

Sex Determination in *Homo sapiens* as a Multi-Step Process: Potential for Development of Variants and Sex Differences in Disease Risk

David A. Hart

Department of Surgery, Faculty of Kinesiology, McCaig Institute for Bone & Joint Health, University of Calgary, Calgary, Canada

Correspondence to: David A. Hart, hartd@ucalgary.ca

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ABSTRACT

Reproduction via cis-binary mechanisms appears to have evolved fairly early in the evolution of complex organisms, and a system committed to prior to evolution of humans. While the evolution of a chromosomal-specific approach has been a successful strategy for survival of a large variety of species including humans, the fidelity of sex determination leading to 100% cis-binary outcomes is not achieved in many species, with evidence for homosexual or bisexual behaviour evident in more than 1500 species. Thus, such outcomes indicates that sex determination is a multi-step process and not a single event, and as such, could lead to the appearance of variants during the process which developed much earlier than humans. Variants could arise either due to intrinsic variation in the steps of determination, or also be influenced by environmental factors of a biological or psychological nature. In contrast to homosexual variants which do not require interventions such as hormone therapy or surgery, expression of gender dysphoria, is more based in psychology, but also has biological underpinnings and can be influenced by such hormonal interventions and surgery. While the numbers of those with gender dysphoria is small (~0.6% - 1.0% of population), the attention given to this issue raises the possibility of biological and psychological environmental factors impacting the emergence of some of those expressing gender dysphoria. Furthermore, transitioning from male-to-female or female-to-male can have consequences regarding disease risks latter in life, including the appearance of autoimmune diseases. This review will attempt to review some of the evidence regarding sex determination, discuss why the system has potentially not been improved upon during evolution, how a potential role for sex chromosome function on neurodevelopment may be central to variation in humans,

and how commitment to the current strategy is likely integrated into other sex-related events such as puberty, pregnancy, and menopause to ensure species survival. It will also discuss how variants in sex determination could contribute to sex differences in disease risk and how epigenetic modifications could play a role in such risk.

1. PURPOSE OF THIS REVIEW

Complex multicellular species have evolved over several hundreds of million years. Originally, they likely reproduced by replication, which is basically cloning. However, somewhere during evolution the concept that recombination via two sexes offered advantages to species survival, possibly via the recombinational process as a generator of heterogeneity or diversity. Such heterogeneity could contribute to species survival via resistance to threats that cloning could not offer. While the development of chromosomes and chromosomal variation between sexes led to insights into how such sex determination was regulated, it cannot account for all of the variation and variants that can arise. The finding of some variants in common between quite diverse species, but including *Homo sapiens*, lends credibility to the concept that sex determination is a process with multiple steps rather than a single event. Certainly, a single event can set in motion unique processes, but “wobble” in the processes could lead to variants. Why a species such as *Homo sapiens* and their predecessors did not improve on the process to enhance the fidelity of the outcome remains unknown. The purpose of this review/perspective is to discuss aspects of the potential steps in the process, the implications of the steps, and potentially why the outcomes have not been overtly improved upon over millennia/eons.

2. INTRODUCTION

Survival of a species depends on the ability to reproduce with fidelity. Early single celled organisms reproduced by dividing with each daughter cell a replicate of the original, and so on. The replication machinery eventually involved an information storage molecule, DNA and associated structural and enzymatic entities required for faithful replication. Such replication is essentially cloning and still evidenced in single celled organisms today including bacteria. Obviously, single celled organisms could generate some heterogeneity in their descendants via mutations and minor errors in DNA replication, so they could and still can adapt to environmental stressors to enhance survival of some descendants.

With the advent of multi-cellular organisms containing cells within their structure with differentiated function now required more sophisticated regulation and additional information capacity in the DNA structure. At this stage, replication would have still been cloning, with all organisms likely similarly sensitive to environmental threats and thus, any particular lineage, or most lineages, at risk for being eliminated by such threats. Thus, resistant to any particular threat would be based on stochastic events or mutations otherwise a threat to the survival of the species. While some complex organisms may still be able to undergo parthenogenesis (a female being able to clone itself), with increasing organism complexity, a different way of generating heterogeneity was needed, one that could enhance the survival of a species in response to any number of environmental threats. The answer appears to be the development of a two-component system that is required to come together to initiate a recombinational event leading to one component laying eggs which mature and hatch leading to a functional descendant (or in the case of many fish, eggs are expelled and the recombinational event is facilitated externally after egg expulsion), or in the case of mammals, one component carrying the replicant internally until functionally viable, or some combination of the above options. For species with a two-component system to generate heterogeneity in reproduction, it required the development of different approaches to facilitate the recombination events. This usually required the evolution of component-specific tissues and organs, and a way to make sure that the abilities of the two individual set of components could be passed on to the descendants with sufficient fidelity to make sure the species survived. This is termed biological sex, with males and females the ele-

ments of the two-component system cis-acting system.

Such 2-component systems likely predate humanoids by millions of years of evolution, but where in evolution such complexity arose and what variations on the 2-component theme have been tried and failed is unknown from the evolutionary record. Clearly, the organization of information in chromosomes rather than one large DNA structure was an advance, as was the sex-determining information on specific X- and Y-chromosomes an advancement that allowed for not only regulation of information defining the sex of the off-spring, but also the inheritance of associated genes on the “sex” chromosomes. Such sex-specific chromosomes appear to have arisen approximately 180 million years ago [discussed in [1]]. However, not all genes associated with sex differences are on the X- and Y-chromosomes as sex differences can be complex involving multiple systems [2, 3], and systems that can be influenced by different stages of the life span [*i.e.* fertilization, puberty, menopause for females] and exhibit heterogeneity in characteristics [reviewed in [2, 3]]. Thus, sex determination is likely not only an event, such as inheritance of two X chromosomes or an X + Y chromosome, but also it is likely a process involving several genes on multiple chromosomes and subject to a variety of influences, as will be discussed below.

3. THE FUNDAMENTALS OF BIOLOGICAL SEX

Successful reproduction not only depends on an ability to conceive of off-spring, but also an ability to carry the viable fetus to term, deliver, and then rear the neonate to an age of semi-independence. While the former is of critical importance and involved a complex process to initiate, the post-conception variables are also very critical. Therefore, many of the variables essential for successful reproduction do not depend on males.

The transition from reproduction in prokaryotes with DNA replication (*i.e.* cloning of the DNA) to organizing the DNA into units such as chromosomes was a complex process that was required as organisms became more complex, but also encouraged generation of biodiversity and enhanced ability of subsets of such organisms to survive exposure to stressors. The evolution of sex chromosomes to accommodate successful reproduction is believed to have originated from a pair of autosomes which then differentiated as sex-determining chromosomes [discussed in [4]]. This process may have occurred several times during evolution [5, 6]. This scenario has led to variation in sex determination systems within the context of chromosomes [7]. Even within and between non-human primates (*i.e.* New and Old World species), differences in the chromosomes and in functions have been noted in primates [discussed in [8]] and other species [9]. Such variation can also be further influenced by epigenetic variation in a sex-dependent manner [discussed in [10]]. Thus, while the basic mechanisms associated with sex-specific chromosomes may be similar in some respects, variation can and does occur to benefit specific species.

Thus, the initial sex determination of a male is initiated by the Y-chromosome, and specifically the SRY gene [11]. Functional inactivation of the SRY gene on the Y chromosome effectively negates the formation of the male genotype/phenotype [12]. Pigs with an inactive SRY gene were males with complete external and internal female genitalia [12]. Recently, the human Y chromosome has been completely sequenced [13], revealing several features of the associated genes besides the SRY gene [discussed in [14-16]]. Interestingly, some species have lost their Y chromosome and the SRY gene but appear to compensate for such loss via male-specific upregulation of Sox9 [17]. As expression of the SRY gene leads to an up regulation of Sox9 [17], such species have compensated for the loss of a Y chromosome via alterations that enhance down-stream effects of the SRY gene. The human Y chromosome also has some unique features regarding variation and the fact that it uniquely represents the male contribution to the fertilization process [discussed in [18, 19]].

The X chromosome also appears to have arisen/evolved ~180 million years ago, similar to the Y chromosome [1], and it contains a variety of genes including those involved in a speciation, sex-associated features, and development [20-22]. As the X chromosome contains ~800 genes, versus ~60 on the Y chromosome [22], it therefore contains a large number of sex-defining genes affecting a variety of tissues [discussed in [21]]. As a female obtains an X chromosome from both the sperm donor and the egg donor

at the time of conception, one X chromosome is inactivated in different tissues either randomly or selectively [23-27], giving rise to mosaic expression patterns.

In this 2-sex system for formation of a fetus, the fetus is effectively a foreign graft to the mother, evoking an immune response that assists placental implantation [discussed in [28, 29]]. If the sperm and egg are from individuals that are histocompatibility similar, there is a higher risk for a failure to successfully carry the fetus to birth [30, 31]. Thus, while the initial sex determination is determined from whether the fetus derives an X or Y chromosome (the SRY gene specifically on the Y chromosome; discussed in [12] from the sperm donor and an X from the egg donor, subsequent events post-fertilization can also influence the survival of the fetus. This system, which appears to drive aspects of heterogeneity in humans [32] appears to operate with many species (*i.e.* humans, pigs], but not rodents such as mice which can be inbred and thus, such species are not representative of humans in this regard.

Based on the above discussion it is fairly clear that sex determination can be initiated by genes such as the SRY gene on the Y chromosome for males, but the process is more complex in females, and that post-events following the impact of the SRY gene means that there is a process associated in part with genes on the Y and X chromosomes, and others that contribute to details regarding how the post-conception events will transpire leading to a potential spectrum of variation in the final phenotype of the sex-specific individual. Thus, sex-determination is a multi-step process in many respects, and as such individual options may be influenced by exogenous environment or stochastic factors. Relevant to this discussion is the known genes on the X chromosome that relate to neurodevelopment [25].

That such a process of sex determination can be influenced by exogenous variables comes in part from observations in certain animal species [33], possibly via their evolution of sex chromosomes [34]. In reptiles such as crocodiles, alligators, lizards and turtles [35-43], as well as fish [44, 45], the sex ratio of off-spring can be influenced by the temperature at which the eggs are incubated. This also includes zebrafish [44], a widely used preclinical model. Interestingly, the direction of the changes in sex ratio can be species-dependent, with some species leading to increases in females with temperature changes, while in others, temperature influences can lead to more males [discussed in [46]]. In contrast, this temperature-dependent loss of males does not occur in birds [discussed in [47]] but other temperature-dependent effects may be operative [48]. Interestingly in many bird species, females are heterogametic (ZW) while males are homogametic (ZZ) [discussed in [49]].

In crocodiles and alligators, the temperature-dependent alterations to the resulting sex ratio of off-spring can be over-ridden by exogenous estrogen [39, 50]. This reversal by estrogen appears to be mediated by estrogen receptor1 (ER1; ERalpha) and not ER2 (ERbeta) [39]. This raises the possibility of influences on sex-determination of xenobiotics in the environment [50], some of which may be estrogen mimics or other types of chemicals that may interfere with estrogen binding to ER or interacting with other steps in the process. There is some evidence for PCB chemicals playing a role for endocrine disruption in a variety of species including humans [reviewed in [51]]. Other chemicals have also been implicated as endocrine disruptors in a variety of species as well [52-56]. Some populations may be at higher risk than others however [57]. Other factors, largely unknown may relate to seasonal variations in male/female ratios [discussed by [58-60]].

Thus, both the initial sex-determining step and subsequent steps in the establishment of sex and other aspects of sex-determination may be influenced by the environment at critical stages of fertilization and development. Clearly, the survival of the humanoid lineage is evidence that such variation has not compromised evolution to the current version, *Homo sapiens*. As sex differences in many tissues such as the musculoskeletal system, the brain, and others [reviewed in [2, 3]], they continue to develop and mature leading to the advent of puberty in preparation for continuing the cycle of reproduction.

4. THE POTENTIAL TO GENERATE NON-CIS VARIANTS OF SEX POST-RECOMBINATIONAL EVENT (PRIMARY, SECONDARY AND TERTIARY STEPS) DURING DEVELOPMENT

If indeed sex determination is a series of events in a process, starting with a primary event (*i.e.* the Y

chromosome for males in humans and expression of the SRY gene) and then secondary and tertiary events or steps in a process, then there could be risk for development of variants in the process leading to a spectrum of biological variants whose impact may depend on where in the process the variant arises and potentially, how far from the primary event the variant arose. The most obvious likely biological variants are those related to homosexuality. While the incidence is fairly low, apparent homosexuality does occur in many species ranging from humans, primates, to birds [~1500 species: [61]]. In addition, some individuals can be bisexual, a category aligning somewhere different on a potential spectrum of biological variants.

To emphasize the concept of sex determination as a process, two examples will be discussed in some detail; one based more on genetics and biology (e.g. homosexuality) and the other based more on psychiatric foundations with some biological underpinning (e.g. gender).

4.1. Non-Cis Behavior in *Homo sapiens*

Non-cis sexual behaviour in *Homo sapiens* may take two general forms, homosexuality which is a preference of a genetically female for other females (Lesbians) or a genetic male for other males (Gay), or gender-dysphoric individuals who perceive themselves as the opposite sex to what their genitalia or genotype would predict. Thus, these two general forms of non-cis sexual behaviour have their basis in different aspects of *Homo sapiens* development and maturation.

As homosexual behaviour has been described for a large number of diverse species, homosexual behaviour likely preceded that of gender dysphoria. Homosexual behaviour may take a variety of forms [discussed in [61]; and others] and thus may exist as a spectrum that could relate to species-specific conditions. However, given the spectrum of species exhibiting homosexual behaviours, it is an indication that the fidelity of the process to allow for survival and evolution of species is sufficient as evidenced by the evolution of complex life over the past millions of years and an estimated incidence of homosexuality in *Homo sapiens* to be ~2% - 8% [62]. If there is a biological basis for homosexuality, it does not appear to have a readily discernable genetic or epigenetic basis [discussed in [62-65]]. However, there does appear to be a biological basis for some aspects of homosexuality [reviewed in [66-68]]. It should be noted that Lesbians can have children via in vitro fertilization in some societies [69, 70], so their sexual preferences may not preclude generating off-spring.

In contrast, the only species in which gender can be ascribed are *Homo sapiens*. As it is likely a cognition-dependent personal assessment of the relationship between their sexual identity based on genotype and a gender dysphoria (e.g. female in the body of a male and vice versa). The American Psychological Association defines gender identity as “a person’s deeply felt, inherent sense of being a boy, a man, or a male, a girl, a woman, or a female, or an alternative gender (e.g. genderqueer, gender non-conforming, gender neutral) that may or may not correspond to a person’s sex assigned at birth or to a person’s primary or secondary sex characteristics” [discussed in [71]]. Thus, while there are likely some contributing biological determinants, including some related to brain development and differentiation, that may influence the appearance of gender dysphoria [72-75], it is likely that many aspects are in the domain of psychology. Based on the above, the expansion of the use of the term gender to be over-arching when describing non-cis behaviour or gender dysphoria is likely incorrect in that one cannot assign gender dysphoria to animals either because one cannot assess it, or their level of cognition may be insufficient to allow for it.

The evolution of cognition to the degree associated with *Homo sapiens*, the current humanoid dominating the planet, is not clear as to when and how various attributes evolved and were integrated into the current function level [discussed in [76-81]]. Furthermore, humans are very heterogenous at multiple levels [discussed in [32]], which is a survival strength, and this also likely extends to various cognitive abilities. In this regard, cognition in *Homo sapiens* is likely a “work in progress” and evolution of this attribute is not complete. Thus, in this context, it is not clear how gender dysphoria represents a significant percentage of the population as it is estimated that this population has an incidence of ~1% [82]. Whether this small population is expanding due to increased recognition or the influence of exogenous environmental

factors or is a constant due to the sex determining process is unknown. However, as gender dysphoria can be experienced by both genetically males and females, it is not due to unique steps in a sex-specific manner.

4.2. Why Has the Process Not Been Improved in *Homo sapiens* and Predecessors?

Based on the above discussion, non-cis behavior is evident across many species and this finding raises a number of issues and questions. One could ask why throughout human evolution was the process not “improved” upon to eliminate homo or bisexual behavior? Certainly, as such behavior is apparently expressed via lineages that likely separated millions of years ago, there would be ample time through evolution to improve upon outcomes unless the incidence rate was sufficiently low as to not compromise survival of the species. Alternatively, there may have been attempts to improve on the fidelity of sexual determination that proved to have negative implications and were “discarded”. Clearly some species have made adaptations which allowed temperature to influence outcomes that benefited the survival of the species for millions of years (*i.e.* crocodiles). Perhaps in humanoids, some modifications to sex determination were not compatible with subsequent events such as those associated with puberty [*i.e.*, epigenetic modifications; reviewed in [2]] or menstrual cycles.

While non-cis biological variants are likely a normal consequence of a lack of fidelity in sex determination processes and likely not based on homosexuality specific genes [82-85] but epigenetics may have a role [56, 57], their roles in society and acceptance have been varied throughout recorded history. Some of the lack of acceptance is rooted in certain religions and in contrast, in some survivalist societies such as subsets of indigenous cultures of North America, such individuals were viewed as “two spirited” and accepted. In the latter, acceptance with roles may not have been due to an “enlightened” interpretation but more pragmatic given the resources committed to such individuals before the homosexuality was evident. Therefore, viewing sex determination as a process allows for species-specific flexibility in influencing outcomes, as well as ensuring that a species such as *Homo sapiens* survive to effectively reproduce and maintain heterogeneity to compete for resources. Where and why species committed to the predominate binary sex determination process still remains largely unknown, but it has been largely effective and the heterogeneity that is generated via such a system in *Homo sapiens* certainly offers advantages over a “cloning” process for reproduction in resistance to environmental threats that could influence the gene pool. Thus, interbreeding with Neanderthals [86, 87], surviving the plagues of Europe [88, 89], or perhaps surviving the exposure of North American Indigenous people to infectious diseases inadvertently brought by Europeans can in large part be attributed to heterogeneity in populations.

4.3. Implications of Biological Sex Determination as a Process

There are a number of implications resulting from the discussion above regarding sex determination as a biological process rather than a single event associated with the expression of the SRY gene in *Homo sapiens*. First, the initial primary step with SRY may initiate of cascade of secondary and tertiary steps in the process leading to cis-binary development in most individuals that are positioned for effective reproduction following puberty. Secondly, biological non-cis variants may arise during the steps subsequent to SRY expression in males. However, where in the process the non-cis lesbian variants arise is not well characterized. However, in both scenarios, the influence of the biological variant on the pre- and post-puberty outcome may depend on how close the variants arose to the initiating events and whether the variation may have led to epigenetic alterations that “fixed” the changes in the genome of specific tissues [63, 64]. Since non-cis Lesbians still have menstrual cycles and experience menopause [90-92], those post-puberty processes are not apparently affected by development of their sexual preference phenotype. If indeed such non-cis biological variants are due to stochastic events influenced by early estrogenic events [93] followed by epigenetic alterations, it is doubtful that psychological interventions would be able to reverse the phenotype. Thus, such non-cis biological variants would exhibit preferences for sexual attraction but not differ with regard functional abilities related to reproduction. Therefore, such preferences may be

the product of multiple variables [66], but the outcomes may reflect the targeting of the variants on neurodevelopment. Interestingly, many of the genes on the X chromosome (and both males and females have a X chromosome) are related to neurodevelopment. Whether the non-cis biological variants exhibit alterations (*i.e.* epigenetic modification) to an otherwise “normal” alleles of a subset of these X-chromosome associated genes, or there is heterogeneous variation in a subset of the genes that are more readily influenced by stochastic events leading to non-cis preferences is not known but could be the target of future study.

5. GENDER AS A TERM FOR THE PSYCHOLOGICAL BASIS OF SEXUAL IDENTITY

The original definition of gender, defined as the behavioral, cultural and psychological traits associated with an individual, has now been expanded and admixed with the term sex and many use the term to include both sex and gender under the unified term, gender [discussed in [94]]. While convenient perhaps from the public perception, the admix of the terms is likely not strictly appropriate since gender is more focused on psychological variants and sex is more focused on biological variants but with some psychological input as well. Furthermore, the use of the term gender to describe animal models is also likely inappropriate as presently animals do not have a gender, but only a sex [94]. Thus, gender may be a consequence of the level of cognition of *Homo sapiens* and some of their predecessors.

While the vast majority of *Homo sapiens* are cis for both sex and gender, only a small subpopulation is non-cis for sex, as well as gender, but not both. Gender dysphoria, or the feeling that an individual may be defined as male or female based on genitalia but actually consider themselves a female in a male body or male in a female body. Thus, there appear to be two sex forms, but a spectrum of multiple gender forms [94]. How many gender forms there are within a possible gender dysphoria spectrum is unknown, but it may vary since it is self-defined and there are no standards.

This gender dysphoria often leads to hormonal and surgical interventions to “correct” the apparent congruence of sex and gender. Transsexualism may be one end of a spectrum associated with gender identity [95]. As the spectrum of gender variants are thus primarily psychological non-cis variants who are self-defined and who require interventions to address the dysphoria, such psychological variants also differ from homosexuals who do not require such interventions. Thus, sexual identity and sexual orientation are likely independent components [96]. However, some aspects of non-cis psychological variants likely have aspects of their origins in biology during the formation and development of the brain [97-100]. As this is likely a multi-step process, with genes from both the X-chromosome [discussed earlier; [101]] and the Y-chromosome [102, 103] involved in the process [104].

Coming out as a someone living with gender dysphoria is a self-defining process, and as such can be influenced by several factors, some of which are related to definition and likely stage of life [105]. Thus, the basis for gender dysphoria before puberty and during early growth and development may be different from that experienced after puberty during which epigenetic alterations occur in multiple tissues including the brain [[106]; discussed in [2]]. Of particular interest is the hypothalamus [100, 107-109]. As transitioning can involve both sex hormone injections and surgery, the background may also play a role in outcomes.

6. EMERGENCE OF GENDER DYSPHORIA AS A FOCUS OF CURRENT RESEARCH, DISCUSSION AND MEDICAL EFFORT

In very recent years the topic of gender dysphoria has emerged as a controversial topic for researchers, politicians, the judiciary, and the public, as well as educators. As gender dysphoria is based primarily as related to non-cis psychological variants, and one self-identifies oneself as dysphoric, this provides an opportunity for the self-identification to be influenced by several factors. These include the following:

- 1) The emergence of individuals experiencing gender dysphoria may be due to the prior suppression of such individuals once identified socially. Thus, removal of this prior suppression of individuals identifying themselves as dysphoric and the associated consequences, now appear to have been lifted in some

countries. However, in some jurisdictions letters from mental health experts are needed in order to proceed with transitioning surgeries [110].

2) Individuals with ambiguity regarding gender may be influenced by the attention in the media and elsewhere to become “unique”. As psychological in part, some individuals who are at a susceptible stage of life may be influenced to make the decision to come out as transgender. It is well known that some individuals are susceptible to the placebo effect (~20% - 40% or more depending on a wide number of reports and studies) and often influenced by spirituality [111, 112], which is the ability to impart a biological response due to receptive conditioning. Others are more susceptible to radicalization than others [113-115], another indication of the success of indoctrination. Finally, there is also ample evidence of “mass hysteria” [116-118] and including the Salem Witch trials [119] where individuals are influenced by belief and not fact. However, if gender dysphoria encompasses a spectrum of underlying causes, then some are likely legitimately seeking sexual transitioning while others may be influenced by other variables.

3) Transition interventions have become “big business”, with an expanding population seeking hormonal and surgical alterations [120]. The numbers of individuals seeking such interventions is in the tens of thousands in North America, the actual numbers remain small in the general population. However, the medical community is attempting to remove barriers to access [110, 121]. Thus, embracing medical and surgical transitioning by the medical community lends an aura of legitimacy to the “correction” of the dysphoria. Interestingly, gender-affirming hormone therapy can lead to epigenetic modifications [122], providing further evidence for the effectiveness of the interventions to induce permanent alterations to correct the dysphoria, but whether such changes can be reversed following a re-evaluation of decision making is unknown presently. Certainly, the surgical modifications cannot be reversed.

4) The emergence of gender dysphoria could also be due to the widespread use and misuse of xenobiotics in the environment which has impacted the psychological background of some individuals during development, and which in concert with Point C could lead to an artificial increase in individuals self-identifying as transexual. In the past, exposure to mycotoxins in food was raised as a possible cause of the Salem witch trial mass hysteria [119]. Certainly, the widespread use of “forever chemicals” [123-125], as well as phytoestrogens [126] in the environment are examples of disruptors of biological regulation.

5) There is the potential of epigenetic modification being involved in biological aspects of gender dysphoria [127, 128]. Epigenetic modifications are known to be involved in the nervous system [129, 130], and may contribute to sex differences in the brain [131] as well as mood disorders [132] and sexual orientation [63, 66]. Rare variations in epigenetic alterations could arise either stochastically or via timely interaction with environmental factors to contribute to conditions leading to gender dysphoria. As many epigenetic alterations are reversible, if this mechanism is involved in gender dysphoria, it may be reversed with an appropriate intervention.

Clearly, gender dysphoria differs from homosexuality at multiple levels, and the field is in need of clarification of definitions, the implementation of additional standards to assist individuals with gender dysphoria or gender ambiguity, and the distribution of accurate and consistent information to educators, politicians, the judiciary, and the general public. If indeed gender dysphoria is a spectrum, there are likely those that are genuinely in need of biological interventions such as hormonal and surgical treatments, plus others that may have their dysphoria based on gender ambiguity and still others that have a self definition based on psychological influences that are in need of a different type of intervention involving psychiatry.

From some aspects, the current state regarding gender definitions and interventions is analogous to the field regarding multiple personalities. Multiple personalities have been known to occur in a small number of individuals for decades [133-135], and these can vary in number of personalities. The number of cases, based on case reports, indicates that the number of individuals affected is not static/year and appears to rise in response to conditions and also having a potential “hysterical” element [136]. Some of the characteristics of multiple personality disorders have also changed over time [137]. Primarily, such patients are treated with complex psychotherapeutic approaches [138]. However, the multiple personality disorder term was replaced over the ensuing years to become “dissociative identity disorder” (DID)

[139-141]. While the concept of DID has been around to decades, and recently modified in the Diagnostic and Statistical Manual of Mental Disorders (DMS-5) [142], the term is still controversial [143-145] which has implications in the medicolegal system [146, 147]. A recent case report has discussed the potential links between gender dysphoria and DID [148]. Thus, similar to DID, gender dysphoria is likely mainly in the realm of psychological disorders, is controversial in its definition and causes, poses issues with the medicolegal system, and may also have a “hysteria” element. Of course, diagnosis of both DID and gender dysphoria is critical, but also deciding on the best treatment interventions for the individual patient is essential as there are many consequences to the hormonal and surgical treatment that can impact aspects of life aside from the sexual identity aspects.

7. CONSEQUENCES OF HOMOSEXUALITY AND GENDER DYSPHORIA ON HEALTH ACROSS THE LIFESPAN

There are health-related consequences regarding homosexuality and gender dysphoria. These include consequences associated with being in those groups, as well as those with gender dysphoria spectrum who have undergone hormonal and surgical interventions. Some are psychological and others are more related to molecular consequences and alterations in disease risk, and thus likely more biological consequences of surgical and hormonal interventions.

Consequences associated with the stresses of being in those groups often leads to eating disorders [149-159], mental health issues [151], and increased rates of suicides [152, 153]. It can also lead to alterations in bone health in transgendered individuals [154-157]. Hormone treatment in gender diverse populations may also contribute to kidney disease risk [158] and cardiovascular disease risk [82, 159-162], but may be different for male versus female transitions [162]. As the transgender population is estimated to be 0.6% - 1.1% of the population [82], the numbers of individuals potentially affected may be small, but the numbers receiving hormonal therapy and surgical interventions is likely rising.

As many autoimmune diseases exhibit sex differences in incidence [discussed in [2, 3, 163]], gender-affirming surgery and hormonal treatments may alter risk for development of such conditions [164, 165]. Systemic sclerosis is an autoimmune condition that affects many tissues, is more common in women, but men have more severe disease possibly for a variety of reasons [166]. Interestingly, a recent review reported the development of systemic sclerosis in three patients who developed the disease after male-to-female transitioning [167]. One patient died from the disease. In addition, Campochiaro *et al.* [167] also discussed two other cases where the same transition led to development of systemic lupus erythematosus (SLE), a condition that exhibits a strong 9/1 female incidence [168, 169]. In one of the SLE cases, the individual developed the disease 20 years post-surgical and continuous hormone therapy [169]. More recently, a case of an individual undergoing a male-to-female transition developed lupus nephritis was reported and symptoms in this individual resolved after cessation of hormone treatment [170]. Pakpoor *et al.* [171] also reported elevated risk to developing multiple sclerosis in male-to-female transitioning. While the above indicate there is likely some risk in transitioning with hormonal treatment, there is also the issue of clinical care for those with rheumatic diseases who transition [172].

Finally, there is the issue of sex differences in diseases of aging [discussed in [2, 3]]. During aging, and after menopause, there appear to be increased incidence of obesity, cardiovascular disease, osteoarthritis, and dementia in the female population than males. In this context, there is likely an interplay between aging and loss of hormones in the females. This concept is supported by the finding that use of hormone replacement therapy (HRT) in post-menopausal females can alleviate some post-menopausal symptoms and diseases [173, 174], including sarcopenia [175], cardiovascular systems [176, 177], lipid metabolism [178], and chronic kidney disease [179]. Therefore, some risk for aging-related diseases may be hormone-responsive and thus, associated with sex differences. However, it remains to be determined if such hormone treatments can overcome age-related loss of other regulatory influences, and thus the aging-sex hormone relationships are likely very complex at the individual level as epigenetic modifications via individual life experiences further complicate the disease risk milieu.

How such diseases would be influenced during aging in transgendered individuals following surgical

and hormone treatments is not known in any detail presently. Questions such as risks associated with continued hormone treatments into advanced aging will need further investigation. However, as the numbers presently are low for this population, as it expands and ages there will be opportunity for addressing such questions.

From the above discussion, surgical and hormonal interventions in gender-affirming interventions have implications beyond sexual orientation. While the numbers currently are fairly small for some of the conditions described, as such interventions become more common, some of these risks will become more recognized as a side-effect of the treatment. This is an area that future research should focus on in some detail.

8. CONCLUSIONS

Commitment of multiple species of complex organisms including humans to a system of reproduction occurred very early in evolution. Thus, commitment to a system facilitating genetic heterogeneity via a male and a female cooperating to yield off-spring was a successful strategy for both survival of a species and generation of genetic diversity. While a successful strategy, in >1500 species, a small percentage of off-spring were not cis in orientation but expressed preference for the same sex and were thus classified as homosexual. Such individuals were thus the likely result of variation in sex determination and an indication that sex determination was a biological process rather than an event. The molecular basis for how homosexuality arises is complex and likely involves hormonal, genetic and environmental influences but has not been characterized in detail. Whether attempts were made to increase the fidelity of reproduction using the existing strategy during evolution is unknown, but the current strategy is sufficient for species survival. The concept of gender differs from that of homosexuality in that it may have some molecular basis in hormonal contributions to brain development, it is likely a more psychological perspective.

While homosexuality is based on preference, gender, originally a sociologic definition, often requires hormonal and surgical interventions which can have significant molecular and disease consequences, although the separation between the two categories may be overlapping in some respects [180]. Gender dysphoria may therefore consist of a spectrum of individuals, and being primarily psychological in nature, could also be influenced by environmental psychological inputs. Thus, while the population of transgendered individuals represents a small percentage of the population (0.6% - 1.0%), there is currently considerable media and medical community, as well as education community attention to gender dysphoria which could contribute to emerging numbers which are increasing. In the medical community, transitioning is becoming a big “business” that is expanding [120]. Whether this is appropriate for all with gender dysphoria should be a topic for further investigation.

In summary, homosexuality and bisexuality are likely a natural consequence of the lack of 100% fidelity of sex determination as a multi-step process. As such, the condition(s) are not abnormal, but due to variation in fidelity of the system employed to ensure survival of the species and generate heterogeneity. In contrast, as a condition more based in psychology, gender dysphoria could be influenced by both developmental variation as well as environmental factors, and thus could be treated by psychological and/or hormonal/surgical interventions. When such interventions should be undertaken (pre or post puberty), and if such interventions should be considered will require significantly more research undertaken outside of any mass hysteria influences. Furthermore, the molecular consequences of hormonal and surgical interventions regarding disease risk should be further investigated. As sex differences in disease risks, such as autoimmune, cardiovascular, dementia-related and musculoskeletal diseases, these are potentially significant consequences of gender-affirming interventions.

If indeed sex determination is a process with some variants expressing clinical phenotypes, there may be many other variants that do not yet express an obvious detectable phenotype but may contribute to sex differences in disease risk in more subtle manners. Thus, such heterogeneity could explain why some subsets of females are at higher risk for certain autoimmune diseases and diseases appearing after menopause. Thus, the two types of variants discussed, homosexual and gender-related may be only examples of the

spectrum of potential variants.

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CONFLICTS OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

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