact with the inherited *hairy (h)* mutant and with mutations in many other genes to result in lethality of mutation heterozygotes during early development, regardless of whether the *wimp* mutation is also inherited [78]. This is a special case of transgenerational effects because the gene product rather than an epigenetic change is inherited through the oocytes.

The second example in *Drosophila* involves the maternally inherited hyperactive $Hop^{Tum\text{-}1}$ allele of JAK kinase. A large number of parental genes interact with the JAK kinase variant to modulate tumor susceptibility. JAK kinase hyperactivity causes heterochromatin changes that were then transmitted to subsequent generations, leading to increased tumor incidence [79]. As with *wimp,* $Hop^{Tum\text{-}1}$ must be inherited through the maternal rather than the paternal germline to show the epigenetically modified tumor susceptibility phenotype.

In mice, a series of pair-wise interaction tests revealed transgenerational epistasis between the $Dnd1^{Ter}$ mutation and all six mutants tested ($Kitl^{Sl/J}$, $Trp53^{null}$, A^{y}, CSS-M19, M19-A2 and M19-C2) affect TGCT risk [80,81]. The *Deadend1* gene (*Dnd1*) is related by DNA sequence to Apobec1 complementation factor (*A1cf*), which is an RNA-binding accessory

protein for the cytidine deaminase enzyme that edits specific sites in specific mRNAs (C to U, read as T) [82]. The DND1 protein also blocks access of specific miRNAs to their mRNA targets [83]. *Dnd1*^{Ter} dramatically increases occurrence of TGCT-affected males, from a baseline rate of 5% in genetically-predisposed strain 129 wild-type males, to 17% in *Dnd1*$^{Ter/+}$ heterozygotes and 94% in *Dnd1*$^{Ter/Ter}$ homozygous mice on the same genetic background [84,85]. If a second modifier is present in either the male or female parent, the prevalence of affected males and of males with bilateral TGCTs (both testes) increases significantly in *Dnd1*$^{Ter/+}$ males, independent of inheritance of the second TGCT modifier [80]. Importantly. in this example, the inherited parental epigenetic change is not restricted to acting through the maternal lineage, demonstrating that the effect cannot be attributed simply to maternal loading of RNA in the oocytes, or to maternal–fetal interactions.

Frequency, magnitude & persistence

Three major questions about transgenerational genetic effects are immediately relevant: are the effects common or rare, are the effects strong or weak, and are the effects long-lasting or transient? In the search for answers to these and other questions, a notorious difficulty in trans-generational epigenetic research is conducting carefully controlled studies to distinguish epigenetic inheritance from conventional genetic effects as well as from environmental factors and social influences.

A recent study, which took advantage of specially engineered strains of mice, assessed the frequency and magnitude of transgenerational genetic effects [86]. An ideal test that could lead to unambiguous results would involve genes that are present in the parental generation but are not transmitted to offspring. The Y chromosome satisfies this criterion, because daughters do not inherit their fathers' Y chromosome. By substituting the Y chromosome from one inbred strain (the donor) onto the background of another inbred strain (the host), a new strain is created that differs from the original host strain only for the Y chromosome [87]. Daughters of males with a substituted Y chromosome and daughters of host strain males are therefore genetically identical and differ only in the genetic constitution of their fathers' Y chromosome, which they do not inherit. A unique test for transgenerational genetic effects is therefore enabled simply by comparing phenotypes among daughters of fathers that have alternative Y chromosomes. In general, these two groups of daughters will be phenotypically similar if transgenerational effects are rare and weak, and different if the genetics of the fathers' Y chromosome transgenerationally affects daughters' phenotypes.

Remarkably, across a broad panel of more than 100 biochemical, physiological and behavioral traits, frequent and large phenotypic effects were found between daughters of fathers with a substituted Y chromosome and genetically identical host strain females [86]. In fact, transgenerational effects among daughters of fathers with a substituted Y chromosome were similar in frequency and magnitude to those found with conventional inheritance of other substituted chromosomes, namely the 19 autosomes and the X chromosome. Thus, in this case where phenotypic differences are unambiguously epigenetic, transgenerational genetic effects rivaled conventional effects in frequency and magnitude. Importantly, the origins of this phenotypic variation remain genetic, but from the previous generation, rather than within the genome of affected individuals.

Three studies in mice address the extent to which transgenerational effects persist across generations. The first involves food intake and susceptibility to diet-induced obesity [88], the second β-cell function [89], and the third testicular cancer [Nelson VR et al., Unpublished Data]. The first study demonstrated that heterozygosity for a randomly selected autosomal segment on mouse chromosome 6 significantly reduced food intake and conferred resistance to diet-induced obesity for at least three generations among sons who did not

inherit this chromosome segment. These effects occurred only with transmission through the paternal germline, and interestingly were fully reversed after transmission for two consecutive generations through the maternal germline. In the testicular cancer case, protection was transmitted for at least three generations through the maternal germline and was reversed after transmission for two consecutive generations through the paternal germline. Studies are now ongoing to test for further persistence, drift or reversion of these phenotypes, to characterize the molecular basis for heritable epigenetic changes, and to identify the mechanisms that are responsible for reversing the phenotypic effects.

Thus, in these early studies, the answers to the three original questions suggest that transgenerational genetic effects are frequent, strong and long-lasting, and appear to rival genetic effects that are inherited in the conventional manner.

Interestingly, the study of the transgenerational effects of the Y chromosome strongly suggests that genetic background affects the phenotypic outcome of inherited epigenetic changes. Epigenetic effects were found on only one of the two genetic backgrounds tested [86]. On the C57BL/6J background, significant changes in anxiety-related phenotypes were associated with substitution of the Y chromosome that was derived from the 129 inbred strain, whereas significant differences were not found for the same Y chromosome on the original 129 background. Perhaps more importantly, the reciprocal test of C57BL/6J-derived Y chromosome on the 129 inbred background also did not reveal evidence for transgenerational effects. Together these results suggest that transgenerational effects result from an interaction between epigenetic changes initiated by specific Y chromosomes in fathers and at least one unidentified, inherited genetic variant in the daughters of specific strains. Because they have not been tested on multiple backgrounds, transgenerational effects may, in general, depend on interactions between genetic background variants among offspring and the epigenetic change inherited from ancestral generations, in other words, from transgenerational epistasis.

Mechanisms

Transgenerational genetic effects raise many old, interesting, hard and important questions, such as the way in which genetic variants trigger epigenetic changes and the ways in which these changes are transmitted to subsequent generations. Genetic variants that lead to trans-generational effects are remarkably diverse both in terms of the functions of the proteins that they encode and the nature of the molecular lesions that induce these effects, (e.g., engineered transgenic alleles [57,70,71,79], spontaneous mutants [72,78,80] and natural variation among inbred strains [Yazbek SN, Nadeau JH, Buchner DA, Unpublished Data [86]). However, parental variants that trigger transgenerational effects may not directly affect epigenetic features in the germline. Instead, these variants may cause a change in the cellular physiology that is sensed by other mechanisms and sometimes in other cells that in turn lead to heritable epigenetic changes in the germline.

Although the molecular signals triggering epigenetic machinery remain largely unidentified, we can speculate based on the nature of the genes and their molecular functions that have been associated with trans-generational effects. Given the still limited number of transgenerational genetic effects reported, it is striking that both the Kit receptor and its ligand have already been implicated. This signaling pathway may provide a mechanism for passing information from somatic cells that express Kit ligand to the germ cells that express the Kit receptor, which is an essential step for transmission of any acquired information through the germline to subsequent generations. Whether this pathway contributes to other transgenerational genetic effects remains to be determined.

Once an epigenetic change is triggered, it must be transmitted from one generation to the next within gametes and maintained in the embryo to generate epigenetically mediated phenotypic effects in offspring. Recent studies have begun cataloging the nature of epigenetic marks and RNAs that are present in gametes [90–94]. Epigenetic features in gametes could then lead to changes in gene expression during early development, which in turn could have substantial and lasting consequences on the organism, thereby providing a pathway for transmitting long-lasting transgenerational effects and maintenance of phenotypic changes in the absence of inherited DNA variants. Interestingly, microinjection of specific miRNAs into fertilized eggs leads to white-tail phenotypes in genetically wild-type mice [57,69–71] and RNA silencing has been shown to initiate transgenerational effects [25,26], suggesting that noncoding RNAs in gametes may be the molecular messenger between generations. However, the functional relevance of RNAs in gametes, the extent to which parental epigenetic marks are maintained, the dependence of these effects on specific genetic variants and the contribution of these parental factors are largely unexplored.

In cases where the transgenerational effect persists in subsequent generations, the epigenetic marks or RNAs must then be maintained in the developing germline, which is one of the earliest cell lineages to be defined in the early embryo. Although maintenance and transmission of DNA methylation and histone modifications has been described [31,75,94–101], mechanisms that allow the maintenance and repeated transmission for RNA signals are less well understood.

Various evidence suggests that RNA granules carried in the gamete may be responsible for transgenerational effects. RNA granules are believed to function in post-translational regulation of gene expression [102–106] and in protection of parental mRNA transcripts that contribute to the developing embryo. These granules, which are abundant in early embryos and germ cells, typically contain translation initiation factors, RNA degradation machinery, apoptotic factors, mRNAs and other small regulatory RNAs [102–106]. Three genes that show transgenerational or parent-of-origin effects, namely *Dnd1* [80], *Apobec1* [Nelson VR, Nadeau JH, Unpublished Data] and *Eif2s2* [Heaney JD et al., Unpublished Data], are found in these granules [102–106]. Moreover, *Dnd1* also shuttles mRNAs between the nucleus and RNA granules [107]. In addition, the Dnd1 protein blocks miRNA-mediated degradation of the bound transcripts [83]. *Apobec1* has also been identified in stress granules, as has at least one of its mRNA targets *Eif4g2* (also known as *Nat1*) [105,106]. *Eif4g2* in turn is part of the *Eif4g* scaffolding subunit of the eIF4 complex that functions together with *Eif2s2* in the eIF2 translation initiation complex in stress granules [105]. However, it remains to be determined whether it is coincidental that many of the proteins involved in transgenerational effects are found in RNA granules.

Normal functions of transgenerational epigenetic effects

Disease, dysfunction and other forms of phenotypic variation are clues to normal biological processes. But what is the relevance of transmitting epigenetic information across generations under normal conditions? Perhaps the basis involves short-term adaptive responses to environmental exposures and genetically based physiological stresses. During the lifetime of an individual, environmental exposures lead to homeostatic responses based on standard physiological principles. These responses, which can include changes in gene expression, can then revert to the normal state after the environmental stress has passed. Similarly, long-term environmental changes can lead to evolutionary adaptations based on changes in DNA sequence. However, during the intermediate term, over several generations, perhaps organisms have evolved epigenetic mechanisms to transmit adaptive gene-expression profiles from one generation to the next to provide progeny an advantage over individuals who are not similarly ‘pre-adapted’. Under this scenario, changes in gene

expression that provide good responses for the parental generation may be appropriate for progeny if the stress conditions persist, and can readily revert and further adapt depending on environmental state. In this context, genetic variants and environmental exposures may represent similar kinds of physiological stresses demanding similar kinds of epigenetic responses. Obviously, this view of heritable epigenetic changes is reminiscent of the original idea of 'inheritance of acquired characteristics' [1,16,17].

Future perspective

Accumulating evidence raises the possibility that transgenerational genetic effects contribute significantly to heritable phenotypic variation. If these transgenerational effects are frequent and strong, the limited success of traditional genome-wide association studies is perhaps not surprising, because in some cases the causative genetic variants are present in previous generations and not necessarily in affected individuals where tests for association between genotype and phenotype are typically made. Despite strong evidence that parental lifestyle and early fetal environment also affect phenotypes in a trans-generational manner [48–55], the study designs needed to control for the various nonsequence-based contributors to phenotypic variation are difficult to arrange in human cohorts. Evidence of transgenerational effects is therefore extremely limited in humans, however, because relatively few multigenerational pedigrees are available for study and replication, and fewer still have been tested specifically for links between phenotype and genotype across generations [108].

The observation that parental genes can affect phenotype independent of DNA inheritance has the potential to transform our views of heritability and our understanding of the relations between genotypes and phenotypes. Modern genetics is based on fundamental discoveries that directly link genetic inheritance and phenotypic manifestations within affected individuals. Passage of DNA between parent and child is thought to account for a substantial portion of heritability and familial history remains one of the best predictors of disease risk. Nevertheless, the correlation between genotype and phenotype is often modest [11,12]. Heritable epigenetic changes resulting from molecules such as RNAs, DNA methylation and histone modifications that act in ways that are perhaps as strong and frequent as direct DNA inheritance has the potential to revolutionize current views of inherited traits. A full understanding of inheritance may be achieved by returning to a broader consideration of the inherited 'particles' and 'factors', and moving forward by incorporating associations between genotype and phenotype across generations as well as between phenotype and epigenetic changes.

Despite a growing literature on trans-generational effects, many questions remain. What are the molecular marks and epigenetic mechanisms through which genetic variants and environmental factors establish trans-generational signals? Do these triggers act directly on the germline, or is the primary target somatic, with signals secondarily transduced to the germline? Do different genetic and environmental triggers use the same epigenetic changes to transmit information across generations? For how many generations are transgenerational effects maintained? In what manner and under what conditions are heritable epigenetic changes reversed? Are transgenerational effects unique to particular genes, pathways or phenotypes? Do transgenerational effects impact human phenotypic variation and disease risk? The answers to these questions will not only reveal insights about the mechanisms of transgenerational epigenetic effects, but will also provide evidence for the relative importance of this exceptional and largely unexplored mode of inheritance.

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Executive summary

- In some cases, phenotypic variation and disease risk in the current generation result from the action of genetic variants in previous generations.
- Transgenerational genetics represents a novel mode of inheritance.
- Recent studies strongly suggest that transgenerational genetic effects are as frequent and as strong as conventional genetic effects, and their effects are long-lasting that can persist for at least four generations.