Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

NaTasha Hollis Date

Genetic Variation in Recombination as a Risk Factor for Nondisjunction

By

NaTasha Hollis
Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences
Genetics and Molecular Biology

_____

Stephanie L. Sherman, Ph.D.
Advisor

_____

David Cutler, Ph.D.
Committee Member

_____

Michael Epstein, Ph.D.
Committee Member

_____

Paula, Vertino, Ph.D.
Committee Member

_____

Michael Zwick, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

Genetic Variation in Recombination as a Risk Factor for Nondisjunction

By

NaTasha Hollis
B.S., Xavier University of LA, 2005

Advisor: Stephanie L. Sherman, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Division of Biological and Biomedical Sciences
Program in Genetics and Molecular Biology
2010

ABSTRACT

Genetic Variation in Recombination as a Risk Factor for Nondisjunction
By NaTasha Hollis

Nondisjunction is the failure of chromosome to properly segregate during meiosis. Nondisjunction of chromosome 21, leading to trisomy 21, is the most common aneuploidy to survive to term. The focus of this thesis research was to better understand altered patterns of recombination in maternally-derived meiotic chromosome 21 errors.

Information on parental origin and stage of nondisjunction, recombinant profiles along the nondisjoined chromosome, variants within genes associated with recombination, and folate supplementation and pathway polymorphisms allowed us to (1) examine the role of genetic variation in recombination and in oocytes with a nondisjoined chromosome 21 and (2) examine the role of folate as a risk factor for nondisjunction of chromosome 21 and how it may interact with recombination.

Variation in recombination rates exists within and among individuals; however, little is known regarding what factors may influence this variation. To determine the role of variation in genomic regions and recombination rates, SNPs in three genomic regions were genotyped in mothers of infants with trisomy 21. We found preliminary evidence for an association of variants in two of the candidate genes/regions, *RNF212* and the 17q21.31 inverted region, and the recombination phenotype in maternal MII nondisjunction errors. No associations were found among maternal MI errors.

Folate deficiency results in aberrant DNA methylation, chromosome breakage, defective chromosome recombination and aneuploidy. The possible role of folate metabolism on the risk of having a child with trisomy 21 remains unanswered. To gain insight, we examined the role of folate supplementation around the time when meiosis is resumed stratified by the type of meiotic error. Our results revealed use of folate supplementation appears to protect against MII errors in the aging oocyte, but not against MI errors. If confirmed, examination of the recombination profile as a covariate in these models may add insight into the role of folate in proper chromosome segregation. To determine the association between risk of trisomy 21 and folate pathway polymorphisms, we narrowed our phenotype to chromosome 21 nondisjunction errors that occurred in the oocyte and examined genetic variants in mothers. We did not find an association between the polymorphisms and chromosome 21 nondisjunction.

Genetic Variation in Recombination as a Risk Factor for Nondisjunction

By

NaTasha Hollis
B.S., Xavier University of LA, 2005

Advisor: Stephanie L. Sherman, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Division of Biological and Biomedical Sciences
Program in Genetics and Molecular Biology
2010

ACKNOWLEDGEMENTS

I would like to thank everyone who has made an impact on my life to help me to become the person that I am today. I would to say a special thanks to the following:

my advisor, Dr. Stephanie Sherman, for her faith and support in my abilities. Her knowledge, guidance, and encouragement have been the driving force for my matriculation through my graduate training.

my committee members, Dr. David Cutler, Dr. Michael Epstein, Dr. Paula Vertino, and Dr. Michael Zwick for your time and guidance have invaluable.

my former undergraduate research program directors, Dr. Rovaris and Dr. Birdwhistell, for introducing research to me and helping me to find that which I am passionate about that helps all mankind; the MARC and McNair Research Programs for providing the structure, support, and exposure to pursue research.

my siblings, LaTonya, Lashonda, and Troy for providing the balance of friendship, love, and support to my life. I would especially like to thank LaTonya for the long conversations and words of encouragement along the way.

my mother, Mattie, for there are no words to express how grateful I am that God blessed me with such a wonderful mother. You are my mom, my best friend, my joy, and my strength. You encouragement, love, and support are insurmountable. For everything, I say thank you.

my angel in heaven, my grandmother Clara, for your life lessons and unwavering faith that you have instilled in me set the foundation from which I grow and navigate through this journey called life. Although years have passed, your memory will always remain. 4/5/1925-5/29/1997

my newest guardian angel, my grandmother Mattie B, for your encouraging words. Just before your transition you told me that you love me, were proud of me, and I can make it. It is those words that have motivated me through this last phase of training. 3/31/1927-3/9/2010

my GOD, for it is because of You and Your favor in me that I have become who I am today, and to You I give all the glory, the honor, and the PRAISE.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

CHAPTER 1

INTRODUCTION

Meiosis is the process by which gametes, egg and sperm, are formed. The process consists of one round of replication followed by two rounds of division. During the first round of division, meiosis I (MI), homologous chromosomes are separated into two daughter cells while the sister chromatids remain attached at their centromeres. During the second division, meiosis II (MII), sister chromatids segregate to opposite daughter cells producing haploid gametes. Failure of chromosomes to properly segregate at either stage during meiosis is known as meiotic nondisjunction.

Aneuploidy that results from these nondisjunction errors is estimated to occur in 5-25% of all human conceptions and is the most commonly identified chromosome abnormality [Hassold and Hunt, 2001]. Aneuploidy, which may occur to all chromosomes, is the leading cause of pregnancy loss and although most aneuploid conceptions end in miscarriage, a clinically relevant percentage survives to term. A few of the most common aneuploid conceptions that survive to term include trisomy 18, trisomy 21, and the 47, XXY and 47, XXX conditions [Hassold et al., 2007]. These liveborn aneuploid individuals generally present with serious clinical consequences, making aneuploidy the most common cause of developmental and intellectual disabilities. Nondisjunction of human chromosome 21 is a key event in Down syndrome (DS) and occurs in about 1 of every 732 live births [Canfield et al., 2006]. DS is the most common aneuploidy to survive to term [Cuckle, 2005]; thus, serving as a model for nondisjunction.

In spite of the striking clinical impact of segregation errors, relatively little is

known about the factors responsible for nondisjunction. When the origin of error leading to aneuploidy is examined, common themes begin to emerge, although subtle differences do exist among the various aneuploid conditions [Hassold and Hunt, 2001]. Meiotic errors in the oocyte predominate, especially errors originating at the first stage of meiosis. This is true in any organism examined as asymmetric meiosis is always more error prone [Zwick et al., 1999]. Two risk factors have been identified for nondisjunction: advance maternal age and altered patterns of recombination. The focus of this thesis research is (1) to examine the role of genetic variation in recombination and in oocytes with a nondisjoined chromosome 21, and (2) to examine the role of folate as a risk factor for nondisjunction of chromosome 21 and how it may interact with recombination. Specifically, I will focus on maternal meiotic nondisjunction errors as over 90% of errors leading to chromosome 21 are maternal in origin [Freeman et al., 2007; Gomez et al., 2000; Mikkelsen et al., 1995]. To begin, I will review the meiotic process and highlight key steps that relate to my thesis work on nondisjunction. Next, I will review research on variation in the rates of recombination and factors that influence these rates and how these may provide insight into to the altered patterns of recombination that we see observe along nondisjoined chromosomes. Last, I will review findings that have shown an association between folate and DS.

Meiosis

Meiosis is the process by which egg and sperm are formed and consists of one round of replication followed by two rounds of division. The first meiotic division is referred to as the reductional stage because chromosome homologs segregate to daughter cells; in humans, this reduces the chromosome number from 23 pairs of duplicated

chromosomes to 23 duplicated chromosomes. The second meiotic division is referred to as the equational stage, as the number of chromosomes stays the same but sister chromatids segregate. The two stages of meiosis, meiosis I (MI) and meiosis II (MII), are each divided further into four stages: prophase, metaphase, anaphase and telophase (for review, see [Handel and Schimenti, 2010]).

A pre-meiotic S phase begins in the nucleus prior to meiosis during which time chromosomes are replicated. During replication, sister chromatid and chromosome cohesin complexes are established to help stabilize the bivalent by "glueing" sister chromatids together. Prophase I, the beginning of MI, is divided into 5 distinct stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. During leptotene, telomeres of chromosomes cluster at the nuclear envelope forming a "bouquet" which aids in proper alignment of homologs. Chromosomes begin to contract and form a lateral scaffold via assembly of axial elements (AE) from cohesin and synaptonemal complex (SC) proteins. Double strand breaks (DSB) in DNA occur via SPO11. Zygotene is marked as the time when homologs pair and synapsis; hence, AEs, now referred to as lateral elements (LE), combine with a central core to form the SC. During pachytene, synapsis completes and DSBs are repaired as genetic material is exchanged between nonsister chromatids of homologous chromosomes. Two major recombination pathways are: (1) the crossover pathway which leads to crossovers between homologous chromosomes that can be visualized as chiasmata, the physical structure which holds the homolog together throughout prophase I when sister chromatid cohesin is removed, and (2) the non-crossover pathway which does not result in physical chiasmata but may appear as gene conversion events which converts the gene sequence of one chromosome to that of its

homologue. The crossover pathway is the focus of my thesis work. The SC dissolves and chromosomes desynapse. Now, bivalents are held together by chiasmata during diplotene with cohesin still existing distal to exchange breakpoints and around the centromere. Finally, during diakinesis, the nucleolus and nuclear envelope breakdown and spindles assemble. Concurrently with prophase I, microtubule organizing centers form at opposite poles of the cell. Upon nuclear envelope breakdown, microtubules attach to chromosomes. During metaphase I, homologs migrate to and align along the metaphase plate. After alignment is complete, chiasmata are resolved, sister chromatid cohesion breaks down but not centormeric cohesion, and homologous chromosomes segregate to opposite poles of the cell. During telophase I, the meiotic spindle breaks down and a nuclear envelope forms around chromosomes at each pole. Cytokinesis, the division of the cytoplasm forming two daughter cells, marks the end of MI.

Meiosis II begins with the disappearance of the nucleoli and nuclear envelope as chromosomes condense in prophase II. Concurrently, outside of the nucleus, the microtubule organizing center form at opposite poles in preparation for the second meiotic division. Following nuclear envelope breakdown, sister chromatids attach to microtubules at opposite poles of the cell. Sister chromatids migrate to and align along the metaphase plate during metaphase II. During anaphase II, sister chromatids separate and segregate to opposite poles of the cell. The meiotic spindle breaks down and a nuclear envelope forms around chromosomes at each pole during telophase II. Cytokinesis marks the end of meiosis II, producing four haploid daughter cells.

Importance of Recombination in Meiosis

Recombination is the mechanism that acts to ensure that homologous

chromosomes properly segregate in MI. I will highlight a few essential proteins involved in the early process of recombination and research examining their association with nondisjunction. The proteins reviewed are in relation to the research of my thesis.

During leptotene of prophase I, a lateral scaffold begins to form along chromosomes by the assembly of axial elements from cohesin (i.e., SMC1β and REC8) and synaptonemal complex (i.e., SYCP3 and SYCP2) proteins. Double strand breaks in DNA initiate recombination. Synapsis of homologous chromosomes begins in zygotene. During pachytene, synapsis completes, the central element of the synaptonemal complex is evident consisting of proteins such as SYCP1, and recombination occurs. Once recombination completes, desynapsis occurs, and sites of crossovers become visible as chiasmata in diplotene (for review, see [Handel and Schimenti, 2010]).

Cohesin

As DNA is replicated in S phase, cohesion is established between sister chromatids by cohesin to holding sister chromatids together until anaphase I. In addition, cohesin is important for recombination between homologous chromosomes, and establishment of the SC. Meiotic cohesin is a mulit-protein complex formed of four core proteins, SMC1β, SMC3, REC8 and STAG3. SMC1β and SMC3 form a heterodimer and provide the structural support of the complex to which REC8 and STAG3 bind [Xu et al., 2005]. In yeast, REC8 binds pericentromeric heterochromatic regions and is important in MII. It also binds at the central core of the synaptonemal complex independent of heterochromatin and is important for monopolar attachment of homologous chromosomes to meiotic spindles at MI [Kitajima et al., 2003]. To ensure proper segregation of chromosomes, cohesin is removed from chromosomes in two steps.

First, arm cohesion between sister chromatids is removed in MI allowing for the segregation of homologous chromosomes followed by removal of centromeric cohesion at MII allowing segregation of sister chromatids [McDougall et al., 2005].

Studies in model systems have shown that deficiency in components of the cohesin complex display aberrant progression through meiosis. Jeffreys et al. [2003] showed defects in proper chromosome segregation of achiasmate chromosomes in *Drosophila* oocytes with increasing age when a defect exists in sister chromatid cohesion.

Hodges et al. [2005] examined the role of meiotic cohesin in oogenesis by examining SMC1β-deficient mice. Null mice displayed a reduction in exchange rate and shorter synaptonemal complexes. This identifies a relationship between the length of the SC and exchange rates in mice. Additionally, altered patterns of exchanges were exhibited: exchanges were more often observed in the distal part of the chromosome. Importantly, they found an increase in univalents, single chromatids, and distally placed recombinant events with increasing age of the mouse (one, two and six months old). This research provides important evidence for aberrations in cohesin as a risk factor for maternal age-associated nondisjunction.

Studies in model systems have revealed the importance of REC8 in the meiotic process. *S. pombe* Rec8 mutants exhibit precocious sister chromatid segregation and defects in the formation of linear elements which are analogous to SCs [Molnar et al., 1995]. In *C. elegans*, depletion of REC8 results in aberrant SC formation and recombination similar to what is seen in yeast [Pasierbek et al., 2001]. Evidence of aberrant synaptonemal formation in the absence of REC8 also exists in mammals as work done by Xu et al. [2005] display similar characteristics as those seen in yeast and worms;

mice null for REC8 displayed aberrant SC formation. They showed that synapsis occurred between sister chromatids rather than between homologous chromosomes which suggests that REC8 has a role in proper synapsis of homologs.

Synaptonemal Complex

The process of synapsis is facilitated by a group of proteins known as the synaptonemal complex. The synaptonemal complex is composed of three types of proteins: two lateral elements (LE), a central element (CE) and transverse filaments (TF). In the process of synapsis, axial elements (AE) are zipped together by the transverse filaments [Yang and Wang, 2009]. AEs form from meiotic cohesins. In mammals, three primary proteins, in addition to meiotic cohesins, comprise the SC: SYCP1, SYCP2, and SYCP3 [Handel and Schimenti, 2010].

Bolor et al. [2009] has shown that mutations in the SYCP3 gene, an essential component to the SC, are associated with recurrent pregnancy loss in humans. Furthermore, studies in human males and in mice display sterility with mutations in this gene. Female mice, although fertile, exhibit aneuploidy in one-third of their litter [Miyamoto et al., 2003; Yuan et al., 2002; Yuan et al., 2000]. A similar phenotype has been observed in mice mutant for *Sycp2* [Yang et al., 2006]. Male mice exhibit meiotic arrest and hence infertility. Female mutant mice have a reduction in litter. AE formation and synapsis does not occur in these mutants. Interesting, SYCP2 localizes to axial chromosome core but SYCP3 does not indicating that SYCP2 is required for the SYCP3 in the formation of the SC. Male mice mutant for *Sycp1* are also sterile due to a meiotic arrest. Additionally, they do not display any crossovers suggesting a role for SYCP1 in recombination [de Vries et al., 2005].

Timing of Meiosis

The timing of meiotic events differs between males and females. Oogenesis is initiated in the human fetal ovary around 12 weeks of gestation (Figure 1.1). It subsequently arrests in diplotene of prophase I in the dictyate stage around 20 weeks of gestation and is not resumed until ovulation some 10-50 years later. At this time, MI completes and a second arrest occurs in metaphase II where the egg remains arrested unless it is fertilized or degenerates. If fertilized, MII is completed. It is important to emphasize that the generation of all cells destined to become oocytes occur *in utero* by 25 weeks of gestation. Human females are born with all the oocytes they will ever have. In contrast, in human males, spermatogenesis begins at puberty and occurs without delay throughout the reproductive lifespan. Due to the prolonged prophase I arrest, the observation that a large percentage of aneuploidy is associated with maternal MI is not surprising. This is further supported by strong association of nondisjunction and advancing maternal age [Handel and Schimenti, 2010; Hunt and Hassold, 2008].

Nondisjunction

Detection of Origin of Meiotic Nondisjunction Error

In the case of a MI error nondisjunction, homologs fail to properly segregate during MI but the sister chromatids segregate properly during MII. Whereas, in MII errors, the homologs may or may not separate correctly during MI, but the defining event results from sister chromatids failing to properly segregate during MII (Figure 1.2). Both MI and MII errors lead to the production of gametes with an abnormal number of chromosomes, aneuploidy. The fusion of egg and sperm, one containing an abnormal

chromosome number and the other with the proper chromosome number, can lead to monosomic and trisomic fetuses.

Using polymorphic markers, the parental origin and the meiotic stage of error can be determined. The contribution of parental alleles of polymorphic genetic markers along the nondisjoined chromosome to the trisomic proband is used to establish the parental origin (Figure 1.3). If two alleles are contributed from the mother at several markers, a maternal error is inferred. A paternal error is inferred when two alleles at informative markers are contributed by the father. If we are unable to obtain a DNA sample from the father, we can infer a paternal error when alleles in the proband are inconsistent with a maternal origin at least two markers. If at least eight informative markers are consistent with a maternal error, we will make this inference knowing that there will be some misclassification errors. Approximately 95% of cases of chromosome 21 nondisjunction are due to free trisomy 21. The remaining 5% are due to translocation and mosaicisms. Based on this algorithm, of the chromosome 21 nondisjunction cases due to free trisomy 21, over 90% of errors are maternal in origin. Approximately 5% of errors are paternal in origin. Mitotic errors are responsible for the remaining errors. However, these percentages change with the maternal age structure of the sample of nondisjoined errors being studied [Allen et al., 2009].

Once the parental origin of the nondisjunction is established, a core set of markers located in the pericentromeric region (13615252 – 16784299 bp) of 21q are used to infer the stage of nondisjunction as MI or MII. Misclassification of the stage of errors may exist because there are no highly polymorphic markers at the centromere. However, this error should be minimal due to the suppression of recombination in the centromeric

region. Irrespective, if heterozygosity of informative pericentromeric markers in the parent in which the errors is established (parent of origin) is retained in the trisomic offspring, an MI error is inferred. If parental heterozygosity is reduced to homozygosity, a MII error is inferred. When all informative markers in the parent of origin are reduced to homozygosity along the length of 21q, the origin of nondisjunction is inferred to be a post-zygotic, mitotic error. Of the maternal meiotic nondisjunction errors, majority occurred in MI (~75%), although, again, this percentage is dependent on the maternal age in the study sample [Allen et al., 2009].

In general, most human trisomies originate during oogenesis with maternal MI errors, however there are exceptions being more common than maternal MII errors [Hassold et al., 2007]. For example, 47, XXY is just as likely to be paternal in origin as it is maternal [Thomas et al., 2001]. Patterns of nondisjunction are similar among acrocentric chromosomes 13, 14, 15, 21, and 22 trisomies [Hall et al., 2007]. However, variation in the origin of nondisjunction exists for nonacrocentric chromosomes. For example, almost all cases of chromosome 16 nondisjunction, the most frequent error observed in humans, are attributed to maternal MI errors [Hassold et al., 1995]. For chromosome 18 nondisjunction of maternal origin, the majority of errors occur in MII [Bugge et al., 1998; Fisher et al., 1993]. Thus, although general patterns do exist, specific chromosome features influence segregation during meiosis.

Risk factors of Meiotic Nondisjunction Errors

Among meiotic nondisjoining errors, two risk factors have been clearly established: advance maternal age and altered patterns of recombination.

Advanced maternal age and nondisjunction

Advanced maternal age is the primary risk factor for human chromosome trisomy. Most, if not all, human trisomies are affected by increasing maternal age, although the magnitude of the effect varies between different classes of trisomy [Morton et al., 1988; Risch et al., 1986]. For example, the frequency of trisomy 16 increases linearly with maternal age as compared to the exponential increase in the frequency of trisomies 15, 18, and 21 with maternal age [Morton et al., 1988; Nicolaidis and Petersen, 1998]. In general, however, the percentage of trisomies among clinically recognized pregnancies climbs from 2% for women under 25 years of age to 35% for women over the age of 40 [Hassold and Chiu, 1985].

Among cases of DS, advanced maternal age is associated with both maternal MI and MII errors (Figure 1.4) [Antonarakis et al., 1992; Yoon et al., 1996]. It is not associated with paternal errors, indicating that it is the age of the oocyte that leads to the increased risk of nondisjunction [Oliver et al., 2009]. After the age of 35, the risk of a DS birth increases exponentially with age [Antonarakis et al., 1992]. Allen et al. [2009] compared maternal age between mothers of infants with DS due to a maternal error (MI or MII) to control mothers of infants without DS. Allen et al. [2009] also compared maternal age between mothers of infants with DS due to maternal MI errors to mothers of infants with DS due to maternal MII errors. Mothers of infants with DS due to maternal MI errors are 4.0 times more likely to be 35-39 years than 20-24 years at the birth of the proband compared to controls. As age increases, mothers of infants with DS due to maternal MI errors are 8.5 times more likely to be ≥40 years than 20-24 years at the birth of the proband compared to controls. Similarly, with advanced maternal age, mothers of infants with DS due to MII errors are 15.1 times more likely to be ≥40 years than 20-24

years at the birth of the proband compared to controls. Additionally, they found an increased proportion in mothers with a MII error compared to MI errors among the youngest (<15) and oldest (40-45) age groups. At this point in time, this intriguing pattern related to MII:MI ratios by maternal age is not understood.

Altered patterns of recombination and nondisjunction

The physical bridge created at the recombination breakpoint, chiasma, is essential to holding homologous chromosomes together during MI, especially during the extended prophase in oogenesis. Absent or reduced levels of recombination, along with suboptimally placed recombinant events, increase the likelihood of nondisjunction in model organisms [Koehler et al., 1996; Krawchuk and Wahls, 1999; Moore et al., 1994; Rasooly et al., 1991; Ross et al., 1996; Sears et al., 1995; Zetka and Rose, 1995]. Exchanges too close to the centromere or telomere seem to confer the most instability.

Importantly, altered amounts and placement in the location of the recombination are a molecular risk factor for human chromosome nondisjunction. The first indication that reduced recombination was a risk factor for chromosome 21 maternal nonsdisjunction errors came from Warren et al. [1987]. Now it is known that significant reduction in recombination is an important risk factor for all MI-derived trisomies studied to date, including trisomies 15, 16, 18, 21 and X of maternal origin and trisomy 21 and Klinefelter syndrome (47 XXY) of paternal origin [Bugge et al., 1998; Hassold et al., 1995; Hassold et al., 1991; Lamb et al., 1997; Lamb et al., 1996; Robinson et al., 1998; Savage et al., 1998; Thomas et al., 2001]. The presence of a distal exchange is a risk factor for maternally-derived nondisjunction of chromosomes 16 and 21 [Cheng et al., 2009; Lamb et al., 1997; Lamb et al., 1996].

Chromosome 21 nondisjunction has been studied the most extensively with respect to recombination risk patterns. Three susceptible exchange patterns are associated with chromosome 21 maternal nondisjunction (Figure 1.5). Previous studies of maternal MI-derived trisomy 21 estimated that 40% of MI cases were derived from oocytes where no meiotic exchange had occurred along chromosome 21 [Lamb et al., 1997; Lamb et al., 1996]. Furthermore, of those maternally-derived MI cases with a single exchange, the majority of exchanges occurred in the distal 6.5 Mb of chromosome 21. A single pericentromeric recombinant event (the most proximal 3.5 Mb of 21q) is a risk factor for chromosome 21 maternal MII errors [Lamb et al., 1997; Lamb et al., 1996]. This suggests that the presence of a pericentromeric exchange might increase the likelihood of chromosome "entanglement" or premature sister chromatid separation at MI, with the resulting disomic gamete having identical centromeres; thus, the case would be scored as originating at MII even though the precipitating event occurred at MI.

Association between maternal age and recombination

Some patterns of recombination along the nondisjoined chromosome are dependent upon the age of the oocyte, whereas others are not as revealed by recent studies of chromosome 21 nondisjunction [Lamb et al., 2005b; Oliver et al., 2008]. Interestingly, the patterns observed differ by the type of meiotic error, MI or MII. The susceptible single telomeric exchange during MI appears to confer the same risk of nondisjunction regardless of age. This leads to a specific maternal age pattern among case of known maternal MI error. The proportion of MI cases with susceptible single telomeric exchanges was highest among young mothers (80%) and decreased significantly in the older mothers (14%). In fact, the pattern of exchanges among the

oldest age group resembled the pattern observed among normally disjoining chromosomes 21 (10%) [Lamb et al., 2005b]. Additionally, MII errors have been examined and displayed the opposite pattern: the susceptible single pericentromeric exchange pattern occurs at higher frequency in older mothers than in younger mothers [Oliver et al., 2008].

Potential Risk Factors for Nondisjunction

Inter-individual Variation in Recombination

In addition to cohesin, the role of chiasmata to ensure proper chromosome segregation at MI is well established. Considering the importance of chiasmata, it would be expected that the number and location of exchanges would be tightly regulated. Surprisingly, studies using direct and indirect approaches have shown that there is significant inter-individual variation in genome-wide recombination rates. For example, Lynn et al. [2002] used antibodies against MLH1 to mark recombination nodules and identify exchanges in pachytene among spermatocytes of 14 control males and found mean values ranging from 46-53 exchanges/spermatocyte. Indirect measures of genome-wide recombination rates using parent-offspring transmission data have also shown inter-individual variation. Initial reports only identified such variation among females and not males [Broman et al., 1998; Kong et al., 2002]; however, more recent data based on the large CEPH and Hutterite datasets have shown inter-individual variation in both maternal and paternal gametes, which suggests that this inter-individual difference is general to human meiotic recombination [Cheung et al., 2007; Coop et al., 2008].

In addition to this inter-individual variation in number of events, Cheung et al. [2007] found that there was significant variation in the location of events. To examine

this question, they began by dividing the genome into 553 bins of 5 Mb each and scored the number of recombinants in each bin separately by female and male meioses. They found that there were genomic regions in each gender that contained significantly more recombinants than expected by chance; these were termed recombination "jungles". Interestingly, in both males and females, these "jungles" were either the most or second most telomeric bins on the chromosomes. They then focused on the five "jungles" that included the most recombination. They detected polymorphic differences among individuals in activity in these recombination "jungles". That is, the meioses from some individuals contributed significantly more recombinant events to a specific recombination "jungles" than those from others

Variation among Recombination within a Gamete

In addition to the inter-individual variation (referred to as the "mother" or "father" effect) discussed above, data from Kong et al. [2002] identified what they called a "gamete" effect. That is, they found that the number of recombinants was positively correlated among chromosomes within the same oocyte, even after adjusting for the "mother" effect. This points to a factor that has a global influence on recombination rates among most chromosomes simultaneously. For example, the *RNF212* gene (details discussed below) has recently been shown to be correlated with genomewide recombination rates in males and females [Kong et al., 2008].

This "gamete" effect was observed in our own chromosome 21 nondisjoined sample (Brown et al. [2000]). We found a statistically significant reduction in genome-wide recombination rates in oocytes with nondisjoined chromosomes 21 that had no detectable recombination (Brown et al., 2000). The reduction was consistent with the

normal variation in recombination observed among oocytes and was predicted by the number of exchanges found on chromosome 21. These data suggest that specific chromosomes may be at higher risk for nondisjunction when the number of genome-wide recombination events is less than some threshold.

Genetic Variation in Genes on Recombination Rates

Variation in genome-wide recombination rates and patterns may be related to functional variation in genes that orchestrate the recombination process or to variation in the environment in which gametes develop. Recently, research has shown that at least two genomic regions, an inversion on chromosome 17q21.31 and variants in the *RNF212* gene, are associated with variation in recombination rates in normally disjoining populations.

Stefansson et al. [2005] identified a 900-kb region inverted region on chromosome 17q21.31 to be associated with genome-wide recombination rates in women in an Icelandic populaiton. Two primary haplotypes, H1 and H2, are associated with this present in genomic region that spans several genes including *MAPT*, *CRHR1*, and *IMP5*. Women with the H2 haplotype have increased recombination and, interestingly, more children. Although the H2 haplotype, under positive selection in the Icelandic population, is found in Europeans at frequency of 20%, it is relatively rare and non-existent in Africans and East Asians.

In another study, Kong et al. [2008] identified variants within *RNF212* that are associated with rates of genome-wide recombination. The function of *RNF212* has not been characterized, but it shares homology with *S. cerevisiae ZIP3* gene and *C. elegans zhp-3*. Both are involved with meiotic recombination and SC assembly, suggesting a

similar role for *RNF212*. Interestingly, they found sex-specific variants in this gene. For females, every copy of the 'C' allele of rs1670533 increased the rate of recombination. Although not significant, the association of rs1670533 with chromosome 21 stood out as having an opposite effect in females. For males, rs3796619 every copy of the 'T' allele of rs3796619 decreases the rate of recombination.

More recently, Chowdhury et al [2009] analyzed genome-wide SNP genotype data in two large study samples, Autism Genetic Research Exchange (AGRE) and Framingham Heart Study (FHS), to identify genetic variants that may influence recombination rates. This group replicated the association of recombination rates and the inversion on chromosome 17q21.31. However, they only identified the association with *RNF212* and recombination rates in males in the two study samples, although they did see the same non-significant pattern in females. Additionally, they identified other genomic regions that associate with the recombination phenotype including SNPs on chromosome 1q21.2 and 10p11.23 with female recombination and SNPs on chromosome 7q36.1 and 9q31.1 with male recombination [Chowdhury et al., 2009].

Folate deficiency

In addition to the accepted risk factors of nondisjunction, advanced maternal age and altered patterns of recombination, environmental factors may also be a risk factor for nondisjunction. One such environmental factor is folate.

Folate is an essential B vitamin that can only be obtained through diet in two forms, as folate in its natural form through foods such as green vegetables or in its synthetic form as folic acid through nutrient supplementation. The folate metabolic pathway plays an important role in DNA and protein synthesis and methylation [Pogribna

et al., 2001]. Folate is important because it provides one-carbon moieties that can be used for aforementioned processes. Two of the major components of the folate pathway are located on chromosome 21 which is one of the most common chromosomes known to nondisjoined; perhaps, aberrations in the folate pathway related to these components may lead to nondisjunction.

Genes in the folate pathway have been studied to gain a better understanding of their roles in nondisjunction. Some of the genes studied thus far include: methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), and methionine sythase reductase (*MTRR*), cystathionine beta synthase (*CBS*), and reduced folate carrier (*RFC1*). Once folate becomes available, via dietary folate or synthetic folate acid, it is reduced in the intestines to tetrahydrofolate (THF) (Figure 1.6). MTHFR catalyzes the conversion of 5,10-methyleneTHF to 5, methylTHF, which is the methyl donor for the remethylation of homocysteine to methionine. This remethylation process is catalyzed by *MTR* and is vitamin B12 dependent; MTR remains functional via reductive activation catalyzed by MTRR. CBS is important for transsulfuration of homocysteine to cystathionine [Scala et al., 2006].

The folate pathway is important for the production of S-adenosylmethionine (SAM), the major intracellular methyl donor which is derived from methionine. If folate levels are low, homocysteine increases because there is a reduction in remethylation of homocysteine into methionine. Thus, homocysteine accumulates in the cells. Furthermore, the ratio of S-adenosylmethionine (SAM):S-adenosylhomocysteine (SAH) decreases because there is a decrease of available 5, methylTHF needed for the

remethylation of homocysteine to methionine, the precursor of SAM. This results in an impairment of methlyation.

Folate and Nondisjunction: What's the link?

A deficiency in cellular folates results in aberrant DNA methylation, chromosome breakage, defective chromosome recombination and aneuploidy [Das and Herbert, 1978; Fenech et al., 1998; Fenech and Rinaldi, 1994; Fowler et al., 1998; Rana et al., 1983]. Studies suggest chromosomal instability and aneuploidy in cancers may be related genome-wide DNA hypomethylation [Duesberg et al., 1998; Matzke et al., 2003; Narayan et al., 1998; Rajagopalan and Lengauer, 2004]. The global hypomethylation seen in cancer patients may be accredited to folate deficiency as patients with colorectal cancer have been shown to have a low folate status and hypomethylation when compared to controls [Pufulete et al., 2003]. Hypomethylation may also play a role in meiotic nondisjunction and aneuploidies [James et al., 1999; Van den Veyver, 2002]. These authors suggest that low folate status may promote centromeric DNA hypomethylation. Hypomethylation of centromeric DNA may be a predisposing factor for abnormal chromosome segregation. Narayan et al. [1998] showed that hypomethylation of pericentromeric DNA in breast cancer is associated with karyotypic instability. In yeast, the centromeric cohesin protein, Rec8, binds to hypermethylated pericentromeric regions; hence, hypomethylation may lead to aberrant binding [Kitajima et al., 2003]. Rec8 is a member of the cohesin complex which is responsible for centromere and sister chromatid cohesion. It is plausible that an interruption of cohesion binding could lead to premature sister chromatid segregation and ultimately nondisjunction.

Evidence for the Association of Folate Defiency and Chromosome 21 Nondisjunction

Studies of genetic variants in the folate pathway

James et al. [1999] was the first to report an association between polymorphisms in genes involved in the folate pathway and chromosome 21 nondisjunction. They showed that the 677C>T polymorphism in the *MTHFR* gene was associated with 2.6-fold increased risk in the chance of having a child with DS. Importantly, they found that mothers of individuals with DS had an elevation in plasma homocysteine levels irrespective of their *MTHRF* genotype. Homocysteine levels are an excellent biomarker of folate status: an increase in homocysteine is a marker of low folate status. Since this initial report, several follow-up studies examining the association of risk of DS and polymorphisms in folate/homocysteine metabolism have been conducted. While some studies found no associations with single genes in the folate pathway and the risk for DS [Biselli et al., 2008; Chango et al., 2005; Takamura et al., 2004], others suggest that it maybe the association between genes that is responsible for the risk of DS [Bosco et al., 2003; Grillo et al., 2002; Hobbs et al., 2000; O'Leary et al., 2002; Rai et al., 2006; Scala et al., 2006].

The understanding of the role of genes in the folate pathway and DS becomes more complex as several of the genes in the pathway are located on chromosome 21, i.e., *RFC1* and *CBS*. Trisomy of these genes may alter the folate load. RFC1 has a role early in the folate pathway as it is the major transport system for the uptake of folate. CBS has a role later in the process for the transsulfuration of homocysteine to cystathionine. Thus, when studying the association between folate and DS the impact of these extra genes must be considered.

Studies of Folate Nutrition: Fortification and Supplementation

In 1992, the US Public Health Service recommended that women of child bearing age consume at least 400µg of folic acid per day to decrease the risk of having a child with neural tube defects (NTD) based on scientific research evidence [CDC, 1992; Czeizel and Dudas, 1992; Yang et al., 2007]. If folate deficiency were a risk factor for DS, as suggested by James et al. [1999] and others, there should be an association of DS and NTD within the same person, within the same family or in rates within populations. To date, no studies have identified this type of association [Amorim et al., 2004; Forrester and Merz, 2004; Torfs and Christianson, 1998].

In 1998, the US Food and Drug Administration began requiring the fortification of foods with folic acid [Yang et al., 2007]. Studies examining the effect of this fortification on chromosome 21 nondisjunction have not seen a decrease in the prevalence of DS since the initiation of fortification [Canfield et al., 2005; Collins et al., 2002; Ray et al., 2003; Simmons et al., 2004]. Yet, others have examined the role of nutritional folic acid supplementation around the time of conception when MI resumes. Botto et al. [2004] did not observe a significant decrease in risk for DS when periconceptional supplementation occurred three or more times a week before conception. However, Czeizel and Puho [2005] showed a decrease in the risk for DS when large doses (~6 mg/d compared with the recommended 400µg/d in the U.S.) of folic acid supplementation occurred within the first month of pregnancy. In this sample, folic acid and iron supplementation occurred concurrently. The authors were able to determine that iron supplementation by itself influence the risk of nondisjunction (OR=0.4, 95% CI (0.1, 0.9)). However, the number of women taking only folic acid was too small to determine its individual effect.

OVERVIEW OF THESIS RESEARCH

The primary focus of this research is to extend previous studies examining recombination as a molecular risk factor for nondisjunction. My initial thesis work began with a project to examine the genome-wide recombination rates in a nondisjoined chromosome 21 population to determine whether trans-acting factors play a role in recombination-based nondisjunction (Chapter 4). Evidence from both individual and inter-individual variation in recombination suggest that the altered recombination patterns that we see associated with chromosome 21 nondisjunction may extend globally. If so, is the genome-wide recombination profile specific to the oocyte with the meiotic nondisjunction event or does it extend to other meiotic events in the same mother?

This project depends on obtaining DNA samples from maternal grandparents and parents of probands with DS, a difficult recruiting task. I was trained by the study personnel to recruit families and conduct maternal interviews of study participants. I have gone to various DS conference to aid in the recruitment efforts for this project as well as conducted several individual interviews. Thus, I gained knowledge and experience in the working in the field of a large epidemiological study. Additionally, I helped write the service grant to the Center for Inherited Diseases (CIDR) for genome-wide linkage studies to be performed. We successfully obtained this grant and genotyping will begin in the fall.

My next project, described in Chapter 2, examined the hypothesis that lack of folic acid supplementation would increase the risk of experiencing a nondisjoining event, i.e., having a child with Down syndrome. Very few studies have examined folic acid supplementation and chromosome 21 nondisjunction [Botto et al., 2004; Czeizel and

Puho, 2005]. We improved on the study design of others by 1) defining specific nondisjunction errors by the parent (maternal or paternal) and stage of origin (MI or MII) and 2) , restricting the window of folate supplementation to around the time of conception, or the time when meiosis I is resumed. The results revealed that older mothers who did not supplement with folic acid had an increased risk of experiencing an MII meiotic error.

My third project, described in Chapter 3, followed-up the folate supplementation project described in Chapter 2 using a small dataset that was available in the laboratory. That is, genotyping data for genes in the folate pathway became available on a subset of our study sample. We looked in this opportunistic sample to determine whether there was an association between genetic variants in the folate pathway and nondisjunction stratified by meiotic error. Our results revealed no association.

Chapter 4 describes data from a growing field of research to examine the roles of genes on recombination rates. This is the first study of its kind among nondisjoining meiotic events; all previous work has been done in normally disjoining meiotic events. Our results reveal that variants in genes that influence rates among properly segregating chromosomes are also influencing recombination rates in our population.

Lastly, Chapter 5 summarizes our findings in respect to the current status of the nondisjunction field and offers future studies to this dissertation.

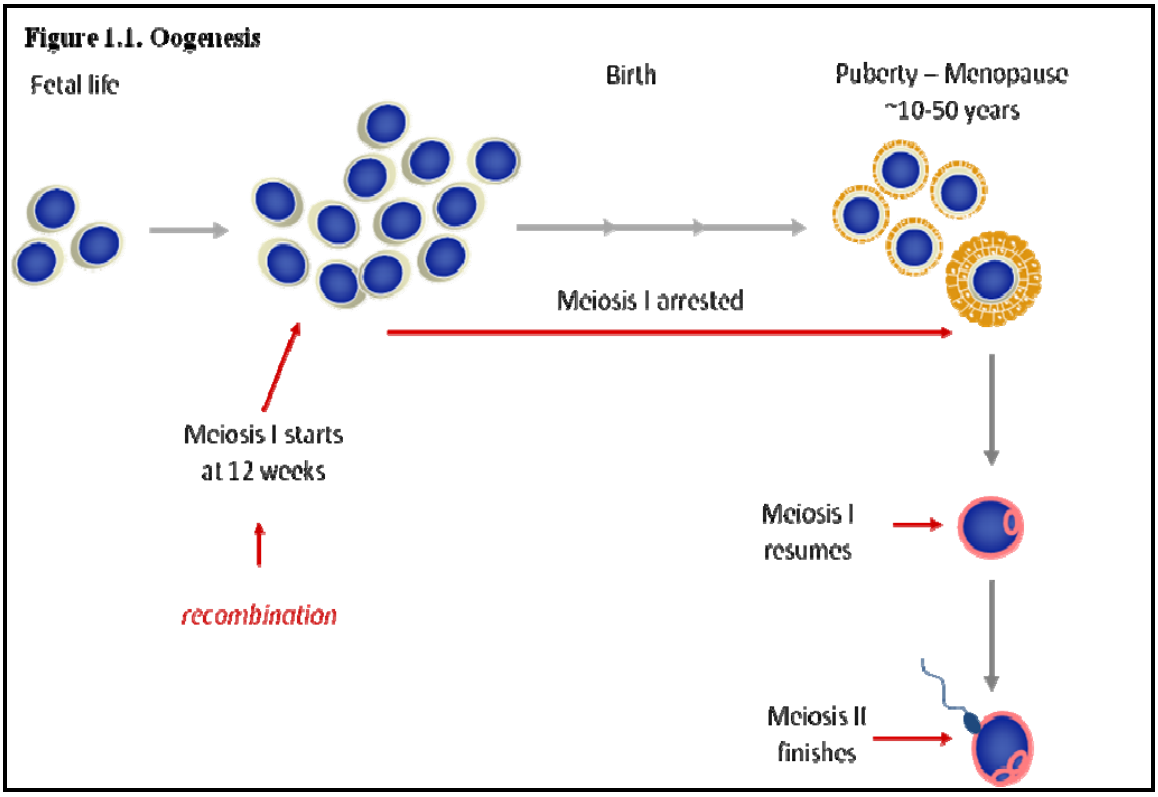


Figure 1.1. Oogenesis
Fetal life
Birth
Puberty – Menopause
~10-50 years
Meiosis I arrested
Meiosis I starts
at 12 weeks
recombination
Meiosis I
resumes
Meiosis II
finishes

Figure 1.2. Types of Chromosome Nondisjunction Errors

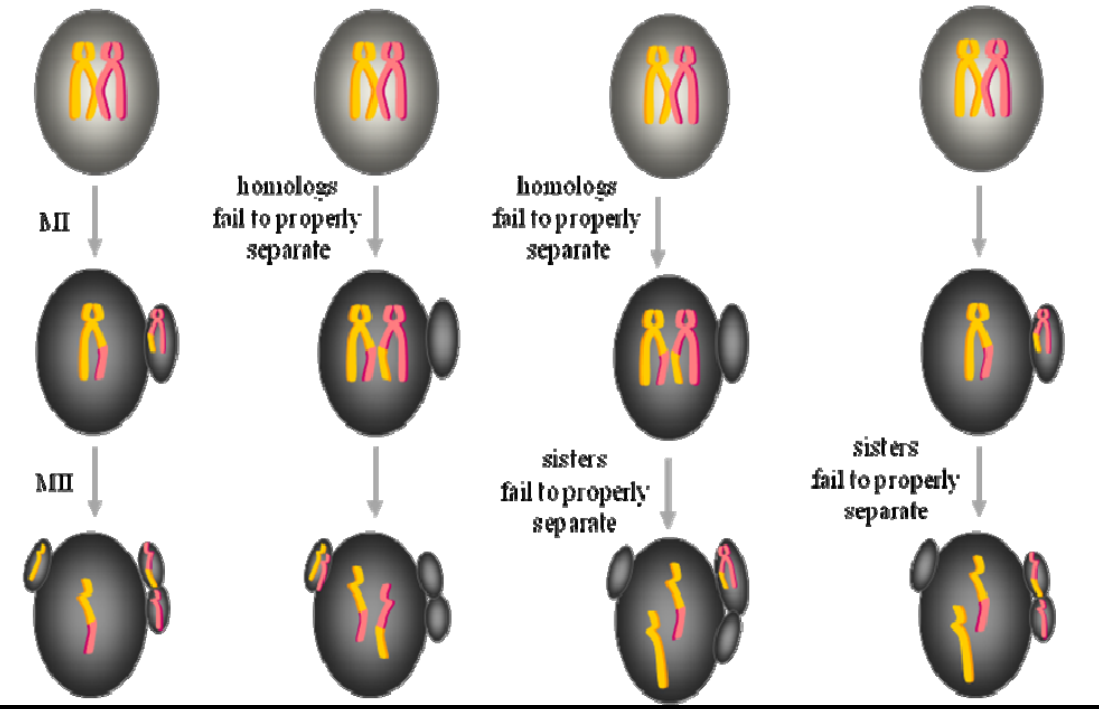


Figure 1.3. Identification of parental origin and stage of recombinant event.
Polymorphic markers along the long arm of chromosome 21 are genotyped for the proband and their parents. Contribution of alleles at informative markers determines parent of origin. Markers close to the centromere (shown at top of chromosome figures) determine meiotic stage of origin. In this example, the error is maternal and occurred in MII (see text). Recombination along 21q is shown by a change in reduction to non-reduction of informative markers from the parent in whom the error occurred or vice versa and is depicted by a change in color.

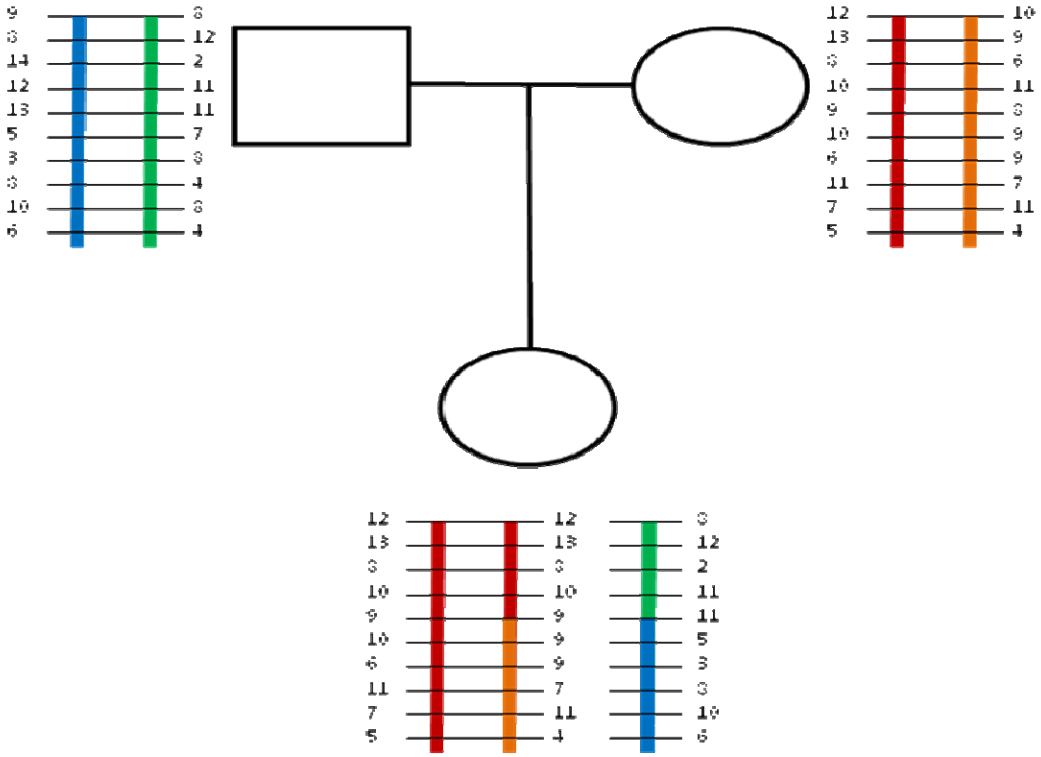


Figure 1.4. Advanced Maternal Age and Down Syndrome

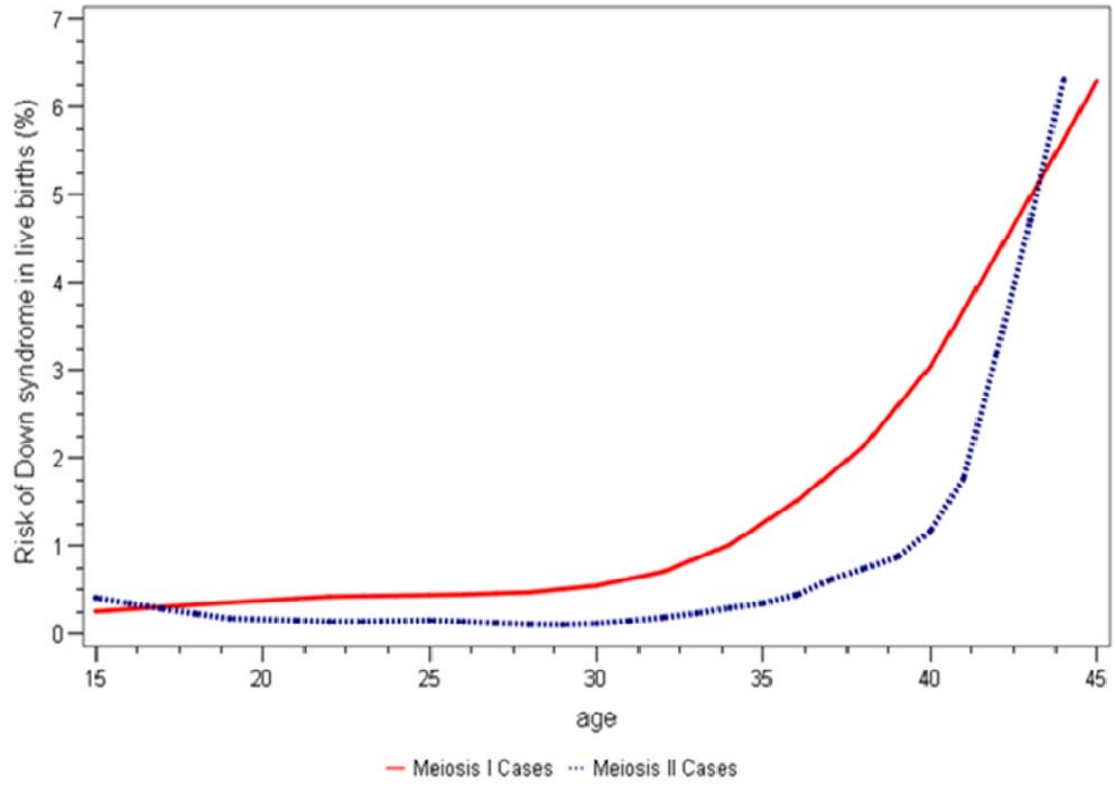
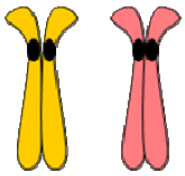


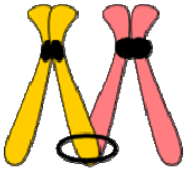
Figure 1.5. Specific Recombination Patterns are Associated with Nondisjoined Chromosome 21



No exchange

↓

MI error



Single exchange
near the telomere

↓

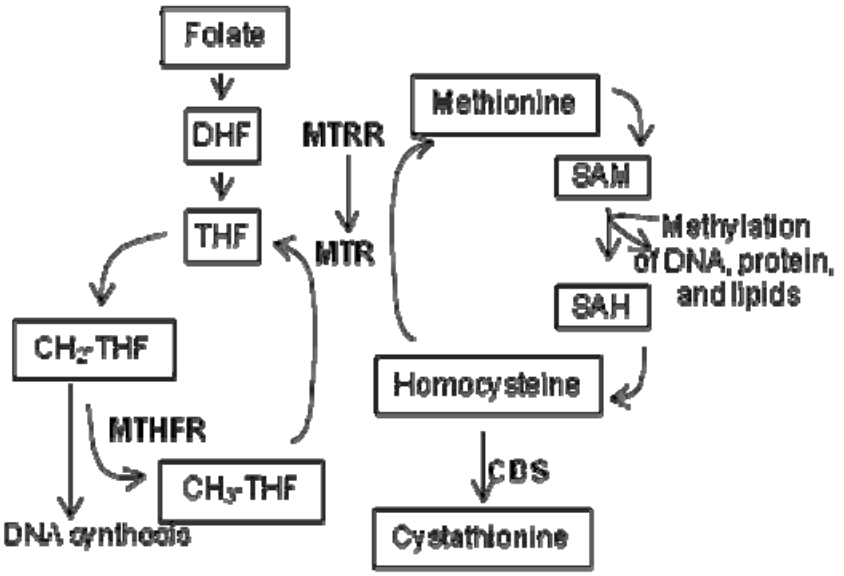
MI error

Exchange near
the centromere

↓

MII error

Figure 1.6. Folate pathway



DHF – dihydrofolate
THF – tetrahydrofolate
MTHFR – 5,10-methylenetetrahydrofolate reductase
MTR – methionine synthetase
MTRR –methionine synthetase reductase
SAM – S-adenosyl methionine
SAH – S-adenosylhomocysteine

CHAPTER 2

Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: a report from the National Down Syndrome Project

NaTasha D. HOLLIS[1], Emily G. ALLEN[1], Stuart W. TINKER[1], Charlotte DRUSCHEL[2], Charlotte A. HOBBS[3], Leslie O'LEARY[4], Paul A. ROMITTI[5], Marjorie H. ROYLE[6], Claudine P. TORFS[7], Sallie B. FREEMAN[1], Stephanie L. SHERMAN[1], Lora J. H. BEAN[1]

[1]Department of Human Genetics, Emory University, Atlanta, Georgia
[2]New York State Department of Health, Troy, New York
[3]College of Medicine, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas
[4]Centers for Disease Control and Prevention, Atlanta, Georgia
[5]Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, Iowa
[6]New Jersey Department of Health and Senior Services, Trenton, New Jersey
[7]Public Health Institute, Birth Defects Studies, Emeryville, California

Running Title: Preconception folate supplementation and Down syndrome

For: *American Journal of Medical Genetics Part A*

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Corresponding author:

Stephanie L. Sherman, PhD

Department of Human Genetics, Emory University

615 Michael St. Suite 301

Atlanta, GA, 30322

Phone: (404) 727-5862

Fax: (404) 727-3949

Email: ssherma@emory.edu

Contribution to Research:

NaTasha Hollis contributed to study design, dissected the maternal questionnaire data on vitamins and supplementation in order to create the folic acid exposure variable, performed all statistical analyses, data interpretation, and primary contributions to the manuscript.

Lora Bean contributed to study design and maternal questionnaire dissection.

Stephanie Sherman assisted with study design, data interpretation, and preparation of the manuscript.

All remaining contributed to the successful recruitment of subjects in order to make this study possible.

ABSTRACT

The folate pathway is important for DNA synthesis, protein synthesis and methylation. Studies, although conflicting, have shown that specific genetic variants in this pathway that lead to decreased serum folate levels and increased homocysteine levels are associated with chromosome 21 meiotic nondisjunction. Dietary supplementation of folic acid can overcome the effect of most genetic variants and thus help maintain normal homocysteine levels and remethylation. Our aim in this study was to determine whether folic acid supplementation specifically around the time of conception influences the risk for chromosome 21 nondisjunction. This time point coincides with resumption of meiosis, a time that is vulnerable to chromosome mal-segregation. Using questionnaire data from the population-based, case-control National Down Syndrome Project, we compared folic acid supplementation among mothers with an infant with full trisomy 21 due to maternal nondisjunction error (n=701) to control mothers, or those with an infant born in the same geographical area with no major birth defects (n=983). We used logistic regression and stratified cases by stage of origin of the meiotic error (meiosis I (MI) or meiosis II (MII)) and by maternal age (younger or older than age 35 years) and adjusting for self-reported race/ethnicity and maternal age Although the odds ratio for lack of folic acid supplementation was not different from 1 among all maternal cases combined (p=0.19) or among those with MI errors (p=0.33), there was a significant increased odds ratio when cases included older mothers with a maternal MII error (OR=1.83 95% CI 1.00-3.34).

KEY WORDS

Down syndrome

Trisomy 21

Aneuploidy

Nondisjunction

Chromosome segregation

Folate

Meiosis

INTRODUCTION

The leading cause of Down syndrome (DS) is the failure of chromosomes 21 to properly segregate during meiosis. More than 90% of the meiotic errors leading to chromosome 21 nondisjunction are maternal; that is, they occur during for formation of oocytes [Freeman et al., 2007; Gomez et al., 2000; Mikkelsen et al., 1995; Yoon et al., 1996]. Of the maternal meiotic errors, the majority occur during meiosis I (MI) (e.g., [Antonarakis et al., 1992; Freeman et al., 2007; Mikkelsen et al., 1995; Yoon et al., 1996]). There are two well-known risk factors for maternal nondisjunction: advanced maternal age and altered patterns of recombination. Advanced maternal age is associated with both meiosis I (MI) and meiosis II (MII) errors [Allen et al., 2009; Antonarakis et al., 1992; Yoon et al., 1996]. Altered patterns of recombination along the nondisjoined chromosomes 21 are also associated with both types of errors, but the patterns differ [Lamb et al., 2005a; Lamb et al., 2005b]. Among MI errors, a single telomeric exchange or no exchange increases the risk of nondisjunction, irrespective of the age of the oocyte (i.e., maternal age). Among MII errors, a pericentromeric exchange is associated with the increased risk of nondisjunction and that association increases with the age of the oocyte [Oliver et al., 2008].

In model systems, a wide variety of genetic and environmental disturbances have been identified to affect chromosome segregation. In humans, cytogenetic and epidemiological studies have identified many candidates for environmental risk factors including smoking [Kline et al., 1993; Torfs and Christianson, 2000; Yang et al., 1999], alcohol [Kaufman, 1983], coffee [Torfs and Christianson, 2000], maternal irradiation [Uchida, 1979], fertility drugs [Boue and Boue, 1973], oral contraceptives [Harlap et al.,

1979; Yang et al., 1999], spermicides [Rothman, 1983; Strobino et al., 1986], and social economic class [Christianson et al., 2004]. However, unequivocal proof is still lacking for these and other intrinsic and extrinsic factors. One factor that has been a focus of many studies is deficiency in folate, an essential B vitamin that provides one-carbon molecules for processes such as DNA synthesis, protein synthesis, and methylation. The first evidence for the association of folate deficiency and chromosome nondisjunction came from James et al. [1999]. They found an increased frequency of the 677C>T variant in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene among mothers with a child with DS compared with controls mothers. This variant is known to decrease folate metabolism. In addition, they found increased levels of plasma homocysteine in mothers with a child with DS compared with controls, irrespective of the *MTHFR* genotype [James et al., 1999]. These authors hypothesized that a decrease in folate and increase in homocysteine lead to hypomethylation, resulting in chromosome instability and abnormal chromosome segregation. The work of James et al. stimulated further investigation into the possible role of folate/homocysteine metabolism on the risk of having a child with DS; however, after 10 years of active research, the question is still unanswered (for review, see Coppede, 2009).

In addition to examination of genetic variants in the folate pathway, others have examined the role of nutritional folic acid supplementation during the key time point of resumption of MI, that is, around the time of conception. For example, Botto et al. [2004] did not observe a significant decrease in risk for DS when supplementation occurred three or more times a week before conception. Yet, Czeizel and Puho [2005] showed a decrease in the risk for DS when large doses (~6 mg/d) of folic acid

supplementation occurred within the first month of pregnancy. In this sample, folic acid and iron supplementation were taken together. The authors were able to determine that iron supplementation by itself influenced the risk of nondisjunction (OR=0.4, 95% CI (0.4, .9)). However, the number of women taking only folic acid was too small to determine its individual effect.

In human females, formation of oocytes is initiated in their fetal life. Meiosis begins around 12 weeks of gestation at which time chromosome arms replicate, pair, and undergo genetic recombination. At approximately 20 weeks, MI arrests in prophase, prior to chromosome segregation to daughter cells. This arrest is not released until ovulation, some 10 to 50 years later. Once MI is complete, MII is initiated and again arrested but only until fertilization. At fertilization, MII is completed. We hypothesize that folate deficiency around this critical time of ovulation and fertilization may influence chromosome segregation.

To test this hypothesis, we have used data collected from the National Down Syndrome Project (NDSP), a population-based case-control study. Our exposure variable was based on supplementation of folic acid prior to conception (MI resumption) at the recommended dose of 0.4mg/day and was based on parent report. We used a nested case-control analysis by restricting our case mothers to those who experienced a maternal meiotic error characterized as occurring in either MI or MII. We found that lack of folic acid supplementation may influence only a subset of errors in aged oocytes.

MATERIALS AND METHODS

Study Population

The National Down Syndrome Project (NDSP) is a large population-based case-control study conducted at six sites in the United States from 2000 to 2004. The details of the protocols and resulting dataset are described in Freeman et al. [2007]. Briefly, each of the six sites used active birth-surveillance systems to identify live births with DS. These sites included: the five-county metropolitan area of Atlanta, Georgia; the states of New Jersey, Iowa, and Arkansas; and selected regions of New York and California.

Cases were defined as live born infants with standard trisomy 21 or mosaic trisomy 21 born to either English or Spanish speaking mothers. Infants with DS due to translocations or those who were born but died before being enrolled in the study were not eligible. Controls were defined as infants without DS or any other major birth defect born to women living in the same geographic areas during the study years.

All sites in this study obtained IRB approval and informed consents were obtained from all participating families. Maternal questionnaires were administered by trained study personnel for case and control families. Mothers were asked about their prenatal vitamins, vitamins, and supplement use before pregnancy, during the first three months of pregnancy, and after the first three months of pregnancy. They were also asked to report their race/ethnicity and age at the time of birth of the proband infant.

Clinical data were obtained on each infant with Down syndrome. For probands with DS only, blood or buccal samples were obtained from the infant and their parents for DNA extraction. The parental origin and stage of the nondisjoining were determined using chromosome 21 specific genetic markers along chromosome 21q as described in Allen et al. [2009].

There were 1481 eligible case families and 1716 eligible control families identified through NDSP. Of the eligible control families, 983 completed the maternal question and were included (57% participation rate). Of the 1481 eligible case families, participation included completing the maternal questionnaire and obtaining a DNA sample for at least the proband and the mother. 907 case families participated (61.4% participation rate). To examine the effect of folic acid use on chromosome segregation at the resumption of meiosis in oocytes, we used a nested-case control design and defined our cases as those due to maternally-derived nondisjunction error in which we could determine the meiotic stage of origin. That is, we excluded cases due to paternal errors (n=32), mitotic errors (n=21), mosaics (n=4), maternal errors for which we could not determine stage (n=21), and those for which we could not establish the origin of the error due to limited DNA samples (n=125). Further, we excluded one mother with a MII error who did not complete the maternal questionnaire and two mothers with MI errors who had other chromosome abnormalities in addition to trisomy 21. Thus, there were 701 maternal meiotic error case families identified, where 525 were MI and 176 were MII.

Preconception Folic Acid Supplementation

Our exposure variable was based on the use of folic acid supplementation around the time of conception, or around two weeks following the last menstrual period. Using information provided from the maternal questionnaire, a mother's folic acid use was defined as follows: 0 for mothers who took folic acid supplementation before pregnancy and up to conception; 1 for mothers who did not take folic acid at all during pregnancy or those that began taking folic acid at any point after conception; and missing for those for whom we could not determine the time point of folic acid supplementation.

Statistical Analysis

Statistical analysis was conducted using Statistical Analysis Software (SAS). Direct comparisons of numbers and percentages were done by chi-square analysis. Comparison of mean maternal ages at the time of birth of the infant was done by t-test analysis. Logistic regression was the primary analysis tool. All models were adjusted for the known confounders of maternal age at the birth of the infant and race/ethnicity of the mother. Maternal age was modeled as a continuous variable. Race/ethnicity was defined as white non-Hispanic, black non-Hispanic, Hispanic, and other. Because of the small number of those defined as other, this group was not analyzed further. Race/ethnicity was modeled as a dummy variable with white non-Hispanic women as the reference group. We first performed logistic regression with all maternal meiotic nondisjunction events to be somewhat comparable to studies that used DS as their case definition [Botto et al., 2004; Collins et al., 2002; Ray et al., 2003]. We then stratified on two variables: maternal age group and meiotic error. Data on the association of maternal age and DS indicate a linear increase in risk prior to the age 35 and an exponential risk thereafter for both MI and MII maternal errors [Allen et al., 2009]. Therefore we dichotomized cases into two groups, mothers <35 and ≥ 35 at the time of birth of the infant. With respect to meiotic errors, we dichotomized maternal cases into MI and MII errors, as our previous studies indicate different mechanisms of nondisjunction by meiotic errors [Lamb et al., 1997; Lamb et al., 1996; Oliver et al., 2008]. For our analysis to test our hypothesis that lack of folic acid supplementation is associated with an increased risk of nondisjunction, p-values are from one-tailed tests. Thus, $p<0.10$ indicates significance at the $\alpha=0.05$ level. All other reported p-values are from two-tailed tests.

RESULTS

Study Population

There were 525 MI and 176 MII maternal case families and 983 control families analyzed. The mean age of case mothers was 33.6 ± 6.8, 33.4 ± 6.6, and 34.2 ±7.3 for all, MI, and MII maternal errors, respectively. The mean age for control mothers was 28.8 ± 6.2 (Table 2.1). As shown previously [Freeman et al., 2007], there was a difference in the frequency distribution of the mother's self reported race/ethnicity between cases and controls. There were more case mothers reporting Hispanic and less reporting black as their race/ethnicity compared to control mothers (Table 2.1). Although supplementation usage did not differ between cases and controls, there was a difference in usage by mothers of different racial/ethnic groups: 44.1%, 14.4%, and 14.5% of white non-Hispanic, black non-Hispanic, and Hispanic mothers took folic acid supplementation prior to conception, respectively. Therefore maternal age and race/ethnicity were included as covariates in all our models.

Preconception Folic Acid Supplementation by Meiotic Error

We first tested the hypothesis that lack of folic acid supplementation around the time resumption of MI and completion of MII (i.e., around the time of conception) increased the risk of chromosome 21 nondisjunction. To test this, we used logistic regression including all case mothers with maternal nondisjunction errors while adjusting for maternal age and race/ethnicity. There was no significant association between the lack of folic acid supplementation and case/control status when all maternal errors were combined (p=0.19, Table 2.2). We then stratified by maternal age group because of the potential different nondisjunction mechanisms [Allen et al., 2009]. We found no effect of

folic acid supplementation on nondisjunction among younger or older mothers with a maternal error (<35 years old, p=0.33; ≥35 years old, p=0.46) (Table 2.2).

Based on our previous studies, we have shown that risk factors associated with MI and MII errors differ for nondisjunction, implicating different mechanisms leading to chromosome malsegregation [Allen et al., 2009; Lamb et al., 1996; Oliver et al., 2008; Yoon et al., 1996]. Thus, we examined the association of lack of folic acid supplementation stratified by meiotic error (Table 2.2). We found no association of lack of folic acid supplementation among all MI errors (p=0.33), nor when stratified we MI errors by maternal age (p=0.19 and p=0.14, younger mothers and older mothers, respectively). In contrast, we found a marginally significant association of lack of folic acid supplementation and risk of MII errors (p=0.08). When we stratified by maternal age, we found that the association was restricted to the risk among older mothers (p=0.03, OR = 1.83; 95%CI 1.00-3.34) (Table 2.2).

DISCUSSION

Nondisjunction of all chromosomes is the leading cause of pregnancy loss, birth defects and intellectual disability [Hassold et al., 2007]. Aneuploidy that results from meiotic errors occurs in an estimated 5-25% of all human conceptions [Hassold and Hunt, 2001]. Most nondisjunction events occur during oogenesis. The two main risk factors are advanced maternal age and altered patterns of recombination. As more women are delaying childbirth until later in age, it is important that we understand the association of advanced maternal age with nondisjunction and determine whether there are factors that may protect against age effects [Gaulden, 1992; Martin et al., 2007].

In 1992, the US Public Health Service recommended that women of child bearing age consume at least 400μg of folic acid per day to decrease the risk of having a child with neural tube defects (NTD) based on scientific research evidence [CDC, 1992; Czeizel and Dudas, 1992; Yang et al., 2007]. Research has shown an association between folate and NTDs as well as between folate and DS. Hence, it is expected that there would be an association between DS and NTDs. However, studies that examined the co-occurrence of DS and NTDS in the general population, within the same family or within an individual did not observe an association [Amorim et al., 2004; Forrester and Merz, 2004; Torfs and Christianson, 1998]. Barkai et al. [2003] found an association between DS and NTDs within the same family, but their study design has been criticized by others [Martinez-Frias et al., 2004; Olsen and Winther, 2003].

In 1998, the US Food and Drug Administration began requiring the fortification of foods with folic acid [Yang et al., 2007]. Studies examining the effect of this fortification on chromosome 21 nondisjunction have not seen a decrease in the prevalence of DS since the initiation of fortification [Canfield et al., 2005; Collins et al., 2002; Ray et al., 2003; Simmons et al., 2004].

We chose to examine the role of folic acid supplementation prior to conception when MI is resumed after many years of meiotic arrest. Our present study focused on the consumption of at least 0.4mg/d of folic acid as has been recommended for women of childbearing age [CDC, 1992]. In addition, we restricted our analysis to maternal meiotic nondisjunction MI and MII errors. Previous studies defined their outcome as DS caused by any type of chromosome error [Botto et al., 2004; Czeizel and Puho, 2005]. This is

important as there are different mechanisms leading to MI and MII errors and potentially different risk factors [Oliver et al., 2008].

We observed no significant association between folic acid supplementation and DS unrestricted by meiotic error. However, we did observe an effect when we stratified the analysis by meiotic error and maternal age: mothers who were ≥35 years of age and had an MII error had a statistically significant association with the lack of supplementation with folic acid around the time of conception compared to controls ($p=0.03$, Table 2.2). No association was observed among young mothers. Our data suggests that older mothers who did not take folic acid supplementation around the time of conception are almost twice as likely to experience a MII error compared to controls.

Our data are consistent with the hypothesis of James et al. [1999]. That is, a decrease in folate may lead to hypomethylation, increasing chromosome instability and abnormal chromosome segregation. A possible mechanism could begin with hypomethylation of centromeric DNA. Work in yeast has shown that the centromeric cohesion protein, Rec8, binds to hypermethylated pericentromeric regions [Kitajima et al., 2003]. Rec8 is a member of the cohesin complex that is responsible for both centromeric and sister chromatid cohesion and helps to ensure proper bipolar orientation of homologous chromosomes and stabilize the bivalent. Cohesin proteins have been shown to degrade with maternal age, and premature sister chromatid segregation increases with age [Hodges et al., 2005; Liu and Keefe, 2008]. The lack of folic acid supplementation may exacerbate hypomethylation conditions as thereby affect chromosome segregation [Cravo et al., 1998; Duthie, 1999]. Together, an interruption of cohesin binding via hypomethylation of centromeric DNA along with age-related

reduction in cohesion could lead to premature sister chromatid segregation and ultimately nondisjunction.

Single exchanges in the pericentromeric region are at an increased frequency among older mothers with MII errors [Oliver et al., 2008]. Perhaps bivalents in older oocytes with a single pericentromeric exchange are already compromised. These configurations may be particularly vulnerable to the lack of folic acid supplementation, supportive of the present data.

The present study had several important limitations. First, we were only able to used analyzed maternal questionnaire data to obtain self-reported maternal history of supplementation use. We were unable to account for the role of other sources of folate, i.e., dietary folate. We included use of multivitamins that not only contained folic acid but also other vitamins that might influence chromosome segregation. In addition, we were unable to genotype variants in the folate pathway that are known to alter folate metabolism. Second, although our study is the largest to date, our sample sizes were relatively small once we stratified by meiotic error and age group. Thus, these data need to be confirmed by an independent sample.

The study also had three significant strengths that improved on previous studies. First, we defined our exposure variable as lack of folic acid use around the time of conception when meiosis is resumed and completed; this is the vulnerable time for chromosome nondisjunction. Second, we increased the homogeneity of our case definition by stratifying by type of meiotic error. Third, we were able to rule out recall bias as an explanation for the positive association as this association was limited to only one type of error leading to DS. If the effect were due to recall bias, we would also expect

to see the effect in mothers of cases experiencing both an MI and MII error. We also compared maternal meiotic errors of nondisjunction to other classifications of nondisjunction: paternal errors and mitotic errors. For the results of those analyses, see Table A.1 in the Appendix.

To summarize, use of folate supplementation appears to protect against MII errors in the aging oocyte, but not against MI errors. If possible, a more precise assessment of the folate status around the time of conception including food frequency questionnaires, biomarkers of the folate pathway (e.g., level of homocysteine), and genotyping of folate genetic variants will help to confirm or refute these findings. If confirmed, examination of the recombination profile as a covariate in these models may add insight into the role of folate in proper chromosome segregation.

ACKNOWLEDGEMENTS

The authors would like to thank the following laboratory personnel for technical assistance: Rupa Masse, Maneesha Yadav-Shaw, and Weiya He. We would also like to thank Helen Smith and Elizabeth Sablón for family recruitment. Additionally, we thank all the personnel at each NDSP site. Special thanks go to the many families nationwide whose participation has made this study possible.

Table 2.1. Characteristics of Mothers of Infants with Full Down Syndrome (cases) and Those with Infants with No Major Birth Defects (controls)

	CASES			CONTROLS
	All	Meiotic Error		
Characteristics		**MI**	**MII**	
N	**701**	**525**	**176**	**983**
Maternal age (mean ± SD)	33.6 ± 6.8	33.4 ± 6.6	34.2 ± 7.3	28.8 ± 6.2
Race/ethnicity				
White	364 (51.9%)	272 (51.8%)	92 (52.3%)	491 (50.0%)
Black	69 (9.8%)	50 (9.5%)	19 (10.8%)	153 (15.6%)
Hispanic	234 (33.4%)	174 (33.2%)	60 (34.1%)	270 (27.5%)
Other	34 (4.9%)	29 (5.5%)	5 (2.8%)	69 (6.9%)
Supplement				
Yes	217 (31.0%)	169 (32.2%)	48 (27.3%)	291 (29.6%)
No	479 (68.3%)	352 (67.0%)	127 (72.1%)	687 (69.9%)
Missing	5 (0.7%)	4 (0.8%)	1 (0.6%)	5 (0.5%)

Table 2.2. Association of Lack of Folic Acid Supplementation with the Birth of an Infant with Down Syndrome Stratified by a MI and MII Meiotic Error and by Maternal Age Group Using Logistic Regression

	Cases		Controls		Adjusted OR[a] (95% CI)	One-sided P value
	n	% of cases lacking folic acid	n	% of controls lacking folic acid		
All maternal meiotic						
All	**662**	68.3%	**910**	70.1%	1.12 (0.87, 1.42)	0.19
Young (<35 years old)	324	70.7%	744	71.4%	1.07 (0.78, 1.47)	0.33
Old (≥35 years old)	338	66.0%	166	64.5%	0.98 (0.64, 1.49)	0.46
MI						
All	**492**	66.9%	**910**	70.1%	1.06 (0.82, 1.38)	0.33
Young (<35 years old)	257	71.2%	744	71.4%	1.16 (0.83, 1.64)	0.19
Old (≥35 years old)	235	62.1%	166	64.5%	0.78 (0.50, 1.23)	0.14
MII						
All	**170**	72.4%	**910**	70.1%	1.34 (0.90, 2.01)	0.08
Young (<35 years old)	67	68.7%	744	71.4%	0.80 (0.45, 1.44)	0.23
Old (≥35 years old)	103	74.8%	166	64.5%	1.83 (1.00, 3.34)	0.03

OR, odds ratios; CI, confidence interval.

[a]Adjusted for maternal age as a continuous variable and race/ethnicity.

CHAPTER 3

Genetic variation in genes in the folate pathway as a risk factor for nondisjunction

NaTasha D. HOLLIS[1], Adam E. LOCKE[1], Lora J. H. BEAN[1], Stephanie L. SHERMAN[1]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia

Contribution to Research:

NaTasha Hollis contributed to study design, and performed all statistical analysis and data interpretation.

Adam Locke and Lora Bean selected SNPs to be genotyped in the population.

Adam Locke assisted with data interpretation.

Stephanie Sherman assisted with study design.

INTRODUCTION

Folate is a vital B vitamin involved in one-carbon metabolism. Naturally occurring folate is found in foods such as green vegetables, meats, beans, and fruits. Synthetic folic acid is found in supplements and fortified foods. Folate is in important for DNA synthesis, protein synthesis, methylation, and conversion of homocysteine to methionine (Figure 3.1). Functions of the folate pathway are catalyzed by enzymatic activity. Polymorphisms within genes in the folate pathway such as methylenetetrahydrofolate reductase (*MTHFR*), methionine synthase (*MTR*), and methionine sythase reductase (*MTRR*) reduce the activity of these genes. Once folate enters the cell, MTHFR catalyzes the conversion of 5,10-methylenetetrahydrolfolate (THF) to 5, methyltetrahydrolfolate, which is the methyl donor for the remethylation of homocysteine to methionine. This remethylation process is catalyzed by MTR which remains active via MTRR. Cystathionine beta synthase (CBS) is important for transsulfuration of homocysteine to cystathionine. Increased activity of this gene alters homocysteine metabolism, such that the folate-dependent resynthesis of methionine is shifted to the synthesis of cystathionine. This promotes a "folate trap," creating a functional deficiency (Figure 3.1). Hence, variants in folate pathway genes or alterations in copy number may alter activity levels and cause disturbances that have clinical implications.

It is known that a deficiency in cellular folates results in aberrant DNA methylation, chromosome breakage, defective chromosome recombination and aneuploidy [Das and Herbert, 1978; Fenech et al., 1998; Fenech and Rinaldi, 1994; Fowler et al., 1998; Rana et al., 1983]. Human chromosome nondisjunction leads to an

increased frequency of aneuploidy. Chromosome 21 nondisjunction occurring during meiosis is the leading cause of Down syndrome (DS) with more than 90% of the meiotic errors maternal in origin [Gomez et al., 2000; Mikkelsen et al., 1995; Yoon et al., 1996]. One known molecular risk factor of nondisjunction is altered patterns of recombination. As defective chromosome recombination and aneuploidy have been shown to be associated with folate deficiency in other diseases and disorders, studies suggest that a deficiency in folate metabolism may be linked to chromosome 21 nondisjunction [Hall, 2004; James, 2004a; James, 2004b].

James et al. [1999] reported the first association between polymorphisms in genes involved in the folate pathway and chromosome 21 nondisjunction. They showed that the 677C>T polymorphism in the *MTHFR* gene was associated with 2.6-fold increased risk in the chance of having a child with DS. This polymorphism is associated with an elevation in plasma homocysteine levels; however, James et al. found that homocysteine levels were increased among case mothers even when they did not carry the T variant. This study prompted several follow-up studies examining the association of DS and polymorphisms in folate/homocysteine metabolism. While some studies found an association between all polymorhpisms examined and DS [Grillo et al., 2002; Hobbs et al., 2000; Rai et al., 2006], others do not found any associations [Biselli et al., 2008; Chango et al., 2005; Takamura et al., 2004], while others found associations with some polymorphisms tested and not others [Bosco et al., 2003; O'Leary et al., 2002; Scala et al., 2006].

There are several crucial genes in the folate pathway that are located on chromosome 21 including the reduced folate carrier (*SLC19A1* (*RFC1*)) and

cystathionine β-synthase (*CBS*). There are increased levels of expression of *CBS* in individuals with DS [Chadefaux et al., 1988; Chadefaux et al., 1985; Ichinohe et al., 2005]. Overexpression of *CBS* removes homocysteine from the folate metabolic pathway. Homocysteine is the precursor for MTR in the remethylation of methionine; hence, affecting the downstream products, *S*-adenosylmethionine and *S*-adenosylhomocysteine. This in turn leads to hypomethylation; hence, creating chromosome instability.

The findings in several studies examining associations between polymorphisms in the folate pathway genes and DS prompted us to do the same. However we narrowed our phenotype to chromosome 21 nondisjunction errors that occurred in the oocyte and examined genetic variants in mothers. We examined five genes using our Emory-based DS sample population: *MTHFR, MTR*, *MTRR*, *CBS*, and *SLC19A1* (*RFC1*).

MATERIALS AND METHODS

Study Population

Subjects were ascertained because they had documented standard trisomy 21 born to either English or Spanish speaking mothers. We drew families from Atlanta Down Syndrome Project (ADSP) and National Down Syndrome Project (NDSP). These studies are described in [Freeman et al., 2007; Freeman et al., 1998]. We also ascertained probands from non-population based studies. Families were drawn from DS clinics, pediatric cardiology units, DS parent support groups and through the internet.

Data Collection

Informed consent forms were obtained for all participating families. All participating mothers completed questionnaires and were self-reported non-Hispanic

white or non-Hispanic black. Clinical data were obtained for each proband with Down syndrome. Blood samples were obtained from the proband and their parents.

Determining Recombination Origin and Stage

The contribution of parental alleles of polymorphic genetic markers along the nondisjoined chromosome to the trisomic proband is used to establish the parental origin (Figure 3.2). If two alleles are contributed from the mother at several markers, a maternal error is inferred. A paternal error is inferred when two alleles at informative markers are contributed by the father. If we are unable to obtain a DNA sample from the father, we inferred a paternal error when alleles in the proband are inconsistent with a maternal origin at >1 genetic markers. If at least eight informative markers are consistent with a maternal error, we inferred a maternal error. Once the parental origin of the nondisjunction is established, a core set of markers located in the pericentromeric region (13615252 – 16784299 bp) of 21q are used to infer the stage of nondisjunction as MI or MII. If heterozygosity of informative pericentromeric markers in the parent in which the errors is established (parent of origin) is retained in the trisomic offspring, an MI error is inferred. If parental heterozygosity is reduced to homozygosity, a MII error is inferred. When all informative markers in the parent of origin are reduced to homozygosity along the length of 21q, the origin of nondisjunction is inferred to be a post-zygotic, mitotic error.

Gene and SNP Selection

Five genes encoding essential proteins in the folate pathway, *MTHFR*, *MTR*, *MTRR*, *CBS* and *SLC19A1*(*RFC1),* were studied in 182 mothers who had a child with a maternally-derived meiotic error. SNPs were selected to efficiently assay common

variation in the genes of interest. The genomic location, known non-synonymous coding variants, and the number of single nucleotide polymorphism (SNP) markers genotyped and analyzed at each locus are shown in Figure 3.1. The majority of our cases and controls self-reported as white (Table 3.1), thus SNP selection was based on the linkage disequilibrium structure of known SNPs in the CEPH (Centre d'Etude du Polymorphisme Humain) dataset using dbSNP build 123. Using the SeattleSNPs Program for Genomic Applications (PGA) Genome Variation Server (http://pga.gs.washington.edu) [SeattleSNPs, May, 2005a], which implements the method of Carlson et al. [2004], we selected SNPs tagging common variation (MAF $\geq$ 5%) at an $r^2 \geq 0.80$ for each gene including 5kb up and downstream of the coding regions. Additionally, non-synonymous coding variants identified using build 126 of dbSNP were also genotyped (http://www.ncbi.nlm.nih.gov/SNP/). Alleles for each SNP are designated "A" for the allele with the highest frequency and "B" for the minor allele frequency based on data in dbSNP for the CEPH pedigrees [Sherry et al., 2001].

Genotyping

All SNPs were genotyped on the Illumina BeadArray platform using the Golden Gate genotyping technology as part of a 384-SNP customized assay. Genotyping was performed by the SeattleSNPs PGA through a service award. Parental genotypes and SNPs located on all chromosomes other than chromosome 21 were scored using Illumina BeadStudio software, and confirmed with 100% concordance using the algorithm developed by Lin et al.[2008]. Genotypes for SNPs located on chromosome 21, where probands were expected to carry three alleles, were called only by the method of Lin et al. [2008]. Because genotyping for rs1801131 initially failed on the Illumina platform, it

was genotyped by the Emory Biomarker Service Center (Emory University) using the GenomeLab SNPStream 48-plex genotyping platform in white families only.

Statistical Analysis

We tested the hypothesis that genetic variants in the folate pathway may increase the risk of DS differentially based on the type of error, either MI or MII error. Thus, we defined our outcome variable as the type of meiotic error (MI=1 and MII=0), and the predictor variable of interest were genotypes of the SNPs. The two covariates in the model were maternal age and race/ethnicity. We modeled maternal age first as a continuous variable and then also as a dichotomous variable (<35 years, ≥35 years) to try to account for the potential different age-related risk factors [Allen et al., 2009]. We included self-reported race/ethnicity (either black or white) to account for population stratification. Our primary analysis was a gene level association test. This test was based on variation of the kernel-based approach of Kwee et al.[2008] extended to case/control data based on the algorithm in Liu et al.[2008]. The kernel-based test was implemented in the R programming language. Reported p-values are at the α=0.05 level.

RESULTS

Study Population

182 families with maternal meiotic errors were initially enrolled and genotyped for this study. Two case families were removed due to absence of maternal age. Thus, there were 136 MI and 44 MII maternal case families analyzed. The mean age of case mothers was 33.1 ± 6.2, 33.2 ± 6.1, and 32.6 ± 6.6 for all, MI, and MII maternal errors, respectively (Table 3.1). There were 145 case mothers reporting white and 35 reporting black as their race/ethnicity. The mean maternal age by race/ethnicity of case mothers was 33. ± 5.3 and 29.4 ± 8.4 for white and black case mothers, respectively (Table 3.1).

Chromosome 21 Candidate Genes

We first tested the two folate pathway genes located on chromosome 21 including the reduced folate carrier *SLC19A1* and the reducing enzyme of homocysteine *CBS.* The gene-specific tests for *CBS* (p=0.86) and *SLC19A1* (p=0.39) showed no association with increased risk for mothers experiencing a MI error compared to a MII error (Table 3.2). Hence, we did not test association of individual SNPs.

Non-Chromosome 21 Candidate Genes

Next, we asked whether the non-chromosome 21 folate pathway genes increased the risk differentially for maternal MI or MII meiotic errors. The gene-level test for all non-chromosome 21 genes revealed no association (*MTHFR*, p=0.37; *MTR*, p=0.66; *MTRR*, p=0.27) (Table 3.2). As a result, we did not perform analysis of individual SNPs in these genes.

DISCUSSION

In 1999, James et al. identified an association between the folate pathway and increased risk of having a child with DS. They were the first to report the observation that the 677C>T polymorphism in the MTHFR gene increased the chance of having a child with DS (OR=2.6). Despite several years and many investigations later into the possible role of folate/homocysteine metabolism and the risk of having a child with DS, the question still remains unanswered. Although, studies to date have analyzed the association between increased risk of DS and variants in genes in the folate metabolic pathway, none have examined the risk based on meiotic error. We took this approach in our current study based on the evidence that different mechanisms are involved in MI and

MII maternal chromosome 21 nondisjunction errors [Lamb et al., 1997; Lamb et al., 1996; Oliver et al., 2008].

As genes for two of the major components of the folate pathway, *CBS* and *SLC19A1*, are located on chromosome 21, we were particularly interested in investigating how variations in these genes could affect the proper segregation of chromosome 21. *CBS* is important because it is responsible for converting homocysteine into cystathionine. Aberrations in *CBS* could potentially lead to a folate deficiency by removing homocysteine from the pathway [Pogribna et al., 2001]. Hence, downstream components that rely on the presence of homocysteine are altered, i.e. methionine, SAM, and SAH. *SLC19A1* is a ubiquitously expressed transmembrane protein responsible for the primary regulated transport of 5-methyltetrahydrofolate, the active form of folate, into the cytoplasm [Chango et al., 2000; Hou and Matherly, 2009; Taparia et al., 2007]. We did not find a relationship between folate pathway genes located on chromosome 21 and increased risk for a meiotic error, MI or MII. Our data support those of other association studies that did not find evidence for an association [Biselli et al., 2008; Chango et al., 2005; Scala et al., 2006].

Additionally, we did not see an association between any of the non-chromosome 21 folate pathway genes and increased risk for chromosome 21 nondisjunction based on maternal meiotic error. Because we did not see an association on the gene level in these genes we did not go on to test on individual SNP any of the common non-synonymous SNPs frequently associated with increased DS risk, e.g., *MTHFR* 677C>T, *MTR* 2756A>G, and *MTRR* 66A>G. However, because these might be functional genes, doing

a gene-level test could dilute the affect of that variant which we did not test individually, a limitation to our study.

There are at least three possible explanations for our results. First and foremost, our limited sample size can only identify large effects that differentiate the risk for an MI versus MII error. The second possibility is that folate acts on both MI and MII errors in the same way. Without controls, we would not be able to identify this overall affect. Similarly, folate may be associated with survival of a fetus with DS. Up to 80% of DS conceptuses are lost prior to birth [Hassold and Jacobs, 1984]. Our sample population consists of mothers who have given birth to a live-born infant with DS or those who survived gestation. Variants compromising genes associated with survival should have their effect on all types of chromosome errors leading to DS including maternal MI and MIIs, paternal errors and translocations. Again, we would not be able to identify such survival variants using our present study design. Two additional controls would be important: 1) mothers with a normal meiotic outcome and 2) those with DS due to errors other than maternal MI or MII.

In summary, we did not find any evidence for a differential effect of folate variants on maternal MI or MII errors. Again, we were limited by our sample size and lack of control mothers to help further determine whether folate variants are associated with chromosome 21 nondisjunction in general. In addition, we only examined one source of folate, i.e., variants in genes in the folate pathway, and its association with maternal meiotic nondisjunction chromosome 21 errors. Perhaps, it is the combination of several factors, including genetic, epigenetic, and environmental that is responsible for the association between folate and chromosome 21 nondisjunction.

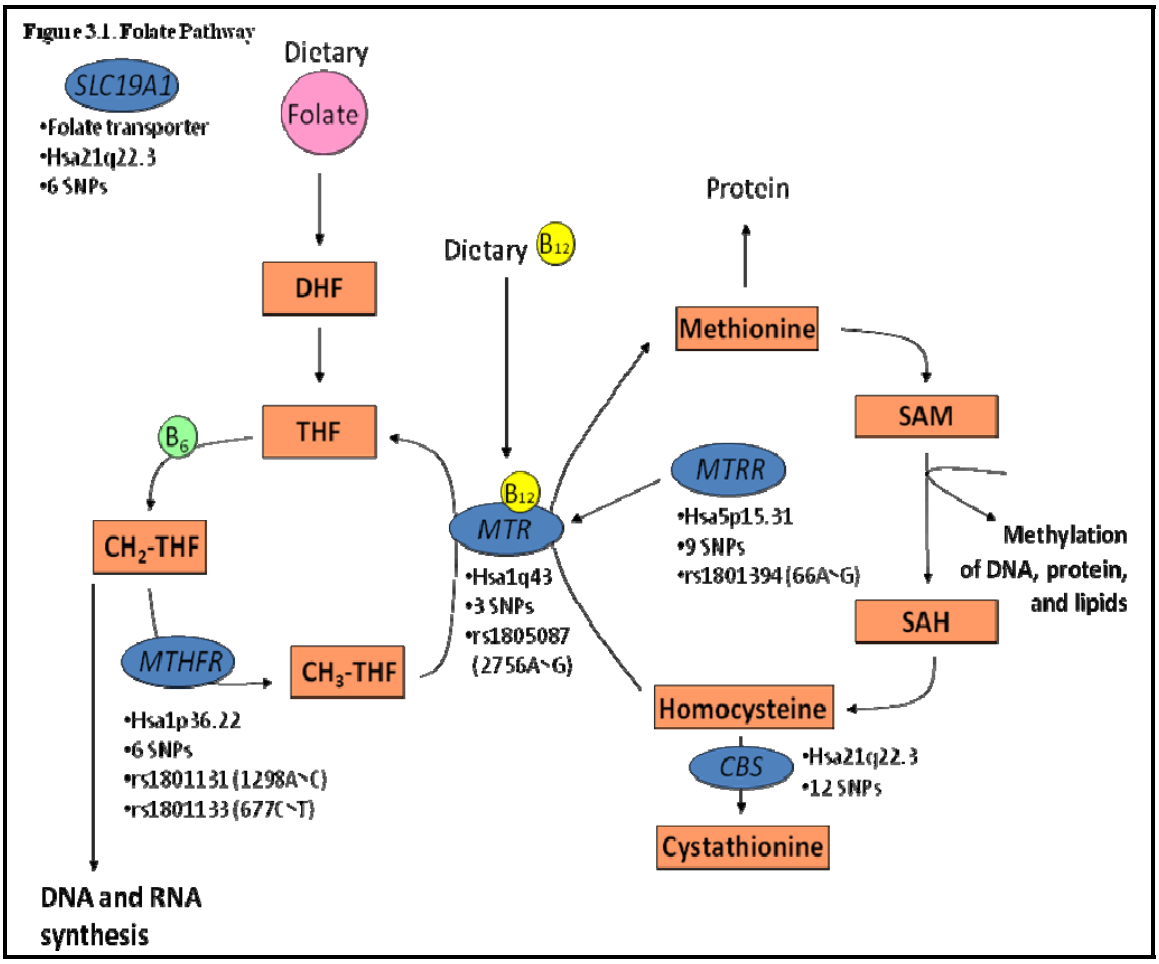


Figure 3.1. Folate Pathway
Dietary
Folate
SLC19A1
•Folate transporter
•Hsa21q22.3
•6 SNPs
DHF
THF
B6
CH2-THF
MTHFR
•Hsa1p36.22
•6 SNPs
•rs1801131 (1298A>C)
•rs1801133 (677C>T)
CH3-THF
DNA and RNA synthesis
Dietary B12
B12
MTR
•Hsa1q43
•3 SNPs
•rs1805087 (2756A>G)
Protein
Methionine
SAM
MTRR
•Hsa5p15.31
•9 SNPs
•rs1801394 (66A>G)
Methylation of DNA, protein, and lipids
SAH
Homocysteine
CBS
•Hsa21q22.3
•12 SNPs
Cystathionine

Figure 3.2. Identification of parental origin and stage of recombinant event.
Polymorphic markers along the long arm of chromosome 21 are genotyped for the proband and their parents. Contribution of alleles at informative markers determines parent of origin. Markers close to the centromere (shown at top of chromosome figures) determine meiotic stage of origin. In this example, the error is maternal and occurred in MII (see text). Recombination along 21q is shown by a change in reduction to non-reduction of informative markers from the parent in whom the error occurred or vice versa and is depicted by a change in color.

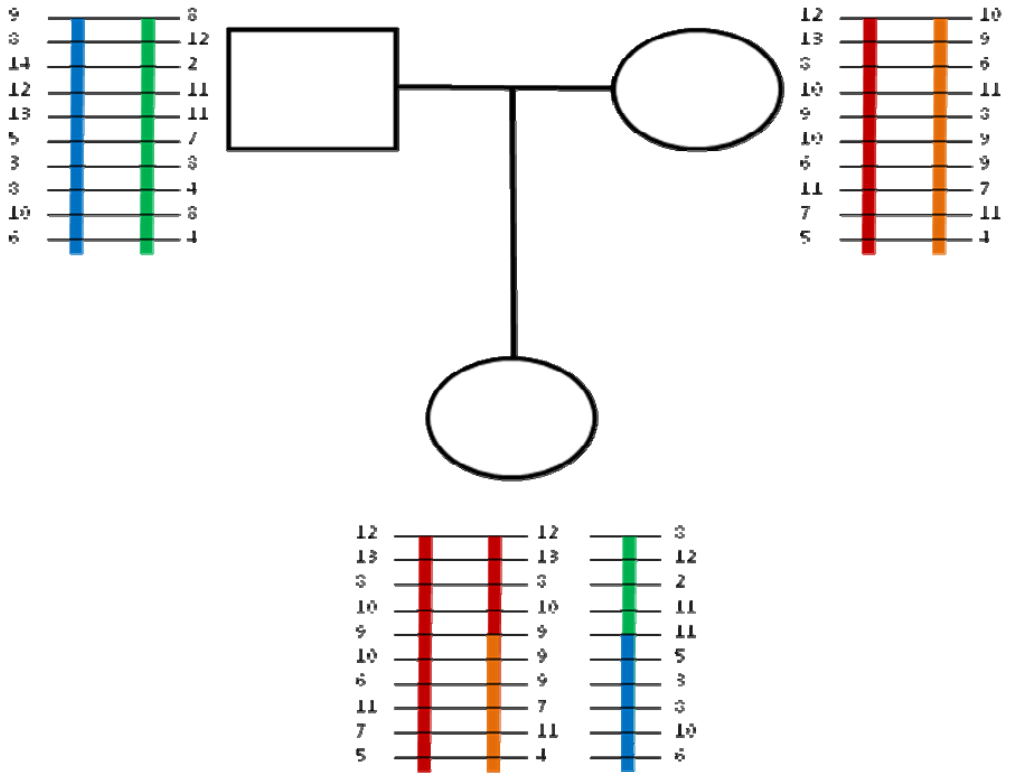


Table 3.1. Characteristics of Mothers of Infants with Down Syndrome Due to a Maternal Meiotic Error, MI or MII			
	Black (n)	**White (n)**	**Total**
All maternal meiotic			
Mean maternal age	29.4 ± 8.4	33.9 ± 5.3	33.1 ± 6.2
Young (<35 years old)	22	72	94
Old (≥35 years old)	13	73	86
Race	**35**	**145**	**180**
MI			
Mean maternal age	30.0 ± 8.0	34.1 ± 5.2	33.2 ± 6.1
Young (<35 years old)	18	54	72
Old (≥35 years old)	11	53	64
Race	**29**	**107**	**136**
MII			
Mean maternal age	26.3 ± 10.2	33.6 ± 5.4	32.6 ± 6.6
Young (<35 years old)	4	18	22
Old (≥35 years old)	2	20	22
Race	**6**	**38**	**44**

Table 3.2. Association of Folate Metabolic Pathway Genes with the Birth of an Infant with Down Syndrome Due to Maternal Meiotic Error Using Gene-Level Association

	n	p-value (age-continuous)	p-value (age-dichotomous)
Non-Chromosome 21 Genes			
MTHFR			
with rs1801131*	133	0.37	0.33
without rs1801131	180	0.23	0.22
MTR	180	0.66	0.64
MTRR	169	0.27	0.27
Chromosome 21 Genes			
CBS	178	0.86	0.88
SLC19A1 (RFC1)	160	0.39	0.42

*includes whites only, rs1801131 originally failed and was re-genotyped in whites only

CHAPTER 4

Genetic analysis of variation in chromosome 21 recombination rates

NaTasha D. HOLLIS[1], Tiffany R. OLIVER[1], Adam E. LOCKE[1], Emily G. ALLEN[1], Stuart W. TINKER[1], Lora J. H. BEAN[1] Eleanor FEINGOLD[2], Stephanie L. SHERMAN[1]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia
[2]Department of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

Contribution to Research:

NaTasha Hollis selected, pulled and quantified all samples from the DNA inventory that were to be sent to PreventionGenetics, Marshfield, WI for genotyping. Additionally, NaTasha strategically chose SNPs to be genotyped, served as the primary contact with PreventionGenetics during the genotyping process, performed data cleaning of the dataset, and performed all analysis.

Tiffany Oliver determined the recombination phenotype (number of chromosome 21 recombinants) based on STR and SNP data available to date.

Adam Locke contributed to the development of statistical analysis.

Eleanor Feingold contributed to the development of statistical analysis.

Stephanie Sherman helped develop the study design and contributed to development of statistical analysis.

All remaining authors contributed to intellectual input in the execution of the project.

INTRODUCTION

Meiosis is the process of cell division which produces haploid gametes. One hallmark feature of meiosis is meiotic recombination. Meiotic recombination between homologous chromosomes acts to ensure the proper segregation of chromosomes. Chiasmata, the physical structures that are build around recombination events link non-sister chromatids and hold homologs together at the sites of crossovers to aid in proper chromosome segregation. MLH1, a DNA mismatch repair protein, localizes to sites of meiotic crossover and can be used as a marker to examine patterns of recombination in gametes. MLH1 foci counts show there is one chiasma per chromosome arm [Hassold et al., 2004]. In addition, Fledel-Alon et. al [2009] recently used pedigree linkage studies to provide evidence that the number of recombinant events is tightly regulated. Their data corroborate those of others and suggest that at least one crossover per chromosome is required for proper disjunction.

The importance of the process of recombination suggests the process would be tightly regulated. However, studies show variation in genome-wide human recombination rates and location between genders as well as within gender [Broman et al., 1998; Cheung et al., 2007; Coop et al., 2008; Kong et al., 2002; Lynn et al., 2002].

The genetic determinants of such variation in human meiotic recombination are not well understood. Stefansson et al. [2005] identified a 900-kb region inverted region on chromosome 17q21.31 to be associated with genome-wide recombination rates in women in an Icelandic populaiton. Two haplotypes were associated within this region that encompasses several genes including *MAPT*, *CRHR1*, and *IMP5*. The H2 haplotype was correlated with increased recombination and having more children. The findings of

this group have been replicated in two additional populations: Autism Genetic Research Exchange (AGRE) and Framingham Heart Study (FHS) [Chowdhury et al., 2009]

Kong et al. [2008] identified gender-specific variants within *RNF212* that are associated with both male and female genome-wide recombination rates, rs3796619 and rs4045481, respectively. The function of *RNF212* has not been characterized, but shares homology with *S. cerevisiae ZIP3* gene and *C. elegans zhp-3.* These genes are involved with meiotic recombination and SC assembly, suggesting a similar role for *RNF212*. Chowdhury et al [2009] confirmed the association of *RNF212* and recombination rates, but the association was only seen in males and not females.

Chowdhury et al [2009] also found preliminary data for other gender-specific genetic variants that may influence the variation in recombination rates in her analysis of the AGRE and FHS datasets. These included alleles on chromosome 1q21.2 and 10p11.23 with female recombination and alleles on chromosome 7q36.1 and 9q31.1 with male recombination [Chowdhury et al., 2009].

Recombination is also facilitated by the formation of the synaptonemal complex (SC), a protein structure that forms between homologous chromosomes. Lynn et al. [2002] showed that inter-individual variation in genome-wide recombination rates is correlated with the length of the SC in both human males and in mice. In addition, inter-sex variation in the SC length is also associated with recombination rates in humans [Tease and Hulten, 2004]. In mammals, three primary proteins, in addition to meiotic cohesins, comprise the SC: SYCP1, SYCP2, and SYCP3 [Handel and Schimenti, 2010]. Understanding how these genes and their products act, and the causes of the variation in the SC can be expected to help explain the role of recombination in proper chromosome

segregation. Recently, Bolor et al. [2009] has shown that SYCP3 plays an essential role in meiosis in humans. They found that variants in this gene are associated with recurrent pregnancy loss. Furthermore, studies in human males and in mice display fertility problems with mutations in this gene [Miyamoto et al., 2003; Yuan et al., 2002; Yuan et al., 2000]. This suggests that mutations in the SC components may have clinical implications as well as influence recombination rates.

Chromosome nondisjunction, the failure of chromosomes to properly segregate during meiosis, is the leading cause of pregnancy loss, birth defects, and intellectual disabilities. In addition to the well accepted advanced maternal age as a risk factor for meiotic nondisjunction, one known molecular risk factor is altered patterns of recombination. Down syndrome (DS), a result of chromosome 21 nonsdisjunction, is one of the most studied human nondisjunction events. Here we tested that hypothesis that variation in recombination rates and patterns may be a predisposing factor to nondisjunction. To this end, we examined the association between the number of recombinants along the nondisjoined chromosomes 21 of maternal origin and the candidate genes/regions described above that influence recombination in normally disjoining samples (*RNF212*, *SYCP3*, and the inversion on chromosome 17q21.31). We performed analysis in two ways. We determined whether variants in these regions were associated with the rates of recombination along the nondisjoined chromosome 21 or whether they were associated with nondisjunction in general.

MATERIALS AND METHODS

Study Population

Subjects for the present study were ascertained through our ongoing Down syndrome research studies including: Atlanta Down Syndrome Project (ADSP), National Down Syndrome Project (NDSP), and Emory Down Syndrome Project (EDSP) and the Down Syndrome Congenital Heart Defects Project (DS-CHD). ADSP and NDSP are described in [Freeman et al., 2007; Freeman et al., 1998]. Those ascertained through EDSP and DS-CHD are not population based, but instead more targeted ascertainment through DS clinics, pediatric cardiology units, DS parent groups and the internet.

Data Collection

Informed consent forms were obtained for all participating families. The study was limited to complete trios (mother, father, and proband). All participating mothers completed questionnaires administered by trained study personnel. From this questionnaire, we obtained the mother's age at birth of the child with DS and the mother's self-reported race/ethnicity of the trio. Participation was limited to trios where all members were reported as the same race/ethnicity as either non-Hispanic white or non-Hispanic black. Only probands with standard trisomy 21 were included. This information was obtained from abstracted medical records. Buccal or blood samples were obtained from each member of the trios.

Recombination Phenotype: number of recombinants along the nondisjoined chromosome 21 of maternal origin

Chromosome 21 genotyping

Chromosome 21-specific STRs were genotyped as described in Lamb et al. [Lamb et al., 2005a] for the majority of trios. In addition, we supplemented these data by comprehensively genotyping chromosome 21-specific SNPs to better define all

recombination events. DNA from trios was sent to Center of Inherited Disease Research (CIDR) to conduct genotyping of 1536 strategically chosen SNPs along chromosome 21 to span the entire length of 21q using the Illumina GoldenGate Assay. Of the chosen SNPs, 1292 SNPs were successfully genotyped. SNPs with >10% of missing data were excluded. RelCheck was used to identify possible sample mix ups. Identified problem samples were re-genotyped if possible or dropped from the study. Parental genotypes were used to calculate the minor allele frequency (MAF), Hardy Weinberg equilibrium (HWE), heterozygosity and frequency of missing genotypes for each SNP in study. SNPs with a MAF ≤ 0.01, test of HWE of $p \leq 0.01$, or heterozygosity ≥ 0.60 were excluded due to potential genotyping errors.

The ability to differentiate between heterozygous and homozygous genotypes is difficult with low SNP genotyping intensity. Probands with >50% of the SNP genotyping intensities ≤5000 were excluded. For disomic parents, SNPs in which the total intensity was ≤5000 were excluded on SNP-by-SNP basis. After extensive data cleaning, approximately 917 21q SNPs were finalized per family for an average density of 1 SNP every 38Kb.

Defining parental and stage of origin of the meiotic error

Our sample consisted of 648 families with data on the complete trio after data cleaning. For each family, we used the combined STR and SNP data to determine the stage and origin of the nondisjunction error. The contribution of parental alleles to the child with DS was used to establish the parental origin (Figure 4.1). We limited the current study to case families of maternal origin. Once the maternal origin of the nondisjunction was established, a core set of markers located in the pericentromeric

region (13615252 – 16784299 bp) of 21q was used to infer the stage of nondisjunction as maternal meiosis I (MI) or maternal meiosis II (MII). If parental heterozygosity was retained in the trisomic offspring, we concluded an MI error. If parental heterozygosity was reduced to homozygosity, we inferred an MII error. In this assay, we could not distinguish between a conventional MII error, in which sister chromatids fail to separate during anaphase of MII, from an error initiated in MI that is not properly resolved in MII. For example, if sister chromatids prematurely separate in MI, some configurations will lead to both sister chromatids segregating to the same pole in MII. Similarly, if homologs pairs fail to separate in MI and then go through a reductional division at MII, sister chromatids will be present in the resulting oocyte. 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.

Defining recombination breakpoints

The location of recombinant events was determined. The breakpoint of a single recombinant event was defined by at least one highly polymorphic informative STR or eight consecutive and informative SNPs flanking the recombination breakpoint. For example, if we use N for nonreduction and R for reduction at informative markers along chromosome 21, a recombinant event would be defined when NNNN…N_8RRRR. An exception to this rule occurred when the most proximal or distal informative markers on 21q (i.e., markers at the very ends of 21q) indicated the presence of a recombinant event. At the ends of the chromosome, a minimum of either one STR or four consecutive and informative SNPs were required to define the breakpoints of recombination. The presence of a double recombinant event was defined by a minimum of 8 consecutive and

informative SNPs flanking the recombination breakpoint on each side (e.g., NNNNNNNNRRRRRRRRNNNNNNNN).

Candidate gene/region variants

SNP selection

Three candidate genes/genomic regions were studied: *RNF212*, *SYCP3*, and the inversion on chromosome 17q21.31. Initial SNPs were chosen because of their reported association with recombination. Remaining SNPs were selected to efficiently assay the common variation in the genes of interest. The majority of our cases and controls self-reported as white, thus SNP selection was based on known SNP variation and linkage disequilibrium structure in parents of the CEPH (Centre d'Etude du Polymorphisme Humain) pedigrees using dbSNP build 123. Using the SeattleSNPs Program for Genomic Applications (PGA) Genome Variation Server (http://pga.gs.washington.edu) [SeattleSNPs, May, 2005b], which implements the method of Carlson et al. [2004], we selected SNPs tagging common variation (MAF $\geq$ 5%) at an $r^2 \geq 0.80$ for each gene including 5kb up and downstream of the coding regions.

SNP genotyping

DNA of parents of trios was sent to PreventionGenetics to genotype the 34 SNPs that tagged *RNF212*, *SYCP3* and the inversion of chromosome 17q21.3. For a complete list of chosen SNPs, see Table A.2 in the Appendix. All SNP genotyping assays were run as submicroliter PCR-based assays on Array Tape (http://www.douglasscientific.com/). Allele-specific PCR assay was conducted as described in Hawkins et al. [2002] and Myakishev et al. [2001] or InvaderPlus reactions from ThirdWave Technologies as designed at http://www.universalinvader.com with standard probe and primer

concentrations as recommended by ThirdWave. Of the chosen SNPs, 20 SNPs were successfully genotyped. Because of the limited number of SNPs, we only excluded those with >20% of missing data. In addition, we excluded individuals with >9 failed SNP genotypes. 17 SNPs were finalized.

Statistical analysis

For the present study, cases were defined as mothers of infants with DS who experienced a maternal meiotic event. Controls were fathers of infants with DS where the nondisjunction event was not a paternal error. For all analyses, we first conducted all analysis using only those cases and controls who self-reported white as their ethnic/racial background due to the small number of those self-reporting black. Next, we conducted analyses including both ethnic/racial groups, including an indicator variable for race in the model. No differences in conclusions were found, thus we present the while only results.

Association with number of recombinants: Case only analysis

We used the Armitage trend test to investigate association between each individual SNP within a gene/genomic region and recombination rates. The minor frequency allele count for each genotype was used as the predictor variable. We performed separate analysis for maternal MI and MII cases. The outcome variable was based on the number of recombinants. For MI cases, it was modeled as dichotomous variable with 0 or >0 recombinants. For MII case, the dichotomous variable was defined as 1 or >1 recombinants, as those with zero recombinants were defined as post-zygotic, mitotic errors (see above). We included maternal age was a covariate in the model.

Logistic-regression was used as implemented in Statistical Analysis Software (SAS) version 9.1.

Case/control analysis

We used a case/control analysis to determine whether there was an association between the candidate genes and nondisjunction of chromosome 21. As described above, cases were defined as mothers of infants with DS who experienced a maternal meiotic event. Controls were fathers of infants with DS where the nondisjunction event was not a paternal error. We compared the allele frequencies of the control fathers with HapMap CEPH (Centre d'Etude du Polymorphisme Humain) frequencies and found that they were comparable and thus can be used as representative of a white population (Table 4.1). We tested individual SNPs, using the Armitage trend test implemented in logistic-regression using Statistical Analysis Software (SAS) version 9.1. We performed separate analysis for all maternal meiotic, MI, and MII errors.

To investigate associations between each individual SNP within a gene/genomic region allele frequency and case/control status, we tested individual SNPs using the Armitage trend test implemented in logistic-regression using Statistical Analysis Software (SAS) version 9.1. We performed separate analysis for all maternal meiotic, MI, and MII errors. Fathers of proband who had not experienced a nondisjoining event represented the general population as allele frequencies were consistent with HapMap CEPH (Centre d'Etude du Polymorphisme Humain) frequencies (Table 4.1). The outcome variable was case/control status. SNP minor allele count was the independent variable in the model.

RESULTS

Study Population

Complete recombination phenotype information was available for 648 trios genotyped by CIDR. Of these 612 were due to maternal nondisjunction errors (28 families with mitotic errors and 8 with paternal errors were excluded). Complete candidate gene SNP information was available on 956 of the original 1064 individuals submitted for genotyping (30 individuals were removed due to possible sample mix-up and 78 were removed due to genotype failure). After merging these two datasets, those with data from CIDR and those with data from PreventionGenetics, we had a total of 325 case mothers and 331 control fathers for analysis. Of these, 285 and 290, respectively, reported their ethnic/racial background as white and were used for the primary analyses presented here (Table 4.2).

Candidate Gene Association with Recombination Phenotype

MI Analyses

Maternal MI chromosome 21 nondisjunction errors are associated with both a lack of recombination along the nondisjoined chromosome 21 and with a single telomeric recombinant [Lamb et al., 1997; Oliver et al., 2008]. When we examined the number of recombinants along the nondisjoined chromosome 21 (0 recombinants vs. >0 recombinants), we found no statistically significant association with *RNF212* (Table 4.3), *SYCP3* (Table 4.4) or the 17q21.31 inversion region (Table 4.5). We found a marginally significant association with rs2732706, located within the chromosome 17q21.31 inverted region (OR=0.50, 95% CI 0.26, 0.97, p=0.04) (Table 4.5): for every copy of the minor allele ‘A,’ recombination is reduced.

MII Analyses

In our previous work on maternal MII errors, we found an increased frequency of single pericentromeric recombinants [Lamb et al., 1997; Lamb et al., 1996; Lamb et al., 2005a; Oliver et al., 2008] and, overall, an increased genetic length of chromosome 21 compared to that generated from normal meiotic events [Lamb et al., 1996]. In our analysis here, we found evidence for an association of two of the candidate genes/regions, *RNF212* and the 17q21.31 inverted region, and the MII recombination phenotype (1 recombinant vs. >1 recombinant) (Table 4.3, 4.5). For *SYPC3*, the gene that is essential in the formation of the SC, we found no association with the recombination phenotype (Table 4.4)

Interestingly, the association with *RNF212* and the 17q21.31 inverted region with recombination appeared to be influenced by maternal age. In *RNF212,* two variants, rs1670533 and rs11939380, showed a statistically significant association with increased recombination among all maternal MII errors (OR=5.26 (95% CI 1.51, 18.31) and OR=3.92 (95% CI 1.20, 12.77), respectively) (Table 4.3). When we stratified by maternal age, the effect appeared to be more pronounced among older mothers (≥35 years of age at the time of birth of the infant with DS) compared with younger mothers (Table 4.3). For the chromosome 17q21.31 inverted region, the observed association with increased recombination rates in three SNPs was restricted to older mothers experiencing a MII meiotic error (Table 4.5). Because of the small sample size in this age group, the confidence intervals were wide, although they did not include 1.0. When we looked at the effect of these SNPs on recombination in younger mothers, two of the three variants (rs4640231 and rs1800547) show the opposite pattern, although the reduced ORs were

not statistically significant (Table 4.5). For rs1078268, there was no association in younger mothers.

Candidate Gene Association with Chromosome 21 Nondisjunction

Using the trend test with logistic regression and adjusting for maternal age, we did not find any statistical significant association of the three candidate gene/regions and nondisjunction when all the data were combined or if we stratified by meiotic error (Tables 4.6, 4.7 and 4.8). We identified one marginally significant variant in *RNF212* (rs4045481: OR=1.40 (95% CI 1.02, 1.93)) and another in chromosome 17q21.31 (rs17660907: OR=1.48 (95% CI 1.01, 2.18)) (Table 4.6). For these two suggestive associations, there were no significant effects on the ORs when the data were stratified by meiotic error or by maternal age as summarized in Table 4.9.

DISCUSSION

Altered patterns in both the amount and location of recombination are known molecular risk factors of nondisjunction. Recombinant events to close to the ends of the nondisjoined chromosome, centromeric or telomeric, reduction in the number of recombinants, and no exchange are associated with nondisjunction [Lamb et al., 1997; Lamb et al., 1996; Oliver et al., 2008; Sun et al., 2008]. There is recent evidence of inter-individual and within individual variation in genome-wide recombination rates in normally disjoining populations [Broman et al., 1998; Cheung et al., 2007; Coop et al., 2008; Kong et al., 2002; Lynn et al., 2002]. Preliminary evidence suggest that this genome-wide variation also exists in oocytes with a nondisjoining error [Brown et al., 2000]. Yet, little is known about the genetic or environmental factors that influence the altered patterns of recombination.

Very recently, a gene, *PRDM9*, was identified to influence the location of recombination. *PRDM9* is a transacting gene that encodes a zinc finger protein that has histone 3 lysine-4 trimethylation activity. It is important in meiotic recombination as it recognizes 13-mer motifs located at human hotspots. Allelic variants of PRDM9 zinc fingers are significantly associated with variability in genome-wide hotspot usage among humans [Baudat et al., 2010; Myers et al., 2010; Parvanov et al., 2010].

Additionally, recent research has identified genomic regions that are associated with recombination rates in a normal disjoining population. An inversion on chromosome 17q21.31 with two distinct haplotypes, H1 and H2, has been identified. The H2 haplotype is associated with an increase in recombination and number of offspring from female carriers [Stefansson et al., 2005]. *RNF212* also influences recombination rates. Two variants in *RNF212* have been identified, one associates with female recombination rates, rs1670533, and the other associates with males recombination rates, rs3796619 [Kong et al., 2008]. Chowdhury et al. [2009] confirmed the effects of *RNF212* on male recombination rates, confirmed the association of the chromosome 17 inverted region on recombination rates, and identified additional regions on chromosomes 1, 7, 9, and 10 that also influence recombination rates.

The study presented here examined the influence of candidate genes/regions on recombination rates in a nondisjoining population, a vulnerable population to altered recombination.

RNF212

Although the function of *RNF212* has not been characterized, shared homology with *S. cerevisiae ZIP3* and *C. elegans zhp-3* suggests a role for *RNF212* with meiotic

recombination and SC assembly [Kong et al., 2008]. Our results complement the previous findings of Kong et al. [2008]: we found an association between rates of recombination and rs1670533 along the nondisjoined chromosome 21 in females. Interestingly, in their study using the Icelandic population, the 'C' allele was associated with increased genome-wide recombination rate, with a nonsignificant decrease in the rate of recombination for chromosome 21. However, we see an increase in recombination rates for every copy of the 'C' allele (OR = 5.26; 95%CI 1.51-18.31). This effect is only observed among mothers who experienced a MII error, not among those with an MI error (OR = 1.16; 95%CI 0.75-1.81), suggesting a MII specific effect. Although based on small numbers, this association strengthens with increasing maternal age (Table 4.3). This suggests a possible age-dependent mechanism for the association of rs1670533 with rates of recombination in chromosome 21 nondisjunction. Additionally, we see a similar pattern with rs11939180 in *RNF212:* there is an increase in recombination rates associated with MII mothers, not MI mothers, and the effect is strengthened among older mothers (Table 4.3). Chowdhury et al. [2009] showed rs11939180 to be the most significant variant of *RNF212* associated with male recombination rates. In their population, a 'T' allele increased recombination, whereas, a 'T' allele in our population decreases recombination rates. This suggests rs11939180 affects both genders but with an opposite effect.

Chromosome 17q.21.31

Stefansson et al.[2005] show that the H2 haplotype is under positive selection in the Icelandic population. They used two diagnostic SNPs to represent the H2 haplotype, rs1800547 and rs9468. The 'G' allele of rs1800547, located in exon 4 of *MAPT*, denotes

the H2 background associated with increased recombination rates. Interestingly, in our nondisjoining population, we see the same results, but the effect is limited to older MII mothers; every copy of the 'G' is associated with increased recombination rates (Table 4.5). This same pattern is observed for two additional SNPs that are also associated with increased recombination rates in our dataset, rs4640231 residing in an intron of *CRHR1* and rs1078268, located near *MAPT*. We must be cautious with our interpretation, as the sample size of older mothers with MII is very small leading to wide confidence intervals.

In our study of *SYCP3,* an essential gene in the formation of the synaptonemal complex, we were unable to capture the majority of the genetic variation due to genotype problems. Thus, we cannot draw any conclusions based on the lack of an association of recombination along the nondisjoined chromosome 21 for the two SNPs we examined.

In summary, we studied the association of recombination rates along nondisjoined chromosomes 21 due to maternal errors in candidate genes known to influence genome-wide recombination rates. Although preliminary, we found intriguing associations for *RNF212* and the inverted 17q31 region, but these were restricted to MII errors. In the future, we will increase our sample size and expand the number of SNPs in these candidate regions to help identify the functional variants. Also, we will continue to study addition genes that are identified in normal meiotic events that affect genome-wide recombination patterns, such as *PRMD9,* to gain insight into their affect on proper chromosome segregation.

ACKNOWLEDGMENTS

The authors would like to thank all the families whose endless efforts makes this work possible as well as to the recruiters for making those initial connections with families.

Figure 4.1. Identification of parental origin and stage of recombinant event.
Polymorphic markers along the long arm of chromosome 21 are genotyped for the proband and their parents. Contribution of alleles at informative markers determines parent of origin. Markers close to the centromere (shown at top of chromosome figures) determine meiotic stage of origin. In this example, the error is maternal and occurred in MII (see text). Recombination along 21q is shown by a change in reduction to non-reduction of informative markers from the parent in whom the error occurred or vice versa and is depicted by a change in color.

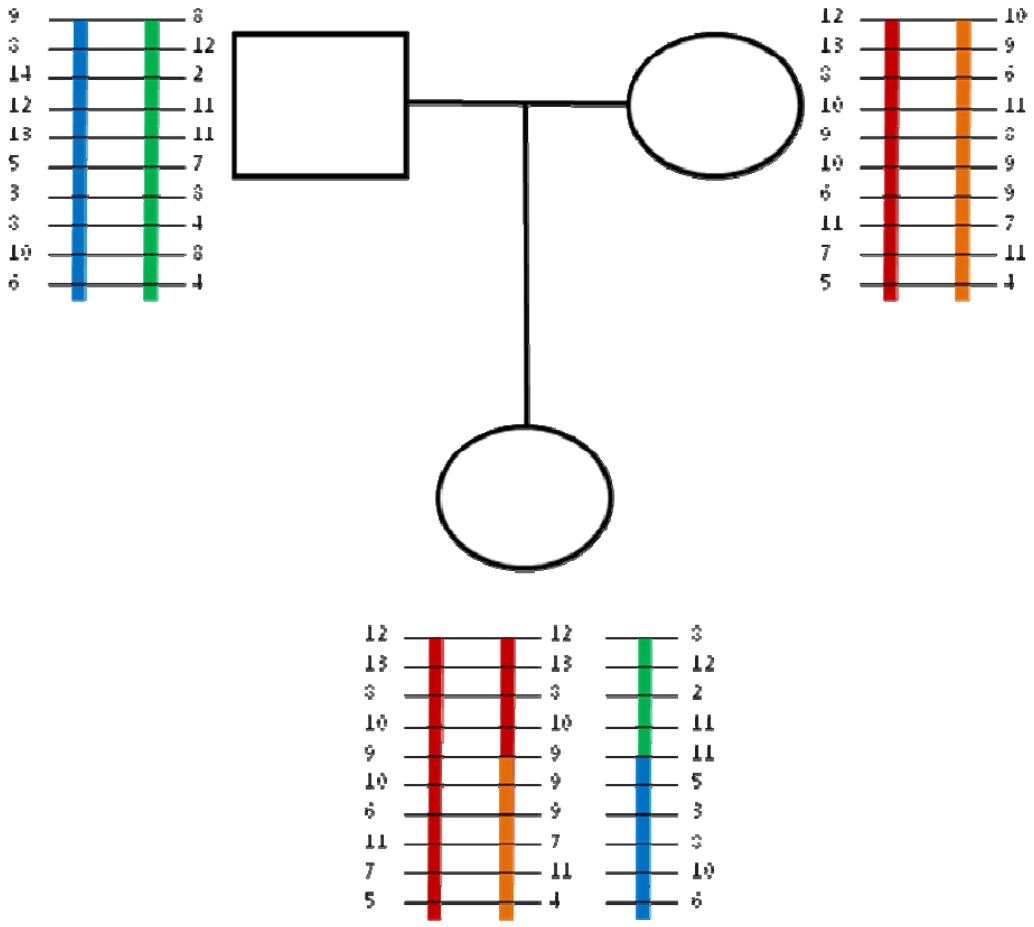


Table 4.1. Minor Allele and Genotype Frequencies of SNPs Analyzed in the Control and CEPH Populations

SNP	Chr	Chr position	Nearest gene	Alleles (A/B)	Minor Allele Frequencies		Control Genotype Frequencies		
					CEPH	Controls	A/A	A/B	B/B
RNF212									
rs1466216	4	1061907	*RNF212*	C/T	0.52	0.52	0.25	0.53	0.22
rs13147452	4	1068124	*RNF212*	A/G	0.52	0.52	0.26	0.53	0.22
rs1670533	4	1068187	*RNF212*	C/T	0.22	0.22	0.03	0.39	0.58
rs17164229	4	1068596	*RNF212*	C/T	0.10	0.13	0.03	0.20	0.77
rs17164235	4	1074061	*RNF212*	C/T	0.15	0.14	0.02	0.24	0.74
rs11939380	4	1076871	*RNF212*	C/T	0.33	0.31	0.07	0.48	0.45
rs4045481	4	1080625	*RNF212*	A/G	0.32	0.33	0.04	0.58	0.38
SYCP3									
rs12296641	12	100648897	*SYCP3*	A/G	0.11	0.11	0.01	0.21	0.79
rs10860779	12	100651393	*SYCP3*	A/C	0.30	0.35	0.08	0.55	0.37
Chr 17q21.31									
rs4640231	17	41268567	*CRHR1*	G/C	0.21	0.19	0.03	0.33	0.64
rs1800547	17	41407682	*MAPT*	A/G	0.21	0.16	0.03	0.27	0.70
rs1078268	17	41431738	*MAPT, STH*	C/T	0.21	0.15	0.02	0.26	0.72
rs9468	17	41457408	*MAPT*	C/T	0.20	0.20	0.03	0.33	0.64
rs17660907	17	41546868	*KIAA1267*	A/G	0.20	0.11	0.03	0.17	0.80
rs12150320	17	41568981	*KIAA1267*	C/T	0.20	0.19	0.00	0.38	0.62
rs2732706	17	41707463	*LRRC37A*	A/G	0.20	0.17	0.00	0.34	0.66
rs2732705	17	41707706	*LRRC37A*	A/C	0.19	0.16	0.01	0.30	0.69

Minor alleles of the sample population are underlined.

Table 4.2. Study Sample Characteristics			
Maternal cases	**Black (n)**	**White (n)**	**Total**
All			
Mean maternal age	31.50 ± 6.41	33.84 ± 5.63	33.55 ± 5.78
< 35 years old	22	154	
≥ 35 years old	18	131	
Race	**40**	**285**	**325**
MI			
Mean maternal age	31.39 ± 6.16	33.50 ± 5.48	33.24 ± 5.60
< 35 years old	18	124	
≥ 35 years old	13	92	
Race	**31**	**216**	**247**
MII			
Mean maternal age	31.89 ± 7.59	34.90 ± 6.02	34.55 ± 6.24
< 35 years old	4	30	
≥ 35 years old	5	39	
Race	**9**	**69**	**78**
Paternal controls	**Black (n)**	**White (n)**	**Total**
Race	**41**	**290**	**331**

Table 4.3. Association between *RNF212* SNPs and Recombination Rates Among Mothers Experiencing a MI or MII Meiotic Error

RNF212	**All**		**Young**		**Old**	
MI	**n (0/>0)**	**OR (95% CI)**	**n (0/>0)**	**OR (95% CI)**	**n (0/>0)**	**OR (95% CI)**
rs1466216	104/67	1.12 (0.70, 1.79)	65/36	1.31 (0.68, 2.50)	39/31	0.96 (0.48, 1.92)
rs13147452	116/70	0.89 (0.57, 1.40)	69/39	1.05 (0.56, 1.97)	47/31	0.72 (0.36, 1.42)
rs1670533	120/81	1.16 (0.75, 1.81)	72/43	1.23 (0.65, 2.30)	48/38	1.13 (0.60, 2.13)
rs17164229	102/62	0.87 (0.44, 1.74)	63/35	1.07 (0.45, 2.57)	39/27	0.62 (0.20, 1.93)
rs17164235	120/80	0.99 (0.53, 1.82)	72/41	1.13 (0.50, 2.54)	48/39	0.82 (0.32, 2.08)
rs11939380	116/80	1.03 (0.67, 1.57)	67/41	0.74 (0.40, 1.39)	49/39	1.38 (0.76, 2.51)
rs4045481	98/64	1.24 (0.75, 2.03)	60/38	0.94 (0.47, 1.86)	38/26	1.67 (0.79, 3.50)
MII	**n (1/>1)**	**OR (95% CI)**	**n (1/>1)**	**OR (95% CI)**	**n (1/>1)**	**OR (95% CI)**
rs1466216	41/13	0.60 (0.24, 1.50)	19/6	0.88 (0.21 3.70)	22/7	0.27 (0.06, 1.19)
rs13147452	41/11	0.64 (0.23, 1.81)	19/5	1.940 (0.35, 10.91)	22/6	0.18 (0.03, 1.07)
rs1670533	**50/13**	**5.26 (1.51, 18.31)**	21/6	2.53 (0.32, 19.69)	**29/7**	**12.16 (1.51, 97.67)**
rs17164229	34/11	0.35 (0.04, 3.23)	14/5	2.42 (0.13, 46.65)	20/6	n. a.
rs17164235	49/15	0.50 (0.10, 2.55)	21/8	1.59 (0.21, 12.02)	28/7	n. a.
rs11939380	**39/10**	**3.916 (1.20, 12.77)**	17/4	1.87 (0.29, 11.96)	**22/6**	**6.36 (1.25, 32.28)**
rs4045481	36/11	2.28 0.69, 7.60)	14/5	0.662 (0.09, 5.19)	22/6	5.35 (0.90, 31.88)

SNPs are listed by position along the chromosome. Significant ($p<0.05$) results are listed in bold.

CI: confidence interval

n. a. = not available due to 0 well among genotypes

Table 4.4. Association between *SYCP3* SNPs and Recombination Rates Among Mothers Experiencing a MI or MII Meiotic E

SYCP3	**All**		**Young**		**Old**	
MI	**n (0/>0)**	**OR (95% CI)**	**n (0/>0)**	**OR (95% CI)**	**n (0/>0)**	**OR (95% CI)**
rs12296641	81/60	1.63 (0.75, 3.51)	49/35	1.20 (0.43, 3.30)	32/25	2.82 (0.80, 9.85)
rs10860779	89/66	1.28 (0.76, 2.16)	54/34	1.56 (0.76, 3.19)	35/32	1.03 (0.48, 2.25)
MII	**n (1/>1)**	**OR (95% CI)**	**n (1/>1)**	**OR (95% CI)**	**n (1/>1)**	**OR (95% CI)**
rs12296641	21/11	n. a.	9/5	n. a.	12/6	n. a.
rs10860779	40/10	0.76 (0.24, 2.42)	20/8	0.94 (0.25, 3.54)	20/2	0.40 (0.03, 4.95)

SNPs are listed by position along the chromosome. Significant ($p<0.05$) results are listed in bold.

CI: confidence interval

n. a. = not available due to 0 well among genotypes

Table 4.5. Association between Chromosome 17q21.31 Inverted Region SNPs and Recombination Rates Among Mothers Ex

Chr 17q21.31	**All**		**Young**		
MI	**n (0/>0)**	**OR (95% CI)**	**n (0/>0)**	**OR (95% CI)**	**n (0**
rs4640231	108/72	0.86 (0.52, 1.43)	64/38	1.00 (0.52, 1.89)	44
rs1800547	97/56	0.69 (0.39, 1.22)	59/31	0.78 (0.38, 1.59)	38
rs1078268	106/71	0.72 (0.42, 1.25)	65/38	0.78 (0.39, 1.57)	41
rs9468	108/71	0.80 (0.48, 1.33)	65/37	0.83 (0.43, 1.58)	43
rs17660907	89/63	0.97 (0.55, 1.70)	56/33	1.01 (0.51, 2.02)	33
rs12150320	67/40	0.64 (0.28, 1.46)	41/22	0.59 (0.20, 1.77)	26
rs2732706	**101/68**	**0.50 (0.26, 0.97)**	60/35	0.50 (0.20, 1.23)	41
rs2732705	100/64	0.70 (0.37, 1.32)	64/35	0.86 (0.40, 1.86)	36
MII	**n (1/>1)**	**OR (95% CI)**	**n (1/>1)**	**OR (95% CI)**	**n (1**
rs4640231	39/13	1.44 (0.52, 3.99)	18/7	0.38 (0.07, 2.08)	**2**
rs1800547	33/12	1.95 (0.68, 5.6)	16/6	0.59 (0.11, 3.17)	**1**
rs1078268	41/11	2.63 (0.84, 8.27)	17/6	1.05 (0.13, 8.44)	**2**
rs9468	38/12	1.46 (0.53, 3.97)	17/5	0.47 (0.08, 2.84)	2
rs17660907	34/8	0.63 (0.11, 3.53)	14/6	0.42 (0.03, 5.33)	2
rs12150320	21/9	1.42 (0.29, 6.95)	9/4	0.08 (0.00, 2.81)	1
rs2732706	34/11	1.80 (0.44, 7.35)	11/6	0.45 (0.05 4.35)	2
rs2732705	37/12	2.03 (0.53, 7.74)	15/6	0.99 (0.12, 8.07)	2

SNPs are listed by position along the chromosome. Significant ($p<0.05$) results are listed in bold.

CI: confidence interval

n. a. = not available due to 0 well among genotypes

Table 4.6. Association of Genomic Regions and Chromosome 21 Nondisjunction Using Armitage Trend Test among All Cases/Controls

	All cases					
	n	MAF	Case Genotype Frequencies			OR (95% CI)
RNF212			**A/A**	**A/B**	**B/B**	
rs1466216	225	0.46	0.19	0.54	0.28	0.77 (0.59, 1.01)
rs13147452	238	0.50	0.22	0.56	0.22	0.91 (0.70, 1.19)
rs1670533	264	0.23	0.07	0.33	0.60	1.07 (0.80, 1.43)
rs17164229	209	0.12	0.01	0.21	0.78	0.92 (0.62, 1.34)
rs17164235	264	0.12	0.01	0.22	0.77	0.83 (0.58, 1.19)
rs11939380	245	0.35	0.13	0.44	0.42	1.22 (0.93, 1.61)
rs4045481	209	0.39	0.12	0.55	0.33	**1.40 (1.02, 1,93)**
SYCP3						
rs12296641	173	0.12	0.00	0.23	0.77	1.07 (0.65, 1.76)
rs10860779	205	0.39	0.11	0.56	0.34	1.18 (0.86, 1.61)
Chr 17q21.31						
rs4640231	232	0.23	0.06	0.35	0.59	1.25 (0.91, 1.71)
rs1800547	198	0.22	0.07	0.30	0.63	1.37 (0.97, 1.95)
rs1078268	229	0.19	0.05	0.28	0.67	1.29 (0.92, 1.81)
rs9468	229	0.24	0.07	0.36	0.58	1.33 (0.97, 1.83)
rs17660907	194	0.16	0.05	0.23	0.72	**1.48 (1.01, 2.18)**
rs12150320	137	0.21	0.00	0.42	0.58	1.18 (0.73, 1.93)
rs2732706	214	0.19	0.00	0.39	0.61	1.19 (0.81, 1.75)
rs2732705	213	0.19	0.01	0.36	0.63	1.31 (0.89, 1.91)

MAF: Minor allele frequencies.

Table 4.7. Association of Genomic Regions and Chromosome 21 Nondisjunction Using Armitage Trend Test among MI Cases/Controls

	MI cases					
	n	MAF	Case Genotype Frequencies			OR (95% CI)
RNF212			**A/A**	**A/B**	**B/B**	
rs1466216	171	0.45	0.18	0.55	0.27	0.75 (0.56, 1.00)
rs13147452	186	0.48	0.20	0.56	0.24	0.84 (0.63, 1.12)
rs1670533	201	0.23	0.08	0.31	0.61	1.06 (0.78, 1.44)
rs17164229	164	0.12	0.02	0.21	0.77	0.96 (0.63, 1.46)
rs17164235	200	0.13	0.02	0.22	0.77	0.87 (0.59, 1.27)
rs11939380	196	0.35	0.13	0.44	0.43	1.20 (0.90, 1.61)
rs4045481	162	0.39	0.12	0.54	0.34	1.38 (0.98, 1.94)
SYCP3						
rs12296641	141	0.12	0.00	0.25	0.75	1.18 (0.70, 1.97)
rs10860779	155	0.38	0.10	0.56	0.34	1.17 (0.84, 1.65)
Chr 17q21.31						
rs4640231	180	0.23	0.06	0.34	0.61	1.21 (0.86, 1.69)
rs1800547	153	0.21	0.07	0.29	0.64	1.34 (0.92, 1.94)
rs1078268	177	0.19	0.05	0.28	0.67	1.28 (0.90, 1.84)
rs9468	179	0.24	0.06	0.35	0.59	1.28 (0.91, 1.80)
rs17660907	152	0.16	0.06	0.21	0.73	1.46 (0.98, 2.19)
rs12150320	107	0.20	0.00	0.39	0.61	1.07 (0.64, 1.81)
rs2732706	169	0.19	0.00	0.38	0.62	1.15 (0.76, 1.73)
rs2732705	164	0.20	0.02	0.35	0.63	1.33 (0.89, 1.99)

MAF: Minor allele frequencies.

Table 4.8. Association of Genomic Regions and Chromosome 21 Nondisjunction Using Armitage Trend Test among MII Cases/Controls

	MII cases					
	n	MAF	Case Genotype Frequencies			OR (95% CI)
RNF212			**A/A**	**A/B**	**B/B**	
rs1466216	54	0.47	0.22	0.49	0.29	0.83 (0.54, 1.27)
rs13147452	52	0.57	0.29	0.56	0.15	1.22 (0.79, 1.90)
rs1670533	63	0.24	0.03	0.41	0.56	1.10 (0.67, 1.79)
rs17164229	45	0.10	0.00	0.20	0.80	0.78 (0.38, 1.60)
rs17164235	64	0.11	0.00	0.22	0.78	0.74 (0.41, 1.36)
rs11939380	49	0.37	0.14	0.45	0.41	1.32 (0.82, 2.13)
rs4045481	47	0.40	0.11	0.60	0.30	1.54 (0.89, 2.69)
SYCP3						
rs12296641	32	0.08	0.00	0.16	0.84	0.68 (0.25, 1.84)
rs10860779	50	0.39	0.12	0.54	0.34	1.21 (0.74, 2.00)
Chr 17q21.31						
rs4640231	52	0.25	0.06	0.38	0.56	1.41 (0.84, 2.35)
rs1800547	45	0.23	0.07	0.33	0.60	1.51 (0.88, 2.62)
rs1078268	52	0.19	0.04	0.31	0.65	1.33 (0.76, 2.31)
rs9468	50	0.27	0.08	0.38	0.54	1.54 (0.92, 2.56)
rs17660907	42	0.17	0.02	0.29	0.69	1.53 (0.82, 2.85)
rs12150320	30	0.25	0.00	0.50	0.50	1.66 (0.75, 3.68)
rs2732706	45	0.21	0.00	0.42	0.58	1.36 (0.72, 2.58)
rs2732705	49	0.18	0.00	0.37	0.63	1.23 (0.66, 2.29)

MAF: Minor allele frequencies.

Table 4.9. Association of Significant SNPs and Chromosome 21 Nondisjunction Using Logistic Regression

	SNP	n	MAF	A/A	A/B	B/B	OR (95% CI)
RNF212							
All cases	**rs4045481**	209	0.39	0.12	0.55	0.33	**1.40 (1.02, 1.93)**
Meiotic Error							
MI case/control	**rs4045481**	162	0.39	0.12	0.54	0.34	1.38 (0.98, 1.94)
MII case/control	**rs4045481**	47	0.40	0.11	0.60	0.30	1.54 (0.89, 2.69)
Age							
Young case/control	**rs4045481**	117	0.37	0.08	0.59	0.33	1.26 (0.85, 1.87)
Old case/control	**rs4045481**	92	0.42	0.17	0.50	0.33	**1.64 (1.09, 2.46)**
Chr 17q21.31							
All cases	**rs17660907**	194	0.16	0.05	0.23	0.72	**1.48 (1.01, 2.18)**
Meiotic Error							
MI case/control	**rs17660907**	152	0.16	0.06	0.21	0.73	1.46 (0.98, 2.19)
MII case/control	**rs17660907**	42	0.17	0.02	0.29	0.69	1.53 (0.82, 2.85)
Age							
Young case/control	**rs17660907**	109	0.17	0.06	0.21	0.72	1.51 (0.97, 2.33)
Old case/control	**rs17660907**	85	0.16	0.04	0.25	0.72	1.44 (0.88, 2.36)

MAF: Minor allele frequencies.

CHAPTER 5

Genome-wide recombination as a risk factor for nondisjunction

NaTasha D. HOLLIS[1], Eleanor FEINGOLD[2], Vivian G. CHEUNG[3, 4], Stephanie L. SHERMAN[1]

[1]Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia
[2]Department of Human Genetics and Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania
[3]Department of Genetics, Emory University of Pennsylvania, Philadelphia, Pennsylvania
[3]Department of Pediatrics, Emory University of Pennsylvania, Philadelphia, Pennsylvania

Contribution to Research:

NaTasha Hollis participated in the recruitment of extended 5-member families for the present study: recruiting maternal and paternal grandparents, as well as, conducting maternal interviews. Additionally, NaTasha helped to write the grant that was reviewed and accepted by CIDR to genotype the samples for this study.

Eleanor Feingold assisted in the development of the statistical methodologies.

Vivian Cheung assisted in the development of the statistical methodologies and contribution of control data.

Stephanie Sherman developed study design and contributed to development of statistical analysis.

INTRODUCTION

Human chromosome nondisjunction leads to an increased frequency of aneuploidy, either too many or too few chromosomes. As such, nondisjunction is a leading cause of pregnancy loss, birth defects, and intellectual disabilities. Although nondisjunction is clinically important, little is known about the mechanisms and risk factors contributing to it. For years, trisomy 21, also known as Down syndrome (DS), has been used as a model to study human nondisjunction. It has been shown that altered patterns of meiotic recombination and maternal age are risk factors for nondisjunction.

Altered patterns of meiotic recombination are specific to the type of maternal error (meiosis I-MII and meiosis II-MII). Significant reduction in recombination along the nondisjoined chromosome has been observed for all MI-derived trisomies studied to date, including trisomies 15, 16, 18, 21 and X of maternal origin and trisomy 21 and Klinefelter syndrome (47 XXY) of paternal origin [Bugge et al., 1998; Hassold et al., 1995; Hassold et al., 1991; Lamb et al., 1997; Lamb et al., 1996; Robinson et al., 1998; Savage et al., 1998; Thomas et al., 2001]. For example, our previous studies of maternal MI-derived trisomy 21 estimated that 40% of MI errors were derived from oocytes where no meiotic exchange had occurred along chromosome 21 [Lamb et al., 1997; Lamb et al., 1996]. Furthermore, of those maternally-derived MI cases with a single exchange, the majority of exchanges occurred in the distal 6.5 Mb of chromosome 21.

Interestingly, so-called "MII" derived cases were highly associated with pericentromeric exchanges, or those that occurred within the most centromeric 3.5 Mb of chromosome 21 [Lamb et al., 1997; Lamb et al., 1996]. This observation of an effect of an MI process – recombination -- on MII nondisjunction prompted us to ask the obvious

question: did these trisomies really originate at MII? We think that the answer is “no”. Instead, we suggest that the presence of a pericentromeric exchange might increase the likelihood of chromosome "entanglement" or premature sister chromatid separation at MI, with the resulting disomic gamete having identical centromeres; thus, the case would be scored as originating at MII even though the precipitating event occurred at MI.

The role of chiasmata to ensure proper chromosome segregation at MI is well established. The importance of this structure in recombination would suggest that the number and location of recombinant events would be tightly controlled. However, studies have shown that there is considerable inter-individual variation in genome-wide recombinant events. Lynn et al. [2002] showed that exchanges in pachytene stage spermatocytes had average values ranging from 46-53 exchanges/spermatocyte. Indirect measures of genome-wide recombination rates have shown inter-individual variation. Initial reports only identified such variation among females and not males [Broman et al., 1998; Kong et al., 2002]. Recent data have shown inter-individual variation in both maternal and paternal gametes [Cheung et al., 2007; Coop et al., 2008]. In addition, data from Kong et al. [2002] identified what they called a “gamete” effect. They found that the number of recombinants was positively correlated among chromosomes within the same oocyte, even after adjusting for the “mother” effect, or the correlation of recombinants among oocytes/offspring of a woman. Perhaps there is a factor that globally affects recombination rates among most chromosomes simultaneously such as *RNF212*, a gene recently shown to affect male and female genomewide recombination rates [Kong et al., 2008].

Cheung et al. [2007] found that there was inter-individual variation in the number

of recombinant events, as well as, inter-individual variation in the location of these events. They detected polymorphic differences among individuals in activity of recombination "jungles," which refer to the location of the recombination. That is, the meioses from some individuals contributed significantly more recombinant events to specific recombination "jungles" than those from others. They began by dividing the genome into 553 bins of 5 Mb each and scored the number of recombinants in each bin separately by female and male meioses. They found that there were genomic regions in each gender that contained significantly more recombinants than expected by chance; these were termed recombination "jungles". They then focused on the five 'jungles" that included the most recombination. Interestingly, in both males and females, these "jungles" were either the most or second most telomeric bins on the chromosomes.

We hypothesize that extreme variation in genome-wide recombination rates and patterns may be a predisposing factor to nondisjunction. This variation may involve genetic variation in genes important for recombination or by environment exposures of the developing gametes. We will test the following hypothesis: altered recombination observed along the nondisjoined chromosome in oocytes with MI and MII errors are dictated by trans-acting factors that influence the risk for nondisjunction. Studies will be done to compare genome-wide recombination characteristics in individuals with nondisjoined chromosome 21 errors to their siblings, as well as, to unrelated individuals with normal meiotic events. If the altered recombination observed along the nondisjoined chromosomes 21 extends to all chromosomes, we will be able to direct our search for factors involved in recombination-associated nondisjunction toward genome-wide phenomena, i.e., trans-acting factors.

MATERIALS AND METHODS

Study Population

A follow-up study of families who have participated in our Emory-based research programs is currently underway to the family units needed to investigate genome-wide recombination levels. Our focus is on maternal errors of nondisjunction; thus, we are excluding paternal and mitotic events and will examine only risk factors associated with the mother of a child with Down syndrome. Anticipated numbers for the study are based on the percent of families where both parental biological samples are available and maternal grandparents are living. We are also collecting biological samples on siblings. If we assume 50% of families with the minimum 5-member family structure will participate, we expect to obtain 99 families with a child with a maternal MI-derived error and 35 with an "MII"-derived error (Table 5.1). Based on our current data, the average number of siblings is 1.3 per family. Thus, we can roughly estimate the number of siblings to be similar to the number of families that participate.

The primary control genotype data will be from the CEPH families, provided by Dr, Vivian Cheung, in which there are currently 283 fully genotyped maternal meioses available for comparison.

Data Collection

DNA extracted from saliva will be used. Saliva samples are collected for maternal grandparents and siblings. We have established cell lines for the majority of probands and parents. However, if there is an inadequate amount of DNA stored from previous studies, blood samples are requested on the original trio.

Genotyping

We will conduct a genome-wide scan of the DS families using the Illumina® Linkage IVb Marker Panel which includes about 5,800 SNPs and has an average coverage of 0.64 cM.

Statistical Analysis

In our attempt to explore the relationship between chromosome 21 altered recombination patterns as a predictor of genome-wide recombination patterns, as well as, if a "gamete" or "mother" effect is responsible for the altered recombination patterns, we will examine three sample groups. We will genome-wide recombination rates from oocytes with nondisjoined chromosomes 21, oocytes with normal disjoined chromosomes 21 from the same mother, and those from unrelated mothers. We will use the same statistical methods as those used in Brown et al. [2000]. Mean and median recombination numbers for each group will be calculated, outliers qualitatively observed, and differences between groups tested using either non-parametric tests or comparable parametric tests. We will also examine any outliers among our nondisjoined samples to determine if these are significantly different from all groups and may be due to major gene/environmental effects that, in themselves, may cause nondisjunction. To analyze the matching between probands and siblings and also for the fact that each proband has a variable number of siblings, generalized estimating equation (GEE) methods are probably most appropriate. Another approach will be to rank preferred sites of recombination in the genome within each study group using the methods outlined in Cheung et al. [2007] to determine if there is more variation among or within groups. The groups for preferred sites analysis are defined according to chromosome 21 meiotic error and location of recombination.

PRELIMINARY RESULTS

Reduction of genome-wide recombination in oocytes with nondisjoined chromosomes 21 that have no detectable recombination: a preliminary study

One of the most intriguing questions regarding the association of altered recombination and nondisjunction involves the extent of the effect; that is, are the alterations in exchange patterns limited to the nondisjoining chromosomes, or are there genome-wide "disturbances" in recombination in nondisjunctional meioses. To begin to address the question of whether or not altered recombination patterns are limited to chromosome 21 or extend genome-wide, we measured the genome-wide recombination rates in an oocyte with non-disjoined chromosomes 21 and no detectable 21q recombination [Brown et al., 2000]. These nondisjoined individuals were chosen to enrich for extremes in recombination. In the final analysis, there were 15 case families which consisted of a DS proband, parents and maternal grandparents of the proband. Genome-wide recombination was measured using 366 markers from the Weber STRP linkage screening set. The control sample contained 91 normal female meiotic events obtained from eight CEPH families.

There was a statistically significant reduction in the total number of recombinant events occurring throughout the genome of the cases compared to control individuals ($p < 0.05$; Table 5.2). The reduction appeared global in nature. The difference between cases and controls could have been due to normal genetic variation in recombination that was revealed because the MI cases with no 21q recombination were selected and compared to a control group unselected for 21q recombination. To examine this, controls were divided into two groups according to the presence or absence of observed 21q recombination. The

inferred number of exchanges per chromosome 21 was estimated to determine if genome-wide recombination increased with increasing chromosome 21 exchange numbers. There was a statistically significant difference among these three groups ($p<0.01$) (Table 5.2, Figure 5.1). It was suggested that the apparent cell-wide reduction observed in case oocytes reflected the low end of normal variation in total recombination occurring in female meiotic events and was because of a trans-acting factor.

Future Directions

Our preliminary results on altered recombination in maternal meiotic errors along the nondisjoined chromosome and genome-wide, taken together with evidence for inter-individual variation in normal meiosis, provide the motivation for further study. As this study is currently on-going, once recruitment is complete and samples are genotyped our first goal is to confirm our previous findings that recombination on a nondisjoined chromosome 21 predicts genome-wide recombination rates (gamete effect) [Brown et al., 2000], similar to that observed among normal meiotic events [Broman et al., 1998; Cheung et al., 2007; Kong et al., 2002].

Once we determine if the altered patterns of recombination are caused by a "gamete" or "mother" effect, we will know how to proceed with this study. For instance, if we see inter-individual variation in meioses of our mothers of affected offspring, we may conclude that there is a "gamete" effect leading to a trisomic child. However, if we conclude, that there is a "mother" effect between offspring in our cases, then we can go on to try and dissect the genetic and environmental factors that lead to the observed recombinant patterns. We can then perform association studies to determine if genes known to play a role in recombination are responsible for the altered patterns seen in

children with nondisjoined chromosomes. Ultimately, this information will provide more insight into the cause(s) of nondisjunction.

ACKNOWLEDGMENTS

The authors would like to thank all the families whose endless efforts makes this work possible as well as to the recruiters for making those initial connections with families.

Table 5.1. Expected number of minimum 5-member families. Numbers are based on current database demographics (a: reference number (sibling reference number) used in text; b: number of chromosome 21 exchanges inferred from the observed data [Lamb et al., 1997].

"Oocyte" description	Reference number (sibling)[a]	Total	Inferred exchanges on chr 21[b]
MI errors		99	--
No observed chr21 recombination	1 (6)	67	0.43
≥1 observed chr21 recombination	2 (7)	32	1.36
"MII" errors (≥1 observed chr21 recombination)	3 (8)	35	1.40
Normally disjoined chromosome		238	--
No observed chr21 recombination	4	124	1.07
≥1 observed chr21 recombination	5	114	1.27

Table 5.2. Comparisons of the total observed number of genome wide recombination events.

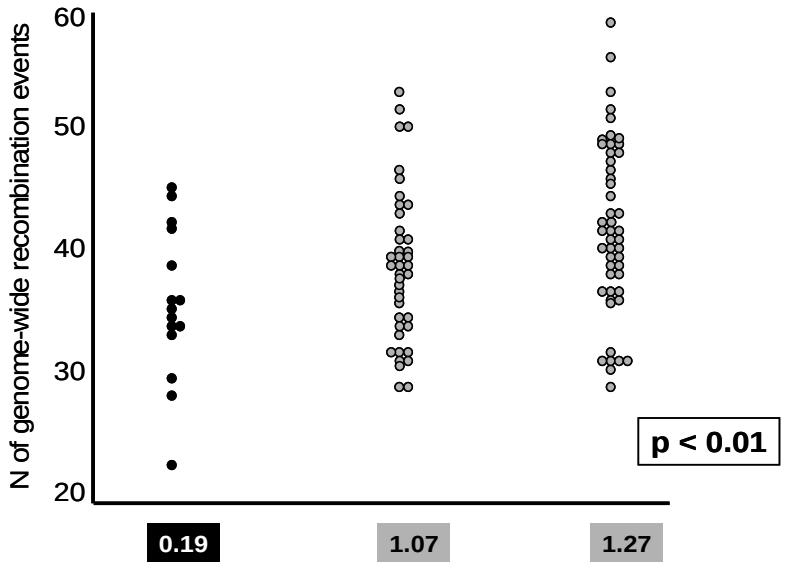
"Oocyte" Description	**N of meioses**	**Inferred N of chr 21 exchanges**	**Total recombination events in: (mean ± S.D.)**
			genome
Cases			
MI errors with no observed CO[a]	15	0.19	35.45 ± 6.30
Controls			
No observed CO[a]	41	1.07	38.13 ± 6.26
≥ 1 observed CO[a]	50	1.27	41.37 ± 6.96
All	91	--	39.91 ± 6.81
Comparisons:			
Cases vs Controls[b]—p-value:			.030
Cases, Controls with 0, and ≥1 rec.[c]—p-value:			.007

[a] CO=crossovers on chromosome 21

[b] Mann-Whitney t-test, two-sided p-values between cases and all controls

[c] Kruskal-Wallis test, two-sided p-values comparing three groups: cases, controls with 0 and ≥ observed recombinants

Figure 5.1. Scatter plot illustrating the total recombination events among the cases (black circles) and controls (gray circles) grouped by the mean of the expected number of exchanges along chromosome 21.



CHAPTER 6

DISCUSSION

The primary focus of this research was to extend previous studies examining recombination as a molecular risk factor for nondisjunction. This thesis was designed to examine four potential risk factors: 1) inter-individual variation in recombination, 2) variation in recombination in a gamete, 3) genetic variation in genes on recombination rates, and 4) folate deficiency. The first two potential risk factors are a part of a project in which active recruitment is still underway, so I will focus on the other potential risk factors here.

As described in Chapter 2, folic acid supplementation taken prior to conception is associated with chromosome 21 maternal MII errors. That is, lack of folic acid supplementation taken prior to conception increased the risk of a maternal MII error. This conclusion was based solely on supplemented folic acid and did not take into account other sources of folate which potentially could impact the results, i.e. diet. Future studies would include examining the combination of both folate (dietary) and folic acid (supplemented) to determine the combined effect of both and the association with DS. If together there is a reduced risk then it would contribute to general knowledge of ways to possibly reduce the incidence of DS among older mothers prior to pregnancy. The optimal threshold of the combination to which the effect is seen would have to be determined. For example, Czeizel and Puho [2005] saw an effect of supplementation when folic acid was taken at high doses (6mg/d), what is the optimal dosage needed in conjunction with diet? To determine which pathways are associated with chromosome nondisjunction and are affected by folate, in vitro studies can be conducted. For

example, Cheng et al. [2009] used MLH1 staining in order to characterize recombination patterns in human fetal oocyte. A similar study should be done where mother's supplementation and diet are known. Fetal oocytes should be stained for different proteins known to be involved with nondisjunction, such as cohesins, and see if different levels of folate affect the ability of said proteins.

The work described in Chapter 3 was an opportunistic study which revealed no association with folate polymorphisms and nondisjunction. Future studies would be to increase our sample size and genotype individuals for common variants in the folate pathway. Additionally, supplementation usage from the mothers of the infants with DS and controls should be available in order to determine if the combination of folate risk factors have a greater effect than both individually.

The work described in Chapter 4 is the first of its kind to look at variation in genes associated with recombination rates in a nondisjoning population where it is known that recombination is a risk factor for the outcome [Lamb et al., 1997; Lamb et al., 1996]. Our results revealed that the examined variants have the same effect on normal disjoining populations and a chromosome 21 nondisjunction population. This suggests, that there are additional factors that may play a role, if not, one would expect the same outcome in both populations (both either normal disjoining or both nondisjoining populations) if that was the only factor involved. Future studies would determine what are the differences impacting the two populations. Does the nondisjoining population have the risk factor of that particular SNP variant plus another factor not? It may be something as simple as a difference in diet that can be detected via questionnaires or it may be something biological and more complex. If the latter, obtaining DNA samples to perform molecular

studies is required.

Additional future studies not mentioned elsewhere include genotyping the families that are currently being recruited for my first project to look at genome-wide recombination patterns in genes that have now emerged to affect recombination rates globally. This will give more information on the way those genes affect recombination globally as compared to my project where I only looked at chromosome 21 recombination.

In summary, there are still many challenges ahead in gaining a better understanding of nondisjunction. While associations are shown between a single risk factor and DS, it is more likely that the single risk factor is associating with another risk factor. The work presented in this thesis provides preliminary evidence for potential risk factors that may affect chromosome 21 nondisjunction, i.e., folate deficiency and variation in recombination, primarily in older MII mothers. It could possibly be an interaction between the factors proposed in this thesis: variation in recombination, variation in genes that influence recombination and folate deficiency. However, due to the clinical implications of nondisjunction in humans, continued effort and research is needed to try to address the possibility of complexity in this process.

WORKS CITED

Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, Royle MH, Torfs CP, Sherman SL. 2009. Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. Hum Genet 125(1):41-52.

Amorim MR, Castilla EE, Orioli IM. 2004. Is there a familial link between Down's syndrome and neural tube defects? Population and familial survey. BMJ 328(7431):84.

Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F, Pangalos C, Raoul O, Slaugenhaupt SA, Hafez M, et al. 1992. The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet 50(3):544-550.

Barkai G, Arbuzova S, Berkenstadt M, Heifetz S, Cuckle H. 2003. Frequency of Down's syndrome and neural-tube defects in the same family. Lancet 361(9366):1331-1335.

Baudat F, Buard J, Grey C, Fledel-Alon A, Ober C, Przeworski M, Coop G, de Massy B. 2010. PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science 327(5967):836-840.

Biselli JM, Goloni-Bertollo EM, Zampieri BL, Haddad R, Eberlin MN, Pavarino-Bertelli EC. 2008. Genetic polymorphisms involved in folate metabolism and elevated plasma concentrations of homocysteine: maternal risk factors for Down syndrome in Brazil. Genet Mol Res 7(1):33-42.

Bolor H, Mori T, Nishiyama S, Ito Y, Hosoba E, Inagaki H, Kogo H, Ohye T, Tsutsumi M, Kato T, Tong M, Nishizawa H, Pryor-Koishi K, Kitaoka E, Sawada T, Nishiyama Y, Udagawa Y, Kurahashi H. 2009. Mutations of the SYCP3 gene in women with recurrent pregnancy loss. Am J Hum Genet 84(1):14-20.

Bosco P, Gueant-Rodriguez RM, Anello G, Barone C, Namour F, Caraci F, Romano A, Romano C, Gueant JL. 2003. Methionine synthase (MTR) 2756 (A --> G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. Am J Med Genet A 121A(3):219-224.

Botto LD, Mulinare J, Yang Q, Liu Y, Erickson JD. 2004. Autosomal trisomy and maternal use of multivitamin supplements. Am J Med Genet A 125A(2):113-116.

Boue JG, Boue A. 1973. Increased frequency of chromosomal anomalies in abortions after induced ovulation. Lancet 1(7804):679-680.

Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. 1998. Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet 63(3):861-869.

Brown AS, Feingold E, Broman KW, Sherman SL. 2000. Genome-wide variation in recombination in female meiosis: a risk factor for non-disjunction of chromosome 21. Hum Mol Genet 9(4):515-523.

Bugge M, Collins A, Petersen MB, Fisher J, Brandt C, Hertz JM, Tranebjaerg L, de Lozier-Blanchet C, Nicolaides P, Brondum-Nielsen K, Morton N, Mikkelsen M. 1998. Non-disjunction of chromosome 18. Hum Mol Genet 7(4):661-669.

Canfield MA, Collins JS, Botto LD, Williams LJ, Mai CT, Kirby RS, Pearson K, Devine O, Mulinare J. 2005. Changes in the birth prevalence of selected birth defects after grain fortification with folic acid in the United States: findings from a multi-state population-based study. Birth Defects Res A Clin Mol Teratol 73(10):679-689.

Canfield MA, Honein MA, Yuskiv N, Xing J, Mai CT, Collins JS, Devine O, Petrini J, Ramadhani TA, Hobbs CA, Kirby RS. 2006. National estimates and race/ethnic-specific variation of selected birth defects in the United States, 1999-2001. Birth Defects Res A Clin Mol Teratol 76(11):747-756.

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. 2004. Selecting a Maximally Informative Set of Single-Nucleotide Polymorphisms for Association Analyses Using Linkage Disequilibrium. Am J Hum Genet 74:160-120.

CDC. 1992. Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Recomm Rep 41(RR-14):1-7.

Chadefaux B, Ceballos I, Hamet M, Coude M, Poissonnier M, Kamoun P, Allard D. 1988. Is absence of atheroma in Down syndrome due to decreased homocysteine levels? Lancet 2(8613):741.

Chadefaux B, Rethore MO, Raoul O, Ceballos I, Poissonnier M, Gilgenkranz S, Allard D. 1985. Cystathionine beta synthase: gene dosage effect in trisomy 21. Biochem Biophys Res Commun 128(1):40-44.

Chango A, Emery-Fillon N, de Courcy GP, Lambert D, Pfister M, Rosenblatt DS, Nicolas JP. 2000. A polymorphism (80G->A) in the reduced folate carrier gene and its associations with folate status and homocysteinemia. Mol Genet Metab 70(4):310-315.

Chango A, Fillon-Emery N, Mircher C, Blehaut H, Lambert D, Herbeth B, James SJ, Rethore MO, Nicolas JP. 2005. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr 94(2):166-169.

Cheng EY, Hunt PA, Naluai-Cecchini TA, Fligner CL, Fujimoto VY, Pasternack TL, Schwartz JM, Steinauer JE, Woodruff TJ, Cherry SM, Hansen TA, Vallente RU, Broman KW, Hassold TJ. 2009. Meiotic recombination in human oocytes. PLoS Genet 5(9):e1000661.

Cheung VG, Burdick JT, Hirschmann D, Morley M. 2007. Polymorphic variation in human meiotic recombination. Am J Hum Genet 80(3):526-530.

Chowdhury R, Bois PR, Feingold E, Sherman SL, Cheung VG. 2009. Genetic analysis of variation in human meiotic recombination. PLoS Genet 5(9):e1000648.

Christianson RE, Sherman SL, Torfs CP. 2004. Maternal meiosis II nondisjunction in trisomy 21 is associated with maternal low socioeconomic status. Genet Med 6(6):487-494.

Collins JS, Olson RL, DuPont BR, Wolff DJ, Best RG, Stevenson RE. 2002. Prevalence of aneuploidies in South Carolina in the 1990s. Genet Med 4(3):131-135.

Coop G, Wen X, Ober C, Pritchard JK, Przeworski M. 2008. High-resolution mapping of crossovers reveals extensive variation in fine-scale recombination patterns among humans. Science 319(5868):1395-1398.

Cravo ML, Pinto AG, Chaves P, Cruz JA, Lage P, Nobre Leitao C, Costa Mira F. 1998. Effect of folate supplementation on DNA methylation of rectal mucosa in patients with colonic adenomas: correlation with nutrient intake. Clin Nutr 17(2):45-49.

Cuckle HS. 2005. Primary prevention of Down's syndrome. Int J Med Sci 2(3):93-99.

Czeizel AE, Dudas I. 1992. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 327(26):1832-1835.

Czeizel AE, Puho E. 2005. Maternal use of nutritional supplements during the first month of pregnancy and decreased risk of Down's syndrome: case-control study. Nutrition 21(6):698-704; discussion 774.

Das KC, Herbert V. 1978. The lymphocyte as a marker of past nutritional status: persistence of abnormal lymphocyte deoxyuridine (dU) suppression test and chromosomes in patients with past deficiency of folate and vitamin B12. Br J Haematol 38(2):219-233.

de Vries FA, de Boer E, van den Bosch M, Baarends WM, Ooms M, Yuan L, Liu JG, van Zeeland AA, Heyting C, Pastink A. 2005. Mouse Sycp1 functions in synaptonemal complex assembly, meiotic recombination, and XY body formation. Genes Dev 19(11):1376-1389.

Duesberg P, Rausch C, Rasnick D, Hehlmann R. 1998. Genetic instability of cancer cells is proportional to their degree of aneuploidy. Proc Natl Acad Sci U S A 95(23):13692-13697.

Duthie SJ. 1999. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull 55(3):578-592.

Fenech M, Aitken C, Rinaldi J. 1998. Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. Carcinogenesis 19(7):1163-1171.

Fenech M, Rinaldi J. 1994. The relationship between micronuclei in human lymphocytes and plasma levels of vitamin C, vitamin E, vitamin B12 and folic acid. Carcinogenesis 15(7):1405-1411.

Fisher JM, Harvey JF, Lindenbaum RH, Boyd PA, Jacobs PA. 1993. Molecular studies of trisomy 18. Am J Hum Genet 52(6):1139-1144.

Fledel-Alon A, Wilson DJ, Broman K, Wen X, Ober C, Coop G, Przeworski M. 2009. Broad-scale recombination patterns underlying proper disjunction in humans. PLoS Genet 5(9):e1000658.

Forrester MB, Merz RD. 2004. Occurrence of neural tube defects and Down syndrome among siblings. Hawaii Med J 63(4):127-128.

Fowler BM, Giuliano AR, Piyathilake C, Nour M, Hatch K. 1998. Hypomethylation in cervical tissue: is there a correlation with folate status? Cancer Epidemiol Biomarkers Prev 7(10):901-906.

Freeman SB, Allen EG, Oxford-Wright CL, Tinker SW, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, Royle MH, Torfs CP, Sherman SL. 2007. The National Down Syndrome Project: design and implementation. Public Health Rep 122(1):62-72.

Freeman SB, Taft LF, Dooley KJ, Allran K, Sherman SL, Hassold TJ, Khoury MJ, Saker DM. 1998. Population-based study of congenital heart defects in Down syndrome. Am J Med Genet 80(3):213-217.

Gaulden ME. 1992. Maternal age effect: the enigma of Down syndrome and other trisomic conditions. Mutat Res 296(1-2):69-88.

Gomez D, Solsona E, Guitart M, Baena N, Gabau E, Egozcue J, Caballin M, Mrosovsky N. 2000. Origin of trisomy 21 in Down syndrome cases from a Spanish population registry. Ann Genet 43:23-28.

Grillo LB, Acacio GL, Barini R, Pinto W, Jr., Bertuzzo CS. 2002. [Mutations in the methylene-tetrahydrofolate reductase gene and Down syndrome]. Cad Saude Publica 18(6):1795-1797.

Hall HE, Surti U, Hoffner L, Shirley S, Feingold E, Hassold T. 2007. The origin of trisomy 22: evidence for acrocentric chromosome-specific patterns of nondisjunction. Am J Med Genet A 143A(19):2249-2255.

Hall JG. 2004. Re: Down syndrome and folic acid deficiency. Am J Med Genet A 131(3):327; author reply 328-329.

Handel MA, Schimenti JC. 2010. Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. Nat Rev Genet 11(2):124-136.

Harlap S, Shiono P, Pellegrin F, Golbus M, Bachman R, Mann J, Schmidt L, Lewis JP. 1979. Chromosome abnormalities in oral contraceptive breakthrough pregnancies. Lancet 1(8130):1342-1343.

Hassold T, Chiu D. 1985. Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. Hum Genet 70(1):11-17.

Hassold T, Hall H, Hunt P. 2007. The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet 16 Spec No. 2:R203-208.

Hassold T, Hunt P. 2001. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2(4):280-291.

Hassold T, Judis L, Chan ER, Schwartz S, Seftel A, Lynn A. 2004. Cytological studies of meiotic recombination in human males. Cytogenet Genome Res 107(3-4):249-255.

Hassold T, Merrill M, Adkins K, Freeman S, Sherman S. 1995. Recombination and maternal age-dependent nondisjunction: molecular studies of trisomy 16. Am J Hum Genet 57(4):867-874.

Hassold TJ, Jacobs PA. 1984. Trisomy in man. Annu Rev Genet 18:69-97.

Hassold TJ, Sherman SL, Pettay D, Page DC, Jacobs PA. 1991. XY chromosome nondisjunction in man is associated with diminished recombination in the pseudoautosomal region. Am J Hum Genet 49(2):253-260.

Hawkins JR, Khripin Y, Valdes AM, Weaver TA. 2002. Miniaturized sealed-tube allele-specific PCR. Hum Mutat 19(5):543-553.

Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, Pogribna M, Rozen R, James SJ. 2000. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet 67(3):623-630.

Hodges CA, Revenkova E, Jessberger R, Hassold TJ, Hunt PA. 2005. SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat Genet 37(12):1351-1355.

Hou Z, Matherly LH. 2009. Oligomeric structure of the human reduced folate carrier: identification of homo-oligomers and dominant-negative effects on carrier expression and function. J Biol Chem 284(5):3285-3293.

Hunt PA, Hassold TJ. 2008. Human female meiosis: what makes a good egg go bad? Trends Genet 24(2):86-93.

Ichinohe A, Kanaumi T, Takashima S, Enokido Y, Nagai Y, Kimura H. 2005. Cystathionine beta-synthase is enriched in the brains of Down's patients. Biochem Biophys Res Commun 338(3):1547-1550.

James SJ. 2004a. Maternal metabolic phenotype and risk of Down syndrome: beyond genetics. Am J Med Genet A 127A(1):1-4.

James SJ. 2004b. Response to letter: Down syndrome and folic acid deficiency. Am J Med Genet A 131A:328-329.

James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL, Gaylor DW. 1999. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 70(4):495-501.

Jeffreys CA, Burrage PS, Bickel SE. 2003. A model system for increased meiotic nondisjunction in older oocytes. Curr Biol 13(6):498-503.

Kaufman MH. 1983. Ethanol-induced chromosomal abnormalities at conception. Nature 302(5905):258-260.

Kitajima TS, Yokobayashi S, Yamamoto M, Watanabe Y. 2003. Distinct cohesin complexes organize meiotic chromosome domains. Science 300(5622):1152-1155.

Kline J, Levin B, Stein Z, Warburton D, Hindin R. 1993. Cigarette smoking and trisomy 21 at amniocentesis. Genet Epidemiol 10(1):35-42.

Koehler KE, Hawley RS, Sherman S, Hassold T. 1996. Recombination and nondisjunction in humans and flies. Hum Mol Genet 5 Spec No:1495-1504.

Kong A, Gudbjartsson DF, Sainz J, Jonsdottir GM, Gudjonsson SA, Richardsson B, Sigurdardottir S, Barnard J, Hallbeck B, Masson G, Shlien A, Palsson ST, Frigge ML, Thorgeirsson TE, Gulcher JR, Stefansson K. 2002. A high-resolution recombination map of the human genome. Nat Genet 31(3):241-247.

Kong A, Thorleifsson G, Stefansson H, Masson G, Helgason A, Gudbjartsson DF, Jonsdottir GM, Gudjonsson SA, Sverrisson S, Thorlacius T, Jonasdottir A, Hardarson GA, Palsson ST, Frigge ML, Gulcher JR, Thorsteinsdottir U, Stefansson K. 2008. Sequence variants in the RNF212 gene associate with genome-wide recombination rate. Science 319(5868):1398-1401.

Krawchuk MD, Wahls WP. 1999. Centromere mapping functions for aneuploid meiotic products: Analysis of rec8, rec10 and rec11 mutants of the fission yeast Schizosaccharomyces pombe. Genetics 153(1):49-55.

Kwee LC, Liu D, Lin X, Ghosh D, Epstein MP. 2008. A powerful and flexible multilocus association test for quantitative traits. Am J Hum Genet 82(2):386-397.

Lamb NE, Feingold E, Savage A, Avramopoulos D, Freeman S, Gu Y, Hallberg A, Hersey J, Karadima G, Pettay D, Saker D, Shen J, Taft L, Mikkelsen M, Petersen MB, Hassold T, Sherman SL. 1997. Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum Mol Genet 6(9):1391-1399.

Lamb NE, Freeman SB, Savage-Austin A, Pettay D, Taft L, Hersey J, Gu Y, Shen J, Saker D, May KM, Avramopoulos D, Petersen MB, Hallberg A, Mikkelsen M, Hassold TJ, Sherman SL. 1996. Susceptible chiasmate configurations of chromosome 21 predispose to non-disjunction in both maternal meiosis I and meiosis II. Nat Genet 14(4):400-405.

Lamb NE, Sherman SL, Hassold TJ. 2005a. Effect of meiotic recombination on the production of aneuploid gametes in humans. Cytogenet Genome Res 111(3-4):250-255.

Lamb NE, Yu K, Shaffer J, Feingold E, Sherman SL. 2005b. Association between maternal age and meiotic recombination for trisomy 21. Am J Hum Genet 76(1):91-99.

Lin Y, Tseng GC, Cheong SY, Bean LJ, Sherman SL, Feingold E. 2008. Smarter clustering methods for SNP genotype calling. Bioinformatics 24(23):2665-2671.

Liu D, Ghosh D, Lin X. 2008. Estimation and testing for the effect of a genetic pathway on a disease outcome using logistic kernel machine regression via logistic mixed models. BMC Bioinformatics 9:292.

Liu L, Keefe DL. 2008. Defective cohesin is associated with age-dependent misaligned chromosomes in oocytes. Reprod Biomed Online 16(1):103-112.

Lynn A, Koehler KE, Judis L, Chan ER, Cherry JP, Schwartz S, Seftel A, Hunt PA, Hassold TJ. 2002. Covariation of synaptonemal complex length and mammalian meiotic exchange rates. Science 296(5576):2222-2225.

Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S, Munson ML. 2007. Births: final data for 2005. Natl Vital Stat Rep 56(6):1-103.

Martinez-Frias ML, Rodriguez L, Lopez-Grondona F, Bermejo E, Rodriguez-Pinilla E, Frias JL. 2004. Frequency of neural tube defects and Down syndrome in the same sibship: analysis of the Spanish ongoing case-control study. Am J Med Genet A 126A(4):430-431.

Matzke MA, Mette MF, Kanno T, Matzke AJ. 2003. Does the intrinsic instability of aneuploid genomes have a causal role in cancer? Trends Genet 19(5):253-256.

McDougall A, Elliott DJ, Hunter N. 2005. Pairing, connecting, exchanging, pausing and pulling chromosomes. EMBO Rep 6(2):120-125.

Mikkelsen M, Hallberg A, Poulsen H, Frantzen M, Hansen J, Petersen M. 1995. Epidemiology study of Down's syndrome in Denmark, including family studies of chromosomes and DNA markers. Develop Brain Dysfunct 8:4-12.

Miyamoto T, Hasuike S, Yogev L, Maduro MR, Ishikawa M, Westphal H, Lamb DJ. 2003. Azoospermia in patients heterozygous for a mutation in SYCP3. Lancet 362(9397):1714-1719.

Molnar M, Bahler J, Sipiczki M, Kohli J. 1995. The rec8 gene of Schizosaccharomyces pombe is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. Genetics 141(1):61-73.

Moore DP, Miyazaki WY, Tomkiel JE, Orr-Weaver TL. 1994. Double or nothing: a Drosophila mutation affecting meiotic chromosome segregation in both females and males. Genetics 136(3):953-964.

Morton NE, Jacobs PA, Hassold T, Wu D. 1988. Maternal age in trisomy. Ann Hum Genet 52(Pt 3):227-235.

Myakishev MV, Khripin Y, Hu S, Hamer DH. 2001. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res 11(1):163-169.

Myers S, Bowden R, Tumian A, Bontrop RE, Freeman C, MacFie TS, McVean G, Donnelly P. 2010. Drive against hotspot motifs in primates implicates the PRDM9 gene in meiotic recombination. Science 327(5967):876-879.

Narayan A, Ji W, Zhang XY, Marrogi A, Graff JR, Baylin SB, Ehrlich M. 1998. Hypomethylation of pericentromeric DNA in breast adenocarcinomas. Int J Cancer 77(6):833-838.

Nicolaidis P, Petersen MB. 1998. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod 13(2):313-319.

O'Leary VB, Parle-McDermott A, Molloy AM, Kirke PN, Johnson Z, Conley M, Scott JM, Mills JL. 2002. MTRR and MTHFR polymorphism: link to Down syndrome? Am J Med Genet 107(2):151-155.

Oliver TR, Bhise A, Feingold E, Tinker S, Masse N, Sherman SL. 2009. Investigation of factors associated with paternal nondisjunction of chromosome 21. Am J Med Genet A 149A(8):1685-1690.

Oliver TR, Feingold E, Yu K, Cheung V, Tinker S, Yadav-Shah M, Masse N, Sherman SL. 2008. New insights into human nondisjunction of chromosome 21 in oocytes. PLoS Genet 4(3):e1000033.

Olsen JH, Winther JF. 2003. Down's syndrome and neural tube defects in the same families. Lancet 361(9366):1316.

Parvanov ED, Petkov PM, Paigen K. 2010. Prdm9 controls activation of mammalian recombination hotspots. Science 327(5967):835.

Pasierbek P, Jantsch M, Melcher M, Schleiffer A, Schweizer D, Loidl J. 2001. A Caenorhabditis elegans cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev 15(11):1349-1360.

Pogribna M, Melnyk S, Pogribny I, Chango A, Yi P, James SJ. 2001. Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet 69(1):88-95.

Pufulete M, Al-Ghnaniem R, Leather AJ, Appleby P, Gout S, Terry C, Emery PW, Sanders TA. 2003. Folate status, genomic DNA hypomethylation, and risk of colorectal adenoma and cancer: a case control study. Gastroenterology 124(5):1240-1248.

Rai AK, Singh S, Mehta S, Kumar A, Pandey LK, Raman R. 2006. MTHFR C677T and A1298C polymorphisms are risk factors for Down's syndrome in Indian mothers. J Hum Genet 51(4):278-283.

Rajagopalan H, Lengauer C. 2004. Aneuploidy and cancer. Nature 432(7015):338-341.

Rana SR, Colman N, Goh KO, Herbert V, Klemperer MR. 1983. Transcobalamin II deficiency associated with unusual bone marrow findings and chromosomal abnormalities. Am J Hematol 14(1):89-96.

Rasooly RS, New CM, Zhang P, Hawley RS, Baker BS. 1991. The lethal(1)TW-6cs mutation of Drosophila melanogaster is a dominant antimorphic allele of nod and is associated with a single base change in the putative ATP-binding domain. Genetics 129(2):409-422.

Ray JG, Meier C, Vermeulen MJ, Cole DE, Wyatt PR. 2003. Prevalence of trisomy 21 following folic acid food fortification. Am J Med Genet A 120A(3):309-313.

Risch N, Stein Z, Kline J, Warburton D. 1986. The relationship between maternal age and chromosome size in autosomal trisomy. Am J Hum Genet 39(1):68-78.

Robinson WP, Kuchinka BD, Bernasconi F, Petersen MB, Schulze A, Brondum-Nielsen K, Christian SL, Ledbetter DH, Schinzel AA, Horsthemke B, Schuffenhauer S, Michaelis RC, Langlois S, Hassold TJ. 1998. Maternal meiosis I non-disjunction of chromosome 15: dependence of the maternal age effect on level of recombination. Hum Mol Genet 7(6):1011-1019.

Ross LO, Maxfield R, Dawson D. 1996. Exchanges are not equally able to enhance meiotic chromosome segregation in yeast. Proc Natl Acad Sci U S A 93(10):4979-4983.

Rothman K. 1983. Spermicide use and Down's syndrome. Am J Public Health 72:399-401.

Savage AR, Petersen MB, Pettay D, Taft L, Allran K, Freeman SB, Karadima G, Avramopoulos D, Torfs C, Mikkelsen M, Hassold TJ, Sherman SL. 1998. Elucidating the mechanisms of paternal non-disjunction of chromosome 21 in humans. Hum Mol Genet 7(8):1221-1227.

Scala I, Granese B, Sellitto M, Salome S, Sammartino A, Pepe A, Mastroiacovo P, Sebastio G, Andria G. 2006. Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet Med 8(7):409-416.

Sears DD, Hegemann JH, Shero JH, Hieter P. 1995. Cis-acting determinants affecting centromere function, sister-chromatid cohesion and reciprocal recombination during meiosis in Saccharomyces cerevisiae. Genetics 139(3):1159-1173.

SeattleSNPs. May, 2005a. NHLBI Program for Genomic Applications, SeattleSNPs. Seattle, WA.

SeattleSNPs. May, 2005b. NHLBI Program for Genomic Applications, SeattleSNPs, Seattle, WA.

Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. 2001. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 29(1):308-311.

Simmons CJ, Mosley BS, Fulton-Bond CA, Hobbs CA. 2004. Birth defects in Arkansas: is folic acid fortification making a difference? Birth Defects Res A Clin Mol Teratol 70(9):559-564.

Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, Baker A, Jonasdottir A, Ingason A, Gudnadottir VG, Desnica N, Hicks A, Gylfason A, Gudbjartsson DF, Jonsdottir GM, Sainz J, Agnarsson K, Birgisdottir B, Ghosh S, Olafsdottir A, Cazier JB, Kristjansson K, Frigge ML, Thorgeirsson TE, Gulcher JR, Kong A, Stefansson K. 2005. A common inversion under selection in Europeans. Nat Genet 37(2):129-137.

Strobino B, Kline J, Lai A, Stein Z, Susser M, Warburton D. 1986. Vaginal spermicides and spontaneous abortion of known karyotype. Am J Epidemiol 123(3):431-443.

Sun F, Mikhaail-Philips M, Oliver-Bonet M, Ko E, Rademaker A, Turek P, Martin RH. 2008. Reduced meiotic recombination on the XY bivalent is correlated with an increased incidence of sex chromosome aneuploidy in men with non-obstructive azoospermia. Mol Hum Reprod 14(7):399-404.

Takamura N, Kondoh T, Ohgi S, Arisawa K, Mine M, Yamashita S, Aoyagi K. 2004. Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan. Eur J Nutr 43(5):285-287.

Taparia S, Gelineau-van Waes J, Rosenquist TH, Finnell RH. 2007. Importance of folate-homocysteine homeostasis during early embryonic development. Clin Chem Lab Med 45(12):1717-1727.

Tease C, Hulten MA. 2004. Inter-sex variation in synaptonemal complex lengths largely determine the different recombination rates in male and female germ cells. Cytogenet Genome Res 107(3-4):208-215.

Thomas NS, Ennis S, Sharp AJ, Durkie M, Hassold TJ, Collins AR, Jacobs PA. 2001. Maternal sex chromosome non-disjunction: evidence for X chromosome-specific risk factors. Hum Mol Genet 10(3):243-250.

Torfs CP, Christianson RE. 1998. Anomalies in Down syndrome individuals in a large population-based registry. Am J Med Genet 77(5):431-438.

Torfs CP, Christianson RE. 2000. Effect of maternal smoking and coffee consumption on the risk of having a recognized Down syndrome pregnancy. Am J Epidemiol 152(12):1185-1191.

Uchida IA. 1979. Radiation-induced nondisjunction. Environ Health Perspect 31:13-17.

Van den Veyver IB. 2002. Genetic effects of methylation diets. Annu Rev Nutr 22:255-282.

Warren AC, Chakravarti A, Wong C, Slaugenhaupt SA, Halloran SL, Watkins PC, Metaxotou C, Antonarakis SE. 1987. Evidence for reduced recombination on the nondisjoined chromosomes 21 in Down syndrome. Science 237(4815):652-654.

Xu H, Beasley MD, Warren WD, van der Horst GT, McKay MJ. 2005. Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell 8(6):949-961.

Yang F, De La Fuente R, Leu NA, Baumann C, McLaughlin KJ, Wang PJ. 2006. Mouse SYCP2 is required for synaptonemal complex assembly and chromosomal synapsis during male meiosis. J Cell Biol 173(4):497-507.

Yang F, Wang PJ. 2009. The Mammalian synaptonemal complex: a scaffold and beyond. Genome Dyn 5:69-80.

Yang Q, Sherman SL, Hassold TJ, Allran K, Taft L, Pettay D, Khoury MJ, Erickson JD, Freeman SB. 1999. Risk factors for trisomy 21: maternal cigarette smoking and oral contraceptive use in a population-based case-control study. Genet Med 1(3):80-88.

Yang QH, Carter HK, Mulinare J, Berry RJ, Friedman JM, Erickson JD. 2007. Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001-2002. Am J Clin Nutr 85(5):1409-1416.

Yoon PW, Freeman SB, Sherman SL, Taft LF, Gu Y, Pettay D, Flanders WD, Khoury MJ, Hassold TJ. 1996. Advanced maternal age and the risk of Down syndrome characterized by the meiotic stage of chromosomal error: a population-based study. Am J Hum Genet 58(3):628-633.

Yuan L, Liu JG, Hoja MR, Wilbertz J, Nordqvist K, Hoog C. 2002. Female germ cell aneuploidy and embryo death in mice lacking the meiosis-specific protein SCP3. Science 296(5570):1115-1118.

Yuan L, Liu JG, Zhao J, Brundell E, Daneholt B, Hoog C. 2000. The murine SCP3 gene is required for synaptonemal complex assembly, chromosome synapsis, and male fertility. Mol Cell 5(1):73-83.

Zetka MC, Rose AM. 1995. Mutant rec-1 eliminates the meiotic pattern of crossing over in Caenorhabditis elegans. Genetics 141(4):1339-1349.

Zwick ME, Salstrom JL, Langley CH. 1999. Genetic variation in rates of nondisjunction: association of two naturally occurring polymorphisms in the chromokinesin nod with increased rates of nondisjunction in Drosophila melanogaster. Genetics 152(4):1605-1614.

APPENDIX

TABLE A.1. Association of lack of folic acid supplementation with the birth of an infant with Down syndrome due to a maternal meiotic error compared to other nondisjoining errors: a test for recall bias using logistic regression.

	Cases		**Internal Controls**		**Adjusted OR[a]**	**p[b]**
	n	% of cases lacking folic acid	**n**	% of controls lacking folic acid		
All maternal meiotic						
All	**662**	68.3%	**48**	70.8%	1.10 (0.55, 2.22)	0.39
Young (<35 years old)	324	70.7%	40	77.5%	0.72 (0.31, 1.65)	0.22
Old (≥35 years old)	338	66.0%	8	37.5%	3.94 (0.84, 18.47)	0.04
MI						
All	**492**	66.9%	**218**	72.0%	0.78 (0.54, 1.13)	0.10
Young (<35 years old)	257	71.2%	107	72.0%	1.14 (0.66, 1.98)	0.32
Old (≥35 years old)	235	62.1%	111	72.1%	0.61 (0.36, 1.02)	0.03
MII						
All	**170**	72.4%	**540**	67.2%	1.35 (0.90, 2.02)	0.08
Young (<35 years old)	67	68.7%	297	72.1%	0.67 (0.36, 1.27)	0.11
Old (≥35 years old)	103	74.8%	243	61.3%	2.00 (1.17, 3.44)	0.005

OR, odds ratios; CI, confidence interval.

[a]Adjusted for maternal age as a continuous variable and race/ethnicity.

[b]one-sided p-values

TABLE A.2. Selection of SNPs for genotyping recombination associated genomic regions.

rs_int	Chromosome	Chromosome position	Nearest gene	SNP
rs1466216	**4**	**1061907**	**RNF212**	**CT**
rs13147452	**4**	**1068124**	**RNF212**	**AG**
rs1670533	**4**	**1068187**	**RNF212**	**CT**
rs17164229	**4**	**1068596**	**RNF212**	**CT**
rs17164235	**4**	**1074061**	**RNF212**	**CT**
rs11939380	**4**	**1076871**	**RNF212**	**CT**
rs608066	4	1077066	RNF212	AG
rs615381	4	1077531	RNF212	AG
rs614945	4	1077617	RNF212	GT
rs4045481	**4**	**1080625**	**RNF212**	**AG**
rs3796619	4	1085281	RNF212	CT
rs17729286	4	1091148	RNF212	AC
rs6840347	4	1095680	RNF212	CT
rs4764651	12	100647352	CHPT1, SYCP3	AC
rs2288379	12	100648050	SYCP3	AC
rs12296641	**12**	**100648897**	**SYCP3**	**AG**
rs11110986	12	100649221	SYCP3	AG
rs10860779	**12**	**100651393**	**SYCP3**	**AC**
rs7976805	12	100652608	SYCP3	AG
rs10778146	12	100654307	SYCP3	GT
rs10431453	12	100657522	SYCP3	AG
rs17426195	17	41188138	CRHR1	AG
rs4074461	17	41210922	CRHR1	GT
rs4640231	**17**	**41268567**	**CRHR1**	**GC**
rs8064870	17	41282569	LOC100128977	CT
rs1800547	**17**	**41407682**	**MAPT**	**AG**
rs1078268	**17**	**41431738**	**MAPT, STH**	**CT**
rs9468	**17**	**41457408**	**MAPT**	**CT**
rs17660907	**17**	**41546868**	**KIAA1267**	**AG**
rs12150320	**17**	**41568981**	**KIAA1267**	**CT**
rs2668695	17	41647903	KIAA1267	AG
rs2732706	**17**	**41707463**	**LRRC37A**	**AG**
rs2732705	**17**	**41707706**	**LRRC37A**	**AC**
rs2668622	17	41707908	LRRC37A	GT

SNPs selected for genotyping are listed. Actual SNPs genotyped and analyzed are in bold. Sample population minor alleles are underlined.