UNDERSTANDING HUMAN ANEUPLOIDY: THE ORIGIN OF TRISOMY AND EFFECT OF RECOMBINATION AND MATERNAL AGE

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The members of the Committee appointed to examine the dissertation of HEATHER ELIZABETH HALL find it satisfactory and recommend that it be accepted

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UNDERSTANDING HUMAN ANEUPLOIDY: THE ORIGIN OF TRISOMY AND EFFECT OF RECOMBINATION AND MATERNAL AGE

Abstract

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The ability to properly segregate chromosomes during meiosis is of immense importance, as aneuploidy is a leading cause of both pregnancy loss and mental retardation. Despite the overwhelming levels of monosomy and trisomy in humans, only two factors have been linked to increased levels of chromosome nondisjunction, aberrant recombination and advanced maternal age. Even with decades of research on human aneuploidy, many questions remain regarding why humans make such an appalling number of meiotic errors. Accordingly, in my thesis research I examined the parental and meiotic origin of several previously understudied human trisomies and explored a possible relationship between the two known predisposing factors, recombination and maternal age. Specifically, I conducted analyses of the origin of nondisjunction for trisomies 13 and 22, with data suggesting that both of these acrocentric chromosomes nondisjoin most often during the first division of maternal meiosis. Additionally, it appears that reduced levels of recombination contribute to increased nondisjunction of both of these chromosomes. The addition of these chromosomes to the pool of studied chromosomes allows for comparison of

nondisjunction origin among different human chromosomes. Evidence suggests that the mechanism of nondisjunction may be conserved among acrocentric, but not nonacrocentric, chromosomes.

To further explore the role of recombination in nondisjunction, the possible role of recombination in the maternal age effect was examined. Two current models that try to explain the relationship between recombination and maternal age are the two hit hypothesis and production line model. Each of these hypotheses makes a different prediction about how levels of recombination will vary with increasing maternal age. Analysis of recombination levels in mothers of varying ages who had trisomic conceptions suggests that the relationship is chromosome-specific with some chromosomes following the predictions of the two hit hypothesis, some the production line hypothesis, and some having a unique pattern.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Dedication

This thesis is dedicated to my mother and father for everything they have done for me throughout my life, as well as during the life of this project

CHAPTER ONE

INTRODUCTION

Meiosis is arguably the most important process taking place in the human body, as the formation of egg and sperm allows our species to survive. Unfortunately, humans make an alarming number of meiotic errors, often leading to chromosome abnormalities. The most common of these abnormalities is aneuploidy, where there are extra or missing chromosomes. Aneuploidy occurs at an extremely high rate in humans as about one-third of all miscarriages have the wrong number of chromosomes [Hassold and Hunt 2001]. Despite this dramatic outcome for many pregnancies and the resulting effect on families trying to conceive, there is very little understanding of the factors that underlie these meiotic chromosome errors. New insight into when and how errors occur in meiosis will be helpful not only in gaining an understanding of one of the most basic processes in life, but also in leading to clues about how to prevent the loss of many pregnancies.

Meiosis: an introduction

The end goal of meiosis is to produce haploid gametes. This is accomplished by two divisions, meiosis I (MI) and meiosis II (MII), which occur without any intervening DNA replication (Figure 1). During MI, homologous chromosomes pair, synapse, and recombine during prophase before lining up on the metaphase plate at metaphase I. Homologous chromosomes segregate to opposite spindle poles at anaphase I, leaving sister chromatids attached. By the end of telophase/cytokinesis, division results in the

formation of two cells. Subsequently, during MII the sister chromatids line up on the metaphase plate and segregate to produce haploid gametes, each with half the number of chromosomes as in the original cell.

While the basic meiotic process is similar in all organisms, there are sex specific differences in human meiosis (Figure 2). Males undergo a specialized process termed spermatogenesis. During spermatogenesis, spermatogonia undergo mitotic proliferation to produce a pool of cells for meiosis. At the time of sexual maturity, these cells undergo two successive divisions to generate four haploid spermatids that will develop into mature sperm capable of fertilization. In males, mitosis continually renews the pool of cells available for meiosis; thus sperm production is maintained throughout the lifetime of the male [Johnson and Everitt 2000].

The circumstances are quite different for females. Female meiosis begins in the fetal ovary, long before puberty. After a round of mitotic proliferation, cells begin meiosis and prior to birth enter a late prophase “dictyate” arrest where they remain until puberty, when approximately one oocyte per month completes MI. Since women can ovulate into their 40s and 50s, cells can remain arrested for decades before completing meiosis. At the end of MI, due to unequal division of the cytoplasm, a secondary oocyte and a polar body are formed. The second meiotic division occurs only if a sperm penetrates the oocyte and produces a second polar body and a mature gamete [Johnson and Everitt 2000]. Thus there are two primary differences between male and female meiosis: the timing and the number of products formed. These male/female differences become critical when considering the disparity in the number of errors made during male and female meiosis.

Making meiotic errors

A basic understanding of meiosis and sex-specific meiotic differences is essential for gaining an insight into meiotic errors. The most common meiotic error is nondisjunction where chromosomes fail to segregate properly, leading to the formation of aneuploid gametes. Aneuploidy is defined as having a number of chromosomes that is not a multiple of the haploid chromosome set. The most common types of aneuploidy are trisomy and monosomy, where one too many or one too few chromosomes are present, respectively. Trisomy, the most common chromosomal abnormality in humans, affects approximately 4% of all clinically recognized pregnancies (CRP) [Hassold and Jacobs 1984]. Monosomies are less common and seen in only about 2% of CRPs [Jacobs 1992]. The rarity of monosomies is likely due to lethality in the early embryo, making detection difficult [Hassold and Jacobs 1984].

Incidence

In humans, the incidence of aneuploidy varies greatly depending on the developmental time point being examined (Table 1) [Hassold and Hunt 2001]. In newborns, for example, 0.3% of liveborns are aneuploid. In stillbirths (i.e., fetal deaths occurring between 20 weeks gestation and birth), the incidence of aneuploidy is 4%, an order of magnitude higher than livebirths. In spontaneous abortions (i.e., fetal deaths occurring from ~6 weeks gestation, when pregnancies are first clinically recognized, to 20 weeks gestation), the incidence is 35%, an additional order of magnitude higher than in stillbirths. Thus, the vast majority of aneuploidy never results in liveborn individuals but rather leads to early pregnancy loss from spontaneous abortions.

While the types of aneuploid conditions seen in stillbirths and livebirths are similar (e.g. +13, +18, +21), many different aneuploidies are observed in spontaneous abortions (e.g. 45,X,+16, +21, +22) [Hassold and Hunt 2001]. The primary reason for the varying incidence of trisomy at different developmental time points is the viability of different trisomic conditions. For example, the most common trisomy, trisomy 16, is seen in over 1% of all clinically recognized pregnancies, and yet never seen in livebirths because such conceptions typically terminate before 20 weeks gestation [Hassold and Jacobs 1984].

Even the high incidence of aneuploidy in spontaneous abortions does not account for all meiotic chromosome missegregation. Many trisomic and virtually all monosomic conceptions are lost before pregnancies are clinically recognized at 6-8 weeks. For example, autosomal monosomies are virtually never seen in CRPs [Hassold and Jacobs 1984]. Several approaches have been used in an attempt to determine the "real" incidence of aneuploidy. Cytogenetic studies of in vitro fertilization (IVF) embryos/oocytes have indicated an aneuploidy rate much higher than that reported in spontaneous abortions. For example, karyotypic studies of 178 diploid human embryos fertilized in vitro found that about 20% of embryos were aneuploid [Jamieson et al. 1994]. Studies of IVF oocytes confirmed these results, indicating that as many as 20-25% of oocytes have extra or missing chromosomes [Jacobs 1992]. However, doubts have been raised about these studies as women undergoing IVF are likely not representative of the general population. This population already has reproductive difficulties, and the drugs used for ovarian stimulation may add to the risk of genetic damage. To circumvent this problem, studies of oocytes from unstimulated

ovaries have been undertaken and these also indicate a rate of aneuploidy around 20% [Volarcik et al. 1998]. The rate in sperm, estimated at around 1-2% [Hassold 1998], is much lower than that seen in oocytes. Overall, the incidence of human aneuploidy is drastically higher than that seen in clinically recognized pregnancies and may be as high as 25%.

This high rate of aneuploidy is apparently unique to humans. For example, in yeast (*Saccharomyces cerevisiae*) and fly (*Drosophila melanogaster*), the rate of missegregation is as low as 1/10,000 and 1/1,700, respectively [Koehler et al. 1996; Sears et al. 1992]. Even other mammals show rates of missegregation much lower than that seen in humans, as the rate in the mouse is only around 1-2%, and other rodent and non-rodent mammals have similarly low aneuploidy rates [reviewed in Bond and Chandley 1983]. This greatly complicates the use of other organisms as models of human aneuploidy, as humans are apparently unique in their high rate of chromosome missegregation. Unfortunately, the rate of aneuploidy in nonhuman primates has never been examined, leaving a major void in the complete picture of nondisjunction.

Origin

Given the high incidence of aneuploidy in humans, numerous studies of the last two decades have been aimed at uncovering the time at which nondisjunction occurs in meiosis or mitosis (Table 2). Based on these studies, several common themes have emerged [Hassold and Hunt 2001]. For the majority of trisomies, the extra chromosome is maternal in origin, indicating errors in oogenesis as the most common source of aneuploidy. Most of these maternal errors arise during the first meiotic division, as seen

in trisomies 15, 16, 21 and 47,XXX [Hassold et al. 1995; Hassold and Sherman 2000; May et al. 1990; Zaragoza et al. 1998].

Despite these broad similarities, there are obvious chromosome-specific differences in the origin of nondisjunction. For example, while the majority of trisomy 18 errors are maternal in origin, they typically occur at the second, not the first, meiotic division [Bugge et al. 1998]. Additionally, the sex chromosome trisomy 47,XXY arises almost equally from maternal and paternal errors [Thomas and Hassold 2003]. Further, a handful of trisomies, including trisomies 7 and 8, show not meiotic, but post-zygotic mitotic missegregation as the primary cause of nondisjunction [James and Jacobs 1996; Karadima et al. 1998; Zaragoza et al. 1998]. Finally, even those chromosomes that share maternal meiosis I as their primary source of nondisjunction display variation in the exact proportion of different types of errors. It appears the study of any one chromosome will not serve as a paradigm for all other chromosomes, as each has its own unique pattern of nondisjunction.

Since the overwhelming majority of nondisjunction occurs at the first meiotic division, it is important to consider that there are several possible mechanisms of MI nondisjunction (Figure 3). “True” nondisjunction implies that chiasmata, the physical sites of exchange, occur between homologous chromosomes but are not properly resolved, causing both homologues to segregate to the same pole at anaphase I. In contrast, in achiasmate nondisjunction no chiasmata are formed between homologues, allowing them to segregate independently at MI, possibly traveling to the same pole. A final mode of nondisjunction is premature sister chromatid separation (PSCS) where the attachment between chromatids is lost, allowing sister chromatids to segregate in MI

rather than MII. Different studies have debated over the relative contribution of each of these types of events, stressing the need for a greater understanding of nondisjunction of individual chromosomes.

What causes nondisjunction?

Despite the high incidence of aneuploidy in humans, there is still limited information available on what underlies these meiotic chromosome errors. To date, only two factors have been linked to increasing levels of nondisjunction: recombination and advancing maternal age. Each of these predisposing factors will be considered independently before turning to the relationship between these two factors.

Recombination and aneuploidy

Recombination is the physical exchange of DNA between homologous chromosomes. Recombination occurs during prophase of meiosis I and when resolved, these exchanges produce recombinant chromosomes with DNA from both the maternal and paternal homologues. While many consider the essential role of recombination to be promotion of genetic diversity, a second role occurs at a much more basic level. The successful establishment and resolution of these exchanges is essential for proper chromosome segregation. Studies in several model organisms have uncovered mutants which affect the recombination pathway, leading to decreased or absent levels of recombination. Virtually all of these mutants have a resulting increase in nondisjunction. For example, several genes involved in double strand break repair in *S. cerevisiae*, such as MSH4 and EXO1, drastically reduce crossover levels and

consequently increase nondisjunction [Khazanehdari and Borts 2000; Ross-Macdonald and Roeder 1994]. Further examples are evident in *D. melanogaster*; for instance, mutations in mei-W68, a homologue of Spo11 that is responsible for inducing double strand breaks in meiosis, have been found to eliminate crossing over and increase levels of nondisjunction [McKim and Hayashi-Hagihara 1998]. The effect of most recombination mutants is shared among different organisms; msh-4 and spo-11 mutants with similar effects have been discovered in *C. elegans* [Dernburg et al. 1998; Zalevsky et al. 1999].

In addition to mutant studies, studies of spontaneous nondisjunction in model organisms demonstrate an association between recombination and nondisjunction. In 1995, Sears et al. used yeast artificial chromosomes, or YACs, to show that pericentromeric gene conversions events increase MI nondisjunction in *S. cerevisiae*. Just one year later, Ross et al. [1996] showed that a YAC with a single distal exchange was more likely to nondisjoin than a chromosome with a more proximal exchange. Similarly, Koehler et al. [1996] observed the same effect in *D. melanogaster*. When examining X chromosome nondisjunction in fly oocytes, MI nondisjunction occurred primarily in oocytes where no exchange had occurred between X chromosomes or in eggs with extremely distal X chromosome crossovers. In contrast, MII nondisjunction occurred in oocytes with proximal X chromosome exchanges.

Studies of nondisjunction and recombination in humans are complicated by the fact that most meiotic errors result in spontaneous abortions [Hassold and Jacobs 1984]. Accordingly, most information on human nondisjunction is from trisomy 21, or Down syndrome, one of the few aneuploidies able to survive to birth. Unlike other

trisomic liveborns, individuals with Down syndrome can survive into adulthood, making them a valuable source of information on the nondisjunction of chromosome 21. Studies of these trisomic individuals have been aided over the last several decades by the advent of DNA polymorphisms. These have allowed not only for analysis of the origin of nondisjunction, but have also made it possible to conduct tetrad analysis and genetic map construction to analyze human recombination levels and placement (Figure 4).

Using this method, human nondisjunction has been found to display a pattern similar to model organisms. Not surprisingly, the first and most extensive studies of recombination were done in individuals with Down syndrome. In comparing normal female meiotic events to meiotic events leading to nondisunction of chromosome 21 in MI or MII, Lamb et al. [1997] made several striking observations. Over 40% of nondisjunction involved chromosomes with no exchange, i.e., achiasmate homologues. However the presence of an exchange was not enough to ensure proper chromosome segregation, as even when recombination occurred the placement of the exchange on the chromosome arm affected levels of nondisjunction. Lamb et al. [1997] discovered that in normal meioses, recombination was not favored in any one particular region of the chromosome. However when chromosome 21 nondisjoined during MI, exchanges were more often placed near the telomere. More specifically, while normally disjoining chromosomes 21 had 40% of single exchanges on the telomeric third of the chromosome, over 80% of single exchanges were located on the distal third of chromosomes that nondisjoined during MI. In contract, for cases with apparent MII nondisjunction, recombination was displaced towards the centromere. Single

exchanges occurred on the proximal third of the chromosome in 63% of MII cases, compared to 35% of normally disjoining cases. Thus studies in both humans and model organisms suggest that it is important to have both an optimal number and placement of exchanges to ensure proper chromosome segregation. Accordingly, when there is too little recombination, or when exchanges occur too proximal or distal on the chromosome, the likelihood of nondisjunction is increased (Figure 5).

Studies have also revealed that the relationship between aberrant recombination and human nondisjunction is chromosome-specific (Table 3). Achiasmate chromosomes play a role in nondisjunction for almost all chromosomes. This is especially evident for the smallest autosome, chromosome 21. This small chromosome is typically held together by only 1-2 exchanges; therefore, the loss of a single exchange results in nonexchange chromosomes, as seen in 40% of maternal MI cases [Lamb et al. 2005]. However, chromosome size is not the sole factor in the genesis of achiasmate chromosomes. Even larger chromosomes that are typically held together by 2-4 exchanges show evidence that nonexchange chromosomes lead to nondisjunction. For example, achiasmate bivalents are associated with 20% of trisomy 15 [Robinson et al. 1998] and 30% of trisomy 18 maternal MI cases [Bugge et al. 1998]. A large proportion of both maternal and paternal MI cases of sex chromosome trisomy are associated with nonexchange chromosomes [Thomas et al. 2001]. In contrast, trisomy 16 is rarely associated with nonexchange bivalents. Reduced recombination, as opposed to absence of recombination, contributes to chromosome 16 nondisjunction [Hassold et al. 1995].

The role of exchange placement in the genesis of human trisomies is not universally shared among chromosomes. While pericentromeric and distal exchanges are associated with the nondisjunction of chromosome 21 [Lamb et al. 1997], there is no evidence that altered exchange placement contributes to trisomies 15 or 18 [Bugge et al. 1998; Robinson et al. 1998]. Extremely distal exchanges have been linked to trisomy 16 [Hassold et al. 1995], while pericentromeric recombination is associated with the sex chromosome trisomies [Thomas et al. 2001]. Thus, aberrant recombination is an important contributor to nondisjunction for all chromosomes, although the contribution is markedly chromosome-specific.

Maternal age

The relationship between maternal age and trisomy was first noted by Penrose in 1933 who noticed that older women were more likely to give birth to Down syndrome children [Penrose 1933]. This was long before it was known that Down syndrome is caused by the presence of an extra chromosome 21 [Lejeune et al. 1959]. The exact nature of the relationship between maternal age and trisomy varies among chromosomes, but for all chromosomes studied, the incidence of trisomy increases with maternal age [Hassold et al. 1980; Hassold et al. 1984; Risch et al. 1986].

The summary of the maternal age effect in all clinically recognized pregnancies (CRPs) is shown in Figure 6. The relationship between maternal age and trisomy increases exponentially, showing a drastic increase around age 35. By age 40, about 35% of CRPs are trisomic. Since many trisomies are lost before pregnancies are clinically recognized, the incidence of trisomy in older women may again be an

underestimate. While all chromosomes appear to display an increased incidence with maternal age, the exact trend varies among chromosomes. For instance, trisomy 16, the most common trisomy in CRPs, has a unique maternal age relationship. Unlike the exponential trend observed for most trisomies, the risk for trisomy 16 increases linearly throughout a woman's childbearing years [Risch et al. 1986].

This striking maternal age effect appears unique to humans. Studies in the mouse have uncovered an increase in nondisjunction with maternal age, but the effect is much less severe than that seen in humans as only certain mouse strains are affected and the effect is less drastic than in humans [Bond and Chandley 1983]. For example, while the CBA strain, which is commonly used to study maternal age in the mouse [Eichenlaub-Ritter et al. 1988], shows no aneuploidy in mice less than five months of age, various studies show levels of nondisjunction in older mice ranging from 0-13.6% [reviewed in Bond and Chandley 1983]. Thus the maternal age effect in the mouse is less pronounced than in humans where 35% of pregnancies in women over 40 years of age are aneuploid, but only 2-3% of pregnancies in women 20-25 years. To date, no other model organism has demonstrated a pronounced maternal age effect.

The importance of the maternal age effect has become increasingly apparent over the last few decades as more women are delaying starting a family [Mathews and Hamilton 2002]. Despite the significant impact of the maternal age effect on women trying to conceive, its basis remains a mystery. There are three time points in meiosis when the errors that underlie the maternal age effect can occur. The first is prenatally in the fetal ovary when recombination is established, as aberrant recombination is a known predisposing factor for nondisjunction. It may be some maternal age-related

factor acting on aberrant recombination events that increases nondisjunction with age. The second probable time point is during the prolonged dictyate arrest when oocytes remain in meiosis I for decades, a significant period of time for the fidelity of meiosis to be compromised. Finally, errors involving the growth and maturation of the follicle may occur around the time of ovulation when meiosis is completed. Despite recent suggestions that follicular renewal does not cease during fetal development but is an ongoing process in the adult ovary [Johnson et al. 2005; Johnson et al. 2004], the protracted dictyate arrest is generally believed to contribute significantly to the maternal age effect. Unfortunately, these ideas are all speculation as the basis of the maternal age effect remains a mystery. However, as discussed below, models have been proposed which implicate each of these time points in the maternal age effect.

Other factors

While maternal age and recombination remain the only known factors associated with aneuploidy, several other genetic and environmental factors have been implicated in nondisjunction. As is clear from Table 4, these factors are controversial, with studies both supporting and refuting an association with trisomy. Further studies will be required to determine any true correlation with nondisjunction

Models of human nondisjunction

Maternal age and recombination remain the only factors definitively linked to increased nondisjunction. Despite extensive research on maternal age and recombination, a possible relationship between the two has not been well-established.

The two hit hypothesis and the production line hypothesis, the only models which propose a link between these two factors and how recombination levels may change with age, will be discussed in the sections below.

The production line hypothesis

The first hypothesis to explain the maternal age effect was proposed by Henderson and Edwards [1968]. Their production line model was based on observations they made when working with female mice of varying ages. They noticed that with increasing age, female mice had reduced numbers of exchanges and increased number of univalents, where no recombination occurred between homologues. They also observed that when a single chiasma was present, it was often terminally located. Based on these observations, Henderson and Edwards proposed their production line hypothesis which has two main tenants (Figure 7). First, germ cells enter meiosis in a sequential order and are released after puberty in the same sequence that they enter meiosis. Therefore, the first cells that enter meiosis will be the first ovulated after puberty, the next into meiosis the next ovulated and so on. The second tenet states that a gradient exists in the fetal ovary such that those cells that enter meiosis first will have a greater number of exchanges than those that enter meiosis later. The first to be ovulated would, therefore, have more exchanges than those ovulated later. Consequently, the frequency of chromosomes with no exchange would increase in older women, leading to increased rates of chromosome nondisjunction.

Since the publication of this model, many efforts have been made to examine the observations of Henderson and Edwards, with mixed success. However, in 1991 Polani and Crolla tested whether or not oocytes are indeed ovulated in the order that they are formed in the fetal ovary [Polani and Crolla 1991]. By radio-labeling oocytes in vitro and transplanting them into spayed females, they were able to examine the proportion of labeled cells before implantation and compare this to the number of labeled cells seen at the time of ovulation. They found that there is indeed a production line whereby the first oocyte formed is the first ovulated. This was re-enforced in 1992 by Hirschfield who used [3H] thymidine labeling of oocytes in the rat; oocytes which entered meiosis early were unlabeled while those entering later were labeled [Hirshfield 1992]. In examining growing follicles of mice from 1-40 days, all were unlabeled, reflecting those cells that entered meiosis early, thus supporting the production line hypothesis.

However, the question of whether a gradient of chiasmata formation exists in the fetal ovary was not addressed by Polani and Crolla [1991] or Hirshfield [1992] and has been highly debated. Unfortunately, there has never been any genetic evidence to support the second part of the hypothesis and consequently there has been much debate over whether there is a gradient formed in the fetal ovary by some developmental factor which causes exchange number to decrease with fetal age, and, ultimately, with advancing age in the adult female [Brook et al. 1984; Speed and Chandley 1983; Sugawara and Mikamo 1986; Tease and Fisher 1989].

A recent paper by Hodges et al. [2005] suggests an alternative explanation for the observations of Henderson and Edwards. Using mice deficient for SMC1β (a meiosis specific component of the cohesion complex), Hodges et al. noticed an

apparent change in the location of exchanges over time (detailed in the section below), with more distal exchanges seen in adults compared to fetal female mice. This suggests that the location of exchanges can be properly established in the fetal ovary and change over time (as seen by Henderson and Edwards) due to maternal age-related loss of cohesion causing "slippage" of exchanges.

The two hit hypothesis

A more recently suggested model of maternal age and recombination is the two hit hypothesis. Proposed by Hassold and Sherman [reviewed in 2000], this model predicts that errors occurring at two different stages of meiosis affect levels of nondisjunction (Figure 8a). The first error or "hit" occurs during prophase when recombination is established. Since the number and location of recombination events can affect levels of chromosome nondisjunction, any susceptible chiasmata configuration will predispose to nondisjunction. As recombination takes place in the fetal ovary, this "first hit" would occur independent of maternal age. The "second hit" occurs during the prolonged dictyate arrest in oogenesis, when oocytes remain arrested for decades. This would be sufficient time for a predicted loss of some meiotic factor, the deficiency of which would affect the efficiency with which susceptible chiasmata could be resolved. The loss of this factor would be dependent on maternal age as the oocytes in older women will have had more time for errors to accumulate.

The question remains, what part of the meiotic process might be compromised as the "second hit." There could, for example, be a breakdown of the spindle checkpoint, failure of defective oocytes to undergo atresia, or other difficulties involving

proper growth and maturation of the egg. One commonly implicated culprit is failure to maintain sister chromatid cohesion [for example, see Warren and Gorringe 2006]. Cohesion is necessary to ensure normal segregation during both mitosis and meiosis, as loss of cohesion leads to the precocious separation of sister chromatids [Kaur et al. 2005; Watanabe and Nurse 1999]. Loss of any of the cohesion components greatly affects chromosome segregation making cohesion a possible "second hit" in the generation of human aneuploidy.

While the mitotic and meiotic cohesion complexes share several components, there are some meiosis specific cohesion proteins. The recent data by Hodges et al. [2005] implicated one of these meiosis specific cohesion proteins, SMC1β, as a cause of age-related nondisjunction. SMC1β is essential for meiosis as male and female mice deficient for this protein are sterile [Revenkova et al. 2004]. Although highly error prone, female meiosis proceeds into meiosis II in mutants, allowing for studies of recombination. Using antibodies to MLH1 (a marker of sites which are thought to be resolved into stable crossovers), Hodges et al. [2005] examined female meiotic prophase cells and discovered decreased levels of recombination in mutant mice compared to wild type; however there was no marked difference in the placement of exchanges along the chromosomes. To confirm that MLH1 foci accurately represented sites of exchange, cells were analyzed at the diakinesis-metaphase I stage. By this stage of meiosis, chromosomes have condensed greatly and sites of exchange can be visually seen as chiasmata. Surprisingly, while chiasmata counts confirmed the reduction in recombination, they also displayed a striking change in the placement of exchanges, as 85% of chiasmata were located on the distal third of the chromosomes.

Thus there is an apparent “slippage” of exchanges over time, indicating that cohesion is necessary for stabilizing sites of exchange. Moreover, when these mice were aged for several months, there was an increase in univalents and single chromatids implicating cohesion as an underlying cause of maternal age-related aneuploidy. When extremely distal exchanges are formed in the fetal ovary (the “first hit”), loss of cohesion over time (the “second hit”) will affect these exchanges to a greater extent than interstitially located exchanges as the only thing holding these homologues together is the cohesion distal to the site of exchange (Figure 8b).

A recent review by Warren and Gorringe [2006] suggested a possible clinical implication of the two hit hypothesis. Within the human population, there are genetic differences that will influence the threshold at which oocytes with a first hit will become sensitive to the effects of the second hit. For example, an individual may have a defect which alters the effectiveness of loading or maintaining cohesion. This would normally have no obvious effect on chromosome segregation; however, for an oocyte with an extremely distal exchange, the likelihood of nondisjunction will be increased. Women with these genetic polymorphisms would be at an increased risk of having an aneuploid conception. This is consistent with clinical studies suggesting that after a trisomic pregnancy, women are 1.6-1.8 times more likely to have another trisomic conception [Warburton et al. 2004]. Therefore, genetic differences in the “second hit” may be an underlying cause of recurrent trisomic conceptions.

A recent study by Lamb et al. [2005] made a very important contribution in uncovering the relationship between maternal age and recombination in humans. They examined recombination levels in trisomy 21 conceptions from mothers of different ages

to examine how recombination levels vary with maternal age. By analyzing 400 trisomy 21 conceptions of maternal MI origin, they found no difference in overall levels of exchange among mothers of different ages, in agreement with the two hit hypothesis. However, they observed a significant difference in the placement of exchanges with age. While pericentromeric and distal exchanges were seen in 34% of cases from the youngest group of women, only 10% of cases from the older women showed this pattern of exchange placement. In fact, the pattern seen in older women resembled that seen for normally disjoining chromosomes 21. One interpretation of this data is that due to the accumulation of age-related errors in the ovary, older women have difficulty segregating not only chromosomes with "susceptible" exchanges, but also those with an "ideally" located exchange. This suggests that sometimes the "second hit" alone may be enough to increase levels of nondisjunction, even without a vulnerable exchange location as the first hit.

Research aims

The overall objective of the studies in this thesis was to investigate the complex relationship among maternal age, recombination and human nondisjunction. Specifically, experiments were designed to study several previously uncharacterized trisomies to determine the predominant origin of nondisjunction, and effects of recombination on aberrant chromosome segregation. Two models of maternal age and recombination were tested to determine factors affecting nondisjunction in humans.

The studies in Chapter Two, published in *American Journal of Medical Genetics* (2007), focus on nondisjunction of chromosome 13. Despite being one of the few

trisomies compatible with livebirth, relatively little is known about the origin of nondisjunction for this chromosome or about the role of recombination in its missegregation. Accordingly, we used DNA polymorphisms to study families with a trisomy 13 conceptus to determine from which parent and at what stage of meiosis (or mitosis) nondisjunction occurred. We further analyzed the number of recombination events to determine what, if any, role aberrant recombination plays in the nondisjunction of chromosome 13.

Chapter Three, published in *American Journal of Medical Genetics* (2007), focuses on the examination of another understudied trisomy, trisomy 22. Despite being one of the most common trisomies in spontaneous abortions, to date almost no information is available about parental and meiotic origin of nondisjunction for this chromosome. We, therefore, undertook studies to examine the origin of this trisomy and any aberrant recombination patterns which predispose to nondisjunction. We compared this chromosome to all other studied chromosomes to examine similarities and differences between chromosomes, focusing specifically on acrocentric and nonacrocentric chromosomes.

Chapter Four focuses on analyses of the two popular models of human age-related nondisjunction, i.e., the two hit hypothesis and production line hypothesis. One complication of studying these hypotheses is the difficulty of testing both events occurring in the fetal ovary and detrimental effects that can accrue over decades in humans. This chapter discusses one method to test how recombination levels vary with maternal age for trisomic conceptions - i.e., do recombination levels increase, decrease, or remain the same with increasing maternal age? We examined both new and

previously published cases of trisomies 13, 14, 15, 16, 18, 21, 22, and the sex chromosome trisomies XXX and XXY to see if the frequency of “vulnerable” exchanges varies between younger and older women.

Conclusions are discussed in Chapter Five along with experiments to further explore human nondisjunction and its relationship to maternal age and recombination.

Figure 1. Overview of meiosis. Prior to the start of meiosis, chromosomes are replicated to produce identical sister chromatids. During meiosis I, *homologous chromosomes* align in metaphase I and are segregated in anaphase I to opposite spindle poles. In the second meiotic division, *sister chromatids* are separated, producing four haploid gametes.

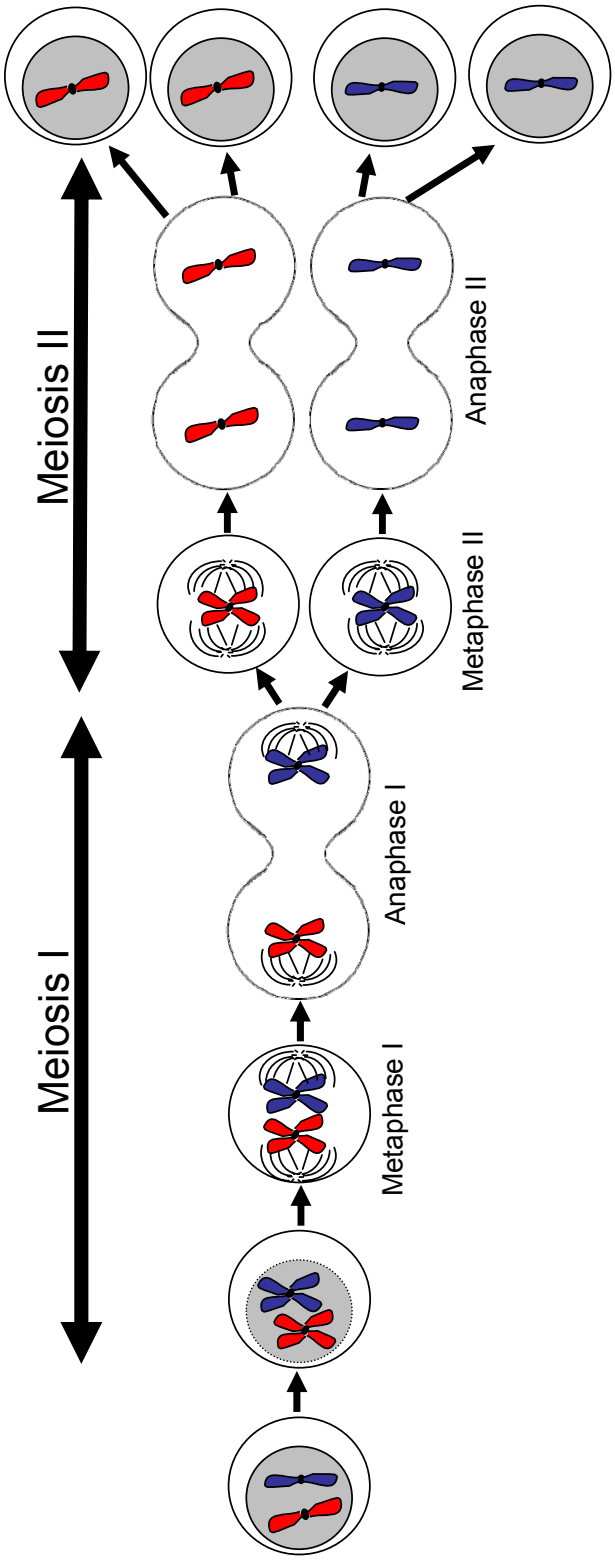

Meiosis I
Meiosis II
Metaphase I
Anaphase I
Metaphase II
Anaphase II

Figure 2. Male/female differences in gametogenesis. [Adapted from Hassold and Hunt 2001] In males, spermatogonia undergo mitotic proliferation to produce a pool of cells for meiosis. At the time of sexual maturity, these cells undergo two successive divisions to generate four haploid gametes which develop into mature sperm. In females, meiosis begins before birth in the fetal ovary. After a round of mitotic proliferation, cells enter meiosis and arrest prior to birth. At puberty, approximately one oocyte per month completes meiosis I. At the end of meiosis I, due to unequal division of the cytoplasm, a secondary oocyte and a polar body are formed.

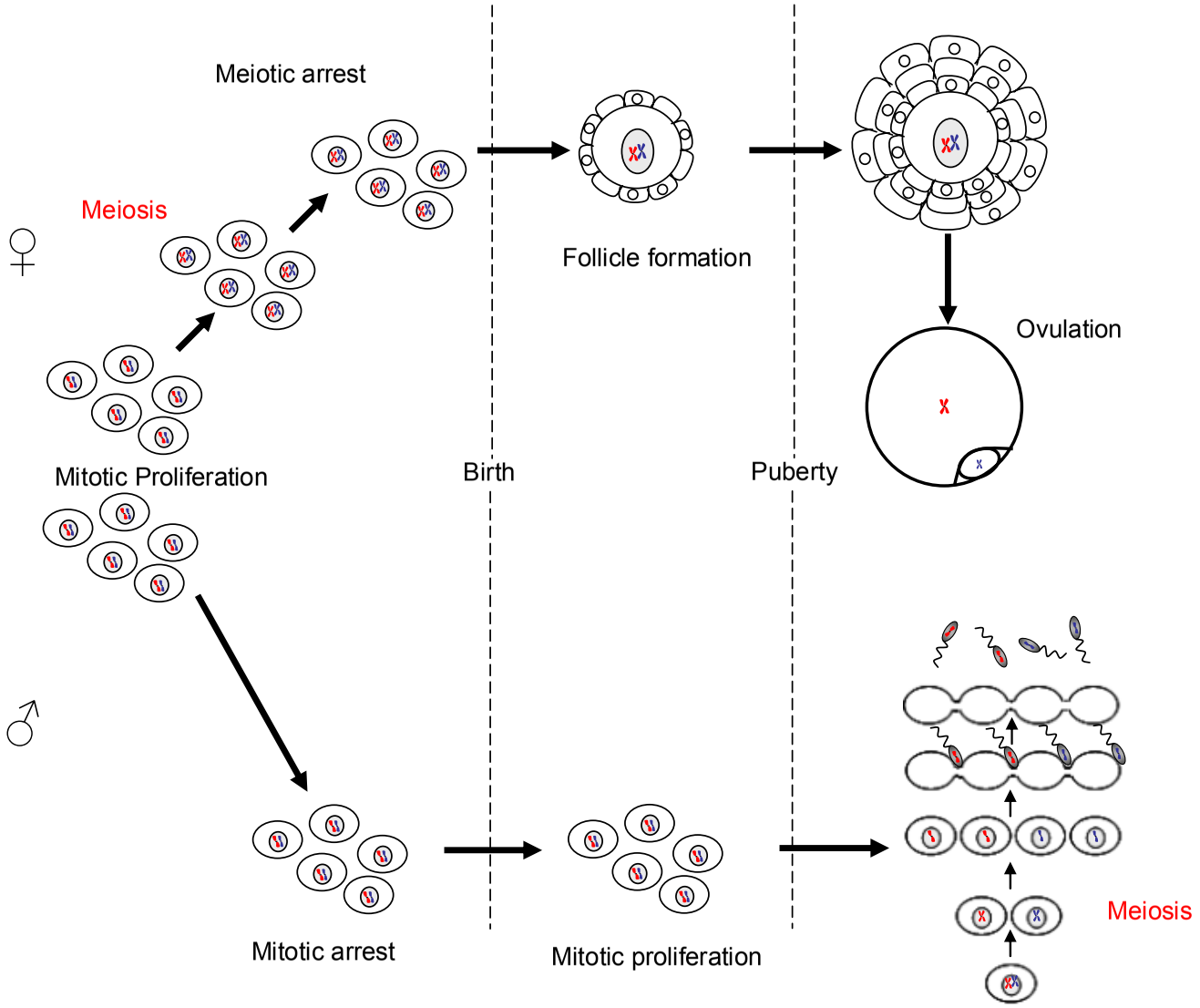
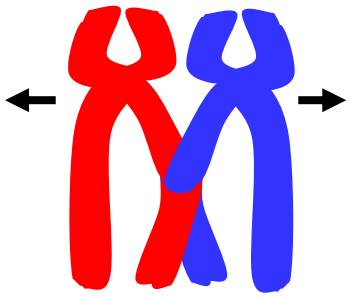
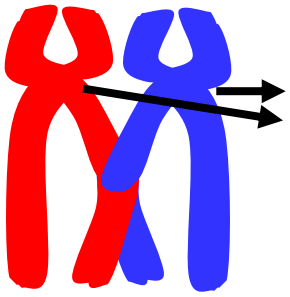

Meiotic arrest
Meiosis
♀
Follicle formation
Ovulation
Mitotic Proliferation
Birth
Puberty
♂
Mitotic arrest
Mitotic proliferation
Meiosis

Figure 3. Possible mechanisms of meiosis I nondisjunction. [Adapted from Hassold and Hunt 2001] Normally, homologues segregate to opposite spindle poles. In “true” nondisjunction, chiasmata are not properly resolved and homologues travel to the same spindle pole. For achiasmate nondisjunction, lack of chiasmata allows homologues to segregate independently, possibly traveling to the same pole. In premature sister chromatid separation (PSCS) loss of cohesion allows sister chromatids to prematurely segregate from each other at MI, rather than at MII.

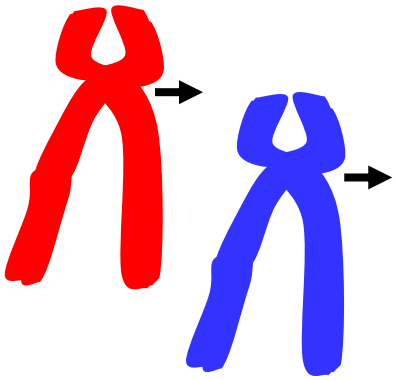
Normal



"True" nondisjunction



Achiasmate nondisjunction



PSCS

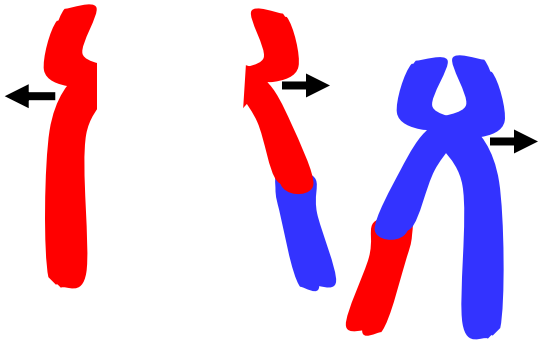
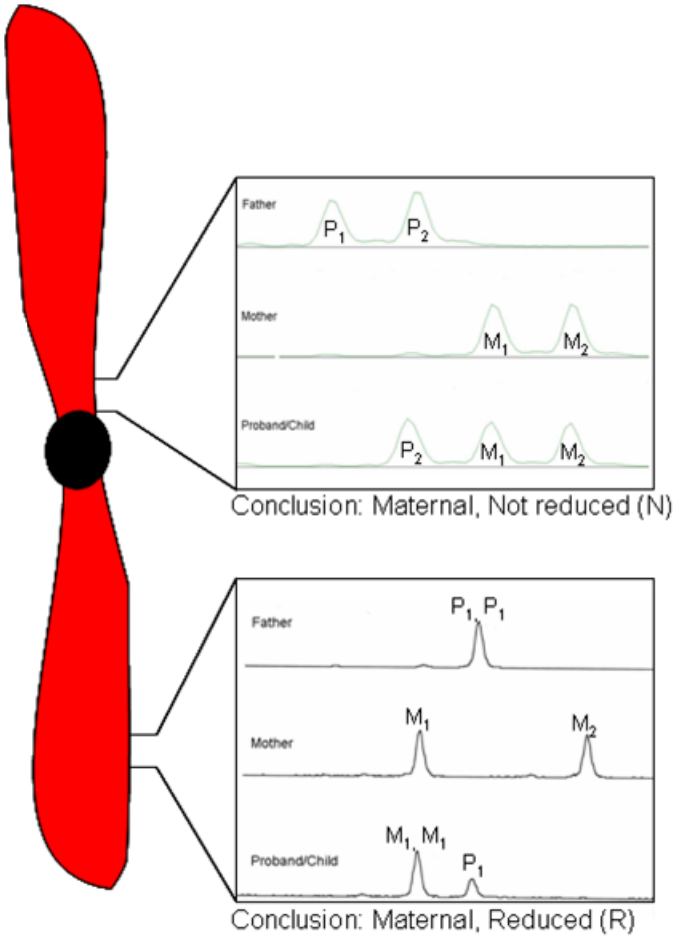


Figure 4. Example of genotyping analysis for a trisomy involving a metacentric chromosome. Markers spaced along the entire length of the chromosome are examined for inheritance of polymorphic DNA markers to determine parental origin and number of exchanges. A) Sample genotyping analysis. In the top example, the trisomic offspring has inherited two *different* maternal alleles and one paternal allele. Because two different maternal alleles were inherited, this locus is "not reduced" to homozygosity (N). In the bottom example, the proband inherited two copies of the *same* maternal allele and one paternal allele. Since two copies of the same allele were inherited from the mother, this locus is "reduced" to homozygosity (R). B) Complete data set for a trisomic family. Markers are spaced along the entire length of the chromosomes in close proximity to ensure no double exchanges occur between markers. Arbitrarily, a minimum of two markers informative for parental origin are required to definitively determine the parent of origin. Markers closest to the centromere reflect meiotic origin. A marker that is not reduced (N) suggests a meiosis I error, while a reduced marker (R) is indicative of an error in meiosis II or mitosis. Markers along the rest of the chromosome are used to differentiate between meiosis II (at least one "N "marker) and post-zygotic mitotic errors (all markers are "R"). Markers along the chromosome arms can also be used to determine the number of recombination events. A change in markers status from "R" to "N", or the other way around, indicates that an exchange occurred within the interval between the two markers. In this example, nondisjunction occurred during maternal meiosis I, with two recombination events.

A



B

Locus	Parental origin	R/N
1	Maternal	R
2		R
3		N
4	Maternal	N
Centromere		
5		N
6	Maternal	N
7	Maternal	N
8		R

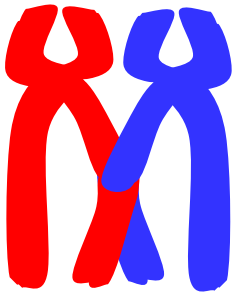
Conclusions:

Origin: Maternal Meiosis I

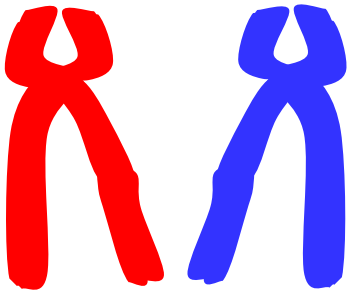
Number of crossovers: 2

Figure 5. Location of exchanges that predispose to nondisjunction of chromosome 21. Most recombination occurs in the interstitial region of the chromosome. However, when exchanges are absent, or too close to the telomere or centromere, the likelihood of nondisjunction is increased.

Optimal exchange



Achiasmate



Distal exchange

Pericentromeric exchange

Figure 6. Increasing incidence of trisomy with advancing maternal age in clinically recognized pregnancies [Adapted from Hassold and Hunt 2001]. The incidence of trisomy increases exponentially with maternal age. By about age 40, about 35% of all clinically recognized pregnancies are trisomic.

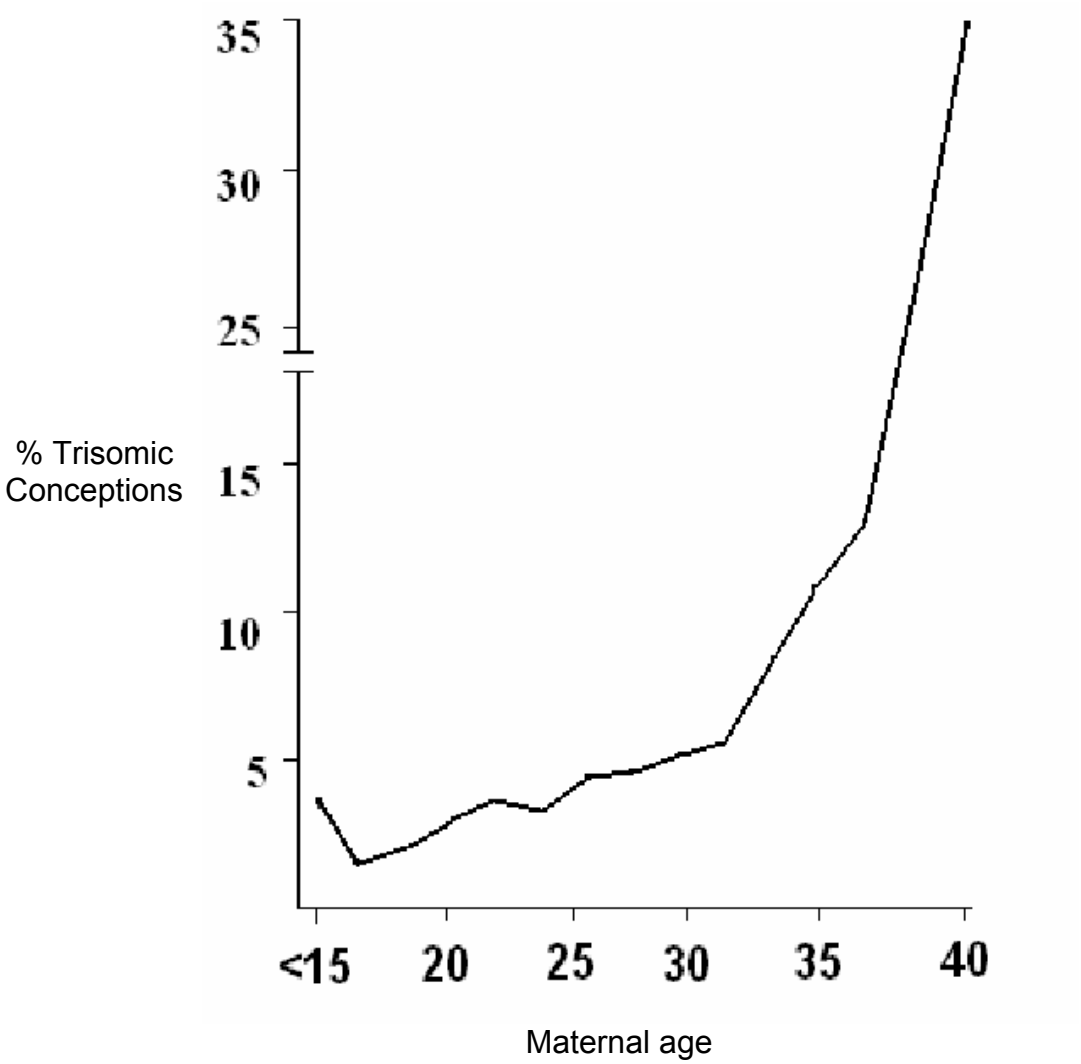

35
30
25
15
10
5
% Trisomic
Conceptions
<15
20
25
30
35
40
Maternal age

Figure 7. Tenets of the production line hypothesis. Cells are assumed to enter meiosis in a sequential order and are ovulated after puberty in the same order. Thus, the first cells to enter meiosis (red arrow) will be the first ovulated after puberty, and those cells that enter meiosis later (blue arrow) will be ovulated later in life. The cells that enter meiosis first are assumed to contain more chiasmata than those that enter meiosis later. Consequently, cells ovulated later in life will have fewer chiasmata and a resulting increased risk of nondisjunction.

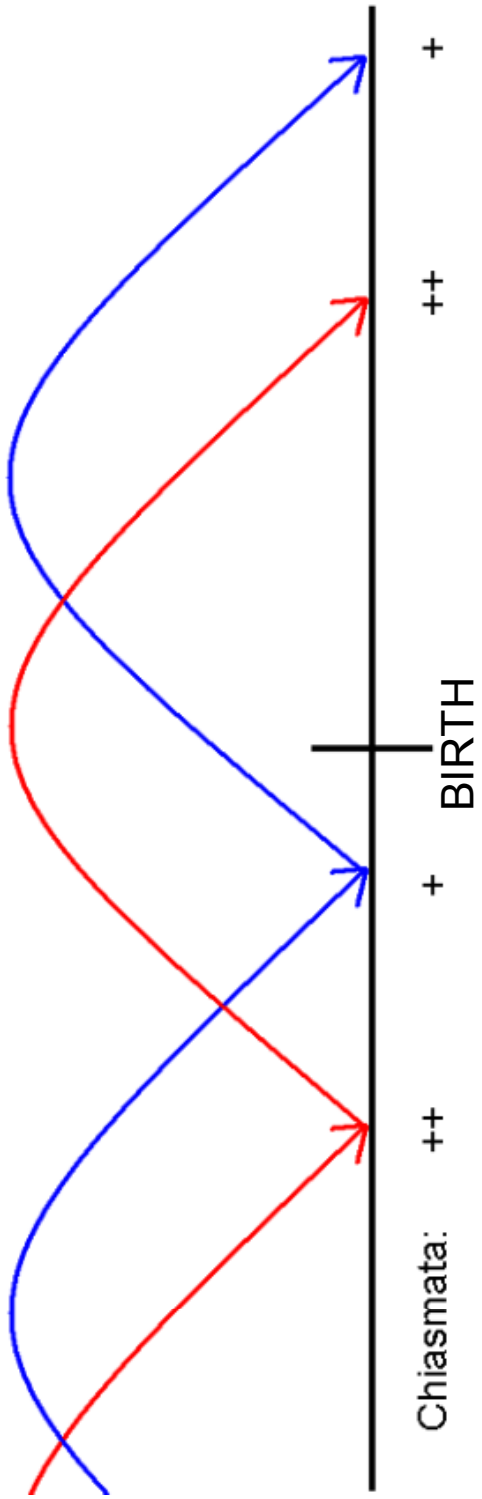

+
++
BIRTH
+
++
Chiasmata:

Figure 8. The two hit model of human aneuploidy. A) This model proposed that the maternal age effect occurs in two steps. In the first step, “susceptible” exchanges are formed in the fetal ovary. A second, maternal age-related hit causes increased levels of nondisjunction. When only one of these two steps has occurred, nondisjunction frequencies will be at a minimum. Having both “hits” will significantly increase levels of chromosome nondisjunction. B) Cohesion and the two hit hypothesis [Adapted from Hawley 2003]. Homologues chromosomes are held together by cohesion located distal to sites of exchange. In the figure on the left, exchanges are ideally located (no first hit) and ample cohesion is holding the homologues together. In the right figure, the extremely distal exchange (the first hit) leaves very little cohesion holding the homologous chromosomes together, predisposing to nondisjunction. If cohesion is lost with maternal age, this exchange may “slip” off the end of the chromosomes.

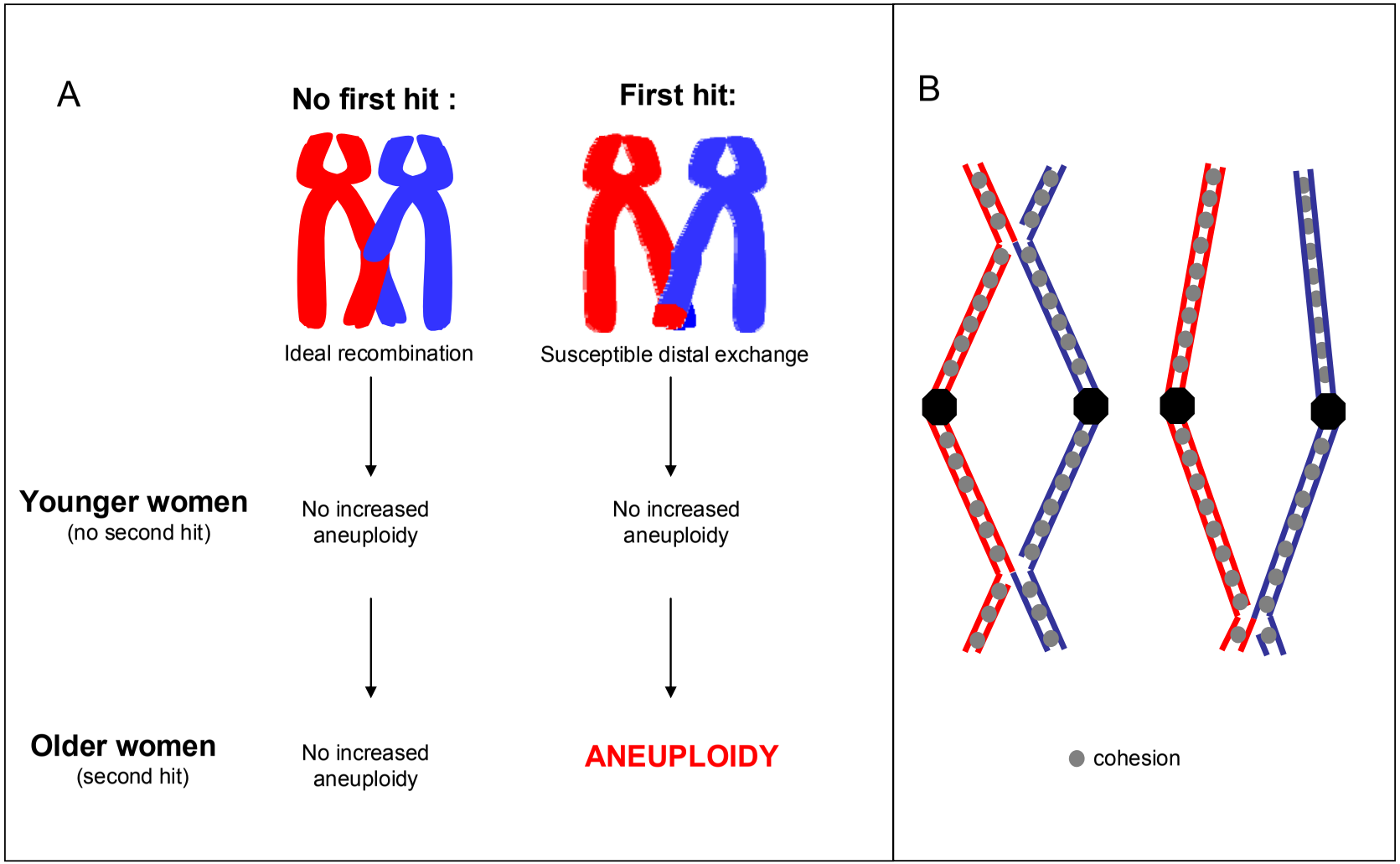

A
No first hit :
First hit:
Ideal recombination
Susceptible distal exchange
Younger women
(no second hit)
No increased aneuploidy
No increased aneuploidy
Older women
(second hit)
No increased aneuploidy
ANEUPLOIDY
B
cohesion

Table 1. Incidence and types of aneuploidy at different developmental timepoints [Adapted from Hassold and Hunt 2001].

	Sperm	Oocytes	Pre-implantation embryos	Spontaneous abortions	Stillbirths	Livebirths
Incidence of aneuploidy	1-2%	~20%	~20%	35%	4%	0.3%
Most common aneuploidies	assorted	assorted	assorted	45,X; +16; +21; +22	+13; +18; +21	+13; +18; +21; XXX; XXY; XYY

Table 2. Origin of nondisjunction for human trisomies. [Data on +2 from Zaragoza et al., 1998; +7 from Zaragoza et al. 1998; +8 from James and Jacobs 1996 and Karadima et al. 1998; +13 from Hall et al. 2007a, in press and Bugge et al. 2007; +14 from Zaragoza et al. 1994 and Hall and Hassold, unpublished observations; +15 from Zaragoza et al. 1998 and Hall and Hassold, unpublished observations; +16 from Hassold et al. 1995 and Hal and Hassold, unpublished observations; +18 from Bugge et al. 1998; +21 from Freeman et al. 2007; +22 from Hall et al. 2007b, in press; XXX and XXY reviewed in Hall et al. 2006]

		Maternal		Paternal		
Trisomy	N	MI[‡] (%)	MII (%)	MI (%)	MII (%)	PZM (%)
2	18	53.4	13.3	27.8	0.0	5.6
7	14	17.2	25.7	0.0	0.0	57.1
8	12	------50.0[#]------		0.0	0.0	50.0
13	163	49.3	39.5	0.8	5.8	4.6
14	34	42.6	42.6	3.7	7.4	3.7
15	104	79.8	8.1	3.7	7.3	1.1
16	206	91.9	4.1	3.4	0.0	0.6
18	161	33.5	59.0	0.0	0.0	7.5
21	907	69.7	23.6	1.7	2.3	2.7
22	130	86.4	10.0	1.8	0.0	1.8
XXX	50	63.0	17.4	0.0	0.0	19.6
XXY	224	25.4	15.2	50.9	0.0	8.5

[‡]MI=meiosis I; MII=meiosis II; PZM=post-zygotic mitotic

[#]These cases were of maternal meiotic origin, but the specific stage could not be determined

Table 3. Role of aberrant recombination in the genesis of nondisjunction for human chromosomes.

Chromosome	Reduced recombination	Achiasmate chromosomes	Pericentromeric exchanges	Distal exchanges
15	☑	☑	☒	☒
16	☑	☒	☒	☑
18	☑	☑	☒	☒
21	☑	☑	☑	☑
X/Y	☑	☑	☑	☒

Table 4. Factors affecting human chromosome nondisjunction. This table provides examples of a few of the many etiological factors suggested to increase the risk of nondisjunction in humans. Consistent with all other putative aneugenic agents, there have been both positive and negative reports; thus maternal age and aberrant meiotic recombination remain the only two known contributors to human nondisjunction.

Factor	Positive	Negative
Folate gene polymorphisms	Hobbs et al. [2000] James et al. [1999]	Hassold et al. [2001] Petersen et al. [2000]
Double Nucleolar Organizing Region (NOR) heteromorphisms	Jackson-Cook et al. [1985] Jones et al. [1988]	Hassold et al. [1987]
Gestational diabetes	Moore et al. [2002] Narchi and Kulaylat [1997]	Pelz and Kunze [1998]
Smoking	Robbins et al. [2005] Yang et al. [1999]	Kline et al. [1995] Rudnicka et al. [2002]
Contraceptives	Harlap et al. [1979] Yang et al. [1999]	Ford and MacCormac [1995]

CHAPTER TWO

THE ORIGIN OF TRISOMY 13

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Note: This chapter is a preprint of a manuscript to be published in *American Journal of Medical Genetics* (2007) and has been reformatted for this document

ABSTRACT

Trisomy 13 is one of the most common trisomies in clinically recognized pregnancies and one of the few trisomies identified in liveborns, yet relatively little is known about the errors that lead to trisomy 13. Accordingly, we initiated studies to investigate the origin of the extra chromosome in 78 cases of trisomy 13. Our results indicate that the majority of cases (>91%) are maternal in origin and, similar to other autosomal trisomies, that the extra chromosome is typically due to errors in meiosis I. Surprisingly, however, a large number of errors also occur during maternal meiosis II (~37%), distinguishing trisomy 13 from other acrocentric and most nonacrocentric chromosomes. As with other trisomies, failure to recombine is an important contributor to nondisjunction of chromosome 13.

Introduction

Trisomy 13, or Patau syndrome, is one of the most common human trisomies, occurring in approximately 0.18% of all clinically recognized pregnancies [Hassold and Jacobs 1984]. Over 95% of trisomy 13 conceptions are lost before birth, with no fewer than 1.1% of spontaneous abortions and 0.3% of stillbirths having an extra chromosome 13 [Hassold and Jacobs 1984]. The rate at birth is estimated at 1/5,000-20,000, making trisomy 13 one of the few trisomies seen in liveborn individuals [Baty et al. 1994; Hassold and Jacobs 1984]. Of those pregnancies that survive to term, about 80% die within the first month due to severe medical complications. Common phenotypic abnormalities associated with trisomy 13 include severe mental retardation, cleft lip and/or palate, hypotonia, skeletal abnormalities, and heart defects [Patau et al. 1960]. Thus, trisomy 13 is of importance as both a common cause of reproductive failure and a cause of severe developmental disabilities.

Despite the clinical importance of trisomy 13, very little is known of the way in which it originates. A few DNA studies of the parental origin of the additional chromosome 13 were undertaken in the 1980-1990s [Hassold et al. 1987; Hassold and Jacobs 1984; Robinson et al. 1996; Zaragoza et al. 1994], but cumulatively, fewer than 50 cases were examined. Further, these studies were conducted before the advent of densely mapped DNA polymorphisms. Consequently, for many of these cases it was not possible to determine the parental origin of the extra chromosome, and the meiotic stage of nondisjunction was specified in only a fraction of cases. Thus, it is not yet clear whether nondisjunction of chromosome 13 shares features with other chromosomes, or

whether chromosome 13-specific factors dictate the ways in which trisomy 13 originates.

The present study was designed to fill this gap, by examining the nondisjunctional origin of a large series of cases of trisomy 13. In our analyses of 78 cases, we were interested in addressing two basic questions: that is, in which parent and at which stage of gametic development does the additional chromosome 13 arise; and does aberrant meiotic recombination play a role in the genesis of trisomy 13, as it does for all other human trisomies that have been examined? Our results indicate that maternal meiosis I errors are the most common source of trisomy 13, closely followed by errors in maternal meiosis II. Further, we observed a decrease in recombination in trisomy 13 generating meioses, indicating that – as for other human trisomies – aberrant meiotic recombination is an important etiological factor.

Materials and methods

Patient populations

The sample consists of 78 trisomy 13 cases, ascertained from England (34 cases) or the Unites States (44 cases). A total of 23 cases was obtained through routine prenatal diagnosis, 37 were identified in cytogenetic analyses of spontaneous abortions, three cases involved stillborn and eight cases liveborn children; in six instances no information on ascertainment was provided and the referring laboratories did not respond to subsequent inquiries.

All studies were reviewed and approved by the appropriate Institutional Review Boards.

Cytogenetic analysis

In the majority of cases, cytogenetic analyses indicated straightforward trisomies, either 47,XX,+13 (30 samples) or 47,XY,+13 (35 samples). However, three cases with atypical chromosome constitutions were identified: one was 45,X/47,XY,+13, one 48,XX,+13,+13 and one 46,XY,+13,der(13,14)(q10;q10). In ten instances, cases were received with a diagnosis of non-translocation trisomy 13, but no further karyotypic information was available from the referring laboratories.

DNA analysis

DNA from probands and parents was extracted and amplified using standard techniques. DNA was available from both parents and the proband in 48 of the 78 cases. In the remaining 30 instances, DNA was available only from the mother and proband; typically, these cases involved analyses of pathological samples from spontaneous abortions, in which DNA was extracted separately from fetal and maternally-derived portions of samples, as previously described [Zaragoza et al. 1998].

For each case, inheritance of DNA polymorphisms spanning chromosome 13 was scored. The approximate locations of the 30 markers used in the study are shown in Figure 1.

Classification of nondisjunctional errors

The parent and meiotic/mitotic stage of origin were determined using the approach previously described by Zaragoza et al. [1998]. These determinations were straightforward in those instances in which DNA was available from both parents and

the proband. However, parental origin assignments were more complicated when DNA was available from only one parent. In these instances, we scored the trisomy as paternal in origin if the trisomic conceptus carried two alleles not present in the mother, or if, on the basis of dosage, the fetus carried two copies of a single, non-maternal allele. However, in these instances it is not possible to unequivocally specify a maternal origin. Instead, a maternal origin was assigned only if the results of five or more markers were consistent with that interpretation.

To determine the stage of origin, chromosome 13 pericentromeric markers (HECH1, D13S1316, or D13S175; see Figure 1) were compared between the proband and parent of origin. If parental heterozygosity was maintained in the trisomic offspring (nonreduction, or "N"), nondisjunction was scored as arising at meiosis I (MI). If parental heterozygosity was reduced to homozygosity (reduction, or "R"), nondisjunction was scored as a meiosis II (MII) error or a post-zygotic mitotic (PZM) error. To distinguish between MII and PZM errors, other noncentromeric markers were examined. If all informative markers were reduced, the case was scored as PZM (although this could technically be a case of MII nondisjunction with no recombination during MI) and if at least one marker was not reduced, the trisomy was scored as a MII error. Some cases were uninformative at pericentromeric loci, so the stage of origin could not be specified.

Nondisjunction mapping

The standard female linkage map of chromosome 13 was constructed using genotypic data from Icelandic families [Kong et al. 2002]. The map was constructed for

the 30 polymorphic markers used to type the trisomy 13 cases, with the markers ordered by physical location in build 35 of the human genome sequence between 17308.183 kb (HECH1) and 110743.481 kb (D13S285). Where genetic locations were not directly known, they were inferred by interpolation between genetic map flanking markers and using known physical locations.

Nondisjunction maps were constructed for trisomies of maternal MI origin using the *map*+ program [Collins et al. 1996]. Heterozygous loci in the mother were classified in the trisomic proband as either N or R. In this way the number and location of transitions between reduced and non-reduced loci could be reliably determined given sufficient coverage by informative markers. In constructing the nondisjunction map, we excluded cases that did not have at least one informative proximal (HECH1-D13S887), medial (D13S801-D13S793), and distal (D13S1271-D13S285) marker.

The theory for the analysis of tetrads follows Shahar and Morton [1986] as implemented in the *map*+ program [Bugge et al. 1998]. Using *map*+ we computed the interference parameter p in the Rao function for the standard female map of chromosome 13 as 0.23, corresponding to a relatively high level of chiasma interference [Rao et al. 1977]. The truncation parameter T, beyond which pairs of loci are separated by more than one recombination value [Bugge et al. 1998], was computed at 55 cM given the estimated level of chiasma interference. The maternal MI nondisjunction map was constructed using the data for which meiotic stage and number of transitions could be reliably determined.

Chiasma distribution

Following Bugge et al. [1998] we re-constructed the chiasma distribution using the program *Exchange* (available on request from Andrew Collins). This enabled the comparison of the estimated proportion of achiasmate chromosomes 13 in normal meioses with trisomies 13 of meiosis I origin.

Results

Parent and meiotic stage of origin

Results on parent and meiotic stage of origin of trisomy are summarized in Table 1. The vast majority of cases (71/78, or 91.0%) were maternally-derived. Most of these cases had at least one non-reduced locus, and were therefore scored as arising in meiosis. Specifically, 31 cases arose during maternal meiosis I, 18 during meiosis II, and in 17 cases the meiotic stage of origin could not be specified because pericentromeric markers were uninformative. In the five remaining maternal cases, we were unable to distinguish between a MII or PZM origin.

Seven cases were scored as paternal in origin. Of these, five arose in meiosis (one MI, two MII, and two either MI or MII), one resulted from a PZM error, and in one case we were unable to distinguish between a MII and PZM origin.

Maternal age and nondisjunction

The mean maternal age for the entire study population was 34.2±5.7 years, elevated by more than seven years over the average maternal age of 27.2 years in 2000 in the United States [Mathews and Hamilton 2002]. In part this was attributable to

the contribution of prenatal diagnosis; that is, a number of cases were ascertained from maternal age-related prenatal diagnoses. Nevertheless, even when these cases were excluded, the overall mean maternal age was still over 33 years (33.7±5.6 years), reflecting the association of maternal age with trisomy 13.

To examine if there were any obvious age-related differences in the origin of nondisjunction, we divided our sample into mothers <35 or ≥35 years of age (Figure 2). While the trends were not drastically different, there was a notable increase in the proportion of MI cases in women younger than 35.

Recombination and nondisjunction

The relationship between aberrant recombination and nondisjunction was examined using two different approaches. First, we generated and compared genetic maps of chromosome 13 based on normal meioses with those based on meiosis I nondisjunctional events that resulted in trisomy 13. Second, we estimated the proportion of "achiasmate" chromosomes 13 (i.e., homologous chromosomes 13 that failed to recombine) in normal meioses and compared that to meioses associated with trisomy 13 of meiosis I origin.

Chromosome 13 maps: comparison of nondisjunction and standard female maps: We generated a meiosis I nondisjunction map as previously described [Bugge et al. 1998] using all maternally-derived trisomies which had at least one proximal, medial, and distal informative marker (see Methods). Because paternal DNA was not available for a large number of cases, we were frequently unable to obtain informative markers in all

three regions. Thus, our genetic mapping was restricted to 28 informative maternal MI trisomies. There were insufficient numbers of maternal MII and paternal cases to construct nondisjunction maps.

The maternal meiosis I nondisjunction map, as well as the standard map based on Icelandic families [Kong et al. 2002], is provided in Figure 3. Our MI nondisjunction map was shorter than the standard map (MI 139.1 cM ± 37.548; standard female map 156.0 ± 3.02), although the difference was not statistically significant. When plotted relative to the standard map, the difference in the slope of the nondisjunction map indicated reduced recombination along all regions of the chromosome. Thus, the maps provide evidence for reduced recombination in maternal MI nondisjunction, but no evidence for specific "cold spots" of recombination in nondisjunctional meioses.

Analysis of the number of exchanges: The genetic map indicated a reduction in recombination levels in maternal MI trisomies. Conceptually, this could result from a failure to recombine in a proportion of cases, from a reduction (but not elimination) in exchanges in some cases, or from a combination of these two factors. To discriminate between these alternatives, we first examined the number of detectable exchanges (i.e., transitions from N→R or R→N) in maternal meiosis I and II trisomies. Exchanges were identified in all 13 MII trisomies but in only 19/28 informative MI cases (p=0.01, [Fisher's Exact Test]), thus implicating achiasmate events in meiosis I errors. Subsequently, we used the program *Exchange* (see Methods) to re-construct chiasma distributions in normal meioses and in maternal meiosis I and II trisomies (Table 2). Approximately

25% of maternal MI trisomies 13 involved achiasmate meiotic events, almost twice the level seen for normally disjoining chromosomes 13.

Discussion

The present study was aimed at addressing two questions regarding nondisjunction of chromosome 13: first, in which gamete and at which stage of development does the extra chromosome 13 originate and how does this compare to other chromosomes and, second, what role, if any, does aberrant recombination play in the genesis of the condition?

Maternal meiosis II errors are surprisingly common in trisomy 13

Similar to reports on other autosomal trisomies, the additional chromosome 13 was almost always maternal in origin, typically due to errors at maternal meiosis I. However, a surprisingly high proportion (37%) of cases was attributable to errors at maternal MII. This estimate is higher than previously reported for trisomy 13, likely because of the scarcity of informative pericentromeric markers in those analyses making it difficult to distinguish between MI and MII errors.

This high frequency of maternal MII errors distinguishes trisomy 13 from other acrocentric trisomies and most nonacrocentric autosomal trisomies for which information on origin is available. That is, nearly 40% of our cases were attributed to maternal MII errors, while comparable estimates for trisomies 15, 16, 21, and 22 are only 9.7%, 0.0%, 22.1%, and 10.0%, respectively [Hassold and Hunt 2001; Hassold and Sherman 2000; Robinson et al. 1993]. Indeed, only trisomy 18 – in which an estimated

60% of cases are associated with maternal MII errors [Bugge et al. 1998] – has a higher proportion of MII errors than trisomy 13. Presumably, this high level of MII errors reflects chromosome-specific factors affecting chromosomes 18 and 13 but not other chromosomes. However, other than the fact that both chromosomes 13 and 18 are relatively gene "poor" [Dunham et al. 2004; Nusbaum et al. 2005], it is not clear that there are any specific genomic features shared by the two chromosomes. Thus, it is not obvious why either chromosome is susceptible to errors at maternal meiosis II, nor is it clear that the underlying nondisjunctional mechanisms are the same for the two chromosomes.

Regardless of the basis of the MII errors, our results provide suggestive evidence that its frequency increases with maternal age. Specifically, the proportion of cases of MII or MI/II origin was approximately two-fold greater in women 35 years of age and older than in those under 35 years. However, as these observations were based on a very small sample, they will need to be confirmed in other series.

Reduced recombination contributes to nondisjunction of chromosome 13

Our results indicate that aberrant recombination is an important contributor to trisomy 13, a feature shared with all other human trisomies for which information is available. Specifically, our estimates suggest that 25% of cases of maternal MI-derived trisomy 13 involve achiasmate chromosomes, while only 12% of normally segregating chromosomes 13 show absence of recombination [Bugge et al. 1998]. This trisomy 13 estimate is consistent with that of a similarly sized acrocentric chromosome, chromosome 15, where 21% of nondisjoined chromosomes are estimated to be

achiasmate [Robinson et al. 1998]. Thus, as for other human trisomies, a leading cause of maternal nondisjunction for chromosome 13 is failure to pair and/or recombine.

The proportion (12%) of achiasmate chromosomes seen for normally segregating chromosomes 13 is somewhat surprising given that 2-4 exchanges would typically be expected for a chromosome that is 156.0 cM in length [Collins et al. 1996; Kong et al. 2002]. Indeed, this is by far the largest achiasmate frequency of any maternal chromosome studied [E. Feingold, personal communication]. One possible reason is that marker coverage is not dense, making the detection of all exchanges difficult and thus underestimating the number of exchanges for normally disjoining chromosomes 13. Alternatively, chromosome 13 may have a larger than expected number of normally disjoining chromosomes that lack any recombination. Regardless, our estimates of twice as many achiasmate chromosomes in nondisjoined versus normally segregating chromosomes indicates that lack of recombination is an important predisposing factor in the genesis of trisomy 13.

Given the role of achiasmate chromosomes in the genesis of trisomy 13, further studies will be needed to explore the possible role of recombination in the variation of maternal MII and MI/II nondisjunction cases with age. Data from trisomy 21 have shown differences in placement of recombination events with age, suggesting an age-related difference in the ability to deal with certain “vulnerable” recombination configurations [Lamb et al. 2005]. It will be interesting to determine if the variation in origin with age for trisomy 13 is due to an age-related difference in recombination, or if other factors affect the types of nondisjunction that occur in younger and older women.

Figure 1. Approximate chromosomal location of the 30 chromosome 13 markers used in this study.

HECH1, D13S1316
D13S175
D13S1236
D13S1275

D13S802

D13S1246

D13S127

D13S894
D13S1248

D13S1227
D13S887

D13S801

D13S800
D13S792
D13S162

D13S170
D13S790
D13S794

D13S1300

D13S793

D13S1271

D13S779

D13S274

D13S797

D13S796

D13S1265, D13S895
D13S1315

D13S285

Figure 2. Origin of trisomy 13 by maternal age. Sample is divided into mothers <35 and ≥35 year of age. MI=maternal meiosis I origin, MII=maternal MII origin; other=either paternal or post-zygotic mitotic origin.

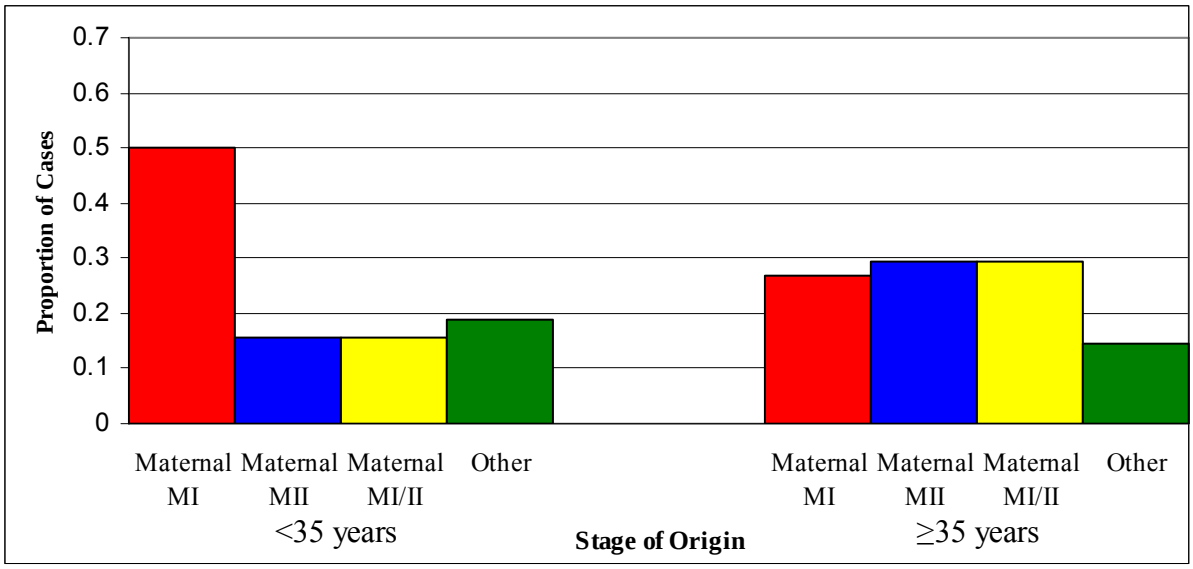
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of Cases
Maternal MI
Maternal MII
Maternal MI/II
Other
<35 years
Stage of Origin
Maternal MI
Maternal MII
Maternal MI/II
Other
≥35 years

Figure 3. Chromosome 13 maternal meiosis I nondisjunction map

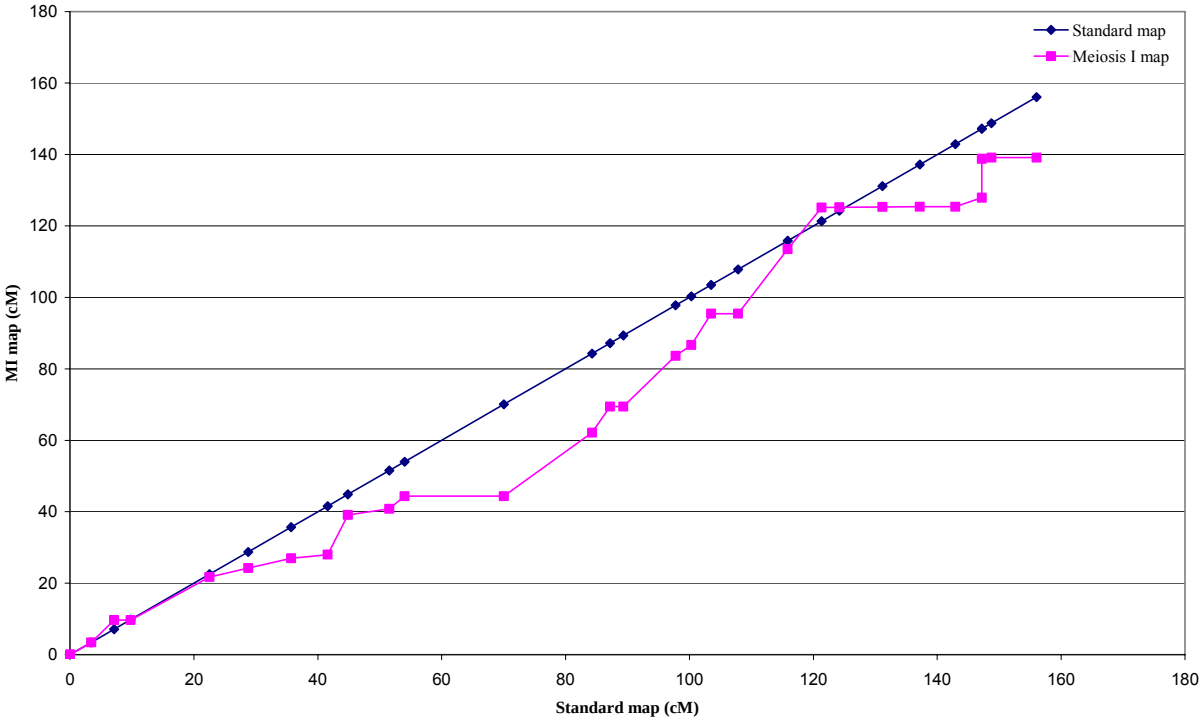
Standard map
Meiosis I map
MI map (cM)
Standard map (cM)
0
20
40
60
80
100
120
140
160
180

Table 1. Classification of cases by parent and meiotic stage of origin of trisomy.

	Maternal				Paternal				
No.	MI	MII	MI/II	Unk	MI	MII	MI/II	PZM	UNK
78	31	18	17	5	1	2	2	1	1

MI=meiosis I; MII=meiosis II; MI/II=meiosis, stage unknown; Unk=unknown; PZM=post-zygotic mitotic

Table 2. Number of exchanges and estimated number of achiasmate bivalents in trisomy generating meioses and normal meioses.

	Number of exchanges							Achiasmate
	0	1	2	3	4	5	6	frequency
Normal	21	27	21	9	0	0	0	12%
Mat MI	9	4	7	4	3	0	1	25%
Mat MII	0	7	2	2	2	0	0	0%

CHAPTER THREE

THE ORIGIN OF TRISOMY 22: EVIDENCE FOR ACROCENTRIC CHROMOSOME-SPECIFIC PATTERNS OF NONDISJUNCTION

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ABSTRACT

Trisomy 22 is one of the most common trisomies in clinically recognized pregnancies, yet relatively little is known about the origin of nondisjunction for chromosome 22. Accordingly, we initiated studies to investigate the origin of the extra chromosome in 130 trisomy 22 cases. Our results indicate that the majority of trisomy 22 errors (>96%) arise during oogenesis with most of these errors (~90%) occurring during the first meiotic division. As with other trisomies, failure to recombine contributed to nondisjunction of chromosome 22. Taken together with data available for other trisomies, our results suggest patterns of nondisjunction that are shared among the acrocentric, but not all nonacrocentric, chromosomes.

Iintroduction

The origin of human trisomy has been extensively studied, with results of parental origin studies now available for over 1,000 trisomic fetuses or liveborns [Hassold and Hunt 2001]. Not surprisingly, most studies have focused on trisomies compatible with livebirth: i.e., trisomies 13, 18, 21 and the sex chromosome trisomies [Fisher et al. 1993; Fisher et al. 1995; Hassold and Sherman 2000; Jacobs et al. 1989; Morton et al. 1988; Sherman et al. 1991]. Based on these analyses, three common themes of human nondisjunction have emerged: first, the majority of trisomies result from errors in oogenesis; second, maternal meiosis I (MI) errors are more common than maternal meiosis II (MII) errors; and third, the proportion of cases of maternal origin increases with maternal age [Hassold and Hunt 2001]. However, while these themes apply to most trisomies, it is also evident that there is substantial variation among chromosomes in the way trisomies originate. For example, although maternal MI errors are the predominant mechanism of origin for most trisomies, trisomy 18 arises most often during maternal MII [Bugge et al. 1998], and for certain trisomies (e.g., the 47,XXY condition) paternal errors are as common as maternal errors [Thomas and Hassold 2003]. Given this variation, fully understanding human meiotic nondisjunction will require studies of each individual trisomy. Unfortunately, to date only a handful of trisomies have been studied.

In the present report, we summarize information on the origin of one of these "under-studied" human trisomies, trisomy 22. Despite the fact that it is not observed in liveborns, trisomy 22 is one of the most common trisomies. It is estimated to occur in

nearly 1/200 clinically recognized pregnancies, accounting for no fewer than 2.7% of spontaneous abortions and 0.2% of stillbirths [Hassold and Jacobs 1984].

In previous studies, we examined the origin of a small series of 43 cases of trisomy 22 [Zaragoza et al. 1994; Zaragoza et al. 1998]. We have extended our analyses to include 87 additional cases and report here results on the total series of 130 cases. Our results suggest that, like other acrocentric chromosomes, the majority of nondisjunctional errors occur during maternal MI. Indeed, it appears that, in contrast to nonacrocentric chromosomes, the pattern(s) of nondisjunction are shared among the acrocentric chromosomes.

Materials and methods

Study population

The study population consisted of 130 spontaneously aborted fetuses with an additional chromosome 22. These samples were collected from four sources: Kapiolani-Children's Medical Center in Honolulu, HI (K series - 9 families), University Hospitals of Cleveland in Cleveland, OH (A series - 23 samples), Northside Hospital in Atlanta, GA (S series - 14 families), and Magee-Womens hospital in Pittsburgh, PA (B series - 84 samples). Samples from Hawaii, Cleveland, and Atlanta were studied as part of cytogenetic surveys of consecutive series of spontaneous abortions. Samples from Pittsburgh were obtained as part of routine clinical examination following a spontaneous abortion. For most samples collected from Hawaii and Atlanta, DNA was extracted from fetal or fetally-derived extra-embryonic tissue, and from blood samples obtained from the father and mother; thus, in these 23 cases ("complete" cases) DNA

samples were available from both parents and the trisomic fetus. For most samples collected from Cleveland and all samples from Pittsburgh, paraffin-embedded blocks were examined for the presence of fetally-derived and maternal (decidual) material, and DNA extracted from each; thus, for these 107 cases ("incomplete" cases), DNA was only available from the fetus and the mother.

All studies were reviewed and approved by the appropriate Institutional Review Boards.

Cytogenetic analysis

Cytogenetic studies of blood and tissue samples were performed using standard procedures; in most cases, a minimum of 5-10 cells were examined. The analyses of the fetuses were consistent with nonmosaic, single trisomy 22 in all but three cases: one a mosaic with a normal cell line (46,XY/47,XY,+22); one a mosaic with trisomy 22 in one cell line and trisomy 12 in the other (47,XY,+12/47,XY,+22); and one a double aneuploid with monosomy X and trisomy 22 (46,X,+22).

DNA studies

DNA was extracted and amplified using standard techniques. As these studies were conducted over a number of years, the chromosome 22 markers used for DNA analysis changed over time. Table 1 shows the approximate location of all 38 polymorphic loci that were utilized; those that were used in the most recent analyses of the 85 B series cases are denoted.

Origin studies

Determinations of parent and meiotic/mitotic stage of origin were made using a previously described approach [Zaragoza et al. 1998]. Briefly, for each family we first determined the parent of origin of the extra chromosome. For "complete" families, parental origin determinations were straightforward, and for these cases, we required consistent results at two informative markers. However, for "incomplete" families (i.e., DNA samples available only from the conceptus and the mother), determinations of parental origin were more complicated. First, while paternal origins could be unequivocally specified (e.g., if the trisomic conceptus carried two alleles not present in the mother) it was not possible to definitively score a case as maternal in origin. Thus, we used rationale similar to that described by Zaragoza et al. [1998], scoring a trisomy as maternal only if the results of five or more markers were consistent with that interpretation. Second, in many of the paraffin blocks involving incomplete cases there was cross-contamination between fetal and maternally-derived material. In these instances the genotyping results were evaluated by two independent observers; if one or both observers were unable to unambiguously interpret the data, the case was discarded.

To determine the stage of origin, centromeric markers were compared between the proband and parent of origin. If parental heterozygosity was retained in the trisomic offspring (nonreduction, or N), the error was scored as a meiosis I (MI) nondisjunction, and if heterozygosity was reduced to homozygosity (reduction, or R), as a meiosis II (MII) error or a post-zygotic mitotic (PZM) error. To distinguish between MII and PZM errors, other noncentromeric markers were examined. If all informative markers (at

least one located in the proximal, medial, and distal region of the chromosome) were reduced, the case was scored as PZM (technically this could also be a case of MII nondisjunction with no recombination during MI) and if at least one marker was not reduced, the trisomy was scored as a MII error. Some cases were uninformative at centromeric loci, so that the stage of origin could not be determined. Most of these cases were nonreduced for at least one locus and were therefore classified as meiotic in origin (MI/II)

Mapping/statistical analysis

Cases were considered informative for recombination if at least one proximal, medial, and distal marker were informative. Our MII and paternal cohorts were too small for meaningful analysis of recombination, but of our 60 maternal MI cases, 45 had enough informative markers spread along the chromosome to allow us to score recombination. We scored each case as having 0, 1, or 2 observed recombinations, and applied the methods described by Lamb et al. [1997] to estimate the percentages of tetrads with 0, 1, and 2 exchanges from the recombination data. The same methods were used to estimate the exchange distribution in the CEPH families based on data reported by Bugge et al. [1998].

Results

Parent and meiotic stage of origin

The results of studies on parent and meiotic stage of origin are summarized in Table 2. No obvious differences were apparent between cases from the four series or

between complete and incomplete families; therefore, the data were pooled for all subsequent analyses.

Of the 130 cases, decisions on parental origin were possible for 110 cases; most of the remaining cases involved incomplete families, where cross-contamination between fetal and maternal samples precluded unequivocal determinations. For 106 of the 110 (96.4%) informative cases, the error was maternal in origin. In subsequent analyses of the stage of origin in these cases, we identified meiosis I (MI) errors in 60/67 (90.0%) instances and meiosis II (MII) errors in 7/67 (10.0%) cases. For the remaining 39 maternal cases, centromeric markers were uninformative and we were unable to specify the meiotic stage of origin. Nevertheless, in each of these cases, nonreduction was observed at one or more markers, indicating that the error was meiotic, not mitotic, in origin.

Only four of the 110 cases were not attributable to errors in oogenesis. Two of these were paternal in origin and resulted from errors in the first meiotic division. The other two cases were attributable to post-zygotic mitotic errors.

Given the well-established relationship between maternal age and trisomy, we were interested in asking whether there were any obvious age-related differences in the origin of nondisjunction. Because of the relatively small sample size, we simply divided the population into two groups: mothers <35 and ≥35 years of age. As is clear from Figure 1, there was no obvious effect of age. A large number of samples in each age group were informative only as maternal meiotic errors (MI/II), with stage of meiotic error unable to be determined due to lack of informative markers near the centromere.

Recombination and nondisjunction

We used two different approaches to examine the relationship between recombination and nondisjunction of chromosome 22. We first examined the overall levels of recombination, and subsequently analyzed the placement of exchanges along chromosome 22.

To measure levels of recombination, we compared the overall genetic map length of our maternal meiosis I nondisjunction sample to map lengths for meioses involving normally disjoining chromosomes 22. The length of the normal female meiotic map of chromosome 22 is currently reported as 74 cM (www.marshfieldclinic.org); similarly, in chromosome 22 recombination data reported for the CEPH families by Bugge et al. [1998] there were 32 individuals with no maternal recombination observed, 39 with one maternal recombination event, and 7 with two maternal recombination events, yielding estimates of 0% nonexchange tetrads, 64% single-exchange tetrads, and 36% double-exchange tetrads, for an overall length of 68 ±7 (S.E.) cM.

The data from our trisomy cases yielded a markedly shorter genetic map. Of the 45 maternal MI trisomy cases in which we were able to score recombination, we observed 25 with no recombination, 14 with one recombination event, and 6 with two recombination events. This yielded exchange frequency estimates of 26% nonexchange tetrads, 49% single-exchange tetrads, and 25% double-exchange tetrads, for an overall genetic length of 49.5 ±9 (S.E.) cM. Thus, the trisomy-based female map was markedly shorter than the normal map, primarily due to trisomies in which no recombination was observed.

Second, we were interested in asking whether there were any obvious hot or cold spots of recombination associated with nondisjoined chromosomes 22. A detailed analysis of recombination distribution was not possible, as the exact location of the events could rarely be determined. Therefore, we divided the cases by stage of origin (MI or MII) and the number of detectable recombination events, and simply charted the regions in which these events must have occurred. As is clear from Figure 2, there was no noticeable region of the chromosome that was either enriched for, or devoid of, recombination.

Discussion

The purpose of the present study was two-fold: first, to compare the mechanisms of origin for trisomy 22 with previously analyzed human trisomies and, second, to determine if – as for other trisomies -- aberrant recombination contributes to the origin of trisomy 22.

Maternal meiosis I errors predominate in trisomy 22

In the current study, the additional chromosome 22 was the result of maternal nondisjunction in 96% (106/110) of informative cases, with the vast majority due to errors in meiosis I. Paternal and mitotic errors played only minor roles, as they each contributed to only 1.8% (2/110) of informative cases. A comparison of these results with those from other acrocentric trisomies reveals several striking similarities (Table 3). As a group, over 80% of errors occur during oogenesis. Further, in each trisomy, the majority of errors originate at maternal meiosis I, with maternal meiosis II errors making

a sizeable contribution and paternal errors typically accounting for fewer than 15% of cases. The only apparent exception, trisomy 14, almost certainly reflects the limited number of informative cases; i.e., specific information on meiotic stage of origin is available for only five cases.

Thus, our data suggest that the mechanism(s) leading to trisomy may well be shared among the different acrocentric chromosomes. In contrast, there is considerable variation in the parent and/or stage of origin of trisomy among nonacrocentric chromosomes. For example, virtually all cases of trisomy 16 result from errors at maternal meiosis I [Hassold et al. 1995], while maternal MII errors predominate for chromosome 18 [Bugge et al. 1998; Fisher et al. 1993], and paternal errors account for approximately one-half of 47,XXY cases [Thomas and Hassold 2003]. Further, limited data from several other trisomies (e.g., trisomies 2, 7, and 8) also suggest considerable chromosome-specific variation in the origin of nonacrocentric trisomies [James and Jacobs 1996; Karadima et al. 1998; Zaragoza et al. 1998]. The reason for the discrepancy between acrocentric and nonacrocentric chromosomes is not entirely clear but, at least in part, may be attributable to chromosome-specific differences in recombination patterns.

Abnormal recombination is an important contributor to chromosome 22 nondisjunction

Our analyses indicate that – like other human trisomies – aberrant recombination is an important contributor to nondisjunction of trisomy 22. Indeed, we estimated that over 25% of all maternal meiosis I-derived trisomies 22 involved achiasmate chromosomes, while we found no evidence for nonexchange chromosomes 22 in

normally segregating female meiotic events. Thus, failure to pair and/or recombine during female meiosis is a leading cause of trisomy 22.

These results are consistent with those for trisomy 21, in which approximately 40% of cases involve nonexchange chromosomes [Lamb et al. 2005]. This similarity is not entirely unexpected. Chromosomes 21 and 22 are the two smallest autosomes and, on average, each is held together by only one or two chiasmata during female meiosis. Thus, unlike larger chromosomes, loss of a single chiasma from chromosomes 21 or 22 frequently yields an achiasmate bivalent.

Chromosome size, however, is not the only factor in the genesis of nonexchange chromosomes, since failure to recombine is a feature of other trisomies as well. Most notably, over 20% of cases of maternal MI-derived trisomy 15 involve achiasmate bivalents [Robinson et al. 1998], despite the fact that chromosomes 15 are joined by 2-4 chiasmata in normal female meiosis. Thus, for the three acrocentric trisomies for which exchange information is available, failure to recombine during maternal meiosis I is a major contributor to nondisjunction.

The contribution of achiasmate bivalents extends to some, but not all, nonacrocentric trisomies. For example, a large proportion of maternal and paternal meiosis I-derived sex chromosome trisomies are associated with nonexchange tetrads [Thomas et al. 2001], as are about 30% of all cases of trisomy 18 [Bugge et al. 1998]. However, nonexchange bivalents are rarely – if ever – associated with trisomy 16 [Hassold et al. 1995]. Thus, at least some of the variation between acrocentric and nonacrocentric trisomies may be related to the incidence of nonexchange chromosomes.

In addition to reduced recombination, the location of recombination events affects chromosome segregation, but here the distinction between acrocentric and nonacrocentric chromosomes is not as obvious. For example, among acrocentric trisomies, there is no evidence that altered recombination placement contributes to trisomies 13, 15 or 22 [Robinson et al. 1998; Zaragoza et al. 1994], but both pericentromeric and distal events have been linked to trisomy 21 [Lamb et al. 1997]. There is similar variation among nonacrocentric trisomies: extremely distal recombination has been linked to trisomy 16 [Hassold et al. 1995] and pericentromeric recombination to sex chromosome trisomies [Thomas et al. 2001], but there is no indication that disturbed chiasma placement is important in trisomy 18 [Bugge et al. 1998]. Thus, the association between placement of recombination and nondisjunction appears to operate on a chromosome to chromosome basis.

Taken together, the results of our and other studies indicate that there is chromosome-specific variation in the importance of factors predisposing to nondisjunction. Some factors appear to affect all chromosomes; e.g., increasing maternal age and reductions in recombination have been linked to all autosomal trisomies and the 47,XXX and 47,XXY conditions. In contrast, some predisposing factors are shared by some, but not all, chromosomes. For instance, our study indicates that as a whole, the acrocentric chromosomes share similar patterns of nondisjunction, with recombination failure likely linked to all acrocentric trisomies; also, previous studies of maternal age indicate similar maternal-age specific curves for acrocentric chromosomes [Risch et al. 1986]. However, other risk factors (e.g., altered exchange placement) appear to be specific to individual chromosomes. Thus, it seems

likely that there are at least three types of nondisjunction-promoting factors in humans: those that increase nondisjunction of all chromosomes, those that apply to groups of chromosomes, and those that are chromosome-specific. The relative importance of each of these factors is uncertain, and can be addressed only by additional analyses of individual chromosomes.

Figure 1. Distribution of origin of nondisjunction in women <35 and ≥35 years of age. (MI=maternal meiosis I; MII=maternal meiosis II; MI/II=maternal meiosis, stage unknown). The four "Other" cases identified in the ≥35 years category consisted of two paternal meiosis I and two mitotic cases.

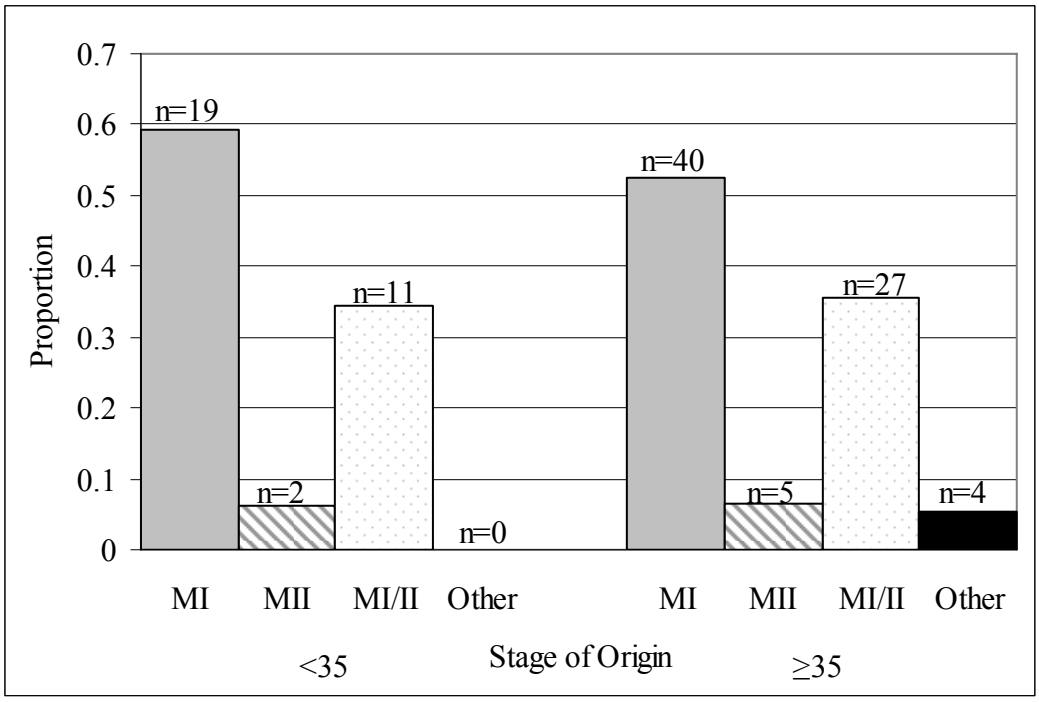
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion
n=19
n=2
n=11
n=0
n=40
n=5
n=27
n=4
MI
MII
MI/II
Other
MI
MII
MI/II
Other
<35
Stage of Origin
≥35

Figure 2. Location of recombination events for nondisjoined chromosomes 22. Locations are shown for chromosomes that nondisjoined in meiosis I or meiosis II and had a single or double exchange. Each line indicates the region in which the exchange occurred.

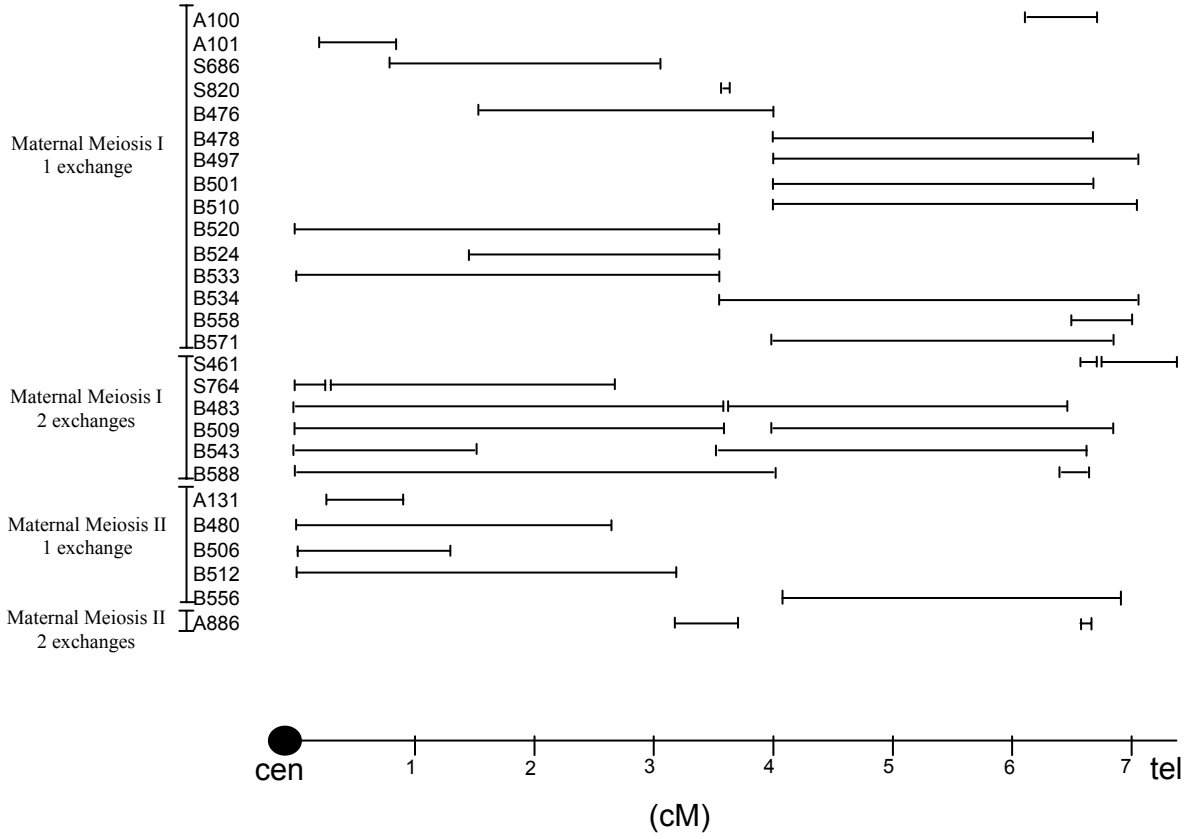
A100
A101
S686
S820
B476
B478
B497
B501
B510
B520
B524
B533
B534
B558
B571
S461
S764
B483
B509
B543
B588
A131
B480
B506
B512
B556
A886
Maternal Meiosis I
1 exchange
Maternal Meiosis I
2 exchanges
Maternal Meiosis II
1 exchange
Maternal Meiosis II
2 exchanges
cen
1
2
3
4
5
6
7
tel
(cM)

Table 1. Approximate cM location of chromosome 22 markers used in this study. Most are from the Marshfield map (www.marshfield.org), although for four markers (indicated by $^{+}$) locations have been interpolated.

Marker	Sex Averaged	Female	Male	Heterozygosity‡
F8VWFP^{+}				0.61
GATA198B05*	1.79	0.00	2.60	0.85
D22S420	4.06	3.32	2.60	0.76
D22S427	8.32	7.83	8.02	0.63
D22S446	13.60	15.35	11.67	0.80
D22S539	14.44	15.35	12.49	0.55
D22S686*	14.44	15.35	12.49	0.79
D22S257	17.71	21.26	12.49	0.62
D22S1685^{+}				0.66
D22S315*	21.47	27.42	14.65	0.81
D22S310	23.37	29.07	16.79	0.64
D22S1154	23.37	29.34	16.79	0.72
D22S1167	24.47	29.59	19.50	0.74
D22S1144	27.48	31.80	22.17	0.76
D22S303^{+}				0.65
D22S275	28.57	32.90	23.24	0.82
D22S689*	28.57	32.90	23.24	0.76
D22S280	31.30	36.23	25.37	0.82
D22S1162	31.84	36.23	26.44	0.80
D22S685	32.39	36.23	27.51	0.79
D22S691	32.39	36.23	27.51	0.79
D22S683*	36.22	40.67	30.71	0.90
D22S283	38.62	45.54	30.71	0.90
D22S692	41.42	51.26	30.71	0.71
IL2RB	42.81	54.11	30.71	0.71
D22S272	45.82	60.34	30.71	0.68
D22S423	46.42	61.58	30.71	0.82
D22S270^{+}				0.75
D22S1157	47.31	63.39	30.71	0.85
D22S1165	48.19	65.20	30.71	0.76
D22S1171*	48.19	65.20	30.71	0.87
D22S274	51.54	67.47	35.04	0.77
D22S928*	52.08	68.55	35.04	0.79
D22S1169*	60.61	71.88	48.68	0.80
D22S526	62.31	74.39	48.68	0.65

*Markers used in the most recent set of analyses of 85 B series cases

‡Heterozygosity of markers provided at www.marshfield.org, with the exceptions of F8VWFP (www.gdb.org), D22S1685 (http://www.bli.unizh.ch), and D22S303 (www.gdb.org)

Table 2. Parental and meiotic origin of trisomy 22 by location of sample ascertainment and family status (i.e., “incomplete” or “complete”; see Maternal and Methods).

		Maternal			Paternal			
	No.	MI*	MII	MI/II	MI	MII	PZM	Unk
Complete Cases[ǂ]:								
K series	9	0	0	4	0	0	0	5
S series	14	10	0	2	0	0	0	2
Incomplete Cases:								
A series	23	5	2	5	0	0	0	11
B series	84	45	5	28	2	0	2	2
Total	130	60	7	39	2	0	2	20

*MI=meiosis I; MII=meiosis II; MI/II=meiosis, stage unknown; PZM=post-zygotic mitotic; Unk=unknown

[ǂ]K series cases were collected at Kapiolani-Children’s Medical Center, Honolulu, HI; S series cases at Northside Hospital, Atlanta, GA; A series cases at University Hospitals of Cleveland, Cleveland, OH; and B series case at Magee-Womens Hospital, Pittsburgh, PA.

Table 3. Summary of studies of parental and meiotic origin of nonmosaic human trisomies.*

Acrocentrics Trisomy	N	Maternal MI‡ (%)	Maternal MII (%)	Paternal MI (%)	Paternal MII (%)	PZM (%)
13	74	56.6	33.9	2.7	5.4	1.4
14	26	36.5	36.5	0.0	19.2	7.7
15	34	76.3	9.0	0.0	14.7	0.0
21	671	67.5	22.1	3.9	4.6	1.9
22	130	86.4	10.0	1.8	0.0	1.8

Nonacrocentrics Trisomy	N	Maternal MI (%)	Maternal MII (%)	Paternal MI (%)	Paternal MII (%)	PZM (%)
2	18	53.4	13.3	27.8	0.0	5.6
7	14	17.2	25.7	0.0	0.0	57.1
8	12	------50.0#------		0.0	0.0	50.0
16	104	100.0	0.0	0.0	0.0	0.0
18	150	33.3	58.7	0.0	0.0	8.0
XXX	46	63.0	17.4	0.0	0.0	19.6
XXY	224	25.4	15.2	50.9	0.0	8.5

*Data on +13 from Zaragoza et al. 1998 and Hall and Hassold, unpublished observations; +14 from Hall and Hassold, unpublished observations; +15 from Zaragoza et al. 1998; +21 from Hassold and Sherman 2000; +22 from present study; +2 from Zaragoza et al. 1998; +7 from Zaragoza et al. 1998; +8 from James and Jacobs 1996 and Karadima et al. 1998; +16 from Hassold and Hunt 2001; +18 from Bugge et al. 1998; XXX and XXY reviewed in Hall et al. 2006

‡MI=meiosis I; MII=meiosis II; PZM=post-zygotic mitotic

#These cases were of maternal meiotic origin, but the specific stage could not be determined

CHAPTER FOUR

TESTING MODELS OF HUMAN ANEUPLOIDY: THE ROLES OF RECOMBINATION AND MATERNAL AGE IN NONDISJUNCTION

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ABSTRACT

Trisomy is the most common chromosomal abnormality in humans, affecting approximately 4% of clinically recognized pregnancies. To date, only two factors have been linked to increasing nondisjunction, recombination and maternal age. As a test of the relationship between these two predisposing factors, we evaluated recombination levels in maternal meiosis I-derived trisomies for over 1300 cases, including both new data and previously published reports. We tested these data on trisomies 13, 14, 15, 16, 18, 21, 22, and the sex chromosome trisomies against two popular models of human aneuploidy: the two hit hypothesis and the production line hypothesis. The two hit hypothesis predicts that levels of recombination are independent of maternal age, while the production line hypothesis predicts decreasing levels of recombination in older women. Our data suggest chromosome-specific differences in levels of recombination with maternal age. While most trisomies, including trisomies 13, 15, 16, 21, and 22 show no variation in recombination levels with age (as predicted by the two hit hypothesis), trisomy 18 and the sex chromosome trisomies demonstrate increased levels of recombination with maternal age, a trend not predicted by the two hit hypothesis or the production line hypothesis. Thus, it appears that no one hypothesis will explain all maternal age-related aneuploidy.

Introduction

Nondisjunction occurs at an alarming rate in humans with as many as 25% of conceptions being estimated to have the wrong number of chromosomes [Hassold and Hunt 2001]. The majority of these errors occur during oogenesis, implicating female meiotic errors as a common cause of reproductive failure [reviewed in Hall et al. 2007b, in press]. It has been known for decades that the frequency of errors occurring during oogenesis increases drastically with maternal age. For example, while the risk of a trisomic pregnancy for a woman 20-25 years of age is only 2-3%, for a woman over 40 years of age the risk is as high as 35% [reviewed in Hassold and Hunt 2001]. Despite the clinical importance of these observations, the basis of this maternal age effect remains a mystery.

There are several possible time points in female meiosis when these errors could occur. The most likely points are: 1) prenatally in the fetal ovary when meiosis is initiated, as some age-related factor may compromise the ability to deal with the exchanges; 2) during the prolonged “dictyate” arrest stage of female meiosis which can last approximately 15-50 years in humans; and 3) at the time of ovulation when meiosis is completed. The first of these three time points is already known to be a common source of meiotic errors, as it is prenatally in the fetal ovary that recombination takes place. Many studies on several different human chromosomes have shown that aberrant recombination increases the likelihood that chromosomes will nondisjoin [Hassold et al. 1995; Lamb et al. 2005; Robinson et al. 1998; Thomas et al. 2001]. For example, studies of nondisjoined chromosomes 21 have shown that absence of

recombination, or recombination occurring too proximal or distal to the centromere predisposes to nondisjunction [Lamb et al. 1997; Lamb et al. 1996].

While nondisjunction has been linked to both recombination and maternal age, the way in which the two are linked remains largely unexplored. Only two hypotheses have fully attempted to propose a relationship between these two predisposing factors: the production line hypothesis and the two hit hypothesis [Hassold and Sherman 2000; Henderson and Edwards 1968].

The production line hypothesis was the original hypothesis linking abnormal recombination to maternal age-related nondisjunction. Henderson and Edwards [1968] proposed this model based on several observations they made when conducting meiotic chromosome studies of oocytes from female mice of varying ages. They noticed that with increasing age there were a decreased number of exchanges and an increased number of univalents, where no recombination occurred between homologues. Additionally, they noticed that when a single exchange occurred in oocytes from older females, it was more often terminally located. Based on these observations, they proposed that a “production line” exists in the fetal ovary so that germ cells enter and exit meiosis in a sequential order, i.e., those cells that enter meiosis first will be the first ovulated after puberty and those cells that enter meiosis later will be the last to be ovulated. Further, they proposed that a gradient exists in the fetal ovary causing those cells that enter meiosis early to have a greater number of exchanges than those cells that enter meiosis late. Consequently, the first cells to be ovulated after puberty will have a greater number of exchanges than those ovulated

later. Therefore, the frequency of chromosomes with no exchanges would increase in older women, leading to increased rates of chromosome nondisjunction.

While the first component of the production line hypothesis has been demonstrated to be true [Hirshfield 1992; Polani and Crolla 1991], the second part has never been demonstrated. Thus, there has been much debate over whether or not a gradient exists in the fetal ovary causing exchange number to decrease with age [Brook et al. 1984; Speed and Chandley 1983; Sugawara and Mikamo 1986; Tease and Fisher 1989].

A more recent hypothesis to explain the relationship between maternal age and recombination is the two hit hypothesis [Hassold and Sherman 2000]. This model predicts errors occurring at two different stages of meiosis cumulatively causing increased nondisjunction. The first error (or "hit") occurs early in meiosis, in the fetal ovary, when recombination is established. Any susceptible exchanges (i.e., reduced recombination or recombination too proximal or distal to the centromere) will increase the likelihood that chromosomes will nondisjoin. As recombination occurs in the fetal ovary, this susceptibility will be independent of maternal age. The "second hit" occurs during the prolonged dictyate arrest of female meiosis. As this stage lasts for decades it would be sufficient time for the loss of some meiotic factor whose deficiency would decrease the ability to resolve susceptible exchanges. As cells in older women have been arrested for a longer period of time than those in younger women, this second hit will be dependent on maternal age as older women will have had more time for errors to accumulate.

These two hypotheses make very different predictions about how recombination will vary with maternal age. The production line hypothesis predicts that recombination levels will decrease in older women [Henderson and Edwards 1968], while the two hit hypothesis predicts that recombination is entirely independent of maternal age [Hassold and Sherman 2000]. Thus, as a test of these two hypotheses, we conducted studies to examine how recombination levels vary in mothers of varying ages who have had a trisomic conceptus.

Materials and methods

Study population

Information on trisomies 18, 21, and the sex chromosome trisomies was collected from previously published reports in which parent/meiotic stage of origin, maternal age, and recombination data are available. In addition to previous reports, new information is presented on four additional trisomies: +14, +15, +16, and +22. All data used in this study, including previously published reports, are summarized in Table 1.

+14: Information on trisomy 14 includes 12 previously published cases [Zaragoza et al. 1994] and 22 new cases ascertained at Magee-Womens Research Institute, Pittsburgh, PA. These new cases were ascertained as part of routine clinical examination following a spontaneous abortion. In all cases, the karyotype of the fetus was consistent with nonmosaic, single trisomy 14

+15: Information on parent and stage of origin was available for 34 cases previously published in Zaragoza et al. [1998]. Information on an additional 70 previously unreported cases is presented here. All 70 new cases were collected at Magee-Womens Research Institute, Pittsburgh, PA as part of routine clinical examination following a spontaneous abortion. In all cases the karyotype of the fetus was consistent with nonmosaic, single trisomy 15.

+16: For 62 trisomy 16 cases, information on parental/meiotic origin, maternal age, and recombination was previously published in Hassold et al. [1995]. In the present report we summarize the results for an additional 144 cases of trisomy 16. Of these new cases, 4 were ascertained at Northside Hospital in Atlanta, GA, 37 were from University Hospitals of Cleveland in Cleveland, OH, and 103 cases were from Magee-Womens Research Institute in Pittsburgh, PA.

For the new cases reported here, the analyses of the fetuses were consistent with nonmosaic, single trisomy 16 in all but two cases: one, a mosaic with a normal cell line (46,XY/47,XY,+16) and the other the carrier of a translocation not involving chromosome 16 (47,XY,+16, t(6;11)). The mother carried this same translocation. In 25 cases, no specific karyotype information was available.

+22: Information on an additional 29 cases of trisomy 22 is presented along with 120 previously published cases [Hall et al. 2007b, in press]. All 29 new cases were collected at Magee-Womens Research Institute, Pittsburgh, PA as part of routine

clinical examination following a spontaneous abortion. In all new cases, the karyotype of the fetus was consistent with nonmosaic, single trisomy 22.

All studies were reviewed and approved by the appropriate Institutional Review Board.

DNA studies/data analysis

DNA was extracted and amplified using standard techniques. For each case, inheritance of DNA polymorphisms was scored. The approximate location of markers for newly studied chromosomes is shown in Figure 1.

For all cases, inheritance of Southern blot probes or microsatellite markers was used to determine parent of origin. Scoring of either three alleles or dosage of alleles in the proband compared to the parent(s) allowed for determination of the parental origin of the extra chromosome.

Available markers located closest to the centromere were used to determine stage of origin. If the parent in which the error occurred was heterozygous, and heterozygosity was maintained in the offspring (nonreduction or N), the error was scored as occurring during meiosis I. If, however, the parent of origin was heterozygous and two copies of the same allele were inherited by the proband (reduction, or R), the error was scored as occurring during the second division of meiosis. Transitions in marker status from nonreduced to reduced, or visa versa, were scored as evidence of a recombination event.

The method for evaluating recombination varied somewhat among reports. Most, including the new cases reported here, examined markers spaced approximately

every 10-15 cM along the chromosome. For almost all studies, the chromosome was divided into intervals (based on size of the chromosome and number of available markers), with one informative marker required in each interval to score recombination [Hall et al. 2007a, in press; Hall et al. 2007b, in press; Hassold et al. 1995; Lamb et al. 2005; Thomas et al. 2001]. The exceptions to this strategy were Denmark-based studies of trisomy 13 [Bugge et al. 2007] and trisomy 18 [Bugge et al. 1998]. In these studies, a minimum of 7-10 markers were required for a case to be used for mapping purposes.

Statistical analysis

In the present analysis, trisomies were grouped into different maternal age categories (<35 years of age or ≥35 years of age), and straightforward goodness of fit tests were conducted to identify any significant age-related differences in recombination.

Results

Parent and meiotic stage of origin

No obvious differences were apparent between the new cases and previously published cases and thus the cases were pooled for the present analyses. A summary of results on parent and meiotic stage of origin is presented in Table 2.

Recombination levels and maternal age

As the majority of meiotic nondisjunction occurs during maternal meiosis I, studies of recombination are limited to errors occurring during this stage. To determine if there was any significant difference in recombination levels with age, mothers of trisomic conceptions were divided into two groups, women <35 years of age and ≥35 years of age. The number of zero-exchange cases was compared to those cases in which at least one exchange was observed (Table 3). For most trisomies, there was no variation in recombination levels in women of different ages, as was true for trisomies 13, 15, 16, 21, and 22 (p=NS). Trisomy 18 showed borderline significance (p=0.08) and the sex chromosome trisomies showed a significant difference in recombination levels in younger and older women (p=0.02).

Discussion

The present study was aimed at exploring the role of recombination in maternal age-related aneuploidy and comparing this relationship to predictions proposed by two hypotheses of human aneuploidy: the two hit hypothesis and the production line hypothesis [Hassold and Sherman 2000; Henderson and Edwards 1968].

The two hit hypothesis predicts recombination patterns for most trisomies

While the production line hypothesis predicts decreasing levels of recombination with maternal age [Henderson and Edwards 1968] and the two hit hypothesis predicts no change in levels of recombination with age [Hassold and Sherman 2000], it appears that no one hypothesis accurately predicts the trend for all trisomies; the relationship

between recombination and maternal age appears to be chromosome-specific. For trisomies 13, 15, 16, 21, and 22, no significant difference was observed between levels of recombination in younger and older women based on a simple goodness of fit test, and thus these trisomies followed the prediction of the two hit hypothesis. In contrast, trisomy 18 and the sex chromosome trisomies showed borderline and true significant differences, respectively, in disagreement with the prediction of the two hit hypothesis (Table 3).

For the two trisomies where recombination levels do vary significantly with age (18 and XXX/XXY), the trend of variation does not follow the predictions of the production line hypothesis. While this hypothesis would suggest an increase in zero-exchange events with increasing age, older women actually showed increased levels of recombination when compared to younger women, the exact opposite of the prediction of the production line hypothesis.

Chromosome-specific patterns of age-related recombination may affect nondisjunction

Further analysis of levels of recombination in women of different ages reveals possible chromosome-specific differences in age-related patterns of recombination. While statistical analyses did not reveal a difference in recombination with age for most trisomies, a simple visual comparison of the proportion of zero-exchange and exchange events in younger and older women suggests several possible age-related trends (Figure 2). It is possible that the limited number of samples for many of these trisomies prohibits statistical significance, and that additional samples may allow many of these trends to reach significance. For example, trisomies 13, 15, and 16 indicate an increase

in the proportion of zero-exchange events in older women, a trend that is in agreement with the fundamental prediction of the production line hypothesis. As the production line hypothesis predicts that decreasing levels of recombination with maternal age are the result of decreasing levels of recombination in the aging fetal ovary [Henderson and Edwards 1968], it will be interesting to see if levels of recombination for trisomies 13, 15, and 16 show the corresponding predicted decrease in recombination with fetal age that is reflected in trisomies from aging women. It is possible that fetal recombination levels will remain stagnant, and it is simply the ability to deal with certain types of exchanges that varies with age for some, or all chromosomes.

Previous studies of trisomy 15 revealed a trend quite different from that seen for our sample. A report by Robinson et al. [1998] found that the proportion of zero-exchange events decreased with maternal age. In contrast, our results suggest that there is no difference, or perhaps even an *increase*, in the proportion of zero-exchange events with increasing maternal age. The reason for the difference between these two data sets is unknown. The majority of the Robinson data set was composed of cases of uniparental disomy, nevertheless, the meiotic bases of these cases should be similar.

In contrast to trisomies 13, 15, and 16, only trisomy 21 shows clear agreement with the two hit hypothesis; recombination levels are unaffected by maternal age for this chromosome [Lamb et al. 2005]. As trisomy 21 is the only trisomy with a large sample size, it will be interesting to see whether increasing sample size for other trisomies will allow them to conform with the two hit hypothesis.

Finally, trisomies 18, 22, and the sex chromosome trisomies show an apparent decrease in the proportion of zero-exchange events with age, a trend in disagreement

with both the two hit and production line hypotheses. It seems likely that for these trisomies, the contribution of a yet to be identified maternal age-related factor is the primary determinant, as older women are often unable to properly segregate even those chromosomes with exchanges.

Many studies have demonstrated the importance of both recombination and maternal age-related effects in the nondisjunction of human trisomies [for example, see Hassold and Hunt 2001]. Based on our study, showing chromosome-specific differences in maternal age-related recombination, it appears that the importance of each of these factors independently, and the effect of unidentified age-related "factors" on aberrant recombination, is highly variable and chromosome-specific, thus further reinforcing the chromosome-specific nature of nondisjunction.

Figure 1. Approximate locations of DNA markers used in this study for previously unpublished chromosomes.

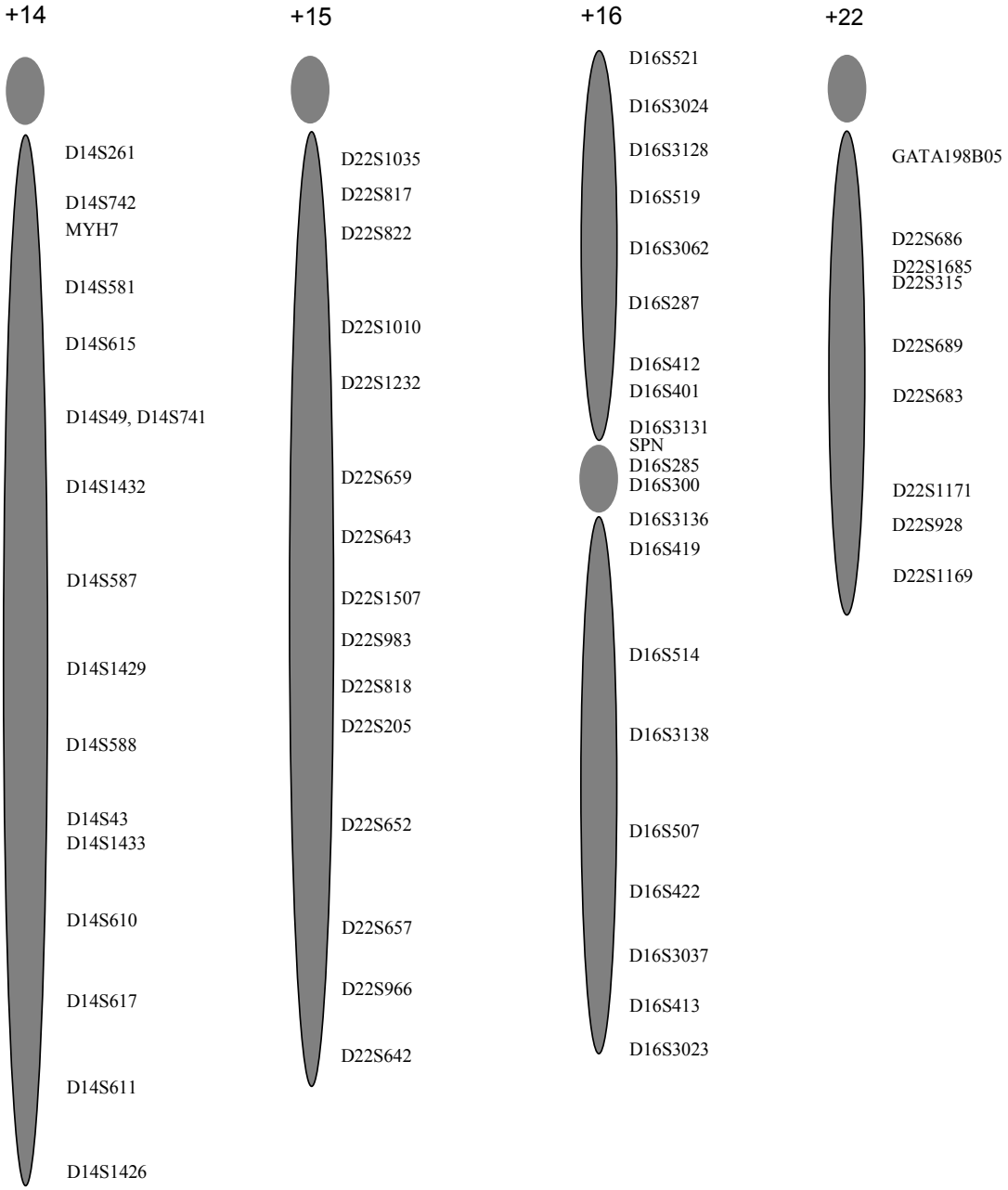
+14
D14S261
D14S742
MYH7
D14S581
D14S615
D14S49, D14S741
D14S1432
D14S587
D14S1429
D14S588
D14S43
D14S1433
D14S610
D14S617
D14S611
D14S1426
+15
D22S1035
D22S817
D22S822
D22S1010
D22S1232
D22S659
D22S643
D22S1507
D22S983
D22S818
D22S205
D22S652
D22S657
D22S966
D22S642
+16
D16S521
D16S3024
D16S3128
D16S519
D16S3062
D16S287
D16S412
D16S401
D16S3131
SPN
D16S285
D16S300
D16S3136
D16S419
D16S514
D16S3138
D16S507
D16S422
D16S3037
D16S413
D16S3023
+22
GATA198B05
D22S686
D22S1685
D22S315
D22S689
D22S683
D22S1171
D22S928
D22S1169

Figure 2. Relative frequency of zero-exchange and exchange events among maternal meiosis I errors in women <35 and ≥35 years of age. For trisomies 13, 15, and 16, there is an increase in zero-exchange events with age. Trisomy 22 shows no change in recombination levels with age, and trisomies 18, 22, and the sex chromosome trisomies show a decrease in zero-exchange with age.

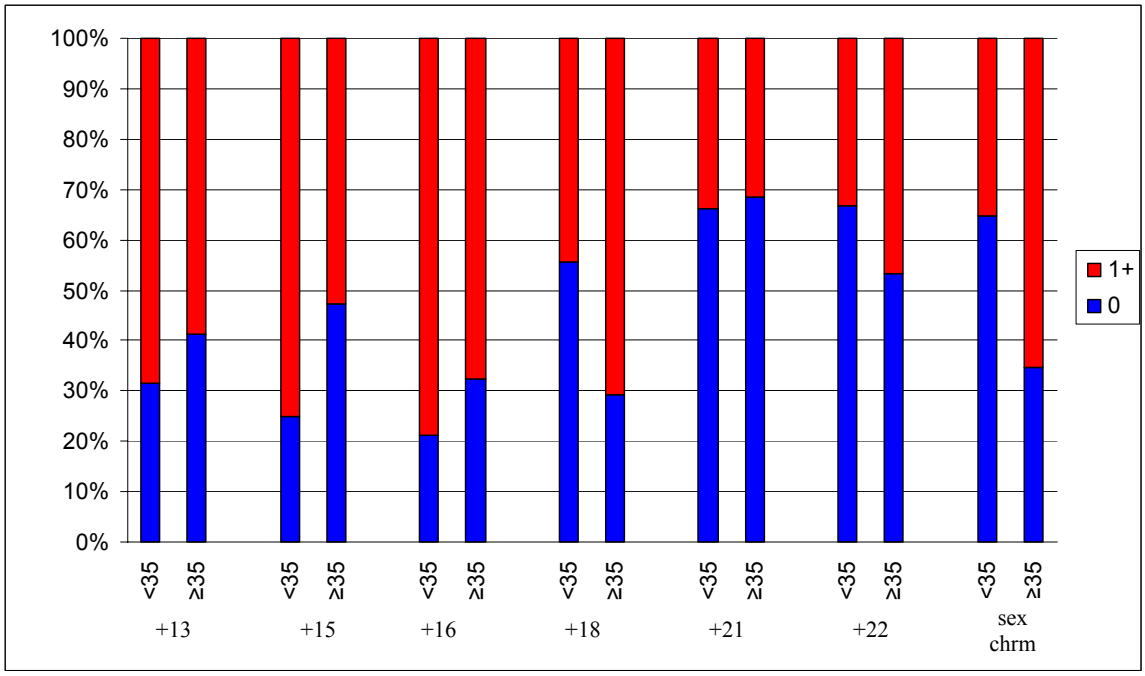
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
1+
0
<35
≥35
+13
+15
+16
+18
+21
+22
sex chrm

Table 1. Summary of new and previously published data sets used in this study

Trisomy	N	Publications
+13	81	Hall et al. [2007a], in press, and unpublished observations
	82	Bugge et al. [2007]
+14	12	Zaragoza et al. [1994]*
	22	Current study
+15	34	Zaragoza et al. [1998]*
	70	Current study
+16	62	Hassold et al. [1995]
	144	Current study
+18	161	Bugge et al. [1998]
+21	400	Lamb et al. [2005]
22	120	Hall et al. [2007b], in press
	29	Current study
XXX/XXY	140	Thomas et al. [2001]

*These studies informative for origin only, not recombination

Table 2. Summary of parental and meiotic origin of human trisomies*

		Maternal		Paternal		
	N	MI(%)	MII(%)	MI(%)	MII(%)	PZM(%)
13	163	49.3	39.5	0.8	5.8	4.6
14	34	42.6	42.6	3.7	7.4	3.7
15	104	79.8	8.1	3.7	7.3	1.1
16	206	91.9	4.1	3.4	0.0	0.6
18	161	33.5	59.0	0.0	0.0	7.5
21	907	69.7	23.6	1.7	2.3	2.7
22	149	86.5	9.9	1.8	0.0	1.8
XXX	50	63.0	17.4	0.0	0.0	19.6
XXY	224	25.4	15.2	50.9	0.0	8.5

*Data on +13 from Hall et al. [2007a], in press and Bugge et al. [2007]; +14 from Zaragoza et al. [1994] and current study; + 15 from Zaragoza et al. [1998] and current study; +16 from Hassold et al. [1995] and current study; +18 from Bugge et al. [1998]; +21 from Freeman et al. [2007]; +22 from Hall et al. [2007b], in press and current study; XXX from MacDonald et al. [1994]; and XXY from Thomas and Hassold [2003].

Table 3. Observed frequency of recombination events for human trisomies

		<35		≥35		
Trisomy	n	0	1+	0	1+	p-value
+13	64	11	24	12	17	0.41
+14	3	0	0	0	3	N/A
+15	27	2	6	9	10	0.28
+16	97	14	52	10	21	0.24
+18	42	10	7	8	17	0.08
+21	400	175	89	93	43	0.67
+22	45	10	5	16	14	0.39
XXX/XXY	63	24	13	9	17	0.02

CHAPTER FIVE

CONCLUSIONS AND FUTURE DIRECTIONS

The studies presented in the previous chapters had two main objectives: first, to provide insight into the origin of nondisjunction for human trisomies and second, to examine the role of aberrant recombination and maternal age in nondisjunction. This chapter summarizes this body of work and discusses approaches to address the many questions that remain about human aneuploidy.

Summary and conclusions

The studies of trisomy 13 presented in Chapter Two explored the parental and meiotic/mitotic origin of this trisomy and whether, as for other chromosomes, aberrant recombination plays a role in nondisjunction of chromosome 13. Trisomy 13 is one of the only trisomies compatible with birth, and yet relatively little information has been available on nondisjunction of chromosome 13. For our study population, the majority of trisomy 13 nondisjunctional errors occurred during maternal meiosis I, as has been seen for most other chromosomes [Hassold et al. 1995; Hassold and Sherman 2000; May et al. 1990; Robinson et al. 1998; Zaragoza et al. 1998]. Surprisingly, a large number of errors also occurred during the second division of maternal meiosis. This level of meiosis II nondisjunction has never been seen for any other acrocentric chromosome and almost no nonacrocentric chromosome, thus distinguishing nondisjunction of chromosome 13 from other chromosomes [Bugge et al. 1998; Hassold

et al. 1995; Hassold and Sherman 2000; May et al. 1990; Robinson et al. 1998; Thomas and Hassold 2003; Zaragoza et al. 1998].

Aberrant recombination predisposed to nondisjunction for chromosome 13. Previous studies of human trisomies, particularly trisomy 21, have shown that if a chromosome does not have enough recombination, or when recombination is too proximal or distal to the centromere, chromosomes are more likely to nondisjoin [Bugge et al. 1998; Hassold et al. 1995; Lamb et al. 1997; Lamb et al. 1996; Robinson et al. 1998; Thomas et al. 2001]. Unfortunately, the size of our study population precluded detailed analysis of exchange placement in trisomy 13. However, when examining overall recombination levels, the number of achiasmate tetrads for nondisjoined chromosomes 13 was more than twice that seen for normally disjoining chromosomes 13. Thus as for other chromosomes, aberrant recombination contributes to nondisjunction of chromosome 13.

Chapter Three explored the origin of nondisjunction of chromosome 22 and the role of aberrant recombination in nondisjunction of this chromosome. Despite being one of the most common trisomies in clinically recognized pregnancies, little information was previously available on the errors in meiosis or mitosis that lead to trisomy 22. Previous studies in our laboratory included fewer than 45 cases, and most of these cases were uninformative for stage of meiotic origin as they were studied before the human genome project had generated densely mapped DNA polymorphisms [Zaragoza et al. 1994; Zaragoza et al. 1998]. Our current analysis of 130 trisomy 22 samples allowed for determination of the parental and meiotic origin of nondisjunction in most cases.

Accordingly, we found that the majority of nondisjunction of chromosome 22 occurs during the first division of oogenesis, and this was true regardless of maternal age.

The well-established relationship between recombination and nondisjunction led us to study the role of recombination in the genesis of trisomy 22. Approximately one quarter of all nondisjunction that occurred during the first division of maternal meiosis involved achiasmate tetrads, indicating that failure to recombine is a major contributing factor to nondisjunction of chromosome 22. There was, however, no obvious difference in the placement of exchanges between nondisjoined and normally disjoining chromosomes.

In our analyses of trisomy 22, we also conducted a comparative study of the origin of all human trisomies. Interestingly, it appears that the mechanism(s) leading to trisomy may be shared among the acrocentric chromosomes, as trisomies 13 (studied in Chapter Two), 14, 15, 21 and 22 all show similar parental and meiotic origins of nondisjunction [Hassold and Sherman 2000; Zaragoza et al. 1998]. For all of these chromosomes, nondisjunction typically occurs during the first division of maternal meiosis, but rarely occurs during paternal meiosis or post-zygotically during mitosis. Further, achiasmate tetrads predispose to nondisjunction for each of these chromosomes. In contrast, nonacrocentric chromosomes show more variation as they do not all share one predominant parental/meiotic origin and the role of achiasmate chromosomes varies [Bugge et al. 1998; Hassold et al. 1995; May et al. 1990; Thomas et al. 2001; Thomas and Hassold 2003].

These studies indicate that while some feature of nondisjunction may be shared by groups of chromosomes, there are still many chromosome-specific factors which

affect nondisjunction. It is essential to acknowledge that each chromosome behaves differently in terms of nondisjunction and thus, a complete picture of nondisjunction can only be fully realized by studying each individual chromosome to determine how it behaves in meiosis.

In Chapter Four, one method to test models of maternal age and recombination was presented. Only two models of human aneuploidy currently exist which purpose to explain how these two factors may be related. The two hit hypothesis predicts that because recombination is established in the fetal ovary, levels of recombination will not vary with maternal age. Rather it will be other maternal age-related factors acting on aberrant recombination configurations that will cause older women to have higher levels of nondisjunction. Alternatively, the production model proposes that there is a gradient in the fetal ovary that causes recombination levels to decrease with fetal age. Studies have shown that the order of cell entry into meiosis in the fetal ovary reflects the order of cells ovulated after puberty. Consequently, if recombination levels decrease with fetal age, they will also decrease with maternal age. To test the predictions of these models we examined levels of recombination in mothers of trisomic conceptions to determine if recombination levels are independent of maternal age (as per the two hit hypothesis), decrease with maternal age (as in the production line hypothesis), or increase with maternal age. After analyzing new and previously reported data on maternal meiosis I- derived trisomies 13, 14, 15, 16, 18, 21, 22, and the sex chromosome trisomies, it appears that the effect of recombination on maternal age-related nondisjunction varies among different chromosomes. While most chromosomes show no significant difference in levels of recombination in younger and

older women, trisomy 18 and the sex chromosome trisomies indicate a possible increase in recombination in older women. One likely explanation for this observation is that older women have increased difficulty segregating these chromosomes, even when exchanges are present.

A visual analysis of the proportion of zero-exchange and exchange events in younger and older women indicated that even more variation may exist among chromosomes with some agreeing with the prediction of the production line hypothesis (+13, +15, and +16), some supporting the two hit hypothesis (+21), and some showing a trend inconsistent with either hypothesis (+18, +22, and the sex chromosome trisomies). These data further reinforce the chromosome-specific nature of human nondisjunction and our lack of understanding about what makes different chromosomes behave differently during meiosis.

Future directions

Despite the fact that human trisomies have been studied for decades, relatively little is known about why humans make so many meiotic errors. By extending the studies described in the previous chapters, we can more fully understand the chromosome-specific patterns of nondisjunction seen in human trisomies. The following sections will focus on four key questions of human nondisjunction: 1) What is the chromosome-specific role of recombination in human nondisjunction? 2) What features make some chromosomes more prone to nondisjunction than others? 3) What other methods can be used to test models of human aneuploidy to examine the relationship

between recombination and maternal age? 4) How is cohesion involved in maternal age-related aneuploidy?

Understanding recombination and human nondisjunction

While the studies presented in this thesis have provided valuable information on nondisjunction of several human trisomies, they are limited by the number of available cases. As a consequence, studies examining recombination have only been extensively conducted for trisomy 21 [Lamb et al. 1997; Lamb et al. 1996; Lamb et al. 2005]. Unfortunately, one glaring truth of human nondisjunction is that it is extremely chromosome-specific. Thus we cannot assume that the relationship between maternal age and recombination for chromosome 21 is true for all other chromosomes. For example, trisomy 21 shows a "normalizing" recombination pattern with age; i.e., in trisomy 21 involving older mothers, "normal" patterns of exchange placement are seen, while aberrant patterns are mostly observed in younger mothers of trisomy 21 cases [Lamb et al. 2005]. However, detailed studies of other trisomies are required to determine whether this pattern extends to other chromosomes. Since the relationship between *levels* of recombination and maternal age is chromosome-specific (Chapter 4), it is likely that studies on *placement* will reveal chromosome-specific patterns different than those described for chromosome 21. Chromosome 21 is a small, acrocentric chromosome which is typically held together by only 1-2 exchanges. Thus, age may play a different role in the placement of exchanges for this small chromosome than for larger chromosomes that are typically held together by more exchanges.

In addition to furthering the studies of previously examined chromosomes, better methods will be needed to detect and examine those chromosomes that nondisjoin less often or terminate too early in pregnancy to be clinically recognized. While it is true that many trisomies are seen less often because they are lost early in pregnancy [Hassold and Jacobs 1984], egg and sperm studies reveal that this is not the only reason some trisomies are seen more commonly in pregnancies than others; some chromosomes do indeed nondisjoin more often than others [Pellestor et al. 2002; Williams et al. 1993]. While studying these additional chromosomes may be useful in gaining a complete picture of chromosome-specific differences in the origin of nondisjunction, it will be even more important to examine the features that cause these chromosomes to nondisjoin less often than other chromosomes. Perhaps chromosomes that nondisjoin less often than those currently studied will show more extreme or different patterns of recombination, helping to unveil the complex relationship between recombination and nondisjunction.

Chromosome-specific nondisjunction

In addition to recombination, what other possible differences might exist among chromosomes that make them nondisjunction “prone” or nondisjunction “resistant”? For example, trisomies 13 and 18 are currently the only chromosomes that show a high proportion of nondisjunctional errors occurring during meiosis II [Bugge et al. 1998]. What similarities do these chromosomes share that make them vulnerable to nondisjunction in meiosis II while most other chromosomes usually segregate normally during this division? Given that meiosis II is essentially a haploid mitosis, it will be

interesting to determine if these chromosomes are also more prone to nondisjunction during mitosis than other chromosomes. For example, fluorescence in situ hybridization could be used to examine nondisjunction of these chromosomes in human cell lines.

One essential chromosome to study is chromosome16. This extremely error prone chromosome is seen in over 1% of all clinically recognized pregnancies [Hassold and Jacobs 1984]. What is it about this chromosome that makes it missegregate so often during meiosis? One unique feature of chromosome 16 is that it has a higher amount of repetitive sequence that most other chromosomes [Martin et al. 2004]. Might this somehow affect proper pairing and segregation of this chromosome? Would chromosomes engineered to contain more repetitive sequence, like chromosome 16, also be prone to nondisjunction. Another possible candidate in chromosome 16 nondisjunction is the centromere. Proper establishment of the kinetochore at the centromere and subsequent formation of a bipolar spindle are essential to ensure proper chromosome segregation [reviewed in Moore and Orr-Weaver 1998]. Thus, further analysis of the chromosome 16 centromere may help to explain why this chromosome is so prone to nondisjunction. Unfortunately the chromosome 16 centromere has yet to be fully sequenced. Hopefully, when this sequence become available, a key to the nondisjunction of chromosome 16 will finally be revealed.

One key to further understanding chromosome-specific differences in nondisjunction in humans is to examine possible chromosome-specific differences in nondisjunction in other species. While model organisms carrying certain mutations show variable frequencies in the rate at which different chromosomes nondisjoin [Gethmann 1984; Hugerat and Simchen 1993], chromosome-specific rates of

spontaneous nondisjunction have only been observed in humans. A study of chromosome-specific differences in nondisjunction in nonhuman primates has never been conducted and would be a major step in understanding why some chromosomes are more likely to nondisjoin than others.

Other methods to examine models of maternal age

This thesis presented one method to examine the relationship between recombination and maternal age. However, many additional tests need to be conducted to fully understand the maternal age effect. Mouse models provide one valuable tool for studying maternal age. An important question regarding recombination is how exactly susceptible exchanges form in relation to maternal age? Are susceptible exchanges established in some temporal order in the fetal ovary that reflects how they are seen in aging women (as predicted by the production line hypothesis [Henderson and Edwards 1968]) or is all recombination established irrespective of fetal age and maternal age itself more directly affects how many susceptible exchanges are seen in younger versus older women (as in the two hit hypothesis [Hassold and Sherman 2000]). In addition to the approach described in Chapter Four, the mouse can be used to directly examine recombination in the fetal ovary. It has already been shown that those cells that enter meiosis early in the fetal ovary will be ovulated in younger women while those that enter meiosis later will be ovulated in older women [Hirshfield 1992; Polani and Crolla 1991]; however, it has never been possible to directly determine whether there is a gradient in the fetal ovary that determines the level and placement of exchanges with fetal age, and consequently adult maternal age. Immunoflourescence analysis of MLH1, a protein

whose localization has been shown to correspond to sites of exchange [Baker et al. 1996], can be directly examined in ovaries from mice of varying fetal ages to test each of the above predictions: 1) do levels of recombination decrease with fetal age as predicted by the production line hypothesis [Henderson and Edwards 1968]; 2) do levels of exchange remain constant in the fetal ovary regardless of age as in the two hit hypothesis [Hassold and Sherman 2000]; or 3) might levels of recombination actually increase with age in the fetal ovary? Indeed a study from deCODE genetics found that in humans, higher recombination levels are seen in older women (though alternative explanations exist for this observation) [Kong et al. 2004]. Examining the localization of this protein in the mouse fetal ovary will finally allow for direct testing of this important question.

Several laboratories are currently undertaking studies to examine recombination in the human fetal ovary [Lenzi et al. 2005; Tease et al. 2002]. As these samples are collected from aborted fetuses, recombination analysis can be conducted on ovaries from fetuses of various ages. These analyses will be far more taxing than in mice, and complicated by inter-individual variation in human recombination levels [Kong et al. 2004; Tease et al. 2002]. However, examination of MLH1 in human fetal ovarian samples will allow testing of the above mentioned question in the human: Do recombination levels increase, decrease, or remain the same with fetal age?

The mouse can also be used for an additional test of the two hit hypothesis. The mouse is questionable as a model to study aneuploidy because meiotic errors are not nearly as common as in humans [Bond and Chandley 1983; Hassold and Hunt 2001]. If mice do not have comparable levels of aneuploidy, how can they be used as models of

human nondisjunction? In an attempt to recreate the human situation in the mouse, studies can be done to recreate the proposed "two hits" of human aneuploidy in a mouse model. To recreate the susceptible exchange, the proposed "first hit," an inversion mouse could be utilized. In an inversion heterozygote, exchanges are suppressed within the region of the inversion [Dresser et al. 1994; Gorlov and Borodin 1995]. Therefore, if an inversion involves all but the distal end of the chromosome, exchanges in a heterozygote will be displaced towards the telomere, recreating the "first hit" of human aneuploidy. One commonly projected "second hit" is age-related loss of cohesion leading to loss or "slippage" of exchanges and increased chromosome nondisjunction [Hodges et al. 2005; Warren and Gorringe 2006]. Thus lowering cohesion levels using heterozygous mice, RNAi or some other method will recreate the "second hit". It will be interesting to see if inversion heterozygote/cohesion deficient mice have increased levels of nondisjunction, recreating the "two hits" of human aneuploidy.

This same approach could additionally be used to study other possible "first hits." For example, work in yeast [Chambers et al. 1996] and more recently in the mouse [Koehler et al. 2006] has suggested that one possible contributor to human aneuploidy is sequence divergence between homologues. A cross between two inbred mouse strains with an estimated 1% sequence divergence, significantly decreased recombination (a proposed first hit of human aneuploidy) and increased nondisjunction to near-human levels. How might aneuploidy levels be further affected by the introduction of a "second hit" which alters levels of cohesion?

The role of cohesion in human aneuploidy

Studies by Hodges et al. [2005] of cohesion deficient mice implicate cohesion as the possible missing link in maternal age-related aneuploidy. Thus, future research needs to focus on how cohesion is maintained/lost over the years it can take to complete meiosis. A review by Warren and Gorringe [2006] suggested that heterozygosity for cohesin subunits could alter the threshold that determines how well "susceptible" exchanges will be tolerated. This implies that cohesion dosage may be important in determining how susceptible different individuals are to the "second hit" of the two hit hypothesis. The mouse can provide a useful model to study the dosage effect of different cohesion proteins. Several mouse knockouts of different members of the cohesin complex exist that can be used to knockdown cohesion individually or in combination to see effects on slippage of exchanges and increases in aneuploidy. Alternate methods like RNAi could also be employed to test the effectiveness of different cohesion dosages in maintaining proper chromosome segregation.

The mouse could further be used to test the importance of maintaining cohesion during dictyate arrest versus replacing cohesion that it is lost over time. Turnover of cohesion in the fetal ovary is not well understood. While cohesion is highly expressed during early prophase in the fetal ovary of the mouse, much lower levels are seen in ovaries from adult mice. Despite lower levels in the adult ovary, they remain consistent in mice from 1-6 months of age, suggesting that cohesion may be replaced in the adult ovary either during dictyate arrest or as the oocyte begins to grow around the time of ovulation [Hodges et al. 2005]. This raises the question whether the cohesion laid down in the fetal ovary is solely responsible for properly maintaining exchanges, or whether

additional cohesion needs to be added at a later time point to stabilize exchanges. A recent paper from the Nasmyth laboratory [Kudo et al. 2006] used Cre recombinase expressed from a zona pellucida promoter to delete a floxed gene specifically in the oocyte of the mouse. Many meiotic proteins (including some cohesion proteins) serve similar roles during mitosis, complicating the use of knock-out mice. Most essential to studies of meiosis, interfering with genes/proteins involved in mitosis will interrupt the mitotic divisions required to produce the germ cells for meiosis. The method of the Nasmyth laboratory [Kudo et al. 2006] circumvents this problem by allowing genes to be knocked out specifically in oocytes. This technique could be expanded to work in a temporal manner allowing the many subunits of the cohesion complex to be established in the fetal ovary, but then conditionally knocked out in the adult ovary, allowing for final determination of the importance of adding cohesion to stabilize sites of exchange.

In summary, many questions remain regarding why humans, particularly females, make so many errors during meiosis. Despite decades of studies on human nondisjunction, we know relatively little about what causes meiotic errors, apart from aberrant recombination and advancing maternal age. A better understanding of these and other factors that predispose to nondisjunction is essential for there to be any hope of one day fixing or preventing the meiotic errors that lead to the unfortunate loss of many pregnancies.

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