Failure to recombine is a common feature of human oogenesis

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Summary

Failure of homologous chromosomes to recombine is arguably the most important cause of human meiotic nondisjunction, having been linked to numerous autosomal and sex chromosome trisomies of maternal origin. However, almost all information on these "exchangeless" homologs has come from genetic mapping studies of trisomic conceptuses, so the incidence of this defect and its impact on gametogenesis are not clear. If oocytes containing exchangeless homologs are selected against during meiosis, the incidence may be much higher in developing germ cells than in zygotes. To address this, we initiated studies of exchangeless chromosomes in fetal ovarian samples from elective terminations of pregnancy. In total, we examined more than 7,000 oocytes from 160 tissue samples, scoring for the number of foci per cell of the crossover-associated protein MLH1. We identified a surprisingly high level of recombination failure, with more than 7% of oocytes containing at least one chromosome pair that lacked an MLH1 focus. Detailed analyses indicate striking chromosome-specific differences, with a preponderance of MLH1-less homologs involving chromosomes 21 or 22. Further, the effect was linked to the overall level of recombination in the cell, with the presence of one or two exchangeless chromosomes in a cell associated with a 10%–20% reduction in the total number of crossovers. This suggests individuals with lower rates of meiotic recombination are at an increased risk of producing aneuploid offspring.

Introduction

Studies conducted in the 1990s and 2000s demonstrated an important link between abnormalities in meiotic recombination and human trisomies of maternal origin (for review, see Nagaoka et al.[1]). Two general categories of recombination abnormality were identified. First, in a proportion of cases of trisomies 16 and 21 and sex chromosome trisomies, the placement of crossovers was altered, with exchanges appearing either too close to, or too far from, the centromere.[2–4] Second, and more importantly, failure of recombination between the nondisjoined chromosomes appeared to account for nearly 50% of cases of trisomy 21 and sex chromosome trisomies, and 20%–25% of other trisomies examined.[1,5–9]

Most of the information implicating "exchangeless" (E0) chromosomes as major contributors to human aneuploidy has come from genetic mapping analyses of trisomic pregnancies. Because these studies are limited to the subset of abnormal pregnancies that survive to clinical recognition, this approach has allowed us to identify the problem but does not provide insight to its magnitude. The availability of cytological approaches to visualize crossover-associated proteins, however, has made it possible to analyze recombination directly in meiocytes. In preliminary studies, we used this approach to determine the level of recombination failure for 10 individual chromosomes in human spermatocytes and oocytes.[10–12] We observed a clear sex-specific difference, with an approximate 10-fold increase in the incidence of E0 chromosomes in oocytes by comparison with spermatocytes.[11] Experimental studies in mice provide compelling evidence that the fate of cells with unpartnered chromosomes at the first meiotic division is also sex-specific: such cells are eliminated by the actions of a robust cell cycle checkpoint in males (reviewed in Nagaoka et al.[1] and Cloutier et al.[13]) but are tolerated in the female and increase the frequency of chromosomally abnormal eggs and embryos. Thus, data to date suggest that recombination failure is a major mechanism of meiotic errors in the human female and a leading cause of aneuploidy.[14]

Our previous analyses were based on the study of a small number of individuals and the assessment of only 10 of the 22 autosomes. We undertook the current large population-based analysis to address important questions about the genesis of E0 chromosomes in the fetal ovary. Specifically, we asked whether there are among-individual differences in the incidence of recombination failure (e.g., due to allelic variation in recombination genes such as *MSH4*, *SYCP3*, or *RNF212*[15,16]); whether the proportion of E0 chromosomes is influenced by gestational age (in accordance with the "production line" hypothesis of human nondisjunction, a model suggesting that the last fetal oocytes to enter meiosis have fewer crossovers and consequently are more likely to non-disjoin[17]); or whether the incidence of E0s is influenced by maternal age (e.g., due to subtle changes in the fetal environment). We identified a surprisingly high level of recombination failure, with nearly 10% of all oocytes containing at least one

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Table 1. Study population	
Category	
No. cases	160
No. fetal oocytes	7,396
Maternal age range (years)	18-42
Maternal age (mean ± SD)	26.1 ± 5.5
Gestational age range (weeks)	15.2-24.0
Gestational age (mean ± SD)	20.4 ± 2.1
Genome-wide MLH1 foci (range)	31-132
Genome-wide MLH1 foci (mean ± SD)	67.3 ± 13.5

exchangeless chromosome pair. Consistent with data from human trisomies,[1,3,5–9] the smallest autosomes (i.e., chromosomes 21 and 22) were most likely to exhibit recombination failure. Thus, our observations suggest that, right from the beginning of oogenesis, a high proportion of human oocytes are "set up" to mal-segregate during the first meiotic division.

Material and methods

Ethics statement

This study was conducted according to the principles expressed in the Declaration of Helsinki. All procedures were reviewed and approved by the University of California, San Francisco, University of Washington, or Washington State University Institutional Review Boards, and written informed consent was obtained from all study participants.

Sample population

The human sample population consisted of 160 fetal ovaries obtained from elective terminations of pregnancy performed at the San Francisco General Hospital Woman's Options Center in San Francisco, California or at clinical facilities associated with the Birth Defects Research Laboratory in Seattle, Washington. The gestational ages of the samples ranged from 15 to 24 weeks, and the maternal ages from 18 to 42 years (see Table 1 for a summary of the study population and Table S1 for detailed information on the samples).

Tissue preparation and immunostaining

The material collected from fetal ovarian samples was processed and immunostained as described previously.[11] Briefly, antibodies were diluted in sterile filtered 1× ADB consisting of 10 mL normal donkey serum (Jackson ImmunoResearch), 3 g BSA (Sigma-Aldrich), 50 μL Triton X-100 (Alfa Aesar), and 990 mL PBS. Incubations were performed in a dark humid chamber at 37°C. Slides were first blocked in 1× ADB for 1 h and incubated overnight in a 37°C humid chamber with CREST (Fisher; human CREST antiserum; 1:500) and MLH1 (BD PharMingen; mouse anti-human; 1:75) antibodies. SYCP3 (Novus; rabbit anti-human; 1:150) was added the next morning and slides incubated for 2 h, followed by 30 and 60 min washes in 1× ADB. Secondary antibodies (Jackson ImmunoResearch) FDAM (fluorescein anti-mouse; 1:75) and ADAH (AMCA anti-human; 1:100) were added for overnight incubation in a 37°C humid chamber, followed the next day by a 45 min incubation with RDAR (rhodamine anti-rabbit; 1:100) and two final washes in 1× PBS for 30 min and 60 min. Slides were fixed with Prolong Gold Antifade reagent (Invitrogen), sealed with rubber cement, and stored at 4°C until analysis.

Cytological analysis of MLH1 foci

MLH1 focus counts were obtained by analyzing pachytene-stage cells as described in Gruhn et al.[11] For each sample, we attempted to score approximately 50 cells, restricting our analysis to cells in which it was possible to unambiguously visualize 23 mature synaptonemal complexes. Exchangeless (E0) homologs were defined as those chromosome pairs in which no observable MLH1 focus was present on a synaptonemal complex. In accordance with human cytogenetic nomenclature recommendations for nonbanded preparations (e.g., McGowan-Jordan et al.[18]), E0 homologs were assigned to one of seven chromosome groups (A, B, C, D, E, F, or G) depending on overall size of the chromosome (as determined by the length of the SC signal) and the location of the centromere (as determined by the chromosomal location of the CREST signal). "A" group chromosomes consist of chromosomes 1–3, "B" group 4–5, "C" group 6–12 and the X, "D" group 13–15, "E" group 16–18, "F" group 19–20, and "G" group 21–22.

The 160 samples were scored over two different time periods. An initial series of 5,883 oocytes from 122 fetal ovarian samples was scored in 2015; preliminary data on the effect of gestational and maternal age on recombination were presented for 60 of these cases,[12] but the data on E0 chromosomes is presented here for the first time. The second series of 1,513 oocytes from 38 samples was scored in 2017–2019. There were no important differences between the two series in the MLH1 scores, the gestational ages, and the maternal ages, but the two groups differed in the overall number of E0-containing cells, with the 2015 group having more E0s (8.3% versus 4.4%) (Figure S1). For each series, cells were scored by at least two independent observers and E0s were confirmed by 1–3 other observers.

Cytological analysis of synaptonemal complex (SC) length

For measurements of synaptonemal complex length, we selected 31 samples with varying levels of E0 cells; for each sample, we scored a minimum of 10 pachytene cells, with 25 cells being scored in most instances. MicroMeasure 3.3 was used to measure synaptonemal complex lengths (taken as the length of the SYCP3 signal) of the 23 bivalents, and the values totaled to yield the genome-wide SC length for each cell.

Results

Exchangeless chromosomes are abundant in human female oocytes

In previous analyses, we combined fluorescence *in situ* hybridization (FISH) with immunofluorescence to examine chromosome-specific levels of recombination failure in human meiocytes.[11,12] The focus of the analysis was on recombination patterns on individual chromosomes and, by analyzing results of a relatively small number of pachytene stage spermatocytes and oocytes, we obtained data on sex-specific differences. In the present study, we

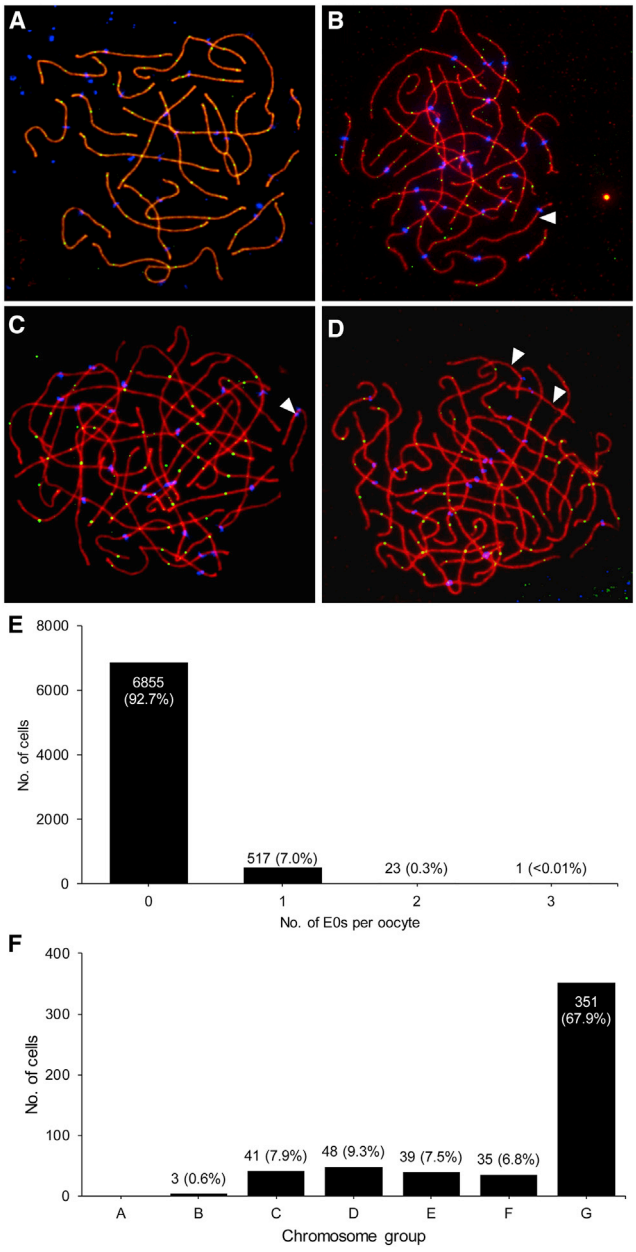

Figure 1. Exchangeless homologs

(A–D) Representative images of pachytene oocytes with (A) no exchangeless homologs, (B) an exchangeless D group homolog, (C) an exchangeless E group homolog, and (D) two exchangeless homologs. For these images, the synaptonemal complex protein SYCP3 is in red, centromere-associated CREST is in blue, and the crossover-associated protein MLH1 is in green.

(E) Summary of results on the total number of cells with 0, 1, 2, or 3 exchangeless homologs.

(F) Distribution of exchangeless homologs by chromosome group.

extend our analysis of human female meiosis to a large population. Using immunofluorescence staining with CREST to identify centromeres and considering both the length of individual synaptonemal complexes (SCs) and the relative ratio of short and long arms, we were able to classify all homologs into the seven human chromosome groups (i.e., A, B, C, D, E, F, and G group chromosomes). Using antibodies to the synaptonemal complex protein SYCP3 and the crossover-associated protein MLH1, we evaluated the total number of MLH1 foci. Because MLH1-mediated repair accounts for ~90% of meiotic recombination events,[19] this allowed us to measure the number of crossovers per cell, evaluate their placement on individual chromosomes in pachytene-stage oocytes, and identify cells with one or more chromosomes that were lacking MLH1 foci; i.e., E0 chromosomes. Representative images of cells with "normal" crossover complements or with one or two E0 chromosomes are provided in Figures 1A–1D.

In total we examined 7,396 oocytes from 160 fetal ovarian samples and, for all scorable cells, found an overall mean number of MLH1 foci per cell of 67.3 ± 13.5 (Table 1); these results are virtually identical to those from our previous analyses of fetal ovarian samples (e.g., Gruhn et al.[11] and Rowsey et al.[12]). We found 541 (7.3%) cells containing E0 homologs; 517 contained a single E0 chromosome pair, 23 cells contained two, and 1 cell contained three E0s (Figure 1E). The vast majority of E0s involved the smallest chromosomes, 21 or 22; i.e., G group chromosomes accounted for 351 of the 517 cells (67.9%) with a single E0 homolog. Cells with exchangeless C, D, E, or F group chromosomes each accounted for 7%–9% of the total, but only 3 exchangeless B group chromosomes were identified and A group E0s were never observed (Figure 1F).

Chromosomes 21 and 22 are unique among autosomes because their female genetic maps are under 100 cM (e.g., Matise et al.[20]), meaning these chromosomes typically are joined by only one or two crossovers in human oocytes.[14,21] By comparison, 5–6 crossovers are typical for the largest human chromosomes and 3–4 are common on mid-sized chromosomes.[11] Thus, it is not surprising that G group chromosomes were the most likely to be exchangeless. However, there is also a possible technical explanation for the high proportion of E0s among G group chromosomes: if sub-optimal immunostaining caused some MLH1 foci to be missed, the chromosomes most likely to be erroneously scored as E0s would be those with the fewest crossovers, G group chromosomes. To examine this possibility, we conducted two analyses to assess potential correlations between sample preparation quality and the occurrence of exchangeless chromosomes. We asked whether the proportion of E0 chromosomes was correlated (1) with the total scorable cells for individual subjects and (2) with the proportion of cells that proved scorable among those captured for analysis. For both measurements a subtle increase in the proportion of E0s was evident in subjects with a moderate number of scored oocytes (Figures S2A and S2B). However, there was no evidence for a simple, monotonic increase in E0s with increasing oocytes scored, as would be expected if sample preparation were an important factor. Further, there was no evidence for a significant correlation between the proportion of E0 chromosomes and either the number of

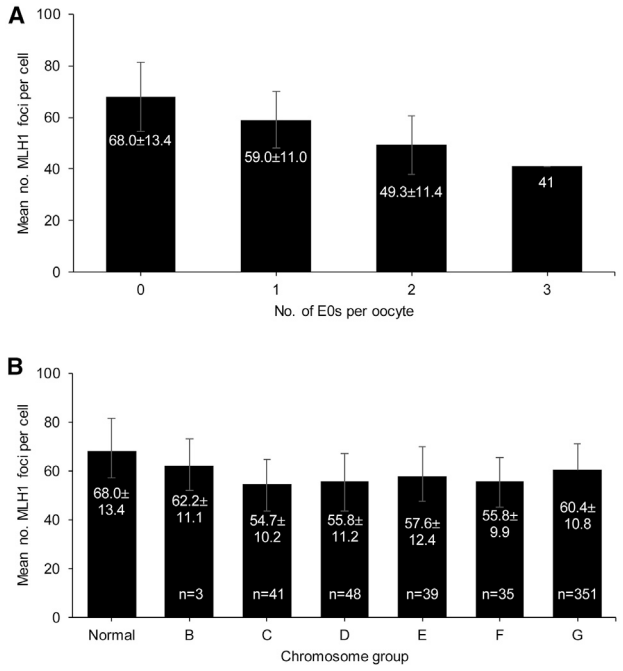

Figure 2. Meiotic recombination and exchangeless chromosomes

Mean MLH1 foci (±SD) per cell (A) for cells with 0, 1, 2, or 3 exchangeless chromosomes and (B) by chromosome group for cells containing a single E0 chromosome.

scorable cells (correlation = −0.04; p = 0.61) or the percentage of scorable cells (correlation = 0.13; p = 0.11). Thus, we conclude that our observations accurately reflect the *in vivo* situation, indicating a remarkably high level of exchangeless chromosomes in human female meiosis.

While the fate of oocytes containing these exchangeless chromosomes is uncertain, two lines of evidence suggest that they are frequently capable of transiting meiosis and being fertilized. First, previous studies of meiosis in mice suggest that pachytene checkpoint control mechanisms are "relaxed" in mammalian females and that, consequently, oocytes with synaptic defects are frequently able to progress past the first meiotic division.[1] Second, a comparison of the frequency of human oocytes containing exchangeless G group chromosomes with the incidence of trisomies 21 and 22 in clinically recognized pregnancies suggests that many of these oocytes can give rise to clinical pregnancies. Specifically, assuming that approximately one-half of E0-containing oocytes yield disomic gametes and that most involve an extra G group chromosome (Figure 2B), we might expect that approximately 2%–3% of fertilized human eggs are trisomic for chromosomes 21 or 22. Indeed, approximately 1%–2% of all clinically recognized pregnancies are thought to be trisomic for chromosomes 21 or 22,[22] with perhaps 25%–50% of these cases originating from E0-containing oocytes.[7,23] Thus, while we cannot know whether some E0-containing oocytes are eliminated during meiosis, it is clear that many survive and are fertilized.

As recombination levels decrease, exchangeless chromosomes increase

Conceptually, there are at least two ways exchangeless chromosomes could arise: i.e., as a result of an error in formation of crossovers on an individual chromosome, or as a consequence of a genome-wide reduction in recombination in some cells. In the first instance we would expect that, with the exception of the E0 chromosome itself, the number and distribution of MLH1 foci would be identical between cells with and without E0 chromosomes. On a practical level, this would mean differences of approximately 3–6 MLH1 foci between normal cells and cells with a "large" E0 chromosome (e.g., involving A, B, or C group chromosomes) and a 1–2 focus difference between normal cells and cells with a "small" E0 chromosome (e.g., a G group chromosome). In contrast, if E0 chromosomes simply represent the extreme ends of the crossover distribution, we would anticipate a more substantial difference in MLH1 values between cells with and without E0 chromosomes.

From Figure 2A it is clear that the latter situation applies. A large, nine focus difference in mean MLH1 values is evident between normal cells and cells with a single E0 chromosome ($t = 17.7$; $p < 0.0001$), and an additional ten focus difference between cells containing one and two E0 chromosomes ($t = 4.0$; $p < 0.0001$). An examination of MLH1 values in cells with different categories of E0 chromosomes supports the conclusion that cells with E0s represent a population of cells at the low end of the recombination distribution for an individual. The mean number of MLH1 foci in cells with a single G group E0 chromosome was 60.4 ± 10.8, approximately eight foci lower than the 68.0 ± 13.4 value for normal cells ($t = 12.7$; $p < 0.001$) (Figure 2B). Thus, the differential between normal cells and G group E0 chromosome-containing cells was far in excess of the 1–2 exchanges that normally occur on chromosomes 21 or 22, and similarly large differentials were also observed for cells with single C, D, E, and F group E0 chromosomes (Figure 2B).

Synaptonemal complex (SC) length has little impact on the likelihood of exchangeless chromosomes

Because recombination levels have been linked to SC length in a variety of organisms, including humans and mice,[11,24,25] we examined the relationship between SC length and the occurrence of E0 chromosomes. For this analysis, we selected a subset of 31 of the 160 fetuses, chosen to include cases with varying levels of cells with E0 chromosomes. For each case, we measured the genome-wide SC lengths (i.e., the combined lengths of the 23 SCs) for at least 10 cells, with 20 or more cells scored for most cases.

In initial analyses, we were simply interested in confirming previous reports that have linked SC length with recombination levels. As expected, there was a positive correlation between SC length and the number of MLH1 foci in almost all samples. In 19 of the 31 samples the

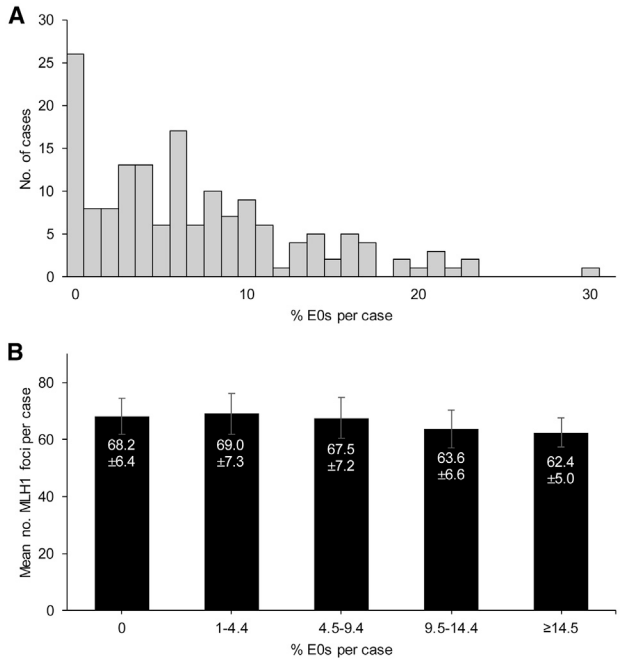

Figure 3. Individual variation in E0 chromosomes
Proportion of cells with E0 chromosomes among 160 fetal ovarian samples (A) and mean MLH1 foci (±SD) per cell for subjects with differing levels of E0-containing cells (B); samples were divided into five groups of approximately equal size.

association was statistically significant, and the failure to find a significant effect in the remaining cases was likely due to the small number of cells that had been analyzed for them (i.e., on average, we scored 20.8 cells for these cases versus 26.6 cells for samples exhibiting a significant correlation; $t = 1.83$; $p = 0.04$). We are presently expanding our sample size and the number of cells per case to further characterize the association between SC length and MLH1 foci in human oocytes.

Subsequently, we asked whether there was a correlation between SC length and the occurrence of E0 chromosomes. Somewhat surprisingly, and in contrast to the negative correlation between mean MLH1 values and the proportion of E0 chromosomes, there was no obvious relationship between mean genome-wide SC values and the level of E0-containing cells per case (Figure S3). Thus, individuals with "shorter" SCs did not appear to have a higher risk of E0 chromosomes. Further, within individual cases there was no obvious difference in SC length between cells containing E0 chromosomes and those with MLH1 foci present on all chromosomes. Thus, our results indicate that SC length per se is not a determinant of the occurrence of E0 chromosomes.

Extensive individual variation in the incidence of exchangeless chromosomes

We observed substantial inter-individual variation in the level of exchangeless chromosomes (Figure 3A). For example, more than 15% (26/160) of cases exhibited no E0 cells, while in a similar number (21/160) we observed E0 chromosomes in approximately 15% or more of cells. To assess whether the variation could be attributable to chance, we calculated the standard deviation of the percent E0s per case and compared it to values obtained when the data on individual cells were permuted across cases. The observed variation was much larger than would be expected by chance ($p < 0.0001$ in 100,000 permutation replicates). Similarly, we observed highly significant individual variation in the occurrence of cells with two or more E0 chromosomes ($p < 0.01$).

Not surprisingly, the incidence of E0 chromosomes per subject was linked to the level of meiotic recombination. For example, in case subjects without E0 chromosomes, the mean number of MLH1 foci per subject was 68.2 ± 6.4, while the mean for case subjects with 14.5% or more cells with E0s was 62.4 ± 5.0 ($t = 3.4$; $p < 0.01$) (Figure 3B). Further, the difference in MLH1 values between these two groups was not solely attributable to cells with E0 chromosomes, since the MLH1 values for normal cells were also significantly different; i.e., 68.2 ± 6.4 for subjects without E0s, and 64.1 ± 5.1 for subjects with more than 14.5% of cells with an E0 ($t = 2.7$; $p < 0.05$). Indeed, the correlation between the proportion of E0 cells and mean MLH1 values in normal cells was highly significant (corr = −0.23; $p < 0.01$) (Figure S4). Taken together, our data suggest that individuals with lower overall levels of meiotic recombination may be susceptible to higher rates of E0 chromosomes and, consequently, to higher levels of nondisjunction.

While we observed significant inter-individual variation in MLH1 scores and E0 cells, one aspect of the occurrence of E0s was shared by almost all samples. That is, within an individual case, E0s almost always were found among cells at the lower range of MLH1 values. Indeed, for 39 of the 160 (24.4%) samples, the cell with the lowest MLH1 count had at least one E0 chromosome (Figure 4). In contrast, we only observed 1 case (1/160 = 0.6%) in which the cell with the highest MLH1 count contained an E0 chromosome.

Exchangeless chromosomes are impacted by fetal but not maternal age

One of the most provocative models of the maternal age effect on aneuploidy in humans—the so-called "production line hypothesis"[17]—proposes that fewer crossovers are formed in the last oocytes to enter meiosis. Accordingly, cells containing E0 chromosomes would be expected to increase with increasing gestational age. Assuming that oocytes are ovulated in the same order that they are formed,[26] the last to be ovulated would be the most likely to contain E0 chromosomes and consequently, at a high risk for non-disjunction.

In previous analyses of the production line hypothesis, we examined the relationship between gestational age of the fetus and MLH1 values in fetal oocytes and, for a limited number of chromosomes, the influence of gestational age on the incidence of E0 bivalents.[12] We observed

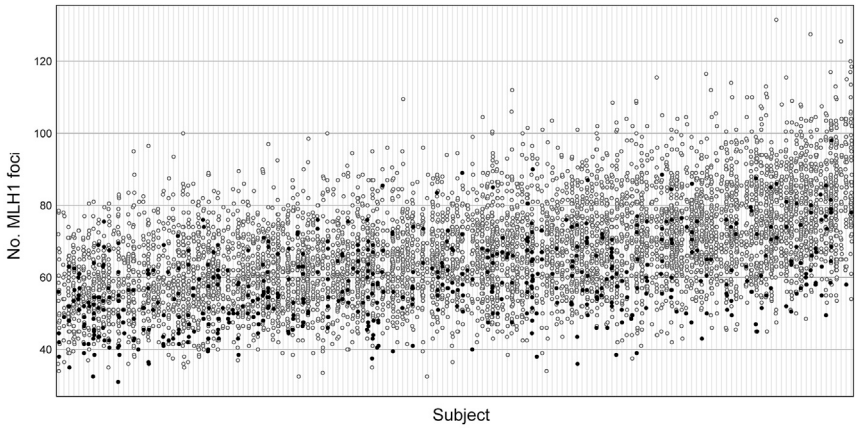

Figure 4. MLH1 values per cell for each of the 160 samples
Filled-in circles denote cells with 1 or more E0 chromosomes; unfilled circles denote cells without E0 chromosomes. Samples are arranged from left to right by increasing mean MLH1 values.

no obvious link between gestational age of the fetus and either the MLH1 values or E0 chromosomes but, importantly, the analysis was not intended to determine the overall incidence of E0-containing chromosomes. Thus, in the present study we extended our analysis to assess the relationship between gestational age and all E0-containing oocytes, not just those on a subset of individual chromosomes.

For these analyses, we first examined the relationship between MLH1 values and gestational age (Figure 5A) and found no obvious change with the age of the fetus. However, surprisingly, we observed a small but significant increase in the proportion of E0 cells with increasing gestational age (corr = −0.20; $p < 0.05$) (Figure 5B). For example, among samples with gestational ages between 15 and 18 weeks, 5.5% of cells per subject contained at least one E0, but among 22- to 24-week-old samples, the value increased to 8.8%.

We also examined the possible association between age of the mother and mean MLH1 values and E0 chromosome frequency in oocytes from her female fetuses (i.e., indicative of a grandmaternal effect on recombination). Intriguingly, we observed a slight but significant decrease in mean MLH1 values per subject with maternal age (corr = 0.20; $p < 0.05$) (Figure 5C), consistent with a decrease of approximately 0.25 crossovers per year. However, there was no obvious effect on the frequency of E0 chromosomes (Figure 5D).

Discussion

Studies of human trisomies have demonstrated the importance of exchangeless chromosomes in the genesis of aneuploidy,[8] possibly attributable to a human female-specific inefficiency in generating crossovers.[25] However, because there have been no attempts to directly measure the incidence of exchangeless chromosomes in a large series of human oocytes, the magnitude of the effect is not clear. Accordingly, we used data from the analysis of 7,396 oocytes from 160 fetal ovary samples to determine the overall proportion of human oocytes containing one or more E0 chromosomes, the extent of chromosome-specific variation in the level of E0s, and the possibility of significant among-individual differences in the proportion of E0-containing oocytes.

Our results indicate a remarkably high level of E0 chromosomes in human oocytes, with approximately 7% of oocytes containing a single E0 and an additional 0.3% two or three E0s. Further, this may be an underestimate of the real frequency of E0s in oocytes, since in previous chromosome-specific FISH analyses we observed even higher values; e.g., 5% each for chromosome 21 and 22,[10,11] approximately 2-fold the level seen for G group chromosomes in the present study. This discrepancy presumably reflects differences in cell selection. That is, in the previous, chromosome-specific, analyses we examined individual SCs regardless of the quality of MLH1 staining for the other SCs in the cell. In contrast, in the present analysis we restricted analysis to cells in which MLH1 staining was uniform throughout the cell and in which all SCs were analyzable. Thus, we suggest that the 7.3% value of the present study represents a minimal estimate for exchangeless chromosomes in human oocytes, and that the "real" value may be as high as 10%–15%.

The consequences of E0 chromosomes to meiotic divisions are also not certain. Classical cytogenetic dogma suggests random segregation of homologs at MI, resulting in 50% normal and 50% aneuploid gametes (i.e., either disomic or nullisomic spermatocytes or oocytes), while recent "MeioMapping" analyses of oocyte-polar body or embryo-polar body trios retrieved in assisted reproductive technology settings indicate that most, if not all, maternally-derived E0 chromosomes mis-segregate.[14] It is not clear which of these predictions is more accurate but, assuming a rate of E0-containing oocytes of approximately 7%, at least 3.5% and possibly as many as 7% of all human conceptuses are aneuploid because of recombination failure in oogenesis. Notably, these estimates are consistent with data from clinically recognized pregnancies, which suggest that at least 5% of human pregnancies involve aneuploid fetuses.[22]

Our results also indicate that more than two-thirds of E0 chromosomes involve the two smallest chromosomes, i.e., chromosomes 21 and 22. Presumably, this is related to the genetic length of these chromosomes since the female maps for each are under 100 cM; i.e., the estimated 81

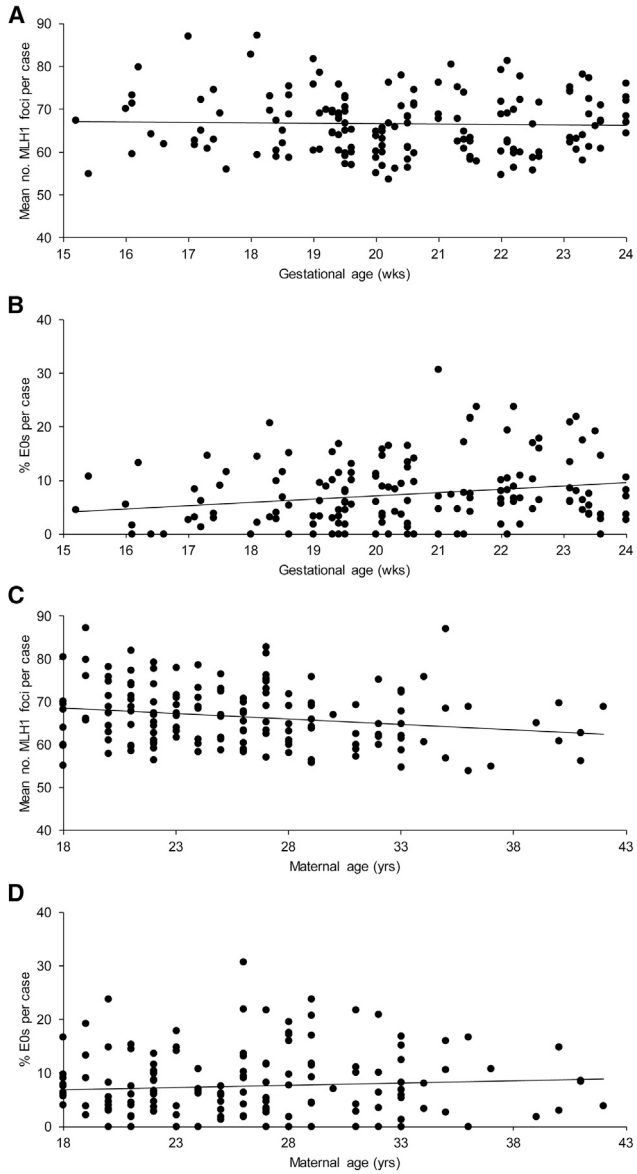

Figure 5. Gestational and maternal age and meiotic recombination

(A and B) Relationship between gestational age of the fetus and (A) mean MLH1 foci per subject and (B) proportion of cells per subject containing E0s.

(C and D) Relationship between maternal age and (C) mean MLH1 foci per subject and (D) proportion of E0-containing cells per subject. Graph axes could be simplified (% E0s per case; mean MLH1 foci per case)

cM and 90 cM for chromosome 21 and 22, respectively,[20] means each is frequently tethered by only one crossover. Thus, unlike chromosomes joined by two or more crossover precursors, loss of a designated crossover[25] will yield a high proportion of E0s. However, genetic length is probably not the only explanation for chromosome-specific variation in the occurrence of E0s. For example, D group chromosomes (i.e., 13–15) had 1.3-fold the number of E0s as E group chromosomes (i.e., 16–18), although the genetic lengths of the two chromosome groups are virtually identical.[20,27] Possibly, the presence of extremely small p arms on acrocentric D and G group chromosomes increases the likelihood of E0 events. However, previous genetic mapping studies of individual trisomies also suggest varying levels of E0s among similarly sized non-acrocentric chromosomes. For example, chromosomes 16 and 18 are of similar genetic length, but while E0s are associated with approximately one-third of cases of trisomy 18, they are rarely if ever involved in the genesis of trisomy 16.[28] Thus, it seems likely that chromosome-specific features other than genetic length also contribute to the occurrence of E0 chromosomes in human oogenesis.

Our observations also provide evidence of significant inter-individual variation in the incidence of E0s (Figure 3A). Intriguingly, this appears unrelated to pairing and/or synapsis, as we found no correlation between synaptic abnormalities (e.g., non-homologous interactions, partial or complete asynapsis, synaptic bubbles or forks) and the level of E0s (data not shown). However, the variation was clearly linked to MLH1 values, suggesting that determinants of recombination level also affect crossover assurance, and implying a genetic background effect on recombination-dependent chromosome mal-segregation in human females.

While our focus in the present study was the incidence of E0s and chromosome-specific and inter-individual variation in the occurrence of exchangeless chromosomes, several unexpected observations are noteworthy. Perhaps most surprisingly, we found a subtle but statistically significant positive correlation between gestational age and the frequency of E0 chromosomes. This is intriguing because it is consistent with a basic tenant of the production line model;[17] i.e., that the last formed eggs are the most likely to have E0 chromosomes. However, the data should be interpreted with caution for several reasons. First, the relationship between gestational age and the level of E0 chromosomes does not fit a simple monotonic model predicted by the production line hypothesis. Although the highest E0 frequency (almost 10% of oocytes) was evident in 21- to 22-week-old fetuses, the frequency decreased to 7.5% in the oldest group (23 weeks of age and older). Second, a biological mechanism for an increase in E0s with gestational age is not immediately obvious. It is not simply due to an overall reduction in recombination levels, since mean MLH1 values did not vary with gestational age (Figure 5B). Further, it does not appear to derive from a relaxation in crossover control in older fetuses. This type of change should alter the distribution of crossovers per chromosome, increasing both E0 chromosomes and higher-order crossover chromosomes. We observed no gestational age-related change in variation in MLH1 values, with only a slight, non-significant correlation between MLH1 standard deviations and gestational age (correlation = −0.05, p = 0.55; 95% C.I. = −0.20 to +0.11). Thus, if the association between gestational age and E0 frequency is real, it likely involves a mechanism that affects crossover assurance, but not other aspects of

recombination control. Lastly, the magnitude of the effect is relatively small. Among the youngest fetuses (15–18 weeks), the incidence of E0s was 5.5% while among the oldest fetuses (22–24 weeks) the value was 8.8%. Clearly, the sample sizes differ markedly between these two age groups, limiting general interpretations. Nevertheless, it is still notable that the observations indicate only a 1.6-fold increase in aneuploidy in the older gestational age group. In contrast, the maternal age-related increase in aneuploidy in clinically recognized pregnancies and preimplantation embryos from ART is closer to an order of magnitude (e.g., Hassold and Jacobs[22] and Gruhn et al.[29]); thus, a gestational age-dependent breakdown in crossover control cannot be solely responsible for the maternal age effect.

Finally, we observed a surprising link between the age of the mother and the mean MLH1 values of the oocytes of her female fetuses; i.e., a "grandmaternal" effect on recombination levels. The effect was modest, with mean MLH1 values per case declining by only about 7%–8% from the youngest to the oldest individuals in the study (Figure 5C). Nevertheless, the trend was virtually the same in the two different series we studied (see Material and Methods), suggesting that the grandmaternal effect is real. Superficially, this conclusion seems at odds with the results of a previous study,[12] in which we reported little relationship between MLH1 counts and maternal age in a sample of 119 case subjects (including 60 from the present report; see Material and Methods). However, a re-inspection of the data suggests few important differences among the various groups of samples. Specifically, if the samples of Rowsey et al.[12] and of present study are divided into three non-overlapping groups—59 samples described only in Rowsey et al.,[12] 60 samples described both in Rowsey et al.[12] and the present study, and 100 samples described only in the present study—the correlations are similarly negative in all three groups (−0.07, −0.18, and −0.17, respectively). Thus, it seems likely that the age of a pregnant woman influences the meiotic recombination profile of her daughter's oocytes.

Assuming that this effect is real, it provides additional evidence for *in utero* influences on the level of recombination in female fetuses. That is, previous studies of pregnant mice have demonstrated a link between exposure to the endocrine disrupting compound bisphenol A (BPA) and increased recombination levels in oocytes of their female offspring,[30] and similar changes have been reported in human cultured oocytes that were exposed to BPA.[31] Our observations suggest that other, as yet unidentified, age-dependent environmental factors can also affect crossover levels, indicating that extrinsic as well as intrinsic agents affect the overall level of meiotic recombination in humans.

Data and Code Availability

Additional data from this study are available upon reasonable request from the corresponding author.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2020.11.010.

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Declaration of Interests

The authors declare no competing interests.

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Web Resources

MicroMeasure 3.3, https://micromeasure.software.informer.com/3.3/

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