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RESEARCH REVIEW

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Chimerism in health and potential implications on behavior: A systematic review

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Abstract

In this review, we focus on the phenomenon of chimerism and especially microchimerism as one of the currently underexplored explanations for differences in health and behavior. Chimerism is an amalgamation of cells from two or more unique zygotes within a single organism, with microchimerism defined by a minor cell population of <1%. This article first presents an overview of the primary techniques employed to detect and quantify the presence of microchimerism and then reviews empirical studies of chimerism in mammals including primates and humans. In women, male microchimerism, a condition suggested to be the result of fetomaternal exchange in utero, is relatively easily detected by polymerase chain reaction molecular techniques targeting Y-chromosomal markers. Consequently, studies of chimerism in human diseases have largely focused on diseases with a predilection for females including autoimmune diseases, and female cancers. We detail studies of chimerism in human diseases and also discuss some potential implications in behavior. Understanding the prevalence of chimerism and the associated health outcomes will provide invaluable knowledge of human biology and guide novel approaches for treating diseases.

KEYWORDS

autoimmune diseases, cancer, microchimerism, women

1 | INTRODUCTION

Originating from Greek mythology, the chimera was known as a creature with a lion's head, goat's body, and a serpent for its tail (van Dijk, Boomsma, & de Man, 1996). The term chimera is commonly used in many scientific disciplines to describe an entity that is comprised of parts from multiple sources to make a whole. For instance, chimeric fusion proteins are made from joining parts of separate genes to produce a fusion protein with domain regions or the function of the original independent proteins. Similarly, a genetic chimera is an individual organism comprised of cell populations from two or more unique zygotes (Race & Sanger, 1975). Sometimes chimerism and genetic mosaicism are interpreted as describing similar phenomena; however, mosaicism refers to unique cell populations that originate from the same zygote. In medicine, the creation of man-made chimeras is the foundation of the lifesaving techniques in transplant and transfusion medicine. Research has explored chimeras in many areas of biology such as genetics, molecular biology, and the generation of model organisms (Eckardt, McLaughlin, & Willenbring, 2011). The studies of spontaneous, natural chimerism are of increasing interest for the consequences that chimerism may have for health, behavior, fertility, and disease.

Known cases of chimerism can be predominantly classified in three categories: (a) artificial, that is, arising after a blood transfusion or bone marrow transplantation; (b) tetragametic, that is, the case of fertilization of two oocytes by two spermatozoa and fusion of the resulting embryos leading to development of one organism; (c) transplacental, as a result of the passage of blood between mother and child, with twin chimerism as a special case. Transplacental microchimerism is bidirectional with the potential for mothers to carry their

children's cells (fetal microchimerism) and children to carry their mother's cells (maternal microchimerism). Fetal cells have been discovered in maternal peripheral blood samples during pregnancy or postpartum, including mesenchymal stem cells, leukocytes, nucleated erythrocytes, trophoblasts, and hematopoietic progenitor cells (Ando & Davies, 2004; Bianchi, Flint, Pizzimenti, Knoll, & Latt, 1990; Bianchi, Zickwolf, Weil, Sylvester, & DeMaria, 1996; Evans et al., 1999; Mueller et al., 1990; O'Donoghue et al., 2003). Studies in several mammalian species have documented that blood exchange during pregnancy can facilitate the transfer of a small number of cells, further discussed in Section 3 (Bianchi et al., 1996; Davies, 2012; Khosrotehrani, Johnson, Guegan, Stroh, & Bianchi, 2005). These specific cases encompass the majority of identified cases of chimerism and are labeled as "microchimerism," defined as a minor cell population contributing to less than 1% of the total cell population. As there is no lower limit that produces microchimerism in an individual organism, the ability to identify a mixed chimerism within an individual sample is ultimately dependent on the sensitivity of the techniques available, as presented in Table 1. In the study of chimerism, there are four primary techniques that have been utilized to detect, quantify, and identify unique cell populations within individual samples.

1.1 | Blood group typing

Many cases of chimerism were initially identified inadvertently by the discovery of blood type discrepancies during routine testing. Mixed red cell populations were originally discovered as a consequence of in utero anastomoses of chorion blood vessels in cattle (Owen, 1945). This was first described in humans by Dunsford et al. (1953) in a blood donor who was revealed to have both type A and type O red cell populations, presumably as a result of chimerism from a twin (Dunsford et al., 1953). Common blood bank antiserum agglutination testing is accomplished by introducing reagent antibodies to the individual's red blood cells and visual agglutination to determine if the cells express the corresponding antigen. Due to the limitations of visual interpretation, this is primarily an effective method for testing the antigen composition of major red cell populations (Mujahid & Dickert, 2015). Additionally, gel column agglutination, another standard blood bank technology, has been reported to detect mixed-field agglutination reactions in an individual with a 10% minor cell population (Hong et al., 2013). Combining anti-sera identification methodology with a fluorescent assay assisted the largest study of blood group chimerism prevalence in 415 DZ twin pairs and 57 sets of DZ triplets (van Dijk et al., 1996). In short, this technique utilizes IgG antibodies targeting specific antigens and subsequently introducing fluorescent microspheres coated with antihuman IgG to visualize cells possessing the antigen (van Dijk et al., 1996). Paired with fluorescent microscopy, this technique reported a sensitivity of one positive cell in 10,000 total cells. At this sensitivity, the study reported 8% of twins and 21% of triplets with detectable red blood cells chimerism.

More recently, studies examining blood group chimerism have exploited flow cytometry to automate the process of counting fluorescently labeled cells. In one case study, a pair of monochorionic DZ twins initially reported type AB and type B blood types. Flow cytometry testing at 3 months of age revealed red cell chimerism of 88%

TABLE 1 Summary of common techniques used in identifying or researching chimerism

Technique/criteria	Primary principle	Target	Detection limit per assay (%)	Advantages	Disadvantages
Traditional blood typing antiserum	Agglutination	RBC antigen	>10	Simplicity Availability	Limited to RBCs Sensitivity
Gel column agglutination	Agglutination	RBC antigen	10	Simplicity Availability	Limited to RBCs Sensitivity
Fluorescent microsphere	Fluorescent labeling	RBC antigen	0.01	Sensitivity	Requires fluorescent microscopy Limited to RBCs
Karyotyping	Microscopy	Chromosome	Limited by nuclei observed	Gross genetic differences	Time consuming Low throughput
Flow cytometry	Fluorescent labeling	Cell surface antigen	Limited by cells observed 0.05	Dynamic Various applications	Complex Availability May require additional samples
Real-time quantitative PCR	PCR amplification	DNA	0.0001	Simplicity Availability Sensitivity	Requires specific target
Short tandem repeat (STR) PCR	PCR amplification	DNA	1–5	Simplicity Availability	Sensitivity
Fluorescent in situ hybridization (FISH)	Fluorescent labeling	DNA	Limited by nuclei observed	Cell level analysis in tissue sections	Requires fluorescent microscopy or automated system

Note: The lowest detection limit is dependent on specific target and application; examples presented from associated literature (Ciolino, Tang, & Bryant, 2009; Cirello et al., 2010; Gilmore, Haq, Shadduck, Jasthy, & Lister, 2008; Greendyke, Wormer, & Banzhaf, 1979; Hong et al., 2013; Kristt, Stein, Yaniv, & Klein, 2007; Sharpe et al., 2014; Summers Jr., Johnson, Stephan, Johnson, & Leonard, 2009; Thiele, Holzmann, Solano, Zahner, & Arck, 2014).

type AB with 12% type B and 99% type B with 1% type AB (Aoki, Honma, Yada, Momoi, & Iwamoto, 2006). In another study, two neonates from a set of triplets produced mixed field reactions in gel card agglutination reactions and flow cytometry was used to establish the presence of both type A and type O cell populations (Chung et al., 2018). Flow cytometry has notable versatility and sensitivity over other blood antigen techniques, including the capacity to measure the prevalence of HLA antigens on white blood cells. Fluorescence-activated cell sorting is built upon flow cytometry and allows the investigator to separate cells based on fluorescent labeling. This has been used to enrich microchimeric cells in blood samples using HLA-specific monoclonal antibodies with minor cell populations as small as 0.01%, allowing for not only detection of these cells but also a further study of this minor cell population (Eikmans & Claas, 2011).

1.2 | Karyotyping

Karyotyping is a common cytogenetic technique utilized for examination of gross genetic changes in an individual's chromosomes including aneuploidy and structural changes. This technique examines an individual's chromosomes for genetic disorders, which has occasionally led to the discovery of chimerism. Karyotyping can identify major differences between the karyotypes of individual cells including the number and type of sex chromosomes. This approach was previously employed for the identification and diagnosis of chimerism in individuals with a mixture of 46,XX/46,XY cells (Chen et al., 2005; Farag et al., 1987; Green, Barton, Jenks, Pearson, & Yates, 1994; Jang, Jung, Kim, Park, & Kim, 2010; Repas-Humpe et al., 1999; Shin, Yoo, Lee, Kim, & Seo, 2012). While this is an effective technique, the approach is limited to the identification of gross chimerism with chromosomal differences such as 46,XX/46,XY and is not able to provide information regarding the potential source of chimerism. Furthermore, this approach does not provide evidence of an individual's alleles making it possible for cases of mosaicism caused by nondisjunction events, as previously reported, to potentially be misdiagnosed as chimerism (Niu, Pan, Lin, Hwang, & Chung, 2002).

1.3 | Polymerase chain reaction

One of the most widely accepted methods for identifying chimerism via genomic DNA is by polymerase chain reaction (PCR) (Mullis, 1990). The PCR technique is based on the fundamental principles of DNA replication where the strands of double-stranded DNA are denatured, primers are annealed to a complementary sequence, and extension is performed by a thermostable Taq polymerase. This process, repeated over multiple cycles, produces exponential growth in copies of the region of interest, which is detected by a variety of different techniques including gel electrophoresis or Sybr Green and TaqMan probes for real-time quantitative PCR. The amplification of signal in PCR is a major reason for the popularity of this technique in microchimerism detection. At a relatively low-cost, researchers can identify a small amount of target genome by amplifying the amount of target available for detection, thereby enhancing the detection signal. It is inherently necessary to examine a target gene that is unique to the chimera cell population or has known discordance in polymorphisms between the minor cell population and host cells. This led to the common use of Y-chromosome genes such as *TSPY1* (*DYS14*) and the sex-determining region Y (*SRY*) as targets for identifying microchimerism in women. Several studies have reported obtaining a sensitivity of one male cell per one million female cells with this technique (Cirello et al., 2010; Gilmore et al., 2008; Peters et al., 2019).

Additional studies have focused on targeting variation and polymorphisms in the genome to identify cells that originate from distinct zygotes and to provide insight into the source of these cells. Researchers are able to exploit known differences between two genomes to identify small populations that have a distinctive characteristic, such as the human leukocyte antigen (HLA) system. As such, the use of HLA-specific PCR assays has been implemented to provide context to the source of microchimeric cells by characterizing the HLA profile of family members to identify the original source (Lambert, 2012). In some studies, it was possible to detect non-inherited HLA sequences representing maternal microchimerism, regardless of the sex of the progeny (Lambert et al., 2004; Nelson et al., 2007). While this is a seemingly significant advantage over other techniques, the need to obtain samples from both zygotes to establish unique HLA markers for chimerism analysis often makes it difficult or not possible to apply. Polymorphic short tandem repeats (STR) are well established in forensic investigations as a PCR target for elucidating the potential sources of genomic material and have also been exploited for chimerism analysis, though with typically lower sensitivity than real-time PCR (Kristt et al., 2007). Cases of chimerism and mosaicism have been reported as a challenge in forensic cases where STR is used to identify the source of the genomic material (Castella, Lesta Mdel, & Mangin, 2009; Chen et al., 2005).

1.4 | Fluorescent in situ hybridization

The techniques described above have primarily focused on the global identification of the presence of chimerism within a sample, consisting of several cells. To study the localization of donor cells on an individual cell level among a background of host cells, researchers may utilize the cytogenetics technique fluorescence in situ hybridization (FISH). The FISH assay technique uses labeled nucleotide probes that hybridize to a specific genomic sequence and are subsequently able to be detected by the presence of a fluorescent signal. FISH has provided notable advantages to studying the biology of chimerism by allowing researchers to identify the localization of nonhost cells within tissue samples (Johnson, Zhen, & Bianchi, 2000). Like PCR, a common technique in tissue samples from women is to use a probe for a Y-chromosome specific sequence, which is often paired with an X-chromosome specific probe using dual-color probe combinations to identify male cells that feature both signals and female cells that

feature two X probe signals. The sensitivity of this technique is dependent on the number of cells examined and can be measured using automated scanning instruments.

The identification of chimeras may lead to new discoveries and implications of chimeric cells on human health and behavior. Considerable progress has been made in testing hypotheses of microchimerism benefits and consequences because of improved techniques with high sensitivity. However, we have yet to establish the definitive role of this phenomenon, identified among several mammalian species. In this systematic review, we seek to examine the current findings in chimera research regarding health, potential for influencing behavior, points of controversy, and discuss questions for future studies.

2 | METHOD

2.1 | Search strategy

We performed a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (Moher et al., 2015). The literature search was conducted by searching PubMed and ISI/Web of Science from inception to April 2, 2019. The search was conducted to include articles with the terms "chimera," "chimerism," and related terms. Excluded terms include "transplant" and "chimeric," which primarily contain material related to man-made chimeras and chimeric fusion proteins, which are outside of the scope of this review. The process for screening and selecting articles for inclusion is provided in Figure 1. The full systematic review strategy can be found in the supplementary material.

3 | ANIMAL STUDIES

Cases of chimerism have been well studied in the animal kingdom and particularly in veterinary science. Several animal taxa have been investigated for the presence of chimerism with a variety of phenotypic outcomes. Among these are cattle, which were the first to be identified as possessing blood cells derived from a co-twin. Monozygotic twinning in cattle is infrequent and similarly, it is not common for full siblings to share blood antigen types across genetically controlled antigens; yet dizygotic twin cattle often share blood type identity (Owen, 1945). Studies of molecular markers in twin bovine calves have found that dizygotic twinning results in bidirectional cell exchange and subsequent chimerism (Ron, Porat, Band, & Weller, 2011). One notable consequence of this exchange in cattle is the generation of freemartin heifers, an infertile female calf, which commonly arise in dizygotic opposite-sex twin pregnancies. It has been suggested that freemartinism is the result of anti-Müllerian hormone exchange from the male co-twin due to intrauterine blood transfusion between the twins via anastomoses of the placental vasculature (Lillie, 1916; Owen, 1945; Vigier, Tran, Legeai, Bezard, & Josso, 1984). The occurrence of freemartinism in other mammals with opposite-sex dizygotic twin pregnancy seems to be rare; although further research is warranted as cases of freemartinism with XX/XY chimerism have been described in ovine, caprine, porcine, equine, Cervidae, and Camelidae families (Padula, 2005). A reported case of freemartinism with XX/XY chimerism in Rocky Mountain Bighorn sheep (*Ovis* canadensis) demonstrated an association with the development of a masculine appearance and behavior (Kenny, Cambre, Frahm, & Bunch, 1992). These observations present an important consequence of chimerism that may have a significant contribution to the understanding of the biological influence of behavior (see Section 4.4).

Primates provide an interesting context to studies in nonhuman mammals due to their phylogenetic proximity to humans; however, in the context of reproduction particular species have unique characteristics. Studies in rhesus monkeys, which share developmental similarities to humans, demonstrated that male microchimerism in maternal tissues can be measured for several years postpartum (Jimenez, Leapley, Lee, Ultsch, & Tarantal, 2005). In the study of chimerism, marmosets are of particular interest due to dizygotic twinning being the principal rule of reproduction for the Callithricidae family (Benirschke, Anderson, & Brownhill, 1962). Marmoset blastocysts undergo fusion that produces anastomoses of the placental vasculature, yet there are no reports of freemartinism in marmoset females. Studies in marmosets have discovered opposite-sex cells within the bone marrow and therefore evidence of prolonged chimerism as a result of prenatal blood exchange (Benirschke et al., 1962). Furthermore, DNA fingerprinting of leukocyte rich-tissues in the common marmoset (*Callithrix jacchus*) produces shared profiles between littermates, whereas leukocyte-poor tissues produce unique profiles supporting the hypothesis of chimerism via intrauterine transfusion (Signer, Anzenberger, & Jeffreys, 2000). PCR amplification demonstrated that sibling-derived chimerism was present in the majority of marmoset twin sets and the associated chimerism was distributed

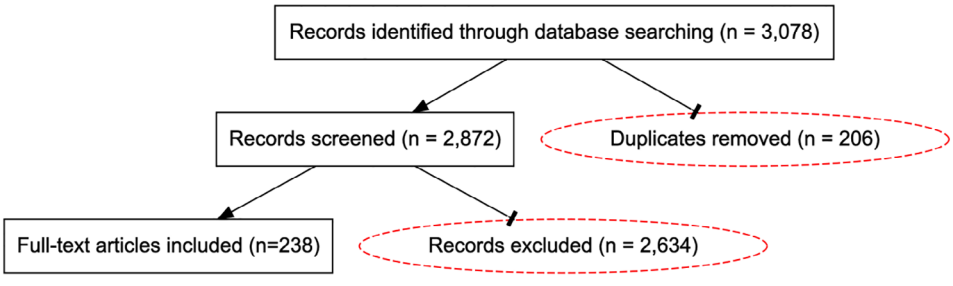

FIGURE 1 Flow diagram of article inclusion. At each stage articles included are in solid black rectangles, excluded articles are in dashed red ovals [Color figure can be viewed at wileyonlinelibrary.com]

across various tissue types including gonadal tissue and sperm samples (Ross, French, & Orti, 2007). Subsequent assessment of behavioral characteristics in relation to kin recognition found that marmoset fathers will carry chimeric infants significantly more than nonchimeric infants, while maternal carrying is significantly lower for chimeric infants suggesting that parental care behaviors may be correlated with allele sharing in the offspring (Ross et al., 2007). These findings in marmosets further suggest behavioral characteristics as a consequence of chimerism and will require future study in other species to understand if behavioral influences of chimerism are similar across mammals.

Among other families of nonhuman mammals, there are reports of natural chimerism in mice, dogs, and cats (Axiak-Bechtel, Kumar, Hansen, & Bryan, 2013; Khosrotehrani et al., 2005; Lyons, 2012). Multiple studies have concluded that female dogs with prior male birth are able to develop persistent male microchimerism with one suggesting that these male cells could explain male chimerism in daughters of subsequent pregnancies (Axiak-Bechtel et al., 2013; Kumar, Hansen, Axiak-Bechtel, & Bryan, 2013). A case report of a dog with ambiguous genitalia was found to have a leukocyte XX/XY chimerism suggesting the potential for phenotypic abnormalities with canine chimerism (Szczerbal et al., 2014). Due to similarities with humans in disease and environment, dogs have been suggested to be useful models for human conditions (Kumar et al., 2013). Mice are a common model of human disease and have demonstrated pregnancy-related chimerism similar to humans (Khosrotehrani et al., 2005; Perlman, 2016). Many studies have investigated the presence of persistent fetal cells in the non-transgenic mother by enhanced green fluorescent protein models for detection (Kara et al., 2012). Both maternal and fetal chimerisms commonly occur in mice with detection in the tissues of all major organs (Khosrotehrani et al., 2005; Su, Johnson, Tighiouart, & Bianchi, 2008). Studies of myocardial injury in pregnant mice have provided evidence of fetal cell migration to sites of cardiac injury and capacity for cardiac differentiation (Kara et al., 2012; Kara, Bolli, Matsunaga, et al., 2012). Fetal microchimerism in the lungs and brain of mice feature various cell types and have greater detection at the site of tissue injury from smoking or excitotoxic lesions, respectively, suggesting a potential protective effect (Pritchard, Wick, Slonim, Johnson, & Bianchi, 2012; Tan et al., 2005; Vogelgesang et al., 2014). It has been proposed that improving our understanding of chimeric cell trafficking across the placenta, blood–brain barrier and migration to sites of tissue injury in mice may provide insights into the properties that also support this behavior in humans (Tan et al., 2005).

4 | HUMAN STUDIES

4.1 | Hermaphroditism

Perhaps the most extreme phenotype that is closely associated with human chimerism is true hermaphroditism, a phenomenon of sexual development defined by the simultaneous presentation of both ovarian and testicular tissue. These cases generally present with ambiguous genitalia of variable external severity. There are several reported cases of 46,XX/46,XY karyotypes with true hermaphroditism; however, these only comprise approximately 10% of total cases of recorded hermaphrodites (Hadjiathanasiou et al., 1994; Niu et al., 2002). Examination of the paternal and maternal genetic contributions have provided a means to establish the origin of these cases; while mostly chimeric, there have been reported cases of mosaicism originating from 47,XXY cells as a result of nondisjunction events (Niu et al., 2002). Several hypotheses have been presented to understand the mechanism that could produce 46,XX/46,XY whole-body chimerism. As this is a developmental anomaly these hypotheses have been focused on zygote fusion before sex differentiation; including separately fertilized ova, fertilization of an ovum and second polar body, or a parthenogenetic division of an ovum fertilized by two sperm (Ramsay et al., 2009; Repas-Humpe et al., 1999; Shin et al., 2012). Due to the overall rarity and variable nature of this condition, it is possible for many cases of hermaphroditism to go undiagnosed or present later in life and therefore the majority of reported cases are young patients with more dramatic phenotypes.

4.2 | Autoimmune diseases

Among the studies of microchimerism in human disease, autoimmune diseases have been a primary focus. In the human population, approximately 5% are affected by autoimmune diseases of which there is a prominent gender bias with an estimated 78% of affected individuals being females (Jacobson, Gange, Rose, & Graham, 1997; Lepez et al., 2011; Wang, Wang, & Gershwin, 2015). Furthermore, several autoimmune diseases are more predominant in women following the childbearing years and share a similar pathology to chronic graft-versus-host disease (cGVHD), a well-studied condition in transplantation medicine as a result of chimerism (Furst, Clements, Graze, Gale, & Roberts, 1979; Lambert et al., 2001). Cumulatively, these characteristics suggest a role for microchimerism in autoimmune disease etiology. The contributions of microchimerism in autoimmune disease etiology have been largely explored for rheumatic diseases and autoimmune thyroid diseases (AITD).

4.2.1 | Rheumatic autoimmune diseases

Rheumatic diseases have been well documented in relation to chimerism. Several studies have examined women diagnosed with systemic sclerosis and have produced findings that indicate a greater prevalence and quantification of male microchimerism in peripheral blood and various tissue samples compared to controls (Burastero et al., 2003; Johnson et al., 2001; Lambert et al., 2002; Ohtsuka, Miyamoto, Yamakage, & Yamazaki, 2001; Rak et al., 2009; Sawaya, Jimenez, & Artlett, 2004). A similar study of women with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) found a greater prevalence of male microchimerism in the peripheral blood compared to controls (Kekow et al., 2013). HLA associations have been

implicated as major risk factors in autoimmune diseases and have therefore been studied extensively with regard to microchimerism. Approximately 80% of patients with RA present with a five-amino-acid motif encoded by *HLA-DRB1* alleles termed the shared epitope (Rak et al., 2009). Microchimerism may contribute to RA disease onset as higher levels of minor cell populations with *HLA-DRB1*04* and HLA-*DRB1*01* in addition to the shared epitope have been identified in women with RA (Rak, Maestroni, et al., 2009; Yan, Aydelotte, Gadi, Guthrie, & Nelson, 2011). These findings indicate disease-associated alleles acquired via microchimerism may contribute to host disease etiology. The implications of HLA associations extend to systemic sclerosis where detectable fetal microchimeric T lymphocytes are associated with a maternal *HLA-DQA1*0501* allele, suggested to facilitate persistent microchimerism (Lambert et al., 2000). Together, these data suggest that the molecular relationship between the host and acquired cells aid in facilitating microchimerism and subsequent autoimmune response; however, more studies are needed to elucidate these interactions and associated immunology.

Autoimmune diseases typically result in tissue destruction and similarity to cGVHD, which has led to studies that have investigated microchimerism within host tissues. SLE is characterized by the production of antinuclear antibodies that have specificity for nuclear components of the cell resulting in extensive organ complications and similarity to cGVHD (Johnson, McAlindon, Mulcahy, & Bianchi, 2001). A case study of a woman who died of SLE complications and previously had given birth to two sons presented with detectable male cells in all of her histologically abnormal tissues (Johnson, McAlindon, et al., 2001). In women that present with Sjögren's syndrome, male cells have been identified in labial salivary glands and bronchoalveolar lavage fluid (36% and 22%, respectively) while none were found in the peripheral blood or in control patients (Kuroki et al., 2002). This localization of nonhost cells to regions of inflammation while simultaneously remaining undetectable in the peripheral blood further illustrates the limitations of studies that exclusively examined the peripheral blood for microchimerism (Toda, Kuwana, Tsubota, & Kawakami, 2001).

4.2.2 | Autoimmune thyroid diseases

Research of microchimerism has further explored the conditions that are categorized as AITD, including Hashimoto's thyroiditis and Graves' disease. This class of diseases is known for being suppressed during pregnancy and subsequently exacerbated postpartum, suggesting that changes incurred during pregnancy facilitate the pathogenesis of these diseases. Studies of thyroid tissues have found detectable male microchimerism to occur more frequently in women with AITD when compared to control women with nodular goiters or follicular adenomas, suggesting a potential role in Hashimoto's thyroiditis and Graves' disease etiology (Klintschar, Schwaiger, Mannweiler, Regauer, & Kleiber, 2001; Renne et al., 2004). Interestingly, flow cytometry of samples from women with AITD demonstrated that fetal cells in Hashimoto's thyroiditis are primarily $CD8^+$ T cells, whereas in Grave's disease the majority of fetal cells are B cells (Lepez et al., 2011). It can be hypothesized that T-cell-mediated cytotoxicity could be directly involved in Hashimoto's thyroiditis; whereas onset of Grave's disease may be dependent on activation via host $CD4^+$ T cells (Lepez et al., 2011). Additionally, HLA typing of the mothers and offspring found that women with detectable male microchimerism more frequently have alleles HLA-DQA1*0501DQB1*0201 or DQB1*0301, which are known to be alleles associated with susceptibility for AITD (Renne et al., 2004).

4.2.3 | Autoimmune diseases of the liver

Primary biliary cirrhosis (PBC) is a disease of the liver that has an autoimmune origin, with clinical and histological similarity to cGVHD. Previous studies of male microchimerism in total female PBC patients (18%) and PBC patients with a male child (70%) found that the prevalence was similar in liver specimens compared to control women with other liver diseases (5% and 72%, respectively)(Schoniger-Hekele et al., 2002; Tanaka et al., 1999). There are significant differences in male microchimerism prevalence between these studies; it is worth noting that the increased percentage of total female PBC patients with male microchimerism compared to other liver disease patients in the study by Schoniger-Hekele et al. (2002) may be due to a larger proportion of women with male children in the PBC group that was unaccounted for. Another study examining male microchimerism of presumed fetal origin found a similar prevalence in the peripheral blood of PBC (36%) and healthy control (31%) women (Invernizzi et al., 2000). Similarly, the potential role of maternal microchimerism in the etiology of PBC has also been explored with a similar report of insignificant findings for any association with PBC (Nomura et al., 2004). Together these studies suggest that the etiology of PBC and other liver diseases are not likely to be influenced by microchimerism.

4.2.4 | Juvenile autoimmune diseases

Similar to adults, rheumatic diseases in youth are a primary focus of research in maternal microchimerism associated diseases. A group of conditions identified as juvenile idiopathic inflammatory myopathies (JIIM) collectively share similar pathology to cGVHD, suggesting a potentially similar mechanism for disease pathogenesis. Juvenile dermatomyositis (JDM) is the most common JIIM condition and presents with lymphocytic perivascular infiltrates, similar to cGVHD, in the muscle and skin lesions. Individuals with JDM have demonstrated a greater prevalence of maternal microchimerism in the peripheral blood and muscle tissue compared to healthy controls (Reed, Picornell, Harwood, & Kredich, 2000; Ye et al., 2012). Likewise, a study of microchimeric HLA-Cw alleles discovered chimerism in 73% of JIIM patients and only 10% in healthy controls (Artlett, Miller, & Rider, 2001). The presence of maternal microchimerism in children with JDM has been associated with the *HLA-DQA1*0501* allele in the

mother (Reed, 2003; Reed et al., 2000). These data present a notable similarity to rheumatic autoimmune diseases in adults and further support the hypothesis of an additional route by which HLA genes might contribute to microchimerism and disease susceptibility, as the *HLA-DQA1*0501* allele is strongly associated with both JIIM conditions across multiple ethnicities and the presence of microchimerism (Lambert et al., 2000; Reed & Stirling, 1995).

Type 1 diabetes (T1D) presents in children and young adults, leading to the hypothesis of a pathogenic mechanism of autoimmunity caused by maternal microchimerism. The peripheral blood and pancreas tissue in patients with T1D have increased maternal microchimerism compared to controls (Nelson et al., 2007; Ye, Vives-Pi, & Gillespie, 2014). Further examination of maternal microchimeric cells in the pancreas has found this to be a common phenomenon among both normal and T1D pancreases; maternal cells of the endocrine, exocrine and vascular endothelial lineages suggest these were derived from a maternal multi/pluripotent progenitor cell (Nelson et al., 2007; Ye et al., 2014). Maternal cells are notably enriched in beta cells in the pancreases of T1D patients; however, the role of these cells in immune balance during development remains uncertain (Nelson et al., 2007; Ye et al., 2014). It is not clear what the role of maternal cells in the pancreatic tissues is, as the timing of cell grafting cannot be derived from retrospective analysis. Therefore, these cells may have been present and involved during disease onset or, alternatively, may have migrated to the tissue after in response to active autoimmunity, which requires further study.

4.2.5 | Contradictory findings in autoimmune diseases

Despite a large body of knowledge that supports a pathogenic hypothesis for microchimerism in autoimmune diseases, there are several studies that have produced contradictory findings. Among these are findings of no difference between cases and controls for the presence of male cells in women with systemic sclerosis, indicating that microchimerism may not be involved with systemic sclerosis pathogenesis (Lambert et al., 2005; Selva-O'Callaghan et al., 2003). These studies have relied on retrospective case–control studies of disease, examining affected populations for detectable microchimerism, assuming that controls will not later develop systemic sclerosis and that no detectable microchimerism at the time of sampling is indicative of negative lifetime microchimerism. A low sample size may have contributed to insignificant findings of circulating male cells and result in inconsistent conclusions.

Studies among groups of women with thyroid autoimmunity have also been plagued by mixed conclusions. A population study of women in diverse age groups by Bülow Pedersen et al. (n = 3,712) found no association between thyroid autoimmunity as indicated by thyroid autoantibodies and previous pregnancy or parity (Bulow Pedersen et al., 2006). However, this is inconsistent with findings by Greer et al. who reported in a study of 17,298 prenatal patients at 20 weeks gestation that thyroid peroxidase antibody levels increased with increasing parity (Greer et al., 2011). Another report found a greater prevalence of male microchimeric cells in healthy control women compared to those with Grave's disease or Hashimoto's thyroiditis (p = .0004 and p = .001, respectively) although male cells were identified in the blood vessels and forming follicles in the thyroid of women with AITD (Cirello et al., 2015). The findings of this study emphasize the need for studies that investigate affected tissue samples for microchimerism, as this will provide insight into the cellular environment of the disease in question. Similarly, some studies of fetal and maternal microchimerism in patients with SLE have reported no significant differences in prevalence compared to healthy controls (Kanold et al., 2013; Mosca et al., 2003). These contradictory findings leave many of the scientific inquiries regarding microchimerism and autoimmune diseases unanswered. However, if these contradictory findings are supported by future studies of affected tissues and sufficient sample sizes, it should be considered to support the null hypothesis that microchimerism is a bystander and does not actively contribute to the onset of autoimmune disease.

4.3 | Cancer

Reports of association between parity and reduced cancer susceptibility have led to the study of microchimerism in the course of cancer diseases (Gadi, Malone, Guthrie, Porter, & Nelson, 2008). Similar to autoimmune diseases, papillary thyroid cancer (PTC) and breast cancer are more prevalent in females; therefore, fetal microchimerism may contribute to the etiology of these diseases. One study of PTC neoplastic thyroid tissue revealed male microchimerism in the tissue of 47.5% of women with previous male pregnancy compared to no detection in female controls with PTC and no previous male pregnancy (Cirello et al., 2008). Subsequent studies have discovered male cells in neoplastic thyroid tissue, despite lower prevalence of male microchimerism in the peripheral blood of women with PTC compared to healthy controls (Cirello et al., 2010; Cirello et al., 2015). Individuals with PTC and peripheral blood male microchimerism have a lower prevalence of extra-thyroidal extension or lymph node metastasis and better overall outcome (Cirello, Colombo, et al., 2015). It has been hypothesized that these findings could be the result of fetal cells protecting the host via a cytotoxic role against preneoplastic cells and as sentinel cells against malignant cells, preventing new tumors and tumor progression, respectively (Cirello & Fugazzola, 2014). Similar study of breast cancer cases and controls has found male microchimerism in 26% and 56%, respectively, while a subsequent case–control study detailed that male microchimerism is less prevalent in the unaffected breast tissue of women with breast cancer (Gadi, 2010; Gadi et al., 2008). Contradictory to the findings in autoimmune diseases, these findings may indicate potential beneficial effects and collectively support the hypothesis that microchimeric cells may be involved in immune surveillance, migrate to damaged or diseased tissue and be recruited for tissue repair. However, further research is necessary to establish the facilitating mechanisms; the discovery of these cells in damaged or diseased tissue may yet constitute null or malevolent effects.

Expanding on these studies, a prospective study of 428 Danish women discovered lower baseline prevalence of male microchimerism in peripheral blood of subjects who later developed breast cancer (40%) compared to controls (70%), whereas women who later developed colon cancer had an overall greater baseline prevalence of male microchimerism (95%) (Kamper-Jorgensen et al., 2012). Survival rates of individuals from the study following the diagnosis of either cancer type were improved in individuals with detectable male microchimerism (Kamper-Jorgensen, 2012). The resulting hypothesis insinuates that microchimerism may contribute to each cancer differently while immunological and repair roles of chimeric cells may contribute to improved survival among women regardless of cancer type. These seemingly contradictory findings were summarized in a hypothesis by Geck (2013) that this is an observation of evolution in action where the positive and negative effects regarding the presence of chimerism via fetomaternal exchange are being weighed in an evolutionary experiment (Geck, 2013). Additional research is needed to specifically examine the mechanism of action for fetal cells to have a protective or pathogenic role in women with cancer.

A variety of other cancers including uterine, melanoma, glioblastoma, and meningioma have found measurable microchimerism in tumor samples via FISH or PCR techniques (Broestl, Rubin, & Dahiya, 2018; Hromadnikova et al., 2014; Nguyen Huu et al., 2009). Male microchimerism, although common in endometrial tissues, was found less frequently in the tissues of type 1 endometrial cancer compared to controls (Hromadnikova et al., 2014). Lower male microchimerism in women with uterine cancer pointed toward better prognoses for factors including histological grade, tumor subtype and stage (Hromadnikova et al., 2014). Melanoma tissues in both humans and mouse models demonstrated that fetal cells selectively migrate to melanoma tissues based on the increased prevalence of chimeric cells compared to nevi (birthmark) sections (Nguyen Huu et al., 2009). Among brain tumors, glioblastoma cases were found to have a greater prevalence of male microchimerism compared to women with meningioma (Broestl et al., 2018). Furthermore, women with glioblastoma and microchimerism had longer average survival time suggesting that the greater prevalence of microchimerism among these patients may have a positive effect on disease outcome (Broestl et al., 2018). The presence of microchimerism in brain tissues introduces several questions including the influence of chimeric cells on brain function and cognition that requires further investigation.

4.4 | Behavior

Studies of chimerism have largely investigated the role of chimerism in somatic diseases, however, associations between physical biology and behavioral traits reasonably suggest a hypothesis for chimerism in individual and social behavior. The presence of chimeric cells in autoimmunity presents the potential for changes in hormone levels and associated behavior due to subsequent disease states. Particularly, diseases of the thyroid have had significant reports of changes in mood and behavior. Current studies of human microchimerism have explored the important potential of involvement in the course of AITD; however, known changes in the endocrine system due to such conditions have been previously established. Hypothyroidism found in Hashimoto's thyroiditis has been known to be associated with deficits in cognitive abilities as well as with depression, while hyperthyroidism in Graves' disease is related to depression in addition to anxiety and irritability (Grigorova & Sherwin, 2012). The relationship between the endocrine system and behavior is complex with bidirectional relationships and studies of the role of chimerism in disorders of the endocrine system are necessary (Garland Jr., Zhao, & Saltzman, 2016). Knowledge of an association between immune dysregulation and the etiology of psychiatric disorders has increased interest in the role of an altered immune system in the pathogenesis and treatment of depression and other major psychiatric disorders (Arteaga-Henriquez et al., 2019; Gibney & Drexhage, 2013). We hypothesize that the currently detailed associations between microchimerism and autoimmune disease (Section 4.2) suggest this pathway may be independent of the endocrine pathway.

Sports and exercise behavior, especially in athletic competition, are largely segregated into male and female domains due to the physiological advantages of males in strength and endurance. Following puberty, males exhibit 15-fold greater circulating testosterone than females that leads to increased interaction with androgen receptors leading to biological enhancement of muscle, bone, and hemoglobin (Handelsman, Hirschberg, & Bermon, 2018). To maintain balanced competition in athletics at the elite level, female competition is protected by rules of eligibility. Recently, sex differentiation has come under significant scrutiny in athletic competition based on the masculine appearance and elevated testosterone of two female athletes in advance of the 2016 Summer Olympics (Genel, Simpson, & de la Chapelle, 2016). Disorders of sex development, including phenotypically female individuals with 46,XY karyotype and androgen insensitivity complicate the established rules that define eligibility for female competition and individuals who present with XX/XY chimerism provide additional challenges for defining males and females based on genetic testing.

Testosterone is involved in social behavior, with bidirectional relationships and Y-chromosomal chimerism potentially having a role in multiple behaviors that are influenced by testosterone: including behaviors associated with survival, reproduction, and dominance (Geniole & Carre, 2018). Developmental studies of psychosocial and psychosexual outcomes have found associations between androgens and generally male behavior in females (Sandberg, Gardner, & Cohen-Kettenis, 2012). It should be considered that male chimerism may be involved in producing changes in sex hormone production and function. It is equally possible that localized chimerism resulting in testosterone production may be sufficient for producing observable traits in females, but further studies are required to understand the scale of chimerism necessary to produce a sufficient change in phenotype. Current studies of disorders of sexual development, including hermaphroditism, have found that women born with atypical genitalia were more comparable to reference men than reference women for psychosocial behaviors, indicating more masculine personality traits.

The relationship of 46,XX/46,XY chimera true hermaphrodites and resulting hormone profiles requires further study (de Neve-Enthoven et al., 2016). Another example involves an individual with a 46,XX/46,XY karyotype who was brought up female, with an ovotestis and prominent phallus in addition to a functional urethral opening and rudimentary vaginal orifice. The person had undergone corrective surgery removing the ovotestis and had a typical hormone profile and feminine gender identity (Farag et al., 1987). These studies suggest that the presence of allogeneic cells of different sex may influence the endocrine function of the gonads, modifying hormone signal profile and subsequent behaviors. Despite known associations of microchimerism in thyroid diseases and T1D, there is a need for future studies to describe the behavioral implications.

As suggested in marmosets, the presence of chimerism can shape social behaviors and manipulate involvement or interaction with offspring (Ross et al., 2007). Maternal microchimerism may indeed be a biological process involved in imprinting oneself on their offspring and, perhaps unintentionally, leading to greater investment and evolutionary implications on survival. Furthermore, increasing maternal exposure to fetal cells during the course of pregnancy may ultimately be involved in actively encouraging processes that support postnatal care of the child, from maintaining maternal health to increased lactation (Boddy, Fortunato, Wilson Sayres, & Aktipis, 2015). Expanding on previous hypotheses presented by Boddy et al. (2015) of microchimerism in an evolutionary framework, maternal and fetal microchimerism may have been selected for during mammalian evolution to have distinct roles, including psychological manipulation, which encourage maternal interaction with their child to facilitate survival. Future longitudinal studies may seek to investigate the role of chimerism in behavior in addition to psychological health.

4.5 | Female-specific diseases

Microchimerism is increasingly inspiring research on other conditions that exhibit a female propensity or clinical similarity to cGVHD. One such group of conditions is pregnancy-related complications. There is an association between experiencing fetal loss and subsequently having detectable microchimerism (Khosrotehrani et al., 2003). It is therefore important to recognize that parity, the number of pregnancies carried to viable gestational age, is potentially less preferable for microchimerism studies than gravidity, which accounts for all pregnancies. It should be noted that research by Gammill et al. established that fetal microchimerism concentration and prevalence do not increase with parity suggesting the possibility of dynamic graft–graft interaction (Gammill, Guthrie, Aydelotte, Adams Waldorf, & Nelson, 2010).

Preeclampsia presents a complex and dynamic relationship with microchimerism as the condition appears to be associated with a specific chimerism source. Fetal microchimerism is seemingly pathogenic and is significantly more prevalent in women with preeclampsia, whereas a protective role is suggested for maternal microchimerism which has been found in lower frequency among women with preeclampsia as well as recurrent miscarriage (Gammill et al., 2011; Gammill, Aydelotte, Guthrie, Nkwopara, & Nelson, 2013; Gammill, Stephenson, Aydelotte, & Nelson, 2014). First-time preeclampsia onset is more common among women with a change in paternity between pregnancies; conversely, a reduction in preeclampsia prevalence is demonstrated for women with preeclampsia in the first pregnancy (Li & Wi, 2000). Exposure to non-shared paternal alleles is hypothesized to be implicated in preeclampsia etiology, potentially exacerbated by fetal microchimerism. Therefore, a change in paternity in women with previous preeclampsia may eliminate exposure to an offending antigen. It is possible that these immunological interactions are exacerbated by fetal microchimerism and further research is warranted to investigate if HLA sharing between maternal and paternal genomes may contribute to the overall risk for preeclampsia.

4.6 | Other human studies

Male microchimerism is common in the female liver and present in fetal, juvenile, and adult liver tissues suggesting a possible role of both maternal and fetal microchimerism in liver diseases (Guettier et al., 2005). In addition to studies in PBC (see Section 4.2), biliary atresia is a neonatal liver disease with maternal chimerism has been observed in the liver tissue of affected infants. Study of these cells found that they include $CD8^+$, $CD45^+$, and cytokeratin positive and are therefore may originate as stem cells capable of differentiation into progenitor lymphocytes, effector lymphocytes, or biliary epithelial cells (Muraji et al., 2008). Additionally, the study of the bile duct epithelium and hepatocytes of female biliary atresia patients has demonstrated the presence of antimaternal HLA class I antibodies (Kobayashi et al., 2007). These findings suggest an immunological role of chimerism in the pathogenesis of biliary atresia; yet distinguishing GvHD or host-vs.-graft disease requires additional study of the mechanisms of disease pathogenesis (Muraji, 2014; Muraji et al., 2008).

Similar to studies in mice (see Section 3), microchimerism of heart tissue had also been seen in humans. The study by Bayes-Genis et al. (2005) investigated the cardiac tissue of two women with male offspring, identifying the presence of male cells via real-time PCR and FISH (Bayes-Genis et al., 2005). The male cells, which were identified via FISH, also demonstrated cardiomyocyte phenotype and protein expression. While this report did not specifically examine the role of microchimerism in cardiac conditions it presents important findings regarding the integration of presumably fetal cells into maternal tissues. This and other studies have demonstrated the plasticity of chimerism and the capacity to differentiate and integrate in a variety of tissue types. It will be important for future studies to investigate this plasticity further and understand the tissue-specific implications of chimerism.

4.7 | Prevalence

Perhaps the most elusive information in the study of human chimerism is its overall prevalence. Limitations of studying chimerism on a

population scale include several of the limitations presented by the current techniques. Limitations of sensitivity and inability to obtain a global sample of chimerism throughout the individual in all tissues make a definitive diagnosis of chimerism complicated. Karyotyping, FISH and PCR approaches based on Y-chromosome detection as an indicator of male microchimerism, limit identification to chimerism originating from a male donor. Remarkably, studies of women without sons have identified detectable male microchimerism. One study that explored male microchimerism in a population of nulliparous 10–15-year-old Danish girls found 13.6% to have male microchimerism in their peripheral blood, supporting the findings of 13% prevalence previously found in healthy nulligravid women (Muller et al., 2015; Yan et al., 2005). We do not know if male chimerism originated from an older brother with fraternal DNA passed through the mother, male miscarriages, vanishing twins, transfusion history or sexual intercourse (de Bellefon et al., 2010; Dierselhuis et al., 2012; Muller et al., 2015; Peters et al., 2019; Utter, Reed, Lee, & Busch, 2007; Yan et al., 2005). Research by Kamper-Jørgensen et al. in a comprehensive study of numerous reproductive traits, health and lifestyle did not establish a model-based prediction of male microchimerism and concluded there is little known about the relationship between exposure to allogeneic cells and maintaining persistent chimerism; although, histocompatibility with transgenerational HLA relationships has been suggested and should be further investigated (Kamper-Jorgensen et al., 2012).

Chimerism has also presented in dizygotic twins. The single largest study of chimerism in twins examined peripheral blood chimerism in 472 individuals and observed chimerism in 8% and 21% of twin and triplet pairs, respectively (van Dijk et al., 1996). Another twin study found that both male and female opposite-sex twins had an increased frequency of thyroid autoantibodies compared to monozygotic twins, supporting the hypothesis that twins may have increased prevalence and risks from microchimerism (Brix, Hansen, Kyvik, & Hegedus, 2009). Observed cases of monochorionic dizygotic twinning have facilitated the hypothesis that chimerism between twins can be the result of twin-to-twin transfusion between dizygotic twins in utero (Ekelund et al., 2008; Le Bras et al., 2016; Rodriguez-Buritica, Rojnueangnit, Messiaen, Mikhail, & Robin, 2015; Shalev et al., 2006; Smeets et al., 2013; Walker, Meagher, & White, 2007). The majority of documented cases of monochorionic dizygotic twin pregnancies have been the result of assisted reproductive technology and a review of monochorionic dizygotic twins found similar risk for perinatal morbidity and mortality in addition to pregnancy loss before 24 weeks when compared to other monochorionic twin pregnancies (Peters et al., 2017). It has been suggested that intrauterine blood exchange may be the cause of genital abnormalities observed in 15.4% of monochorionic dizygotic twin cases. In a recent study of Mayer–Rokitansky–Küster–Hauser syndrome, a phenotypically analogous condition to freemartinism in animals, we unexpectedly found that male microchimerism in the peripheral blood was less frequent in adult patients with this condition compared to healthy controls (Peters et al., 2019). An important consideration of the findings in monochorionic dizygotic twin pregnancies is that many dizygotic twin pregnancies have been assumed to be dichorionic and therefore scientists may be underestimating the overall prevalence of this phenomenon. A more detailed assessment of chronicity in dizygotic twin pregnancies in future studies will be necessary to provide more comprehensive insight into associations with health complications.

5 | FUTURE RESEARCH

Despite improvements in molecular techniques, achieving a sensitivity of one in one million genome equivalents, studies continue to be limited when attempting to identify the presence or absence of single chimeric cells on a scale as grand as a human organism (Peters et al., 2019). It is possible that several studies have an inadequate sample size to properly identify an appropriate number of individuals with microchimerism due to the limited sensitivity of modern molecular and cytological techniques, as well as insufficient knowledge of the minimum level of chimerism required to produce pathology if these pathogenic hypotheses are correct. A better understanding of the individual interactions of donor cells with the host environment is needed to establish the role of single allogeneic cells in pathology. As chimerism may be transient and therefore become undetectable following pregnancy, it cannot be excluded that chimeric cells contribute to pathology after they are no longer detected. Current approaches for assessing the implications of chimerism are dependent on the sensitivity of modern techniques, such that only measurable microchimerism can be directly assessed for association with disease etiology. The modern techniques described in the current literature are unable to provide a noninvasive approach to comprehensively assess global chimerism status in humans. However, given these limitations, it must be considered that alternative approaches may be needed to compare non-shared genomic traits between a mother and offspring, which may provide a better understanding of underlying mechanisms of disease pathogenesis than direct measurement of microchimerism. Moreover, we hypothesize that the study of non-shared (paternal) alleles between the mother and offspring, using readily available genotyping platforms, may be useful to identify non-inherited genes that have a previously undefined role in fetal or maternal disease.

The majority of studies have obtained samples for analysis of chimerism status after disease onset at which point chimerism level may have decreased to undetectable levels. As a consequence of such retrospective case–control study designs, investigators cannot firmly establish a causal role for transient microchimerism in human health. Furthermore, preferential selection or elimination of allogeneic cells may be distorting the associations made in current studies without examining longitudinal chimerism burden. It will be critical that future studies investigate the role of both transient and persistent chimerism as a factor in both diseases listed in this review and those to be later established. To address this missing component in the current body of knowledge, prospective cohort studies examining chimerism status in women and offspring from the time of fetomaternal exposure to the time of disease onset are needed. These studies will provide an

opportunity to understand the biology that facilitates persistent chimerism and association with human health. However, such prospective study designs may be difficult and expensive to implement. Twin studies have provided significant insight into various omics studies, controlling for confounders that complicate other research designs (van Dongen, Slagboom, Draisma, Martin, & Boomsma, 2012). The disease-discordant monozygotic (MZ) twin design is of particular interest as it controls for age, sex, and genetic contributors, allowing for investigation of environmental contribution (Finnicum et al., 2018; Li, Christiansen, Hjelmborg, Baumbach, & Tan, 2018; van Dongen et al., 2012). The discordant MZ twin design may be able to help establish both patterns of allele sharing and associated risk of persistent microchimerism in disease onset.

There may be different forms of each disease that have a unique etiology, such that one form includes a mechanism of pathogenesis activated by chimeric cells and another does not. A study by Rak et al. (2009) compared the presence of male microchimerism in blood samples from females with two different clinical presentations of systemic sclerosis to come to the conclusion that male microchimerism in whole blood occurs more frequently in limited cutaneous systemic sclerosis than diffuse cutaneous systemic sclerosis; however, the opposite was found in peripheral blood mononuclear cells despite no notable difference from controls (Rak, Pagni, et al., 2009). Subsequent study of the HLA compatibility between the maternal and fetal cells for HLA-DRB1 found having an HLA-DRB1 compatible child was significantly associated with limited cutaneous systemic sclerosis, but not diffuse cutaneous systemic sclerosis (Rak, Pagni, et al., 2009). These findings suggest that different clinical presentations of systemic sclerosis may be the result of different mechanisms of onset, which may also translate to other conditions associated with chimerism. Despite evidence indicating an association between HLA-DQA1*0501 and microchimerism in autoimmune diseases (see Section 4.2), others did not find an association between HLA-DQA1*0501 and the presence of microchimerism in patients with systemic sclerosis or JIIM; proposing that discrepancies may be due to methodology, population, study size or confounding association of HLA-DQA1*0501 and autoimmune diseases (Artlett et al., 2003). To control for several of these confounders, future studies should consider utilizing a discordant twin design, as described above; this may be able to provide further clarity to these ambiguous conclusions.

Currently, chimerism has been most widely explored in women with XX/XY male chimerism due to the simplicity and sensitivity achieved using PCR for targeting the Y-chromosome, leaving chimerism in males understudied. The use of HLA typing via PCR and flow cytometry has demonstrated excellent sensitivity and capacity for the study of chimerism, not limited by sex chromosomes. Similarly, blood group chimerism has been shown to be effective for the study of chimerism that is not reliant on sex chromosomes (van Dijk et al., 1996). Published results have also used FISH to identify and quantify the sex chromosomes of cells in tissue sections providing an opportunity to study maternal microchimerism in male subjects (de Bellefon et al., 2010; Nelson et al., 2007; Ye et al., 2012). Researchers must address current challenges in studying mixed chimerism samples by developing techniques with adequate sensitivity and versatility to provide confidence in defining cell sources. A relatively new technology that has shown promise for application in the field of microchimerism is droplet-digital PCR (ddPCR), which one group demonstrated an assay limit of detection and limit of quantification of 0.008% and 0.023%, respectively (Kliman et al., 2018). Future studies will require a comprehensive assessment of subject samples within pedigrees to define the source of microchimerism within the proband. Approaches may rely on panels of informative polymorphisms for PCR or targeted sequencing to identify similarities with potential cell sources. Improvements in the sensitivity, specificity, and costs of these techniques may provide the tools necessary to better understand chimerism in the general population. Furthermore, larger studies that examine different types of chimerism among various tissue sources will be necessary to elucidate the overall prevalence of human chimerism and better understand the biological relationships facilitating health and disease. Obviously, all techniques and study designs face questions about the appropriate tissue to use for analysis and the comparability of findings across different tissues.

Significant progress has been made in advancing the current understanding of chimerism and the implications hypothesized for human health and wellness. However, two significant questions remain which warrant future study. First, what are the mechanisms by which chimerism produces disease and tissue-specific protection or pathogenic consequences? These studies will most likely include experiments of chimerism in animal models for longitudinal study of chimerism in disease progression or tissue repair. To truly understand the role of chimerism, it will be necessary to study chimerism at the tissue level instead of exclusively via noninvasive approaches, such as blood samples. The primary focus of most research to date has been to establish an association with disease. While these studies have produced exciting findings, they have not defined the mechanisms that drive disease pathogenesis. As chimerism has been hypothesized to have beneficial, pathogenic or null effects on health and disease, hypotheses of a role in disease etiology warrants further validation of the proposed biological mechanisms. Second, what is the proportion of the population exposed to allogeneic cells via microchimerism? It will be exceedingly important to establish the prevalence of lifetime chimerism in the human population to understand the positive and negative effects of chimerism in human biology and possibly psychology. These studies will require large population studies to examine the epidemiology of chimerism and systematic collection of samples at the time of chimerism incidence, for example, during pregnancy. Furthermore, to facilitate this aim it will be necessary to develop high throughput approaches to identifying microchimerism in mixed chimerism samples. Prospective studies are desperately needed in the study of chimerism and will subsequently illustrate biological and behavioral changes in subjects longitudinally. By addressing these questions future research will detail the diverse implications and mechanisms underlying chimerism in human biology. This knowledge will facilitate new insight into complex diseases and behavior, potentially inspiring novel approaches to proactive screening, diagnosis, and treatment including novel strategies and therapeutics. Similarly, as chimerism is

the direct result of medical transplantation and transfusion medicine, it is of increasing importance that we have a comprehensive understanding of the implications of natural chimerism to better prevent long-term consequences, including graft rejection. This research will be able to provide additional context to our understanding of alloimmunization, immune tolerance, the need for immunosuppressants, guide clinical decisions and inform subsequent diagnostic screening. For example, understanding the mechanisms that produce protective and pathogenic consequences of chimerism will provide invaluable information to promote better outcomes in patients receiving allogeneic transplants.

6 | CONCLUSIONS

Chimerism in humans has been documented for over 60 years; however, we have only recently begun to elucidate the sources, prevalence, and relationship of chimerism in human behavior and disease. The continuous improvements in sensitivity and specificity of techniques for chimerism detection has provided researchers with the means of detecting minute levels of allogeneic cells and explore hypotheses relating chimerism to various human conditions. Also, with increases in assisted reproductive technology more dizygotic twin pregnancies are seen; creating a potential increase in chimerism prevalence and stressing another need for understanding the implications of chimerism. The study of chimerism in human behavior and health appears to have a significant amount of complexity that we have only just begun to explain. Current findings suggest that chimerism may have a pathogenic role in autoimmune diseases through mechanisms similar to cGVHD. Similarities in the pathophysiology of autoimmune diseases and cGVHD observed in transplantation patients provides further evidence to support a pathogenic consequence for sequestering allogeneic cells. Understanding the potential roles of allogeneic cells in the health of a host organism has direct implications in defining the long-term influences on the health of recipients of allogeneic transplants and transfusions. In contrast, among cancer patients, chimerism has primarily been found to be less prevalent in the peripheral blood compared to controls. In addition, chimeric cells appear to migrate to tumor tissues and result in better overall outcomes suggesting a protective role. However, there is an immense need for understanding the underlying mechanisms that may be driving contribution to disease etiology. As such, we cannot exclude that allogeneic cells sequestered in disease tissues may also have no direct role in disease prevention or pathology. Studies of chimerism in PBC have shown no significant association, yet biliary atresia has demonstrated a cGVHD role in pathogenesis. Due to the variety of findings that support pathogenic, protective or neutral roles for various human diseases, it is also possible for chimerism to have different roles and mechanisms depending on the associated host conditions. Future studies will be necessary to explain the intricacies that underlie these various conditions and describe the prevalence of microchimerism among human populations to establish additional associated phenotypes.

Elucidating the prevalence of chimerism, both in males and females, and associations of chimerism with health and behavior may facilitate better care and quality of life in individuals with disorders characterized by significant sex differences in prevalence or unknown etiology. Ultimately, these studies will provide information to improve our understanding of human health and behavior.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTION

B.N.J. performed systematic review design, literature search, article screening, and drafting of manuscript. D.I.B., E.A.E., and G.E.D. performed supervision of review design and drafting of manuscript. All authors provided critical feedback, read and approved the final manuscript prior to submission.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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