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Assessment of cis and trans-acting factors for involvement in the altered recombination patterns associated with Trisomy 21

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Abstract

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Altered recombination count and placement along chromosome 21 have been identified as risk factors for nondisjunction (NDJ) which causes Trisomy 21. Hence, we performed two studies to determine: 1. whether cis-acting factors (genomic features) differ between Trisomy 21 probands and controls at sites of recombination 2. whether trans-acting factors that regulate genome-wide recombination number are dysregulated in Trisomy 21 probands.

In our study of trans-acting factors, we sought to determine if genome-wide recombination patterns in MI error probands differed from that of normal individuals. Studies have found that there is significant correlation between the number of observed recombinants on a specific chromosome and the number of recombinants in the rest of the genome and this is called the "gamete effect". We stratified by meiotic outcome group (MI error, MI error siblings, and controls) and used linear regression models to test for the "gamete effect" in each group. We found strong evidence for a gamete effect among the autosomes of the normal meiotic outcome group as well as the MI siblings, but not in the MI error group. These data indicate two things: there is a disruption in the "gamete effect" across the autosomes of the MI error group and this disruption is oocyte-specific (it does not extend to their siblings).

In our second study, we sought to determine if cis-acting factors (GC content, CpG fraction, Poly (A)/Poly (T) fraction and gene density) or the number of hotspots in a genomic region are significant predictors of the proportion of recombination in bins across disjoined versus nondisjoined chromosomes 21. We used univariate linear regression to determine the relationship between the proportion of recombination in bins across 21q and the quantity of each of our variables of interest. Results from our analysis of normal meiotic events and MI errors showed, as expected, that recombination placement was correlated with hotspots on 21q. In contrast, among MII errors, correlation was not significant between the proportion of recombination and number of hotspots per bin.

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Chapter 1

Introduction

1.1 History

Prior to the late 1800s, individuals with Down Syndrome (DS) were grouped into the broad category of "imbeciles." This designation also encompassed what we now know to be autism, fragile X syndrome and various other disorders of intellectual development. Individuals born with DS were thought of as "unfinished children" and it was believed that DS was the result of a tuberculosis-stricken parent(Down, 1866). It was not until John Langdon Down classified intellectual developmental disorders that the syndrome was given a name and a clinical description. He noted that individuals with Down Syndrome had distinct physical characteristics which distinguished them from other individuals with intellectual disabilities(Down, 1866). He noted that their eyes were obliquely placed and distant, they had abnormally large tongues and their faces were flat and broad(Down, 1866). They also seemed to have similar mental capacities. Based on their slanted eyes, he erroneously concluded that the disease was the result of degenerate evolution to the "inferior" Mongolian race(Down, 1866). This misconception was perpetuated in the literature for many years as many clinicians and research scientist referred to these cases as Mongols or Mongoloids.

One of the first elucidated risk factors for DS was discovered using epidemiologic techniques. Lionel Penrose, a British geneticist, determined that

the incidence of Down Syndrome increased exponentially in women after age 30 (Penrose, 1933). He attributed this association to deterioration of ova over time. He noted that an age-related effect was not present in mothers aged 15-19 nor was there an evident age effect in cases of paternal origin. He suggested that there were two distinct groups of individuals with DS: those who resulted from an age-dependent mechanism and those who resulted from an age-independent mechanism(Penrose, 1954). He attributed the age-independent mechanism to various, complex etiologies that would require teasing apart in order for the disease to be fully understood.

Nearly a century after Langdon Down's clinical description of the syndrome, Waardenburg (an ophthalmologist) suggested that Down syndrome was the result of an extra chromosome (G. Allen, 1974). He never performed the research to determine if his hypothesis was true. In 1959, a French geneticist named Lejeune confirmed Waardenburg's theory by karyotyping (a technique used to fix chromosomes to a microscope slide) the genomes of individuals with Down syndrome (Jacobs, Baikie, Court Brown, & Strong, 1959; Lejeune, Gauthier, & Turpin, 1959; Lejeune, Gautier, & Turpin, 1959). He concluded that individuals with Down syndrome had an extra chromosome 21. Soon after this discovery, a group of geneticists at a World Health Organization meeting proposed a more politically correct and scientifically accurate name for the disease: Down's syndrome or Trisomy 21 (G. Allen et al., 1961). The "s" in Down's syndrome was later dropped. After the landmark discovery by Lejeune et al., over

two decade passed before the source of the extra chromosome 21 was associated with meiotic error.

1.2 Meiosis and gamete formation

Meiosis is a biological process in which germ cells divide in order to form gametes. These divisions require precise partitioning of cellular components to daughter cells. Failure to properly execute this process can result in infertility, loss of genomic integrity, and the production of abnormal offspring. One such failure occurs when chromosomes do not properly segregate during one of the two meiotic cellular divisions and this phenomenon is termed nondisjunction (NDJ). This section will outline the key features of normal meiosis and the differences between male and female meiosis.

Key Components of Meiosis

Meiosis is initiated after the DNA in the germ cell has been replicated. DNA replication results in two pairs of sister chromatids that are linked by a circular complex called cohesin (SMC1/ SMC3/REC8)(Bannister, Reinholdt, Munroe, & Schimenti, 2004; Revenkova et al., 2004; Xu, Beasley, Warren, van der Horst, & McKay, 2005). At this point, homologous chromosomes must be tethered together to ensure proper segregation into daughter cells. A component of this chromosome exchange process is homologous recombination, which allows for genetic variation of gametes. The first stage of meiosis (MI), Prophase I, is comprised of several sub stages (Leptotene, Zygotene, Pachynema, Diplotene and Diakinesis) that mark various steps of the chromosome exchange process.

During Leptotene, telomeres attach to the periphery of the inner nuclear envelope by interacting with the nuclear envelope proteins Sad1 /Unc84 (SUN) and Klarsichct/ ANC-1/ Syne-1 homology (KASH)(Fridkin, Penkner, Jantsch, & Gruenbaum, 2009; Penkner et al., 2009). The telomeres organize into loops at the nuclear periphery and this process is aptly called bouquet formation as the loops are said to resemble a bouquet of flowers (Chikashige et al., 1994; Scherthan, Bahler, & Kohli, 1994). Recruitment of telomeres to the nuclear envelope periphery is believed to aid in homologous chromosome pairing by bringing homologous sequences into close proximity (Penkner et al., 2009). At this time, a proteinaceous scaffold called the synaptonemal complex begins to form between homologous chromosomes. Axial elements (SYCP2 and SYCP3)(de Vries et al., 2005; Yang et al., 2006) associate with the sister chromatids of univalents and will later be linked by another synaptonemal complex protein(von Wettstein, Rasmussen, & Holm, 1984). In parallel, homologous recombination is initiated by SPO11 which introduces double- stranded breaks in the DNA (Maleki, Neale, Arora, Henderson, & Keeney, 2007; Romanienko & Camerini-Otero, 2000). Recombinase A related protein (RECA), DMC1, and RAD51 bind along the developing axial elements(Plug, Xu, Reddy, Golub, & Ashley, 1996).

At Zygotene, the axial elements of homologous chromosome are brought into close contact with each other and central elements form a link between them(von Wettstein et al., 1984). The axial element is now called a lateral element and homologues are now paired. Pairing extends across the homologues at which point the chromosomes are considered to be synapsed.

During Pachynema, homologue arms exchange genetic material. Once this has occurred, de-synapsis begins but the chromosomes remain connected at the recombination site(s). The visible manifestation of the recombination crossover is called a chiasma. The germ cells are then ready to proceed through the remaining meiosis I stages: Metaphase I, Anaphase I and Telophase I.

The microtubule organization center (MTOC) forms shortly after germinal vesicle formation. The microtubule organization center is brought into close contact with the condensed chromosomes(Vogt, Kirsch-Volders, Parry, & Eichenlaub-Ritter, 2008). A protein called γ-TuRC nucleates the polymerization of microtubules at the organization center (Taylor et al., 2007). During the metaphase to anaphase transition, the MTOC migrates to the oocyte cortex(Vogt et al., 2008).

Metaphase I is marked by the aligning of homologues along the metaphase plate. During Anaphase I, homologues are pulled to separate poles of the dividing cell after the cohesins between sister chromatids are phosphorylated and targeted for cleavage by securase. However, cohesions at the centromere of sister chromatids are spared from this fate by shugoshin (Sgo1) which recruits PP2A (a phosphatase that dephosphorylates the REC8 subunit of cohesin) (Clift, Bizzari, & Marston, 2009; Gregan, Rumpf, Li, & Cipak, 2008; Ishiguro, Tanaka, Sakuno, & Watanabe, 2010). At Telophase I, the nuclear membrane envelops each new diploid genome and this process results in two daughter cells, each containing a diploid genome.

The second stage of meiosis (MII) mimics mitosis with a few exceptions. Prophase II marks the condensation of chromosomes and the breakdown of the nuclear envelope. During Metaphase II, sister chromatids align on the metaphase plate. The centromere is cleaved as well as the REC8 subunit of the cohesin complex which allows the sister chromatids to be pulled to opposite poles during Anaphase II (Katis et al., 2010). Telophase II is marked by uncoiling of the chromosomes and reforming of the nuclear envelope followed by either cell cleavage or cell wall formation. This results in four spermatocytes in males or three polar bodies and one oocyte in females. There are several additional differences in the meiotic process between males and females. These differences will be outlined in the following section.

Male versus Female Meiosis

One of the hallmarks of the human meiotic process is the difference in duration and outcome of meiosis between males and females. Spermatogenesis in males begins in an embryo with a gonocyte which is a primordial germ cell that goes through mitosis to develop into spermatozoa. This process begins 10-13 years after birth in humans(Dym, Kokkinaki, & He, 2009). The gonocytes develop into spermatogonial stem cells, which are stem cells that produce more gonocytes, or spermatogonia. Spermatogonial stem cells are self-renewing and this allows for continued sperm production throughout the reproductive lifespan of a male (puberty up until death) (Yoo, Lim, Ko, Lee, & Kim, 2010). When the male reaches prepubescence, the spermatogonia begin to divide mitotically (Caires, Broady, & McLean, 2010). During puberty, these cells undergo the first and

second meiotic divisions in succession, producing a primary and secondary spermatocyte, respectively(de Kretser, Loveland, Meinhardt, Simorangkir, & Wreford, 1998). An additional mitotic division yields mature, elongated spermatids. This process takes about 64 days (Heller & Clermont, 1963).

Human female gametogenesis is characterized by meiotic pauses, less gametes per meiotic event, a limited oocytes pool and elevated formation of chiasmata. Oogenesis begins in the fetus at around two months post conception (Gondos, Westergaard, & Byskov, 1986). Mitotic proliferation starts around this time and continues until around month seven post conception and germ cells that initiate this phase are considered to be oogonia. These divisions result in approximately seven million oogonia but approximately 80% of this population is lost during periods of atresia or apoptotic cell death (T. G. Baker, 1963). Meiosis begins around two months post conception in germ cells that have become oogonia (T. G. Baker, 1963). Prophase I results in paired homologues that synapse and exchange genetic information and oogonia that have reached this stage are now considered to be primary oocytes (B. S. Baker, Boyd, et al., 1976). However, the diplotene stage is arrested during a prolonged stage termed dictyate. Oogonia that have not entered prophase I by seven months post conception undergo atresia (T. G. Baker, 1963). Primary oocytes, may also undergo atresia during the pachytene or diplotene stage of Prophase I. Hence, atresia causes loss of both oogonia and oocytes at different stages of oogenesis (T. G. Baker, 1963).

Oocytes cannot self-renew as does spermatocytes. At birth, a human female will have two million germ cells of which 400,000 survive to puberty and 500

will be ovulated (T. G. Baker, 1963). Meiotic arrest of the oocyte continues until it is recruited for ovulation (approximately 10-40 years depending on an individual's age of menses and menopause)(Eichenlaub-Ritter, 1998). Luteinizing hormone levels mount during ovulation, stimulating the primary follicles (primary oocyte surrounded by granulose cells) to mature and resume meiosis. Chromosomes condense and align with the metaphase plate during Metaphase I and segregate to opposite poles at Anaphase I. Cellular division results in two daughter cells: one daughter cell forms the functional germ cell while the other becomes meiotic wastage called a polar body. Meiosis II is initiated immediately with spindle formation and alignment of sister chromatids at the spindle equator. Once again, meiosis is arrested and is not resumed until fertilization. Meiosis will resume if the oocyte, which is now considered a secondary oocyte, is fertilized by a sperm. Fertilization triggers completion of MII, which results in an oocyte and a second polar body.

1.3 Nondisjunction

Nondisjunction and Human Reproduction

Nondisjunction is the failure of homologous chromosomes or sister chromatids to separate during cellular division (Figure 1.1). This event can occur during Meiosis I (MI error), Meiosis II (MII error), or Post Zygotic Mitosis (PZM). These errors result in aneuploidy or gametes with too many or too few chromosomes. This section will provide an overview of the impact of nondisjunction on human reproduction and studies that have provided insight into its etiology.

Common themes among different types of nondisjunction

Nondisjunction is purported to occur in at least 5% of clinically recognized pregnancies (Angell, Sandison, & Bain, 1984; T. Hassold et al., 1996; T. Hassold & Hunt, 2001; Warburton & Fraser, 1964) and accounts for approximately 35% of spontaneous abortions (T. Hassold & Hunt, 2001). Notably, nondisjunction occurs at a significantly higher rate in oocytes than in sperm. Nondisjunction occurs in approximately 1-2% of sperm (T. J. Hassold, 1998) and 20-25% of oocytes (Jacobs, 1992; Marquez, Cohen, & Munne, 1998).

The initial studies of aneuploidy and nondisjunction focused on characterizing trisomy which is the presence of a third copy of a chromosome in an organism that is normally disomic. This particular type of chromosomal abnormality is compatible with live birth while monosomies almost always spontaneously abort. Trisomies that are compatible with live-birth include trisomy 8, 13, 16, 18, 21, 47XXX and 47 XXY(Canfield et al., 2006; Goldstein & Nielsen, 1988; T. Hassold, Hall, & Hunt, 2007; Riccardi, Hassler, & Lubinsky, 1977; Warkany & Rubinstein, 1962; Wolstenholme, 1995) (although trisomy in aborted fetuses have been reported to occur in additional chromosomes). Infants that are diagnosed with trisomies 8, 13, 16 and 18 are typically mosaics (contain two different populations of somatic cells of which only one is trisomic) which means that nondisjunction occurred during mitosis (Nicolaidis & Petersen, 1998). However, a majority of infants diagnosed with trisomy 21 have full trisomy (all somatic cells are trisomic for chromosome 21)(E. G. Allen et al., 2009).

Among clinically recognized autosomal trisomies, maternal meiotic error accounts for a majority of NDJ events (Hall et al., 2007). Among the most common autosomal trisomies in live births (13, 18 and 21) this figure is upwards of 90% (Hall et al., 2007). Specifically in Trisomy 21, maternal MI error, maternal MII error and PZM accounts for ~69.6% , ~23.6% and 2.7% of cases, respectively (Hall et al., 2007; T. Hassold et al., 2007). Maternal meiotic error is prevalent in trisomies 8, 15 and 16 as well (Nicolaidis & Petersen, 1998)(Figure 1.2). Hence, maternal meiotic error is the leading cause of nondisjunction and aneuploidy in humans. It has been suggested that the temporal differences between female and male gametogenesis explain the disparate rate of meiotic error between the two counterparts.

Infants born with these trisomies have moderate to severe intellectual development disabilities, physical defects and a shortened lifespan. Infants with Trisomy 18 and 13, which are the second and third most common trisomies in live births, respectively, rarely live beyond the first year of life (Nicolaidis & Petersen, 1998). As Trisomy 21 is the most common trisomy in live births (1/700) (Canfield et al., 2006), it is more commonly used in studies of nondisjunction.

Nondisjunction Etiology: Determining a molecular mechanism

As was previously mentioned, there are key differences between oogenesis and spermatogenesis. One of these differences may help explain the elevated rates of nondisjunction during oogenesis. A prolonged meiotic process has been identified as a potential underlying reason for age-dependent mechanisms of

NDJ. One of the leading theories suggests that the oocytes that are ovulated later in a female's reproductive life time suffer from protein degradation of components of the cohesin complex. This would explain malsegration of chromosome during MII and the observation that this error type is six times more susceptible to age-related malsegregation (Yoon et al., 1996).

As for MI, lack of recombination along the nondisjoined chromosomes explains a large proportion of nondisjunction in this group (Lamb et al., 1997; Warren et al., 1987). In addition, altered recombination placement has been associated with MI as well as MII error. Recombination-related risk factors will be deferred until the discussion on recombination (see section 1.4, page 22); Hence, this section will focus on the nonrecombination-related mechanisms related to MII errors.

With regard to MII error, nondisjunction is associated with the loss of the proteins that maintain sister chromatid cohesion. A study performed in *Schizosaccharomyces pombe* found that a Shugoshin (Sgo1) is a protector of cohesin Rec8 and prevents cohesin cleavage during MI (Kitajima, Kawashima, & Watanabe, 2004). Depletion of Sgo1 through RNA interference allows homologous chromosomes to segregate properly during MI but leads to random segregation of chromatids during MII (Tang, Sun, Harley, Zou, & Yu, 2004). This study also found that Sgo1 interacts with Bub1, a conserved centromere-associated kinase that localizes Sgo1 to centromeres (Tang et al., 2004).

A study in humans found that older women that have had a trisomic 21 child due to an MII error have greater telomere loss than women of a similar age that

have had a normal meiotic event or an MI error (Ghosh, Feingold, Chakraborty, & Dey, 2010). It was suggested that these women are "genetically older" (Ghosh et al., 2010) than their age-matched controls and this occurrence is in some way related to meiotic NDJ. However, this theory does not explain the increased rate of NDJ in oocytes when compared to spermatocytes, as males are also subject to telomere loss as they advance in age. To date, there has not been any published evidence that shows that an increased rate of telomere loss is specific to females.

1.4 Homologous Recombination and Nondisjunction

As recombination is the process that leads to chiasma formation and stable chiasmata are associated with proper chromosome segregation, features of recombination have been attractive candidates for studies in NDJ. Additionally, genome-wide recombination rates are significantly higher in females than males (Chowdhury, Bois, Feingold, Sherman, & Cheung, 2009; Kong et al., 2002) which may be indicative of a difference in recombination pathway regulation. To explain the various epidemiologic features of oocyte NDJ, such as advanced maternal age and disparate NDJ rates between oocytes and sperm, it has been suggested that altered recombination may be the initiating event. These studies have resulted in several lines of evidence that suggest that the number and placement of recombination on the nondisjoined chromosome have an influence on chromosome segregation. This section will review: known features of the homologous recombination pathway, the features that are associated with normal recombination followed by the aberrant recombination patterns observed in Trisomy 21.

Homologous Recombination Pathway

Homologous recombination is the exchange of genetic material between homologous regions of the genome. Its proximate mechanism is to ensure that bivalents, or tethered homologous chromosomes, segregate into separate daughter cells. On evolutionary time scales, recombination can help speed the spread of advantageous mutations through populations via positive natural selection, while also aiding in the elimination of deleterious alleles through purifying selection. Mammalian homologous recombination begins during the pachytene stage of Prophase I. Homologous regions of chromosomes begin to align but are not yet paired. SPO11 induces double strand breaks (DSB) in the DNA that lead to the recruitment of repair machinery that recognizes this particular aberration in the DNA (Maleki et al., 2007; Murakami & Keeney, 2008; Romanienko & Camerini-Otero, 2000). The DNA is resected followed by localization of proteins RECA, DMC1 and RAD51 to the DSB site (Bishop, 1994; Bishop, Park, Xu, & Kleckner, 1992; Jensen, Carreira, & Kowalczykowski, 2010; Katis et al., 2010; Kitajima et al., 2004; Maleki et al., 2007; Plug et al., 1996; Xu et al., 2005). Complementary strands are pried apart and invade the adjacent homologous chromosome, annealing with complementary regions. If RNF212 is available at these sites, it may stabilize cross-overs which promotes chiasma formation (Reynolds et al., 2013). The regions where the strands have crossed over migrate along the chromosomes, resulting in an extended region of incorporation of the opposing homologous DNA. The bivalents (tethered homologous chromosomes) are maintained until Anaphase I. At this point, DNA

strands are cleaved and repaired, releasing the recombined homologues to opposing daughter cells.

Recombination: An incomplete puzzle

Homologous recombination is widely studied as its dysregulation leads to many phenotypic consequences. Studies in model organisms have revealed proteins involved in this pathway and their functions. An indication of a biological pathway's importance is conservation across taxonomic groups. In the case of homologous recombination, there is conservation in Eukaryotes and Prokaryotes. Amongst Eukaryotes, the conservation extends beyond the general process of recombination to the proteins involved in recombination. *Caenorhabditis Elegans* have orthologues to human proteins (MRE11, RAD51, DMC1, Spo11RNF212) that are similar to their counterparts in genetic sequence and protein structure (Bishop, 1994; Jensen et al., 2010; Maleki et al., 2007; Reynolds et al., 2013). Each of the aforementioned orthologous proteins are involved in a different aspect of the recombination process, showing similarity between these species at each step of the pathway. Additionally, studies in model organisms using knock out mutants have demonstrated that these proteins are functionally analogous to human proteins (Bishop et al., 1992; Maleki et al., 2007; Murakami & Keeney, 2008; Romanienko & Camerini-Otero, 2000; Rubin, Ferguson, & Holloman, 1994; Shinohara, Ogawa, & Ogawa, 1992). These findings have not only helped to better understand how the pathway is regulated, but also provide clues as to what may be causing abnormal recombination events. However, there are many limitations in current technology that hinder the discovery of

novel proteins. Many of the null mutations in mammalian recombination genes lead to embryonic lethality in offspring or sterility(Bannister et al., 2004; Revenkova et al., 2004; Watson, 1969; Xu et al., 2005). Furthermore, studies in non-mammalian organisms may miss genes that influence meiosis in humans. For this reason, studies in models systems are limited. Due to the importance of this pathway, as evidenced by its conservation and the consequences of its dysregulation, it is likely that there are many more genes that are yet to be discovered. Until there is a way to approach this problem, it will be difficult to identify and test new candidate proteins for their involvement in NDJ. Epidemiological studies may allow a more broad testing method to identify significant molecular contributors to NDJ. Identification of specific factors using molecular methods would then be warranted.

Genome-Wide Recombination (GWR): Features in meiotically normal individuals

Studies in recombination location and frequency have been performed in meiotically normal, control data sets. Considering the importance of recombination in gamete formation, it was expected that the number and placement of recombinations genome-wide would be tightly regulated. While there are similarities in the recombination patterns across a population, genome-wide recombination frequency and location are variable between individuals based on genetics, sex and ethnicity differences.

Recombination distributions across the genomes of different populations have been shown to cluster in regions of the genome called hotspots. Historically

defined hotspots have been identified using linkage disequilibrium data from the hap map study. These regions can also be identified by phasing genotype data from multi-generational pedigrees or molecularly by observing MLH1 foci in sperm typing studies. Characterization of these regions has shown that there are genomic features found to be associated with hotspots. For example, Kong et. al. found that regions of high sex-averaged recombination rates have elevated CpG content, low GC and Poly AT content and high gene density (Kong et al., 2002). Another studied found that 41% of these regions contain a 13 mer motif that does not confer hotspot activity, but can increases its activity if present (Myers, Freeman, Auton, Donnelly, & McVean, 2008). It can be deduced that sequence changes in these motifs or in the genes that code for proteins that interact with these regions may alter recombination frequency and location. Hence, linkage and association studies have been performed to identify genetic loci that explain differences in recombination frequency and placement between individuals.

Variation between male and female recombination profiles is most pronounced in genome-wide recombination rate. Cheung et. al. found the average genome-wide recombination rate in females to be 38.4 (range 27.5 - 46.4; SD 5.3) and 24 (range 16.9-28.9; SD 2.7) in males in the CEPH data set (Cheung, Burdick, Hirschmann, & Morley, 2007). Comparable studies had similar findings in the average genome-wide recombination rates and there was congruence in the female to male ratio of genome-wide recombinants (1.6) (Broman, Murray, Sheffield, White, & Weber, 1998; Cheung et al., 2007; Kong et al., 2002). Additionally, it has been shown that recombination is elevated at the telomeric

portion of chromosomes in males and is interspersed in females (Broman et al., 1998; Kong et al., 2002). These difference may be due to a difference in recombination regulation between females and males (Chowdhury et al., 2009).

Several studies have noted that at the individual level, the number of recombinations observed genome-wide between a person's gametes is significantly different between individuals (Cheung et al., 2007; Chowdhury et al., 2009; Kong et al., 2002; Kong et al., 2010). Analysis of variance (ANOVA) was used to show that despite differences in recombination rates between an individual's gametes, these rates were more similar between sibling gametes than across gametes from different mothers (Cheung et al., 2007; Kong et al., 2002). This suggests that variation between mothers can be explained by some inherent genetic characteristic or an environmental factor. Additionally, a study found correlation of the number of recombinants among chromosomes within a gamete and they termed this phenomenon the "gamete effect" (Kong et al., 2002). The aforementioned studies suggest that there is a trans-acting factor that can regulate recombination counts by acting across gametes of the same mother or across chromosomes within a gamete.

Factors associated with Variation in Recombination

Several studies have identified genomic loci and genes that contribute to variation in recombination frequency and placement. Alleles in *RNF212* were the first to be associated with gamete-wide recombination counts in both males and females; This result was confirmed in a subsequent study (Chowdhury et al., 2009; Kong et al., 2008). A molecular study of RNF212 in mice found that this

protein is essential for crossing over and works by stabilizing recombination proteins(Reynolds et al., 2013). A heterozygous mutation in this gene leads to reduced recombination(Reynolds et al., 2013). Hence this gene is believed to work in a concentration-dependent manner where mutations that lead to haploinsufficiency cause a reduction in genome-wide recombination. An inversion at genomic region 17q21.31 was also found to be associated with elevated recombination as well as increased fecundity (Stefansson et al., 2005) in carriers versus noncarriers (Chowdhury et al., 2009; Stefansson et al., 2005).

Currently, the most widely studied gene that relates recombination variation with a genetic component is PRDM9. PRDM9 is a zinc finger protein that has been shown to have three protein domains, one of which can interact directly with DNA and does so in a sequence-specific fashion (Axelsson, Webster, Ratnakumar, Ponting, & Lindblad-Toh, 2012; Baudat, Buard, Grey, & de Massy, 2010; Baudat, Buard, Grey, Fledel-Alon, et al., 2010; Berg et al., 2010a; Berg et al., 2011). It contains variability in both the number of zinc finger repeats and the DNA sequence within the repeats and this variation has been shown to lead to differential hotspot usage (Baudat, Buard, Grey, Fledel-Alon, et al., 2010; Berg et al., 2010b; Berg et al., 2011).

Altered recombination patterns associated with Trisomy 21

Recombination studies in meiotically normal individuals have provided information on recombination characteristics (average recombination counts per chromosome and per gamete as well as the distribution of recombination along chromosomes) that are associated with proper chromosomes segregation. These

properties include: the occurrence of at least one recombinant per chromosomes arm (T. Hassold et al., 2004; Pardo-Manuel de Villena & Sapienza, 2001) and recombinants that are medially placed along the chromosome arm (Cheng et al., 2009). Studies in NDJ have used these findings to determine if individuals with a meiotic error display recombination patterns that deviate from normal recombination events.

MI errors

Studies that have examined recombination count and placement along nondisjoined chromosomes 21 in humans have identified differing patterns for individuals with MI errors when compared to meiotically normal individuals. With regard to recombination count, a study sought to determine if MI errors are the result of premature unpairing of chromosomes or failure to pair, both of which would prevent recombination and chiasma formation (Warren et al., 1987). It was shown that there is reduced recombination on chromosomes that nondisjoin during MI and this indicates a failure in chromosome pairing (B. S. Baker, Carpenter, Esposito, Esposito, & Sandler, 1976; Warren et al., 1987). It is estimated that at least 45% of MI errors are achiasmate which is in sharp contrast with the estimate for normal meiotic events (0-20%)(Lamb et al., 1997; Oliver et al., 2008).

A pilot study examined the number of cell-wide recombinants in oocytes that had an MI error and no observed recombination along the nondisjoined chromosome 21. It showed that an absence of recombination on chromosome 21 predicted a reduced number of cell-wide recombinants in these oocytes(Brown,

Feingold, Broman, & Sherman, 2000). The control data, which consisted of oocytes that were meiotically normal, also displayed a linear correlation between chromosome 21 recombination counts and cell-wide recombination counts. This phenomenon was later found in larger control data sets and attributed to variation in a trans-acting factor that regulates cell-wide recombination counts (Cheung et al., 2007; Chowdhury et al., 2009; Kong et al., 2002). Correlation of recombination counts between chromosomes seemed to be characteristic of recombination in oocytes and not specific to trisomic oocytes. However, the MI error group appeared to have reduced genome-wide recombination when compared to controls.

From these studies, two alternative hypotheses emerged: 1. Reduced exchanges on chromosome 21 are related to a cell-wide decrease in exchanges that is a part of the normal variation of exchange rates. For the MI error group, this rate has fallen below a critical level where chromosome 21 fails to have an exchange. 2. The genome-wide recombination count among MI errors is abnormally low and is not a part of the normal variation of genome-wide recombinants counts. Instead, it represents dysregulated recombination due to genetic or environmental risk factors. As this study was a pilot study conducted with a small number of samples and non-parametric tests, confirmation using larger data sets is required to confirm these findings.

Studies in models systems support the theory that MI errors that result from achiasmate chromosomes 21 are the result of variation in a trans-acting factor that regulates cell-wide recombination. The Mei-S282 mutation in *Drosophila*

melanogaster shows a decrease in global recombination and increased rate of chromosomal nondisjunction during MI (Parry, 1973). Furthermore, nondisjunction events within a gamete were associated with an increase in achiasmate heterologous chromosomes within that same gamete(Parry, 1973). Similar phenotypes have been found in *Caenorhabditis elegans* that are *him-6/him-4* null in which genome-wide recombination was found to be decreased and nondisjunction rates increased in these mutants(Zetka & Rose, 1995).

With regard to recombination placement, studies have shown that maternal MI errors are associated with a single distal exchange (Lamb et al., 1997; Lamb et al., 1996). A mutation in Drosophila melanogaster gene called *nod* displays a similar phenotype. This mutation leads to impaired meiotic spindle assembly and function and malsegregation of chromosomes during MI (Hawley, Frazier, & Rasooly, 1994). This gene codes for a kinesin-like protein that is required for disjunction of chromosomes (Theurkauf & Hawley, 1992). The recombination profiles of these mutants are similar to those that are associated with MI nondisjunction where recombination is elevated at the distal region of the chromosome and reduced at the proximal region. Zwick et al. showed that there is variation in rates of nondisjunction, and that common alleles in the *nod* gene account for much of the variation in nondisjunction rates(Zwick, Cutler, & Langley, 1999).

MII errors

MII errors appear to be driven by different age and recombination traits: MII errors are associated with a recombination event near the centromere of 21q (Lamb et al., 1997) and this association increases with increasing age of the oocyte (Oliver et al., 2011). As recombination is an event that occurs during meiosis I, yet altered recombination is a risk factor for MII, it has been suggested that these events are initiated during MI and manifested during MII (Lamb et al., 1997). Nonetheless, mechanisms have been posed that may help explain the interaction between maternal age and recombination placement in this error group. First, a pericentromeric event may initiate a "two-hit" mechanism where age-related risk factors act additively or synergistically. Second, a pericentromeric event may protect against the age related risk factors associated with MI allowing for proper segregation of homologues, but not the age related risk factors associated with MII error. For nonrecombination-related risk factors that are associated with MII, please refer to section 1.3, page 20.

1.5 Trisomy 21: A model for Nondisjunction

Trisomy 21 is the most commonly found aneuploidy in live births and occurs at a rate of 1/700 live births in the United States (Canfield et al., 2006). Although there are subtle differences in this rate between ethnic groups, it has been suggested that these differences are confounded by differential access to prenatal screens which may influence the decisions related to pregnancy termination (Canfield et al., 2006). The majority of these events are the result of nondisjunction events occurring in oocytes. Maternal meiotic error accounts for 93.2% of Trisomy 21 cases while paternal error and post zygotic mitosis account

for 4.1% and 2.7%, respectively (Hall et al., 2007). These statistics have warranted more extensive study in the area of maternal meiotic error.

Amongst maternal errors, approximately 70% occur during MI and 30% in MII (Figure 1.2) (E. G. Allen et al., 2009; T. Hassold et al., 2007). Studies have shown that maternal MI error is associated with a telomeric exchange(Lamb et al., 1997; Lamb et al., 1996) or absence of an exchange(Warren et al., 1987) while MII is associated with a pericentromeric exchange(Lamb et al., 1997; Lamb et al., 1996; Oliver et al., 2008; Warren et al., 1987). Considering that recombination is an MI-associated event, it has been argued that MII errors are actually MI errors that are improperly resolved during MII(Lamb et al., 1997; Sherman, Freeman, Allen, & Lamb, 2005)).

Maternal age has been identified as the most highly correlated risk factor of Trisomy 21(E. G. Allen et al., 2009; Huether et al., 1998; Lamb, Yu, Shaffer, Feingold, & Sherman, 2005; Penrose, 1951, 1967) with recombination being the first molecular correlate(Antonarakis et al., 1986; Lamb et al., 1997; Lamb et al., 1996; Warren et al., 1987). These risk factors have also been associated with other trisomies. As Trisomy 21 is the most commonly identified autosomal Trisomy in live births and shares risk factors with other types of NDJ (Bugge et al., 2007; T. Hassold, Merrill, Adkins, Freeman, & Sherman, 1995; Houge, Boman, Lybaek, Ness, & Juliusson, 2006; Matsubara, Murakami, Nagai, & Ogata, 2011), it is often used as a model for NDJ. Hence, the findings from Trisomy 21 research may have broad applicability to NDJ and help women assess their risk for having a child with trisomy.

1.6 Research Overview

As was previously stated, altered recombination has been identified as a risk factor for maternal meiotic errors that lead to Trisomy 21. The frequency and placement of recombination on nondisjoined chromosomes 21 has been examined in several studies. However, there are no works to date that have examined the genomic features found at locations of recombination along the nondisjoined chromosome or whether variation in trans-acting factors contribute to altered recombination. This work outlines two studies: one that examines the potential for variation in a trans-acting factor that acts in chromosome 21 NDJ and one that characterizes the cis-acting factors present in regions of recombination along nondisjoined chromosomes 21.

Chapter 2 outlines a study where we assessed MI and MII errors for GWR characteristics that have been found in meiotically normal individuals. A study in normal individuals has found that the number of recombinants on individual chromosomes can predict the GWR count. This is called the gamete effect. Another study found that the GWR counts among the offspring of a mother are more similar to each other when compared to the offspring of other mothers. This is called the mother effect. We tested to see if these patterns are perturbed in individuals with meiotic errors. Recombination count and location were assessed in oocytes using genotype data from extended pedigrees. The number of recombinations on a specified chromosome was used as a predictor for the number of recombinants in the rest of the genome for four groups: MI errors, siblings of probands with an MI error, MII errors and meiotically normal

individuals. The presence or absence of recombination in the telomeric or centromeric portions of 21q was used as a predictor for elevated telomeric or centromeric recombination genome-wide. We found that oocytes with an MI error and their sibling oocytes have significantly reduced genome-wide recombination counts when compared to controls. However, a weakened gamete effect was observed only among oocytes with MI errors which suggests that the factors that regulate the gamete effect are altered in this group.

Chapter 3 outlines a study performed to characterize the genomic features of recombination events that occur along disjoined and nondisjoined chromosomes 21. As elevated CpG content, low GC, and Poly AT content and high gene density have been shown to be correlated with elevated sex-averaged recombination rates in normal individuals, these features were assessed for correlation with recombination on nondisjoined chromosomes 21. In addition to the aforementioned genomic features, hotspot usage was also examined for a potential role in altered recombination. While the proportion of recombination per bin was positively correlated with hotspot density for Normal and MI error meiotic events, there was no evidence of correlation in the MII group.

Figure 1.1 Meiotic errors that result in nondisjunction

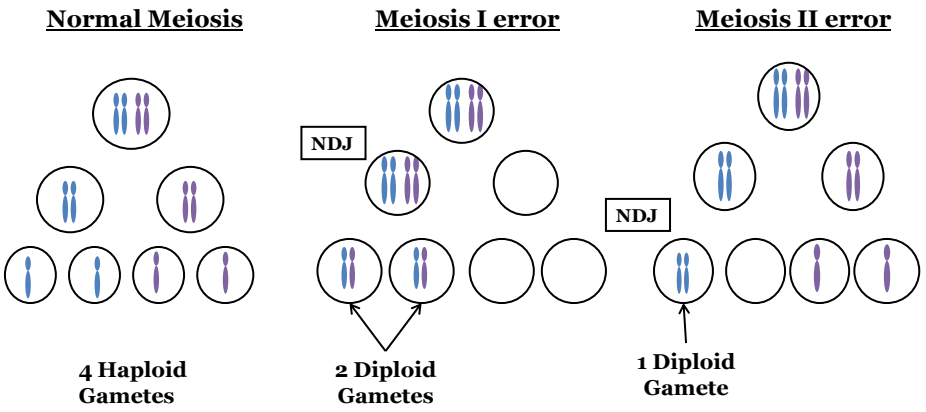
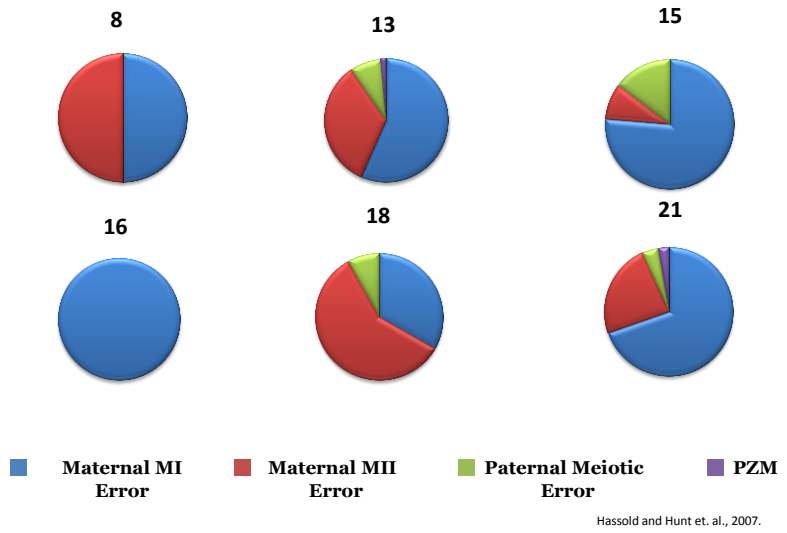


Figure 1.2 Origins of nondisjunction for various trisomies



Hassold and Hunt et. al., 2007.

Chapter 2

Evidence for dysregulation of recombination count in oocytes with nondisjoined chromosomes 21.

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2.1 ABSTRACT

In oocytes with nondisjoined chromosomes 21 due to a meiosis I (MI) error, recombination is significantly reduced along chromosome 21. A previous study suggested that those oocytes also had reduced recombination genome-wide. However, it was unclear whether the reduced genome-wide recombination counts were related to dysregulation of recombination or were within the range of normal variation. In the current study, we assessed genome-wide recombination phenotypes among oocytes with nondisjoined chromosomes 21 to gain insight into these alternative explanations. Information on recombination in trisomic oocytes and their siblings were derived using parents and grandparents of probands with trisomy 21 due to a maternal nondisjunction error. These data were compared with publically available genome-wide association data. We examined both "gamete" effects (effects leading to a correlation of the number of recombinants among chromosomes within a gamete) and "mother effects" (effects that lead to a correlation of recombination counts among oocytes from a single mother) to determine whether these properties were perturbed in oocytes with nondisjunction. Among normal meiotic events (n=2723), we found a statistically significant gamete effect. We found a much weaker gamete effect in MI nondisjunction events (n=94). Among their siblings (n=64), genome-wide recombination rates were similar to probands, however, the gamete effect was similar in magnitude to normal meiotic events. Thus our data suggest that the

reduced genome-wide recombination count in oocytes with MI errors appears to be related to dysregulation of the components that act in the gamete effect.

2.2 INTRODUCTION

Nondisjunction is the failure of homologous chromosomes or sister chromatids to segregate to separate daughter cells during cellular division. When this type of error occurs during meiosis, some of the resulting gametes will have too many or too few chromatids compared with the expected haploid number (aneuploidy). After fertilization, these aneuploid embryos will be significantly compromised, leading to early fetal loss or significant defects at birth including intellectual and developmental disabilities, physical defects and a shortened life span. Aneuploidy is purported to occur in at least 5% of clinically recognized pregnancies (Angell et al., 1984; T. Hassold et al., 1996; T. Hassold & Hunt, 2001; Warburton & Fraser, 1964) and accounts for approximately 35% of spontaneous abortions (T. Hassold & Hunt, 2001). Notably, aneuploidy occurs in approximately 1-2% of sperm (T. J. Hassold, 1998) and 20-25% of oocytes (Jacobs, 1992; Marquez et al., 1998).

Trisomy 21 is the most commonly observed type of aneuploidy in live born infants and occurs at a rate of 1/700 live births in the United States(Parker et al., 2010). As with the majority of autosomal trisomies, trisomy 21 is most often the result of errors in the oocyte (referred to as maternal errors), and accounts for about 90% or more of cases (E. G. Allen et al., 2009; Freeman et al., 2007)

Among those meiotic maternal errors, about 70% are classified as MI errors and 30% as MII errors. It may be that many of the MII errors are initiated in MI, but end with an oocyte containing sister chromatids (Lamb et al., 1997).

Maternal age has been identified as the strongest risk factor for trisomy 21. The first molecular correlate found to associate with the occurrence of trisomy 21 is altered recombination along 21q. Studies have shown that maternal MI errors are associated with a single distal exchange or absence of any exchange on nondisjoined chromosomes 21 while MII is associated with a proximal pericentromeric exchange (Lamb et al., 1997; Oliver et al., 2008; Warren et al., 1987). The basis for these associated patterns of recombination is unclear. However, studies in normal meiotic outcomes (Cheung et al., 2007; Chowdhury et al., 2009; Kong et al., 2002; Kong et al., 2010; Kong et al., 2008) and oocytes with nondisjoined chromosomes 21 (Brown et al., 2000) have provided evidence for factors that act globally (i.e., trans-acting factors) on the gamete to influence the variation in recombination counts across the genome.

Studies in recombination location and frequency in normal meiotic outcomes have used publically available data sets genotyped for genome-wide variants. Considering the importance of recombination in segregating chromosomes during gamete formation, it was expected that the number and placement of recombination genome-wide would be tightly regulated. While each study has used different assessment tools for phasing and linkage, a common theme has emerged: genome-wide recombination frequency and location are variable based on individual, sex and ancestry differences (Cheung et al., 2007; Chowdhury et

al., 2009; Kong et al., 2002; Kong et al., 2010). This suggests that variation may be explained by some inherent genetic characteristic or an environmental factor. For example, genomic loci and genes have been shown to contribute to variation in recombination frequency and placement. RNF212 was the first gene to be correlated with recombination count in males (Chowdhury et al., 2009; Kong et al., 2008). An inversion at genomic region 17q21.31 was also found to be associated with elevated recombination in carriers versus non-carriers (Chowdhury et al., 2009; Stefansson et al., 2005). PRDM9 is an example of a gene known to be involved in the placement of recombinant events. (Baudat, Buard, Grey, Fledel-Alon, et al., 2010; Berg et al., 2010b; Berg et al., 2011).

As recombination frequency and placement is altered in trisomy 21, it is of interest to assess whether trans-acting effects are involved in the mal-segregation of chromosomes. In a previous pilot study, we examined the number of genome-wide recombinants in oocytes that had an MI error and no observed recombination along the nondisjoined chromosome 21 (Brown et al., 2000). We found that this chromosome 21 pattern significantly predicted a reduced number of genome-wide recombinants in these oocytes and, similarly, that the chromosome 21 recombinant number among normal meiotic events predicted genome-wide recombinant counts. These findings suggest a factor that regulates the overall number of genome-wide recombinants per oocyte (Brown et al., 2000).

Two alternative hypotheses emerged from the pilot study,: 1. Reduced exchanges on chromosome 21 are related to a cell-wide decrease in exchanges

that is a part of the normal variation of exchange rates. For the MI error group, this rate has fallen below a critical level where chromosome 21 fails to have an exchange. 2. The genome-wide recombination count among MI errors is abnormally low and is not a part of the normal variation of genome-wide recombinants counts. Instead, it represents dysregulated recombination due to genetic or environmental risk factors.

In the current study, we first sought to confirm the findings from our previous study (Brown et al., 2000) using a larger sample size. We also expanded our sample to include oocytes with MII errors. We then sought to distinguish between the two possible hypotheses noted above. For each type of meiotic outcome—MI error, MII error and normal meiotic events—we assessed both recombination number and location along the nondisjoined chromosomes 21 as predictors for genome-wide recombination patterns. Using the terms coined by Kong et al.(Kong et al., 2002), we examined the "gamete" effect, or the effect that leads to a correlation of the number of recombinants among chromosomes within a gamete, and the "mother" effect, or the effect that leads to a correlation of recombination counts among oocytes from a single mother. We confirmed that oocytes with an MI error and their sibling oocytes have significantly reduced genome-wide recombination counts when compared to meiotically normal outcomes. However, a weakened gamete effect observed only among oocytes with MI errors suggests a disruption of normal variation of recombination patterns.

2.3 SUBJECTS AND METHODS

Ethics Statement

All recruitment sites obtained the necessary Institutional Review Board approvals from their institutions.

Sample Sets

Trisomy 21 Sample Set

Families of probands with standard trisomy 21 were recruited by various sites using a common protocol, with the goal to identify risk factors of nondisjunction. Blood and/or buccal samples were collected from probands and their families for the genetic studies. The minimal family unit required for this analysis included the proband with trisomy 21, parents, and maternal grandparents (5-member family). In some cases, we were able to ascertain siblings of the proband. Genome-wide genotyping was performed at the Center for Inherited Disease Research using the Golden Gate linkage panel. This panel consists of 6,056 SNP markers tiled across the genome and spaced on average .63 cM apart. The Center for Inherited Disease Research assessed several metrics of data quality in order to exclude low quality data. As a result of their quality control assessment, 358 markers were dropped due to low genotyping rates (<.98) and atypical intensity plot cluster patterns (forming greater than two clusters). This resulted in a final marker count of 5,698 SNPs. The remaining markers had a mean genotyping call rate of .9988 (min=.9805, std=.0021). At the person level, there were 896 samples sent for genotyping and 57 were dropped. These samples either failed genotyping because they were of poor quality or were removed as a result of

having genotyping call rates that fell within the 5% area of the lower tail of a normal distribution. Following genotyping quality control measures, families were removed if probands were not found to have an MI or MII error. Nine families were removed due to the following: four were found to be paternal in origin, one due to failure to identify stage or origin and four were likely to have resulted from post-zygotic mitosis. The final sample set contained 114 families of which 94 had probands with MI errors and 20 had probands with MII errors (Table 2.1).

Normal meiotic outcome sample set

Recombination profiles from normal meiotic outcomes were obtained from 2762 families that were genotyped through the following three studies: the Framingham Study (FHS), GENEVA Dental Caries Study (GENEVA) or Autism Genetic Resource Exchange (AGRE) study groups. These families were genotyped for at least 500,000 SNPs across the genome and assessed for uniformity in recombination distribution. The AGRE samples were genotyped for 520,017 SNPs tiled across the genome using the Infinium(R) HumanHap550-Duo Bead Chip. However, 11,473 markers were excluded from the analysis due to deviation from HWE ($p<10^{-7}$). After quality control measures were completed, the AGRE dataset contained genotype information for 512,207 markers across the genome. The FHS samples were genotyped for 500,568 markers across the genome using the Affymetrix Genome-Wide Human SNP Array 5.0. However, 22,000 markers were excluded from the analysis due to deviation from HWE ($p<10^{-7}$). After quality control measures were complete, there was genotype

information for 478,658 markers across the genome for the FHS dataset. The GENEVA samples were genotyped for 620,901 markers using the Illumina 610-Quad Array. There were 58,610 markers that were excluded from the analysis due to deviation from HWE ($p<10^{-5}$) and a MAF < 0.02. After quality control measures were complete, there was genotype information for 562,291 markers across the genome for the GENEVA population. The final data set contained 2723 control samples (Table 2.1). All SNP locations were based on human NCBI Build 36 (hg18).

Determination of the Nondisjunction Error Type

The origin of a nondisjunction event was categorized by both the parent from which the extra chromosome originated and meiotic stage of origin as described previously (E. G. Allen et al., 2009; Freeman et al., 2007). Briefly, to determine which parent contributed the disomic gamete, we assessed the chromosome 21 allelic contribution from each parent to the child. We included only those cases found to be of maternal origin. The meiotic stage (MI or MII) was inferred using pericentromeric markers. For the most proximal pericentromeric marker heterozygous in the mother, allelic heterozygosity (nonreduction) in the proband led to the classification of an MI error. Allelic homozygosity (reduction) in the proband, led to the classification of an MII error. MII errors could result from classical MII errors where sister chromatids fail to separate at MII or to errors initiated in MI followed by abnormal segregation in MII. Additionally, when all markers were found to be reduced to homozygosity, indicating an MII error with no recombination on chromosome 21, the origin of nondisjunction was inferred

to be a post-zygotic mitotic error. These cases were excluded from this study. However, this allelic configuration may also have been the result of an MII error in which no recombination had occurred.

Determining Recombination Phenotypes

Trisomy 21 Sample Set

For the trisomic data set (probands), two methods were used to identify recombination location and number. Trisomic chromosomes 21 were analyzed separately because tri-allelic genotype data do not meet the assumptions used in available haplotyping software. For trisomic chromosomes 21, SNP and STR data were combined from our previous studies (Oliver et al., 2011), and used to define the location of recombination events along 21q. The breakpoints of a single recombinant event were defined by a minimum of either one STR or eight consecutive informative SNPs flanking the recombination breakpoint. However, when the most proximal or distal informative markers on 21q indicated a recombinant event, a minimum of either one STR or four consecutive informative SNPs were required to define recombination break points.

For the disomic chromosomes, we used our own method implemented as a Python program to call recombination events. Our method works on similar principles as that of Coop et al.(Coop, Wen, Ober, Pritchard, & Przeworski, 2008) and Chowdhury et al.(Chowdhury et al., 2009), except that it is tailored towards three-generation pedigrees also allowing for the use of informative marker density as a quality control measure for calling recombination break points.

First, for each SNP and a three-generation pedigree, the proband's maternal allele is assigned a grandparent-of-origin status or which grand-parent the allele is inherited from. The grandparent-of -origin statuses are then ordered by the SNPs' physical positions to demarcate segments along the chromosomal that alternate between the two grandparents of the proband. The region between two consecutive segments, known as a recombination interval, represents the location of a recombination event. In our analysis, each recombination interval that was supported by flanking segments both containing at least two consecutive informative markers, was scored as a separate recombination event.

Normal meiotic outcome sample set

For the AGRE, FHS and GENEVA datasets, genotype data from members of two-generation families with three or more children were used to infer the location and number of recombinants of autosomal chromosomes using the method developed by Coop et al.(Coop et al., 2008) and Chowdhury et al. (Chowdhury et al., 2009) In this method, markers on the parental chromosomes are assessed for identity by descent allele sharing (IBD) between two siblings, and each sibling assigned either the same haplotype phase or different haplotype phases depending on the IBD status. In the event where there was a switch from shared to unshared phase, a putative recombination event is noted. The haplotype phases of two or more siblings are then compared to identify which sibling inherited the recombinant chromosome. This method as well as the datasets are described in detail in Chowdhury et al. (Chowdhury et al., 2009)

Statistical Analyses

Genome-wide recombination count

We first performed t-tests to compare the mean number of genome-wide recombination counts between specific meiotic outcome groups defined by meiotic error (MI error, MI-sibling and MII error vs. normal meiotic outcome) and, in subsequent comparisons, by chromosome 21 recombination count. For the analysis of MI error probands or of their siblings vs. the normal meiotic outcome group, the chromosome 21 recombination groups were 0, 1 or > 1 recombinant on chromosome 21. For the comparison between MII errors vs. the normal meiotic outcomes, the chromosome recombination groups were 1, 2 or >2 recombinants on chromosome 21. We did not assess MII errors with 0 recombinants because these cases are most likely the result of a post-zygotic mitotic error. A significance level of .05 was used for this test as well as for all other tests in this study.

We then used linear regression models to assess the relationship between the number of recombinants on chromosome 21 (predictor variable) and the total number of autosomal recombinants less those that occurred on chromosome 21 (outcome variable). We stratified by meiotic outcome group (MI error, MI error siblings, MII error and normal meiotic outcome) as each group has a different ability to detect observed recombination along chromosome 21 and each is associated with a different chromosome 21 recombination profile. The latter may reflect distinctive mechanisms of recombination regulation. We adjusted the regression model for maternal age at the birth of the proband for the MI and MII data sets, as it is an important covariate for nondisjunction(E. G. Allen et al.,

2009; Antonarakis et al., 1992; Muller, Rebiffe, Taillandier, Oury, & Mornet, 2000; Yoon et al., 1996) However, we did not find maternal age to be a significant predictor of genome-wide recombination count and it was therefore omitted from the final model. Maternal age at birth was not available for the normal meiotic outcome group. However, current data suggest its effect on genome-wide recombination is very small(Kong et al., 2004). To further analyze the gamete effect, or the correlation of recombination counts among chromosomes within the same oocytes, we conducted the same regression model described above for each of the other autosomes. In these models we used the number of recombinants on each autosomal chromosome as the predictor variable and the aggregate count of recombinants in the remaining autosomes as the outcome variable.

We then sought to determine whether oocytes that have had a nondisjunction event (resulting in the proband with trisomy 21) showed similarity in genome-wide recombination count with oocytes from the same mother (proband's siblings). In order to assess this, we performed an ANOVA of the genome-wide recombination counts of siblings from different mothers. We accounted for the effect of random sampling by using a random effects ANOVA.

Genome wide recombination location

To assess the location of recombination, each arm of each chromosome was split into three regions: proximal, medial and distal. The proximal and distal regions were defined as the 20% most proximal and distal of a chromosome arm defined at the base level, the same definition used by Przeworski et al.

(2011)(Fledel-Alon et al., 2011). We then performed a linear regression analysis where our outcome measure was the proportion of proximal or distal recombinants genome-wide and our predictor was the presence or absence of a recombination in the proximal or distal region of chromosome 21. Again, we analyzed each meiotic outcome group separately.

2.4 RESULTS

Genome-wide Recombination Count

Meiosis I nondisjunction

We first sought to confirm the results seen in Brown et al.(Brown et al., 2000) In the aforementioned study, we obtained genome-wide recombination counts from 15 oocytes with an MI error and no observed recombinants on chromosome 21. We further refined this group to better represent lack of recombination along chromosome 21 using somatic cell hybrid studies to remove hidden, single complimentary chromosome 21 recombinant events as described in Brown et al. We compared the genome-wide recombination counts from these MI errors to normal meiotic outcomes that were obtained from the CEPH pedigrees and stratified by having no observed recombination on chromosome 21 or having one or more. We found that there was a significant positive association with the inferred number of chromosome 21 exchanges and genome-wide recombination count. That is, we detected a gamete effect.

In our new data set, we were not able to conduct somatic cell hybridization to exclude single complimentary recombinants; thus we have an increased

frequency of chromosomes with an undetectable exchange in our group of no observed recombinants compared with Brown et al. Irrespective, we know that a large subset of MI nondisjoined chromosomes 21 result from bivalents with no exchanges(Lamb et al., 1997). We hypothesized that chromosome 21 recombination count predicts the genome-wide recombination count due to the gamete effect. Thus, we predicted that oocytes with MI errors and no detectable chromosome 21 recombinants would have reduced genome-wide recombination counts compared with normal meiotic outcomes with no detectable chromosome 21 recombinants, similar to our previous study. Using a t-test, we found that there was a significant reduction in the mean genome-wide recombination count among all MI errors compared with that of the normal meiotic outcome group (37.9 (95% CI 36.3-39.4) vs. 42.61 (95% CI 42.3-43.0) ($p<.0001$), Table 2.2). When we restricted the sample to only those with no observed recombinants on chromosome 21, the difference remained significant (37.6 (95% CI: 35.5-39.8) vs. 40.87 (95% CI 40.3; 41.4), $p<.006$, Table 2.2). Further examination of the mean genome-wide recombination counts of the normal meiotic outcomes by 0, 1 and >1 recombinants showed the predicted pattern for a gamete effect (Figure 2.1): the mean genome-wide recombination count increased with increasing number of chromosome 21 recombinant events. Among MI errors, this pattern was perturbed, as there was no difference between means for those with 0 and 1 observed chromosome 21 recombinants, although the mean genome-wide recombination count among those with >1 chromosome 21 recombinants was increased.

Next we compared genome-wide recombination counts of MI error siblings with MI error probands and with the normal meiotic outcome group to examine the "mother" effect. As with MI error probands, we found that there was a significant reduction in the mean genome-wide recombination count among MI errors siblings compared with that of the normal meiotic outcome group ((38.5 (95% CI 36.5-40.5) vs. 42.6 (95% CI 42.3-43.0) ($p<.0001$)), Table 2.2). Upon examination of the MI siblings mean genome-wide recombination counts stratified by 0, 1 and >1 chromosome 21 recombinations, we found that all means were reduced when compared to the normal meiotic outcome group (Figure 2.2). However, unlike the MI error probands, the mean genome-wide recombination count increased with increasing chromosome 21 recombination, suggesting a gamete effect.

We next examined the other autosomes to determine whether the observed gamete effect was restricted to chromosome 21 among controls and MI siblings, but not among MI error probands. We used linear regression to determine the relationships between the number of recombinants on an autosome (predictor variable) and the genome-wide recombination count minus that autosome's count (outcome variable) (Table 2.3). First, we found a highly statistically significant association of genome-wide recombination counts with the observed number of chromosome 21 recombinants among normal meiotic outcomes (beta coefficient=2.90, standard error= 0.27, $p<0.001$) but not MI errors (beta coefficient=.14, standard error= 1.10, $p=0.30$), which reflects the pattern of means discussed above. Upon examination of the other autosomes, we found that

there was strong evidence for a gamete effect in the normal meiotic outcome group. As the number of recombinants on each individual chromosome increased by 1, the genome-wide recombination count increased by 1.44 to 2.06 recombinants, as indicated by the beta coefficient ($p<0.001$) (Table 2.3). The sample size difference causes a larger variance in betas among the chromosomes (-0.07 – 2.29 for cases vs. 1.44 – 2.06 for controls), but does not affect the mean beta. (Table 2.3). The mean beta coefficient was reduced compared with normal outcomes (1.23 vs. 1.75, Figure 2.3). We next assessed siblings of probands with an MI error to determine whether their autosomal beta coefficients were more similar to MI error probands or the normal meiotic outcome group. Despite the small sample size (N=64), we found that mean beta coefficient for MI siblings was more similar to the normal meiotic outcome group than to MI error probands: 1.72 vs. 1.75 for controls and 1.23 for MI error probands (Figure 2.3). However, the range of beta-coefficients was large (0.58-3.35, Table 2.3), most likely due to the small sample size. This shows that our method for detecting the gamete effect was successful even when used for a reduced sample size and provides evidence that the effects seen in the MI error probands is not due to low power.

We next tested for the mother effect by determining whether there was a correlation between the genome-wide recombination counts among offspring of the mothers of MI error probands. We conducted ANOVA using the mothers (N=38) as the group variable (Table 2.4). We found no evidence for a mother effect ($p=0.12$, graphically shown in Figure 2.4). This result is inconclusive. We

cannot rule out a mother effect as the number of sibships per mother is less than those used to detect the mother effect in other studies (Cheung et al., 2007; Kong et al., 2002)

Meiosis II nondisjunction

In the preliminary analysis performed by Brown at el., only MI errors were compared to individuals with normal meiotic outcome for differences in genome-wide recombination rate(Brown et al., 2000). MII errors have been shown to have an elevated number of recombinants as well as an increased rate of pericentromeric recombinants on chromosome 21q when compared to the MI error or normal meiotic outcome group (Lamb et al., 1996). Here, we asked whether these patterns extended to genome-wide recombination patterns. Although the following studies are limited by the number of MII errors in our sample (n=20), they provide, for the first time, preliminary results on recombination regulation in oocytes of this type of error.

We first compared the genome-wide recombination rates of the MII error group with that of the normal meiotic error group. As MII errors with no observed recombinants on chromosome 21 likely represent post- zygotic errors in mitosis, we restricted our comparison to the samples in the normal meiotic error group to those with at least one observed recombinant on chromosome 21 (N=1712) . The MII group had a mean genome-wide recombination count of 40.45 (95% CI 36.61-44.28) and the normal meiotic outcome group had a mean genome-wide recombination count of 43.35 (95% CI 42.84-43.85) (Table 2.2). These values were not significantly different (t-test=.111, α>.05).

Next, we performed linear regression to determine whether the number of recombinants on chromosome 21 predicted the genome-wide recombination counts. Although we found that the beta coefficient was higher than that of the normal meiotic outcome group (2.70 vs. 1.98) it was not a statistically significant predictor because of the small sample size (standard error=3.32 p= 0.43) (Table2. 3). As in our MI cases, we then assessed the beta coefficients of the other autosomes to determine whether there was evidence for a gamete effect. As in the MI error group, the beta coefficients of the chromosomes varied widely because of the small sample size, ranging from -2.63-4.65 (Table 2.3). However the average beta coefficient (1.54) was closer to what was found in the normal meiotic outcome group (1.75) than to what was found in the MI error group (1.23) (Figure 2.3). There were not enough siblings of probands to examine the mother effect among the MII error group.

Genome Wide Recombination Location

To determine whether the altered recombination location pattern associated with MI and MII errors extends to the entire genome, we assessed the relationship between the presence or absence of a proximal or single distal recombination event on chromosome 21 (predictor) and the proportion of genome-wide recombination in the proximal or distal 20% region of the chromosomes (outcome variable). We included genome-wide recombination count as a covariate in the model to correct for the potential effect of interference (Weber et al., 1993). That is, an increased number of multiple recombinants per arm due to an overall increase in genome-wide recombination counts could lead

to recombinants localized nearer to the ends of chromosome arms. Among normal meiotic events, we found that the presence of a single distal chromosome 21 recombinant or the presence of proximal chromosome 21 recombinant was significantly correlated with a greater proportion of distal (Table 2.5) or proximal (Table 2.6) genome-wide recombination events, respectively. The beta coefficients are reported in Tables 2.5 and 2.6 for the MI and MII regression models. There was no evidence that the location of recombination on chromosome 21 predicts recombination location genome wide for these nondisjunction error groups. This finding is inconclusive because we had a reduced sample size for this test compared to other tests performed in this study (we could only include samples that had at least one recombinant).

2.5 DISCUSSION

Altered recombination patterns have been identified as a risk factor for nondisjunction (Lamb et al., 1997; Lamb et al., 1996; Warren et al., 1987). As the number or placement of recombination on chromosome 21 are major determinants of proper chromosome segregation, it is important to identify factors that regulate recombination on chromosome 21. Our previous study showed that reduced number of exchanges on chromosome 21 was associated with reduced exchanges genome-wide (Brown et al., 2000). This suggested that variation in factors acting globally (trans-acting factors) may be involved in controlling recombination counts on chromosome 21 and elsewhere.

Here, in our expanded study, we found similar evidence to Brown et al(Brown et al., 2000) when we simply compared genome-wide recombination counts

between MI nondisjunction errors and normal meiotic samples. MI errors with zero observed chromosome 21 recombinants had significantly reduced genome-wide recombination counts compared with normal meiotic outcomes (Table 2.2). This is most likely due to the fact that there is a large class of chromosomes 21 that lack an exchange among the MI error group compared with the normal meiotic outcome group (Lamb et al., 1997; Lamb et al., 1996; Oliver et al., 2008). However, on further examination of samples with 1 or >1 chromosome 21 recombination events, the gamete effect was less evident among the MI error oocytes (Figure 2.1). This weakened gamete effect was also observed in the analyses of other autosomes in the nondisjoined oocytes (Figure 2.3).

Siblings of MI error probands provide some insight into the type of factors that may influence this pattern. Siblings of MI error probands showed a similar reduction in genome-wide recombination counts as did the MI error probands (Table 2.2.), although they showed evidence of a gamete effect based on all autosomes, including chromosome 21 (Figures 2.2 and 2.3). We suggest a two-step model that leads to dysregulation of genome-wide recombination count observed in the MI error oocyte. The first step leads to a predisposition to reduced genome-wide recombination perhaps due to a variant in a gene that controls recombination number or to an environment exposure to the fetus. This would suggest a mother effect; however, we did not observe a mother effect among offspring of the 38 mothers with an MI error. We speculate that this may be due to the second step, which sets the MI error proband apart from their siblings with respect to recombination. That second step would be a gamete-

specific factor that leads to dysregulation of recombination in that single oocyte. This factor is unlikely to be environmental as all gametes would be affected. Hence, it is more probable that a *de novo* mutation has occurred in the MI error oocyte.

With regard to the mother effect, studies in model systems have previously identified genetic variants that result in reduced genome-wide recombination counts among sibling gametes and, in some cases, concomitant nondisjunction of susceptible chromosomes. For instance, the Mei-S282 mutation in *Drosophila melanogaster* leads to a decrease in global recombination and increased rate of chromosomal nondisjunction during MI (Parry, 1973). Furthermore, nondisjunction events within a gamete were associated with an increase in achiasmate heterologous chromosomes within that same gamete (Parry, 1973). Similar phenotypes have been found in *Caenorhabditis elegans* that are *him-6/him-4* null in which genome-wide recombination was found to be decreased and nondisjunction rates increased in these mutants (Zetka & Rose, 1995). Taken together, these results indicate that variation in a trans-acting factor can lead to reduced recombination among sibling-gametes. Additionally, this factor can lead to nondisjunction in a subset of gametes, much like what was seen in this study

Genetic variants that lead to variation in genome-wide recombination counts have also been identified in human genes. RNF212 is an ortholog of the *Caenorhabiditis elegan* gene zhp-3 and is required for crossover recombination in this organism. Several genome wide association studies have shown that variants in RNF212 are associated with increased recombination number in

human males (Chowdhury et al., 2009; Fledel-Alon et al., 2011; Kong et al., 2008) with two of these studies finding an association in females(Fledel-Alon et al., 2011; Kong et al., 2008). Most recently, a molecular study of RNF212 in mice found that this protein is essential for crossing over and works by stabilizing recombination proteins (Reynolds et al., 2013). A heterozygous mutation in this gene leads to reduced recombination (Reynolds et al., 2013). Hence this gene is believed to work in a concentration-dependent manner where mutations that lead to haploinsufficiency cause a reduction in genome-wide recombination. The effects of RNF212 on cross-over number may be influenced by an interaction between chromosome size and protein concentration (there may be a lower probability of protein binding along smaller chromosomes when compared to larger chromosomes in low protein concentrations). Also, a common inversion on chromosome 17q21.31 has consistently been shown to be correlated with elevated recombination counts in females (Chowdhury et al., 2009; Fledel-Alon et al., 2011; Stefansson et al., 2005) This inversion carries two haplotypes (named H1 and H2)(Pittman et al., 2004). These haplotypes have differing gene expression profiles (de Jong et al., 2012). Our data show that chromosome 17 is a strong predictor of genome-wide recombination count, as it is highly significant even in the MII group (N=20) (Table 2.3). Potentially, the presence of the inversion on chromosome 17 effects recombination in two ways: by influencing the number of recombinants on chromosome 17 when acting in cis and by influencing the genome-wide recombination count by acting in trans (haplotype variation causing differential expression of genes that influence recombination).

In summary, we have confirmed that oocytes with MI errors as well as their sibling-oocytes have reduced recombination when compared to meiotically normal oocytes. This cell-wide reduction in recombination found in oocytes with an MI error does not appear to be within the normal limits of recombination rates and is associated with a disturbance in the gamete effect. This disturbance in the gamete effect does not extend to the meiotically normal offspring of a mother. This study is the first to identify global dysregulation of recombination in MI error oocytes. In future studies, it will be important to identify trans-acting factors that regulate the gamete effect. As variants in RNF212 and region 17q21.31 have been found to associate with increased or decreased genome-wide recombination counts in females, these may serve as the first candidate genes for genotyping in mothers of MI error probands. Determining whether these trans-acting factors influence the gamete effect would also help identify other candidate genes/molecules that contribute to the altered recombination patterns seen in MI error oocytes.

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Figure 2.1 Average genome-wide recombination counts for MI errors and Controls.

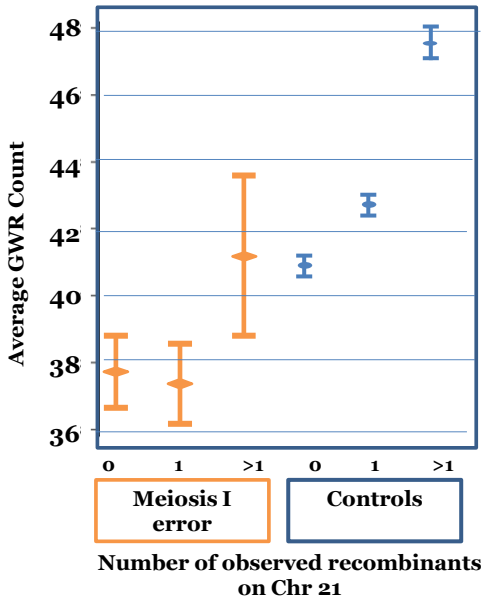

Figure 2.2 Average genome-wide recombination counts for MI error siblings and normal meiotic outcomes.

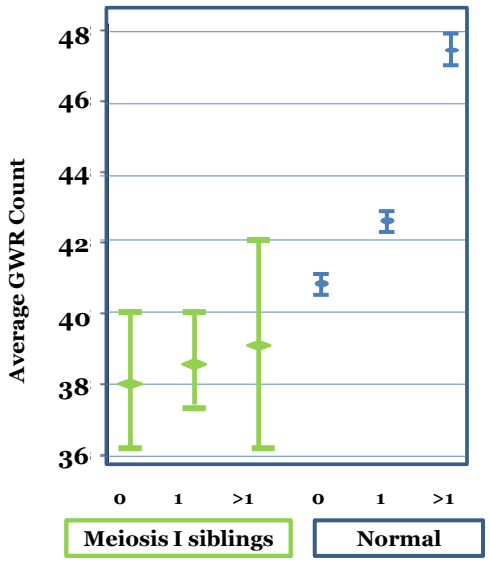

Figure 2.3 Average beta coefficients.

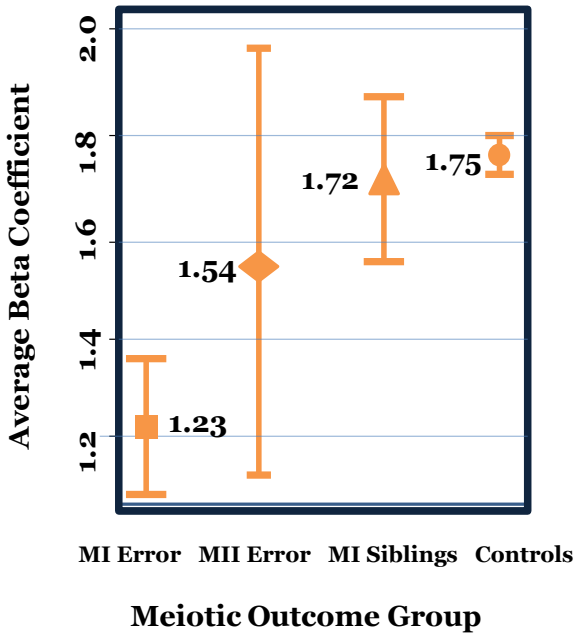

Figure 2.4. MI error proband and sibling genome-wide recombination counts.

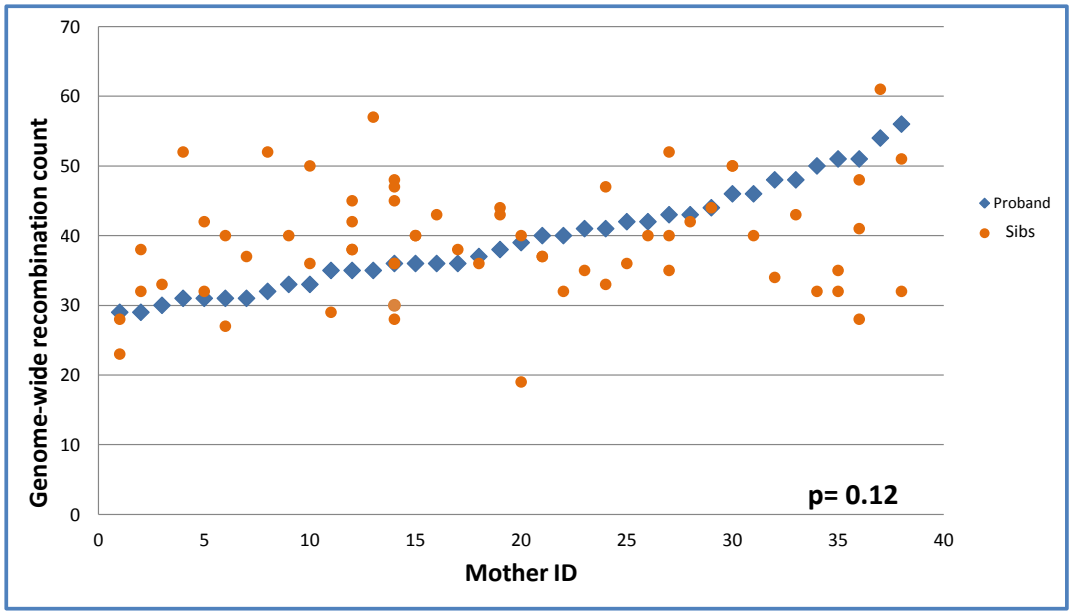

Table 2.1 Samples sizes stratified by meiotic outcome group and number of observed recombinants on chromosome 21

Meiotic outcome group	Number of observed recombinants on chromosome 21			Number of siblings
	0	1	> 1	
Meiosis I Error	56	28	10	64
Meiosis II Error	n/a	15	5	n/a
Normal	1044	1319	360	n/a

Table 2.2 Genome-wide recombination count t-tests.

Meiotic Outcome Group	Mean	95% CL Lower	95% CL Upper	Difference From Controls	p-value
MI comparisons:					
Controls (N=2723)	42.61	42.27	42.95	n/a	n/a
MI (N=94)	37.89	36.35	39.44	-4.72	<.0001
MI siblings (N=64)	38.52	36.4976	40.5337	-4.10	0.0003
Controls with 0 recombinants (N=1044)	40.87	40.34	41.40	n/a	n/a
MI with 0 recombinants (N=56)	37.63	35.48	39.77	-3.25	0.0066
MII comparison					
Controls >0 recombinants (N=1679)	42.61	42.27	42.95	n/a	n/a
MII >0 recombinants(N=20)	40.45	36.62	44.28	-2.16	0.29

Table 2.3 Relationship between number of recombinants on a specified autosome and the sum of all other autosomal recombinants. The beta coefficient, standard error and p-values of each autosomal chromosome for MI and MII errors, MI error siblings and Controls. Significant p-values are marked with an asterisk*.

Chr	MI (N=94)	MII (N=20)	MI Siblings (N=64)	Controls (N=2723)
	Beta coefficient, SE, p-value	Beta coefficient, SE, p-value	Beta coefficient, SE, p-value	Beta coefficient, SE, p-value
1	2.29, 0.44, <0.001*	2.32, 0.80, 0.009*	1.06, 0.63, 0.10	1.78, 0.09, <.0001*
2	0.51, 0.53, 0.34	0.11, 1.15, 0.92	1.25, 0.74, 0.10	1.83, 0.10, <.0001*
3	0.65, 0.65, 0.31	0.08 1.07, 0.94	1.16, 0.62, 0.07	1.73, 0.11, <.0001*
4	1.13, 0.53, 0.04*	-1.64, 1.60, 0.32	1.35, 0.72, 0.07	1.86, 0.11, <.0001*
5	0.47, 0.70, 0.50	3.26, 1.06, 0.01*	2.48, 0.77, 0.002*	1.88, 0.12, <.0001*
6	1.88, 0.54, <0.001*	2.48, 0.83, 0.01*	2.10, 0.73, 0.006*	1.97, 0.13, <.0001*
7	2.00, 0.54, <0.001*	0.39, 1.59, 0.81	2.03, 0.93, 0.03*	1.85, 0.13, <.0001*
8	1.36, 0.67, 0.05*	1.48, 1.39, 0.30	1.27, 0.93, 0.18	1.57, 0.12, <.0001*
9	1.34, 0.63, 0.04*	1.67, 1.95, 0.40	1.22, 0.79, 0.13	1.88, 0.14, <.0001*
10	1.88, 0.63, 0.004*	3.12, 1.77, 0.10	0.87, 0.96, 0.37	1.74, 0.13, <.0001*
11	1.74, 0.66, 0.01*	2.12, 1.72, 0.24	2.02, 0.87, 0.02*	1.68, 0.14, <.0001*
12	0.43, 0.72, 0.56	-1.34, 1.68, 0.44	1.67, 0.96, 0.09	1.95, 0.14, <.0001*
13	0.98, 0.92, 0.29	1.53, 2.44, 0.54	2.35, 1.08, 0.03*	1.44, 0.17, <.0001*
14	0.83, 0.89, 0.36	3.41, 1.73, 0.07	1.80, 1.17, 0.13	1.75, 0.17, <.0001*
15	0.87, 0.87, 0.32	-0.83, 2.56, 0.75	2.41, 1.02, 0.02*	1.57, 0.17, <.0001*
16	1.07, 0.77, 0.17	3.41, 1.74, 0.07	2.79, 1.01, 0.008*	1.62, 0.15, <.0001*
17	2.00, 0.71, 0.006*	4.66, 1.11, <0.001*	3.35, 0.88, <0.0001*	1.34, 0.17, <.0001*
18	1.32, 0.93, 0.16	2.44, 1.85, 0.20	1.43, 1.15, 0.22	1.84, 0.17, <.0001*
19	-0.07, 1.06, 0.95	1.58, 2.02, 0.45	0.58, 1.19, 0.63	1.62, 0.19, <.0001*
20	1.89, 0.99, 0.06	-2.63, 2.72, 0.35	2.46, 1.38, 0.08	2.06, 0.18, <.0001*
21	1.14, 1.10, 0.30	2.70, 3.32, 0.43	0.58, 1.70, 0.73	1.98, 0.24, <.0001*
22	1.40, 1.13, 0.22	3.70, 2.69, 0.19	1.49, 1.46, 0.31	1.58, 0.23, <.0001*

Table 2.4 Number of siblings broken down by family

Number of siblings	Number of families
1	21
2	13
3	2
4	1
5	none
6	none
7	1

Table 2.5 Relationship between distal recombination on chr21 and the proportion of genome wide distal recombinants. The beta coefficients for the presence or absence of recombination in the distal portion of chromosome 21 (predictor) and the proportion of all other autosomes with a distal recombinant (outcome) for MI and MII errors and Controls. GWR was included as a variable to adjust for its effects on telomeric recombination. Significant p-values are marked with an asterisk*.

	Multivariate model for proportion of genome-wide CENTROMERIC events					
	M1 (N=41)		M2 (N=20)		Normal (N=1679)	
Predictor	Param. est.	p-value	Param. est.	p-value	Param. est.	p-value
GWR rate	-0.00049	0.74	0.00079	0.52	0.00031	<.0001
chr 21 centromere (y or n)	-0.00136	0.96	-0.03544	0.08	0.02612	<.0001

Table 2.6 Relationship between proximal recombination on chr21 and the proportion of genome wide proximal recombinants. The beta coefficient for the presence or absence of recombination in the proximal portion of chromosome 21 (predictor) and the proportion of all other autosomes with a proximal recombinant (outcome) for MI and MII errors and Controls. Genome wide recombination count was included as a variable to adjust for its effects on proximal recombination. Significant p-values are marked with an asterisk*.

	Multivariate model for proportion of genome-wide TELOMERIC events					
	M2 (N=20)		M1 (N=41)		Normal (N=1712)	
Predictor	**Param. est.**	**p-value**	**Param. est.**	**p-value**	**Param. est.**	**p-value**
GWR rate	-0.000415	0.8139	0.00193	0.2102	0.00041071	0.0018
chr 21 telomere (y or n)	-0.01414	0.7692	-0.00132	0.9477	0.03428	<.0001

Chapter 3

Differential hotspot usage is observed among recombinant events on nondisjoined chromosomes 21.

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3.1 ABSTRACT

Trisomy 21, or Down Syndrome (DS), is the most common autosomal trisomy among live-born infants and is caused mainly by nondisjunction (NDJ) of chromosome 21 within oocytes. Risk factors for maternal cases of trisomy 21 include increased maternal age and altered recombination. Studies of normal meiotic events in humans have shown that recombination clusters in regions referred to as hotspots. In addition, GC content, CpG fraction, Poly(A)/Poly(T) fraction and gene density have been found to be significant predictors of the placement of sex-averaged recombination in the human genome. These observations led us to ask whether the altered patterns of recombination associated with NDJ of chromosome 21 and DS could be explained by differences in the relationship between recombination placement and recombination-related genomic features (i.e. GC content, CpG fraction, Poly(A)/Poly(T) fraction or gene density) on 21q or differential hot-spot usage along the nondisjoined chromosome 21. We did not find a significant association between any of our genomic features of interest and recombination. However, we found different patterns of Linkage Disequilibrium-based (LD-based) hotspot usage when we stratified by the type of meiotic error. Among MI errors, the proportion of single recombinants along 21q was correlated with hotspot density, but occurred in different regions of 21q than normally disjoining chromosomes. Our data suggest that factors other than those related to hotspot usage are affecting recombination among MI errors. With regard to MII errors, single recombinants as well as the proximal recombinant of a double event are not positively correlated with LD-based hotspot density. These findings suggest that the MII error group has

acquired a new set of hotspots that are distinct from historical hotspots. For MII errors, more data are needed to determine whether altered recombination location may be controlled by variation in factors that control recombination in normal meiotic outcomes. As genetic factors that regulate hotspot usage have been identified and variation in these genes are associated with altered recombination, this variation may contribute to dysregulation of recombination in oocytes with an MII error.

3.2 INTRODUCTION

Trisomy 21, or Down Syndrome (DS), is the most common autosomal trisomy among live-born infants, occurring in approximately 1 in 700 live-births, and is caused mainly by the failure of chromosome 21 to properly segregate during oogenesis (Freeman et al., 2007). Increased maternal age and altered number and location of recombination events have been found to be associated with maternal meiotic errors involving chromosome 21 (Lamb et al., 1997; Oliver et al., 2008). Specifically, the absence of recombination (Warren et al., 1987) or the presence of a single recombinant event near the telomere of 21q (Lamb et al., 1997) are associated with maternal meiosis I (MI) errors and these associations appear to be independent of the age of the oocyte (i.e., maternal age at the time of birth of the infant with trisomy 21) (Oliver et al., 2011). Meiosis II (MII) errors appear to be driven by different age and recombination traits: MII errors are associated with the placement of a recombinant event near the centromere of 21q (Lamb et al., 1997) and this association increases with increasing age of the oocyte (Oliver et al., 2011).

Studies of normal meiotic events in humans show that the placement of recombination is not a random event. Rather, both cis and trans-acting factors have been found to be associated with the placement of recombination. Specifically, GC content, CpG fraction and Poly(A)/Poly(T) fraction have each been found to be significant predictors of placement of sex-averaged recombination events in the human genome (Kong et al., 2002). In addition, sequence variation in the zinc-finger domain of the gene *Proline Rich Domain Containing 9* (PRDM9) has a major impact on the location of recombination in humans (Berg et al., 2010a; Berg et al., 2011; Hinch et al., 2011; Kong et al., 2010). Specifically, allelic differences in the zinc finger binding domain of *PRDM9* explain approximately 80% of the heritable variation in "hotspot usage" " (i.e. the frequency at which recombination occurs within linkage disequilibrium (LD) or "historically"-defined hotspots)(Baudat, Buard, Grey, & de Massy, 2010; Fledel-Alon et al., 2011; Hinch et al., 2011). The observation that both cis and trans-acting factors are associated with the placement of recombination led us to question whether the altered patterns of recombination associated with NDJ of chromosome 21 could be explained by differences in the relationship between recombination and genomic features (i.e., GC content, CpG fraction, Poly(A)/Poly(T) fraction or gene location) on 21q or differential hot-spot usage. We did not find a difference in the association between recombination and recombination-related features between normally and abnormally segregating chromosomes. However, we found different patterns of hotspot usage among MII nondisjoined chromosomes compared with normally disjoining chromosomes. As for MI, the proportion of recombinants within a bin along 21q was correlated

with hotspot density, however those recombinants occurred in different regions of 21q than normally disjoining chromosomes. We provide possible explanations for these observations.

3.3 RESULTS

To assess the relationship between recombination placement and each of our variables of interest, 21q was divided into 500kb bins. We chose this bin size based on our level of refinement of recombination break-points. The proportions of each bin occupied by each genomic feature of interest (i.e. GC content, CpG fraction, Poly(A)/Poly(T) fraction and gene location) were the independent predictor variables. The outcome variable was defined as the proportion of all chromosome 21 recombinant events that occurred within the bin. Univariate linear regression was then used to determine if there was correlation between each predictor and the proportion of recombination in each bin. Because the genomic features, particularly CG content, differed greatly by region of the chromosome, we performed additional analyses where 21q was stratified by chromosome region, designated as proximal (13.6 -18.6 Mb), medial (18.6 - 23.6 Mb) and distal (23.6 - 48.5 Mb) (Figure 3.1). We also stratified analyses by chromosomes with single and double recombinant events, as mechanisms of chromosome 21 nondisjunction may differ based on the number of recombinant events on 21q (see Table 3.1 for the distribution of recombinants by region on 21q).

No association with genomic features along chromosome 21 and location of recombinant events

We did not find statistically significant or noteworthy associations between any of our genomic features of interest and recombination among any of our meiotic outcome groups (MI, MII and normal). This lack of association among maternal chromosomes 21 differs from an analysis of the whole genome combining both maternal and paternal chromosomes where associations were first described (Kong et al., 2002) While the smaller sample size of our case group may have led to a reduced power in our ability to detect these relationships, our control group was of similar size to that of Kong et al. Hence, this difference in results is likely due to a difference in study design (we examined only maternal meiotic recombination events along chromosome 21 while the study performed by Kong et al. examined all chromosomes for paternal and maternal events).

Hotspot usage among normally disjoining chromosome 21 events

We examined historically-defined hotspots first among normally disjoining chromosomes (normal meiotic outcome group) with one recombinant event and those with two recombinant events. Among those with one detectable event, we found a strong association between the number of hotspots per bin and the proportion of recombination per bin for our analysis of the entire long arm of chromosome 21 ($p < .0001$) (Figure3.2 and Figure 3.3) as well as in the analysis by region of 21q ($p=0.01$ or less for all regions) (Fig.3.4). Similarly, among those with two detectable events, we found that the locations of the proximal and distal recombinant events were significantly associated with the location of historically-

defined hotspots (p =0.03 and <.001, respectively) (Figure 3.5). Thus, as expected, the amount of recombinant per bin is positively correlated with historical hotspot density suggesting that historical hotspots are used for recombination along normally segregating chromosomes 21.

Hotspot usage among nondisjoining chromosome 21events due to MI errors

We first focused on the analysis of the entire long arm of chromosome 21. We found hotspot density to be a significant predictor of the proportion of recombination within a bin, similar to normally segregating chromosomes (p=0.02) (Figures 3.2 and 3.6). We next tested if the slope of the line for the MI error group was significantly different from that of controls and found they were not significantly different (p=.43). Next we examined single recombinant events by region of 21q. We found a significant positive association between hotspot density and the proportion of recombination per bin in the distal region, (p=0.001) (Figure 3.4) similar to normally segregating chromosomes. There was no significant association in the medial region and there weren't enough recombinants in the proximal region to perform this analysis for this error group (Figure 3.4 and Table 3.1).

Among nondisjoining chromosomes with two detectable recombinants we separated analyses by the proximal and distal event. We found a significant, inverse relationship between hotspot location and recombination in the proximal region of 21q, suggesting that these proximal recombinant events occur preferentially in regions relatively void of historically-defined hotspots (p= 0.04) (Figure 3.5). However, we are cautious in our interpretation, as it may be due to

three influential data points that, when paired with a relatively small sample size, resulted in this inverse relationship. Furthermore, when we adjusted the regression models by including bin location as a covariate (see Methods): 1) the inverse relationship was no longer significant (p= 0.34) and 2) inclusion did not alter the relationship between hotspot count and proportion of recombination for any other chromosome region among our MI meiotic events. For distal recombinant events, we found that recombination was significantly associated with the location of historically-defined hotspots (p=0.004).

Hotspot usage among nondisjoining chromosome 21events due to MII errors

As for MII, there was no evidence that the proportion of recombinants per bin was significantly correlated with hotspots across the long arm of chromosome 21 In other words, the slope of the line was not significantly different from zero. (Figures 3.2 and 3.7). This group is the only group where hotspot density was not a significant predictor of the proportion of recombination along the length of 21q. We next tested if the slope of the line for the MII error group was significantly different from that of controls and found a significant difference (p=.05). In the regional analysis, we did not find an association between hotspot density and the proportion of single recombinant events per bin for the proximal (p=0.63) or medial region (p=0.77) (Figure 3.4). However, hotspot density was a significant predictor in the distal region (p<.01).

Among MII errors with two recombinant events, again, there was no significant correlation between the proportion of recombination per bin and density of historically-defined hotspots in the proximal region (p=0.62) (Fig. 3.5). Again, we

must be cautious as inclusion of bin location in this model led to a significant association (p=0.02). For MII distal events, there was a significant positive association with hotspot usage and this was robust to exclusion or inclusion of bin location in the model (p=0.01 and p=0.03, respectively).

3.4 DISCUSSION

Our previous studies suggested that patterns of recombination on nondisjoined chromosomes 21 differ from that of normally segregating chromosomes (Lamb et al., 1997; Oliver et al., 2011). These unique patterns are observed among normal meiotic outcomes, but at significantly lower frequencies. We hypothesized that in some cases, these events may result from dysregulation of recombination and in other cases they may simply be a low frequency event that happens normally. For those due to dysregulation of recombination, we may expect to see a lack of association with genomic features associated with normal recombination or a lack of usage of historically defined hotspots.

We did not find noteworthy associations between the proportions the genomic features of interest and amount of recombination within a bin along 21q in any of our meiotic outcome groups, which differs from the study performed by Kong et al. in 2002. As we had approximately the same number of controls samples (1,272) as was used in the Kong et. al study (Kong et al., 2002), we attribute this difference to a difference in study design. Two key differences in our study design when compared to Kong et al. may explain our difference in analysis results. First, we restricted our analysis to 21q, whereas the original associations were found through the analysis of the entire genome. Second, the Kong et al. study

included 1,257 meiotic events, combining maternal and paternal outcomes while we only examined maternal recombination events.

Our findings with regard to LD-based historical hotspots do differ between our meiotic outcomes groups and provide some insight into recombination-associated nondisjunction. First, we gain confidence that our analyses are able to identify associations with hotspot usage, as our findings from normally disjoining chromosomes 21 are consistent with expectation. That is, using our subset of normal meiotic events, we confirmed in all analyses and stratifications that recombinant events on 21q occur in previously defined regions of elevated recombination or LD-based hotspots. This was expected as samples with normal meiotic events, which are more likely to have resulted from normal recombination patterns, were used in the identification of historical hotspots.

Among MI errors, our analysis of the entire chromosome indicates this group has a recombination placement pattern that reflects the distribution of LD-based hotspots. For our regional test, we found that there was a significant correlation between the proportion of recombination per bin and hotspot density in the distal region for both chromosomes with single and double events. Elevated recombination in the distal region is associated with this error type (Lamb et al., 1997; Lamb et al., 1996), yet these results indicate that this is not due to dysregulation of factors involved in hotspot usage. We offer two explanations for these results. First, this phenotype may be due to inhibition of recombination in the proximal and medial region or recombinants that are less stable when they occur in these regions. Perhaps differences in chromatin structure that would

suppress recombination in the more proximal regions explain these results. Second, single exchanges located in these more distal hotspot locations in maternal meiosis may lead to unstable bivalents. Our previous studies show that the location of the distal recombinant differs significantly between MI and Controls: among single events, the average location of recombination along 21q among MI errors is 37.56 Mb compared with 27.53 Mb in normal events (Oliver et al., 2011). Among chromosomes with two detectable recombinants, the distal event is more medially placed among MI errors compared to normal meiotic outcomes (35.28 vs.38.86 Mb, respectively (Oliver et al., 2011). Our current study provides evidence in support of our prior hypothesis; when an exchange is too far from the kinetochore, it prevents the bi-orientation of homologues on the meiotic spindle leading to MI errors (Hawley et al., 1994; Koehler et al., 1996; Nicklas, 1974; Ross, Maxfield, & Dawson, 1996). However, it should be noted that historically-defined hotspots do not distinguish maternal and paternal meiotic events or single and multiple events. They are a summary of recombination across many, many generations. Therefore, it is possible that the more distally defined hotspots are associated with either paternal meiotic events or multiple events.

For MII errors, we found that single recombinants across 21q are not significantly correlated with historically-defined hotspots. A comparison of regression slopes between these two groups revealed that their slopes are significantly different from each other. This suggests that the reduced correlation observed between hotspot density and the proportion of recombination per bin is due to a significant difference in the relationship between these two variables for

MII errors. Our regional analysis and examination of double recombinants indicate that the proximal region seems to be the source of this lack of correlation as we only found significant relationships in the distal region. This observation differed from that of normal meiotic events. More data are needed to determine whether this difference in relationship is the result of variation in factors that control recombination in normal meiotic outcomes. Again, it is important to note that the average location of the proximal events among MII nondisjoined chromosome is much closer to the centromere than the normal events (Oliver et al., 2011). Perhaps variation in factors that affect recombination placement, such as the gene PRDM9 (Baudat, Buard, Grey, Fledel-Alon, et al., 2010; Myers et al., 2010; Parvanov, Petkov, & Paigen, 2010) or factors that alter chromatin confirmation, allow for recombination that is more proximal to the centromere.

3.5 MATERIALS AND METHODS

Ethics Statement

All recruitment sites obtained the necessary Institutional Review Board approvals from their institutions.

Trisomic Population

Study Sample

Families with an infant with full trisomy 21 were recruited through a multisite study of risk factors associated with chromosome mal-segregation (Freeman et al., 2007; Lamb et al., 1997; Lamb et al., 1996). Parents and the infant donated a biological sample (either blood or buccal) from which DNA was extracted. Only families in which DNA was available from both biological parents and the child with trisomy 21 were included, leading to a total of 785 maternal MI and 283 maternal MII cases of trisomy 21; 416 of the maternal MI and 141 of the maternal MII cases were included in a previous study reported by Oliver et al. 2008 (Oliver et al., 2008).

Genotyping and Quality Control

Samples were genotyped at 1536 SNP loci on 21q by the Center for Inherited Disease Research using the Illumina Golden Gate Assay. The most centromeric SNP was rs2259403 and the most telomeric was rs46909248. Mendelian

inconsistencies and sample mix-ups were identified using RelCheck among the trios. In addition, parental genotyping data were used to identify poorly performing SNPs. SNPs that met the following criteria were excluded from our analyses: minor allele frequency (MAF) <0.01, deviation from Hardy Weinberg Equilibrium (HWE) ($p<0.01$), heterozygosity >0.60 or > 10% missingness. We also excluded SNPs on a family-by-family basis if >50% of the genotype data for a proband had low intensity levels.

Determining Stage and Origin of Meiotic Chromosome Mal-Segregation

The parental origin of the meiotic error was determined by establishing the contribution of parental alleles to the proband with trisomy 21. Only cases of maternal origin were included in our analyses. Once the maternal origin of the meiotic error was established, markers located in the pericentromeric region (13,615,252 bp – 16,784,299 bp) of 21q were used to infer the stage of the meiotic error, MI or MII. If parental heterozygosity was retained in the trisomic offspring, we concluded a MI error. If parental heterozygosity was reduced to homozygosity, we concluded a MII error. In this assay, we cannot distinguish between the different types of underlying errors that might lead to an MII error. For example, sister chromatids that fail to separate during anaphase of MII or an error that is initiated in MI and not resolved properly in MII both lead to the contribution of sister chromatids to the gamete. Also, if sister chromatids prematurely separate in MI, some configurations will lead to both sister chromatids segregating to the same pole in MII. Lastly, when all informative

markers in the parent of origin were reduced to homozygosity, the origin of nondisjunction was inferred to be a post-zygotic, mitotic error and excluded from the study.

Identifying the Location of Recombination

After genotyping quality control measures were implemented and SNP data were combined with STR data from our previous studies (Oliver et al., 2008), we defined the location of recombinant events. The breakpoints of a single recombinant event were defined by a minimum of either one STR or eight consecutive, informative SNPs flanking the recombination breakpoint. An exception to this rule occurred when the most proximal or most distal informative markers on 21q indicated the presence of recombinant event. In these instances, a minimum of either one STR or four consecutive, informative SNPs were required to define the breakpoints of recombination. The presence of a double recombinant event was defined by a minimum of either one STR or 8 consecutive, informative SNPs flanking the recombination breakpoint on each side for both events.

Euploid Population

Study Sample

SNP genotyping data for normally segregating chromosomes 21 were taken from families recruited for 1) the Autism Genetic Research Exchange (AGRE) (N=743) (Weiss et al., 2008), 2) the Framingham Heart Study (FHS) (N=764) (Dawber, Meadors, & Moore, 1951) and 3) the GENEVA Dental Caries Study

(N=107) (Polk et al., 2008). All families were two-generation families with a minimum of three children. This was necessary to define specific recombination profiles for each parent child transmission.

Genotyping and Quality Control

The AGRE samples were genotyped for SNPs genome-wide using the Infinium (R) HumanHap550-Duo BeadChip. The AGRE data included genotypes at 520,017 markers genome-wide, however 11,473 markers were excluded from the analysis due to deviation HWE ($p<10^{-7}$). After quality control measures were completed, there was genotype information for 7,810 SNPs on 21q for the AGRE dataset. The FHS samples were genotyped for SNPs genome-wide using the Genome-Wide Human SNP Array 5.0. The FHS data included genotypes at 500,568 markers. However, 22,000 markers were excluded from the analysis due to deviation from HWE ($p<10^{-7}$). After quality control measures were completed, there was genotype information for 6,705 SNPs on 21q for the FHS dataset. The GENEVA samples were genotyped using the Illumina 610-Quad Array. The GENEVA dataset included genotypes at 620,901 SNPs. 58,610 markers were excluded from the analysis due to deviation from HWE ($p<10^{-5}$), a MAF < 0.02. After quality control measures were completed, there was genotype information for 8,189 SNPs on 21q from the GENEVA population. All SNP locations were based on human NCBI Build 36 (hg18).

Identifying the Location of Recombination

For the AGRE, FHS and GENEVA datasets, genotype data from members of two-generation families with three or more children were used to infer the location of recombination along the maternal chromosome 21. Our approach and software are described in Chowdhury et al. (Chowdhury et al., 2009). Briefly, parental genotypes were used to identify informative markers. Then, using these markers, genotypes of the children were compared to identify alleles inherited identical-by-descent from the mothers and fathers. Between two sibs, a switch from sharing the same maternal allele to the different maternal allele was scored as a maternal recombination event.

Statistical Analyses

Examining the Relationship Between Genomic Features and Recombination

Linear regression was used to determine whether there was an association between the genomic feature of interest and the amount of recombination. Each genomic feature was analyzed separately. In our approach, chromosomes with a single recombinant event were analyzed separately from those with two recombinants on 21q since the mechanism of nondisjunction may differ depending on the number of recombinants on 21q (Oliver et al.). This led to the sample sizes shown in Table 1. For chromosomes with two recombinant events, the proximal event was examined separately from the distal event. Interference (a phenomenon in which multiple recombinants are spaced a minimal distance apart) (Weber et al., 1993) is likely to have forced double recombinants to occur in the centromeric and telomeric portions of 21q. Hence it was important to adjust for this possible confounder in the regression analysis. Our regional

stratification and binning methods allowed us to adjust for interference. By examining the proximal and distal regions separately, we were able to determine if there are factors in addition to interference that cause elevated recombination in specific bins within these regions.

Since the distribution of GC content, one of our genomic features of interest, differed greatly by region of the chromosome (Figure 3.1), we further divided our analyses based on regions designated as proximal (13.6 -18.6 Mb), medial (18.6 - 23.6 Mb) and distal (23.6 - 48.5 Mb). Each region was then divided 21q in 500kb bins and univariate linear regression was conducted with the outcome variable being the proportion of recombinant events in each bin and the predictor variable being the proportion or count of each genomic feature per bin. We also examined a multivariate model in which our genomic feature of interest and the location of the bin along 21q were used as predictors of the amount of recombination. All analyses were conducted for our three meiotic outcome groups: normal, MI and MII meiotic errors. Accounting for location did not significantly alter the results of the linear regressions models, hence, it was omitted from the formal regression analyses unless otherwise specified. An association between the quantity of the genomic feature and the amount of recombination was identified if the beta coefficient for the predictor variable (i.e. the feature of interest) was different from zero at $p<0.05$. Data on genomic features were based on the hg18 build of the human genome and retrieved from the following tables within the USCS Genome Browser: gc5Base, CpGIslandExt and rmsk (repeat master), UniGene_3 and RefGene. Data on the location of hotspots on 21q was inferred from genome-

wide Phase II HapMap linkage disequilibrium (LD) data by Myers et al. 2008 (Myers et al., 2008).

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Conflict of interest statement. None declared.

Figure 3.1 Distribution of GC Content along 21q. The centromere region of 21q consists of intervals 1-12, the medial region intervals 13-24 and the telomeric intervals 25-67. 21q was broken down into these three regions as the relationship between interval location and GC content, a factor associated with recombination, was not linear. This allowed us to fit a linear model to each region of 21q. The colored dotted lines represent the three regions examined which are based on the relationship between interval and percent GC, red = centromeric, black= medial and green=telomeric.

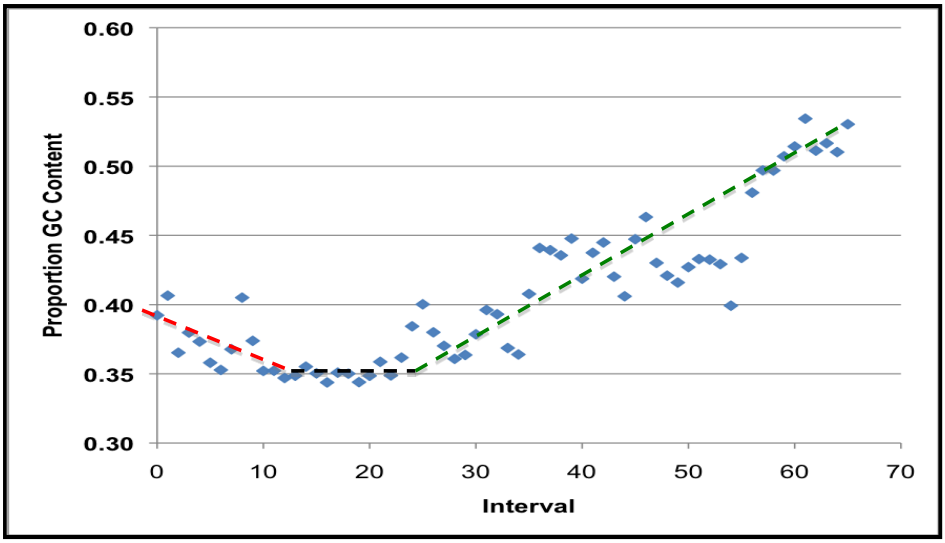
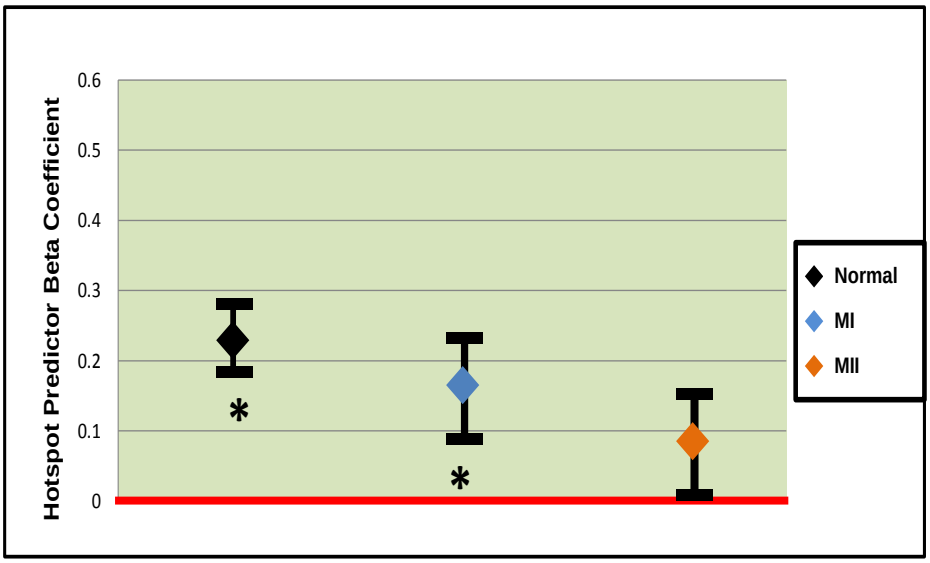


Figure 3.2 Beta Coefficients for Single Recombinant Events. Beta coefficients and 1 SD error bars represent the relationship between the number of hotspots and percent of recombination. Beta coefficients significantly different from zero are indicated by the presence of an asterisk.



15

Figure 3.3 Relationship Between Hot Spot count and Recombination Across 21q for CONTROLS

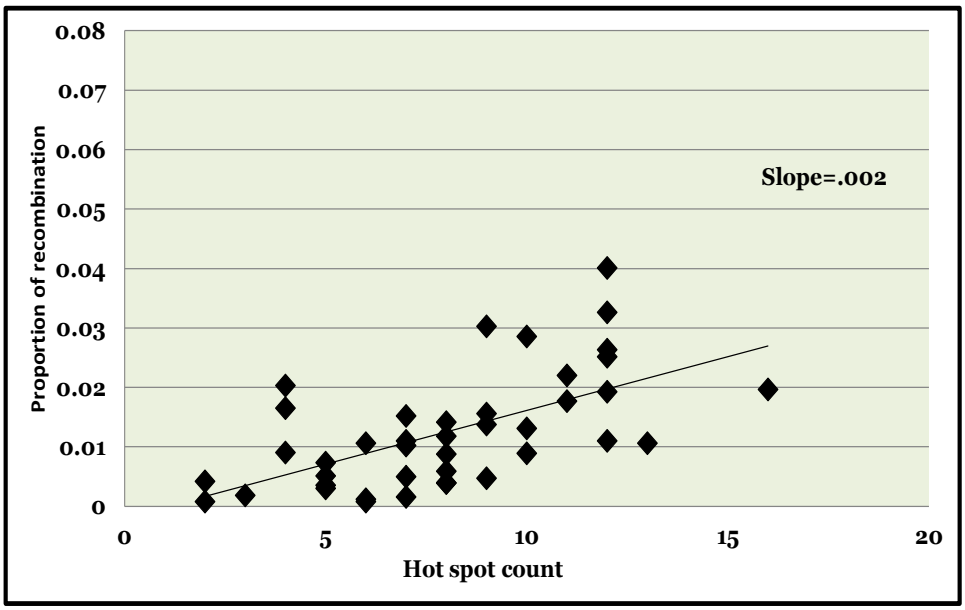


Figure 3.4 Beta Coefficients for Regional Analysis of Single Recombinant Events. Beta coefficients and 1 SD error bars represent the relationship between the number of hotspots and percent of recombination. Beta coefficients significantly different from zero are indicated by the presence of an asterisk.

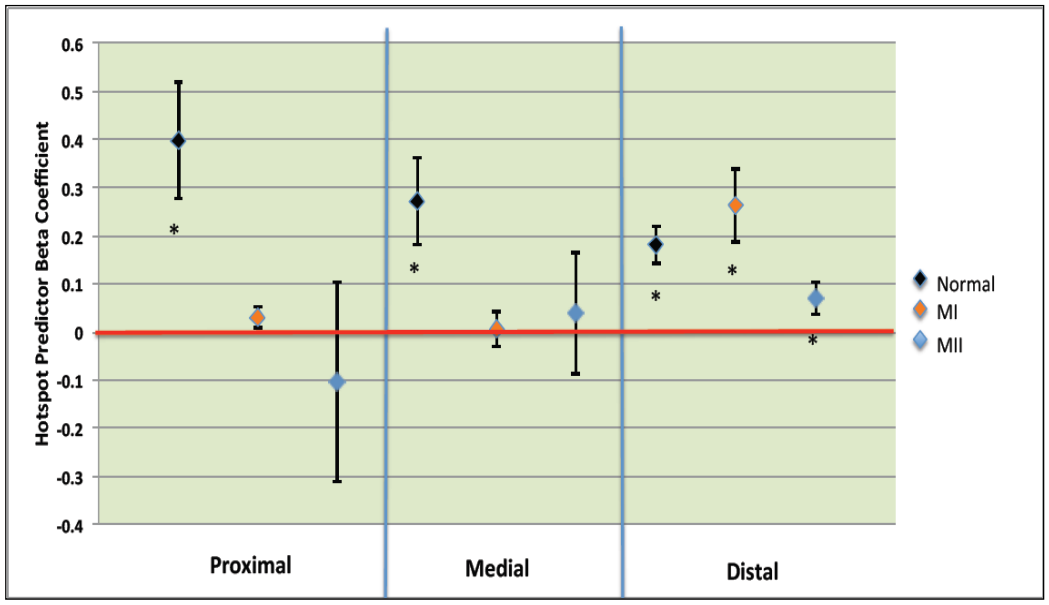


Figure 3. 5 Beta Coefficients for Double Recombinant Events. Beta coefficients and 1 SD error bars represent the relationship between the number of hotspots and percent of recombination. Beta coefficients significantly different from zero are indicated by the presence of an asterisk.

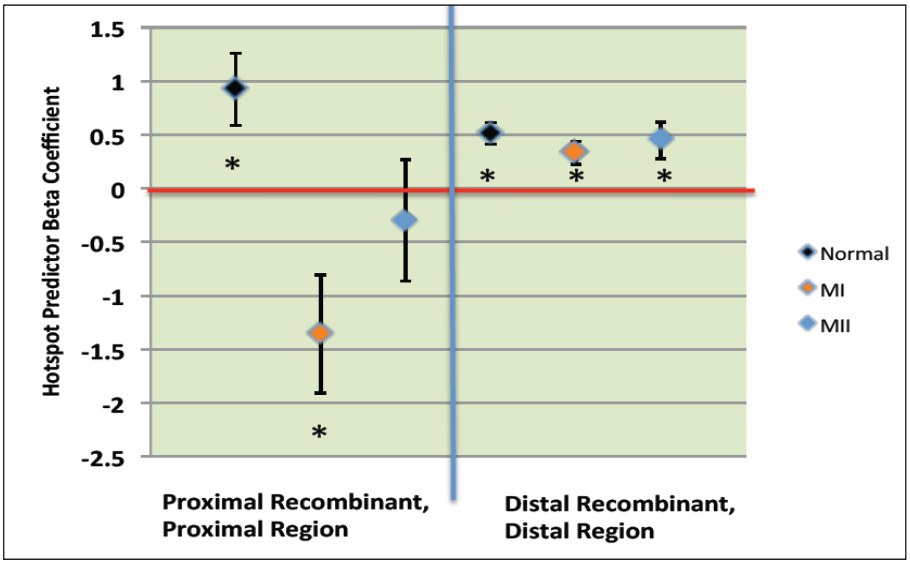


Figure 3.6 Relationship Between Hot Spot count and Recombination Across 21q for MI ERRORS

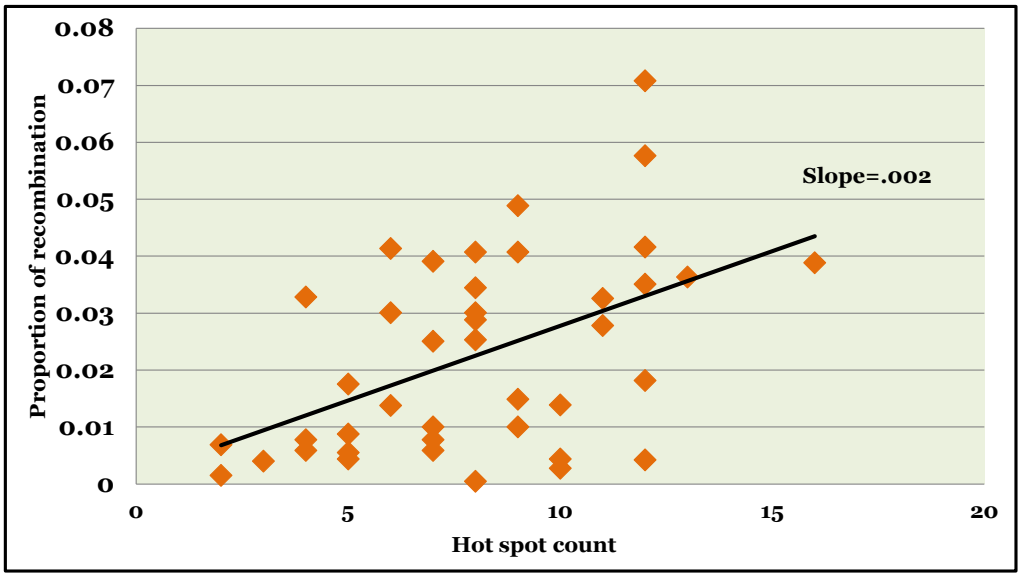


Figure 3.7 Relationship Between Hot Spot count and Recombination Across 21q for MII ERRORS

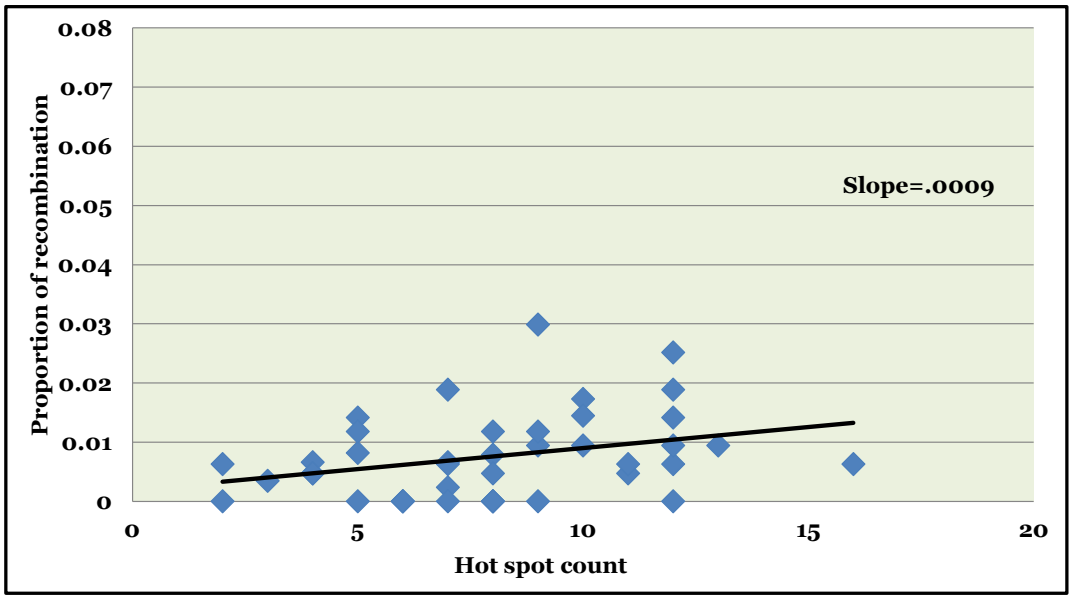


Table 3. 1 Population Sample Sizes and Distributions of Recombination by Location on 21q. Values presented are expressed as a percentage

	N	Centromere	Medial	Distal
MI Single	222	1.50	3.29	95.21
MI Proximal	75	68.67	6.00	25.33
MI Distal	75	2.00	4.00	94.00
MII Single	202	37.57	25.11	37.31
MII Proximal	75	67.33	13.94	18.73
MII Distal	75	0.00	0.00	100.00
Normal Single	1272	21.16	13.63	65.21
Normal Proximal	342	32.74	11.65	55.60
Normal Distal	342	1.02	0.58	98.39

Chapter 4 Discussion

4.1 Applicability of Trisomy 21 research to other trisomies

Nondisjunction is reported to occur in at least 5% of clinically recognized pregnancies (Angell et al., 1984; T. Hassold et al., 1996; T. Hassold & Hunt, 2001; Warburton & Fraser, 1964) and accounts for approximately 35% of spontaneous abortions (T. Hassold & Hunt, 2001). Trisomy is the most common type of nondisjunction in live births as monosomies almost always spontaneously abort (T. Hassold et al., 2007). Infants born with these trisomies have moderate to severe intellectual and development disabilities, physical defects and a shortened lifespan. As a majority of these conceptuses result in spontaneous abortions or a shortened live span, Trisomy 21 is used as a model for nondisjunction as it is the most common in live births (Canfield et al., 2006). Trisomy 21 shares risk factors with other types of nondisjunction. Hence, Trisomy 21 research is applicable to several other trisomies.

4.2 Scope of research

Altered recombination has been identified as a risk factor for nondisjunction (NDJ). The frequency and placement of recombination on the nondisjoined chromosome have been examined in several studies. Specifically for Trisomy 21, the absence of recombination or the presence of a single recombinant event near the telomere of the long arm of chromosome 21 (21q) are associated with maternal meiosis I (MI) errors. Meiosis II (MII) errors are associated with the placement of a recombinant event near the centromere of 21q. The focus of this

thesis research was to determine whether cis and/or trans-acting factors that are associated with recombination in normal meiotic events are altered in meiosis I or II errors.

Chapter 2 presented a study where we sought to determine whether "gamete" effects (trans-acting factors that influence the correlation in recombination number between chromosomes within the same gamete) or "mother" effects (trans-acting factors the influence the correlation between genome wide recombination counts of oocytes from the same mother) are involved in abnormal recombination placement and/or frequency along nondisjoined chromosomes 21. Chapter 3 presented a study where we examined genomic features found at locations of recombination along nondisjoined chromosomes 21 to determine if they differ from chromosomes that have disjoined.

4.3 Contribution to nondisjunction research: Variation in trans-acting factors contribute to nondisjunction

In our study of trans-acting factors, we assessed MI error probands for recombination properties found in normal individuals. Studies have found that there is significant correlation between the number of observed recombinants on a specific chromosome and the number of recombinants genome-wide and this is called the "gamete effect"(Kong et al., 2002). Studies have also shown that the genome wide recombination counts in oocytes are more similar between sibling oocytes from the same mother when compared to the offspring of other mothers and this is called the "mother effect" (Cheung et al., 2007; Kong et al., 2002). We used linear regression models to test for the "gamete effect", stratified by meiotic

outcome group (MI error, MI error siblings and controls). We used the beta coefficients (indicates the increase in GWR count for every unit increase in recombination on an individual autosome) of each autosome to summarize our findings. We found that there was strong evidence for a gamete effect among the normal meiotic outcome group as all autosomes were significant predictors ($p<0.001$) of GWR count. As the number of recombinants on each individual chromosome increased by 1, the GWR count increased on average by 1.75 recombinants. In contrast, chromosome specific analyses among the MI error group showed a reduced average beta coefficient of 1.23. We then assessed the meiotically-normal MI error siblings to see if their patterns were more similar to their siblings or the controls. We found that they had an average beta coefficient of 1.72 which is very close to what was seen in the control group. These data indicate two things: there appears to be a disruption in the "gamete effect" across the autosomes of the MI error group and there is no evidence for this disruption in the "gamete effect" in the siblings of MI error probands.

In future studies, it will be important to identify trans-acting factors that regulate the gamete effect. As variants in RNF212 and region 17q21.31 have been found to associate with increased or decreased genome-wide recombination counts in females, these may serve as the first candidate genes for genotyping in mothers of MI error probands. Determining whether these trans-acting factors influence the gamete effect would also help identify other candidate genes/molecules that contribute to the altered recombination patterns seen in MI error oocytes.

Perhaps there are also environmental factors that alter the maternal effect by causing greater variation in the GWR counts between sibling oocytes. Studies in BisPhenol A (BPA), which is an organic compound found in many packaging materials such as plastic, has been shown to be a potent mutagen of meiosis (causes aneuploidy)(Hunt et al., 2003). It was also found to alter the number of chiasma (visual manifestations of homologous recombination) after exposure(Brieno-Enriquez et al., 2011) as well as alter gene expression(Brieno-Enriquez et al., 2012). A study examined the effects of BPA exposure on developing fetuses as well as the oocytes that these fetuses later produced. While the fetuses were found to display aberrations in meiotic prophase and high GWR counts, the oocytes produced by these fetuses had high rates of aneuploidy (Hunt et al., 2003; Susiarjo, Hassold, Freeman, & Hunt, 2007). In addition, the effect of BPA on oogenesis was found to be modified by the diets of the mothers (Muhlhauser et al., 2009). Taken together, differential BPA (or some other meiosis mutagen) exposure of oocytes and variation in the diet of a mother may explain why gametes with an MI error display an altered gamete effect when compared to their siblings.

4.4 Contribution to nondisjunction research: MII errors may be caused by factors that influence hotspot usage

In our second study, we sought to determine if genomic features (GC content, CpG fraction, Poly (A)/Poly (T) fraction and gene location) or the number of hotspots in a genomic region are significant predictors of the proportion of recombination for disjoined versus nondisjoined chromosomes 21. To assess the

relationship between recombination placement and each of our variables of interest, we subdivided 21q into bins. We then used univariate linear regression to determine the relationship between the proportion of recombination in a given bin and the quantity of each of our variables of interest. We stratified by meiotic outcome group (MI, MII and normal meiotic outcome) and examined chromosomes 21 with single and double recombinants separately. Results from our analysis of normal meiotic events and maternal meiosis I NDJ errors showed, as expected, that recombination placement was correlated with hotspots on 21q. In contrast, among maternal meiosis II errors, there was no significant correlation between recombination placement and hotspots. Recombination along nondisjoined chromosomes was not associated with the other genomic features assessed in this study. This finding suggests that the factors that regulate hotspot usage may be dysregulated in MII errors.

Our results based on differences in usage of historically-defined hotspots draw attention to the recent work on *PRDM9,* a gene identified as a regulator of meiotic recombination hotspots in humans and mice (Baudat et al.; Myers et al.; Parvanov, Petkov, & Paigen). *PRDM9* contains a zinc finger array that, in humans, can recognize a short 13-base pair sequence associated with hotspots (Myers et al., 2008). Allelic variation in the zinc finger repeat has been shown to affect hotspot activity (Baudat, Buard, Grey, Fledel-Alon, et al., 2010; Berg et al., 2010a). Using human sperm samples, Berg et al. (Berg et al., 2010a) also showed that subtle changes within the zinc finger array creates hotspot non-activating or enhancing variants that can trigger the appearance of a new hotspot. Thus based on the results presented here, we hypothesize that the frequency distribution of

PRDM9 genotypes among women with chromosome 21 nondisjunction errors may differ significantly by the type of meiotic error, those with MII errors having an increased frequency of non-activating variants, enhancing the potential of a random pericentromeric event. Alternatively, specific *PRDM9* variants may increase the usage of binding sequences that differ from those associated with historically-defined hotspots. Among MI errors, telomeric events were associated with historically-defined hotspots and thus the frequency distribution of *PRDM9* genotypes may not differ from those with normal meiotic outcomes. Instead, specific *PRDM9* variants may interact with additional genomic features in these subtelomeric regions, promoting these unusual recombinant events. We also speculate that the class of errors due to a lack of recombination may be due to carriers of non-activating *PRDM9* variants. If differences in *PRDM9* genotype are associated with altered patterns of recombination on 21q and differential hotspot usage, this knowledge may be helpful in predicting the risk for increased pregnancy loss and nondisjunction-associated birth defects.

4.5 Clinical applicability

As many women are electing to delay child birth and nondisjunction is associated with advanced maternal age, identification of factors that are associated with nondisjunction will help women assess their risk for having a child with trisomy. The two studies describe in this dissertation potentiate several biological factors (PRDM9, RNF212, 17q21.31) and an environmental factor (BPA) as contributors to nondisjunction of chromosome 21. If an allele within the aforementioned genes is found to associate with altered recombination and an MI or MII error, genotyping of these alleles can be used to aid in trisomy

21 risk assessment. Furthermore, if BPA is found to contribute to altered recombination, exposure prevention methods may be developed. As was previously mentioned, these finding may also be applicable to other types of nondisjunction. If the associations found in Trisomy 21 are confirmed in other trisomies, these results may have a greater impact on nondisjunction research.

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