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APOLIPOPROTEIN E AS AN HEREDITARY RISK FACTOR FOR NON-DISJUNCTION - A FEASIBILITY STUDY

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APOLIPOPROTEIN E AS AN HEREDITARY RISK FACTOR FOR NON-DISJUNCTION - A FEASIBILITY STUDY

By

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ABSTRACT

APOLIPOPROTEIN E AS AN HEREDITARY RISK FACTOR FOR NON-DISJUNCTION - A FEASIBILITY STUDY

By

Nicole M. Jones

Chromosomal trisomy is a major contributor to pregnancy loss. Although it has been 40 years since the discovery of the first human trisomy, maternal age is the only well documented risk factor. There is a large variation in the frequency of different types of chromosomal trisomy sampled at different times in pregnancy. Through the use of molecular markers, it is possible to determine the parent in which the nondisjunction event occurred and the cell division of error.

Both Alzheimer's disease and Down syndrome have been associated with the allele Apolipoprotein ε4. We conducted a feasibility study aimed at developing methods for a larger study guided by the hypothesis that Apolipoprotein ε4 is a risk factor for non-disjunction and Alzheimer's disease. Our feasibility study was designed to develop methods for measuring family history of Alzheimer's disease and stage of non-disjunction error among parents of trisomy pregnancies. We designed a case-control study with cases matched to controls on ethnicity and frequency matched on age. A total of 29 cases and 61 controls participated in our feasibility study. During our feasibility study, we identified a collection of potential cases, archived trisomy DNA samples, refined our interview and laboratory instruments through field-testing, and developed and debugged a Microsoft-Access database capable of storing our interview data.

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ABSTRACT

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Chromosomal trisomy is a major contributor to pregnancy loss. Although it has been 40 years since the discovery of the first human trisomy, maternal age is the only well documented risk factor. There is a large variation in the frequency of different types of chromosomal trisomy sampled at different times in pregnancy. Through the use of molecular markers, it is possible to determine the parent in which the nondisjunction event occurred and the cell division of error.

Both Alzheimer's disease and Down syndrome have been associated with the allele Apolipoprotein ε4. We conducted a feasibility study aimed at developing methods for a larger study guided by the hypothesis that Apolipoprotein ε4 is a risk factor for non-disjunction and Alzheimer's disease. Our feasibility study was designed to develop methods for measuring family history of Alzheimer's disease and stage of non-disjunction error among parents of trisomy pregnancies. We designed a case-control study with cases matched to controls on ethnicity and frequency matched on age. A total of 29 cases and 61 controls participated in our feasibility study. During our feasibility study, we identified a collection of potential cases, archived trisomy DNA samples, refined our interview and laboratory instruments through field-testing, and developed and debugged a Microsoft-Access database capable of storing our interview data.

are defined by a cutoff value. In a population with a normal distribution of maternal age, a cutoff of 1:250 will detect 59% of DS pregnancies with a false positive rate of 5%[7].

The quadruple test is designed to improve the screening detection rate over the triple test. The quadruple test measures the sub-units of hCG (free α-hCG and free β-hCG), AFP and uE_3[8]. This combination of four markers can detect 65% of DS pregnancies with a false positive rate of 5%[7]. Women who screen positive on the triple or quadruple test may chose to have a diagnostic amniocentesis or chorionic villus sampling (CVS).

History

In 1912, the number of human chromosomes was first reported as 48[9]. This falsity held for over forty years until Tjio and Levan observed 46 chromosomes[10]. Later that year, Ford and Hamerton confirmed that 46 was the true number[11]. Today it is known that 46 is the normal number of chromosomes for a human to possess.

In 1959, it was discovered that some deviations from 46 chromosomes were compatible with survival[12]. Lejeune et al. showed that trisomy of a small acrocentric autosome was the cause of Down syndrome, a previously well-described syndrome. Later, in 1960, trisomy 18 and trisomy 13 were described (Edwards and Patau syndrome respectively)[13, 14].

Prevalence

The prevalence of trisomy varies at different stages of pregnancy. Trisomy is more frequent among the earlier stages of pregnancy with 26.8% of spontaneous

abortions, 3.8% of stillbirths, and 0.3% of live births being trisomic[15]. A total of 4.3% of all clinically recognized pregnancies are trisomic[15].

There is a large variation in the frequency of type of chromosomal trisomy sampled at different times in pregnancy (Table 1). This inequality could either reflect a difference in selective disadvantage or it could represent differences in the frequency of non-disjunction among the chromosomes.

Upon examination of table 1, three main points are apparent. First, across the different chromosomes the prevalence of specific trisomies among spontaneous abortions varies greatly. The frequency ranges from zero for chromosomes 1 and 19 to 7.5% for chromosome 16. Second, there is a large amount of selection that occurs before birth. The only autosomal trisomies compatible with survival to term are 13, 18, and 21. Third, even among these three trisomies, selective intrauterine mortality occurs. Only 3% of trisomy 13, 5% of trisomy 18, and 22% of trisomy 21 pregnancies survive to birth.

Table 1: Frequency of chromosomal trisomy

Chromosome	Population			Probability of survival to term*
	Spontaneous abortions n=4088	Stillbirths n=624	Livebirths n=56952	
1	-	-	-	-
2	1.1	-	-	0
3	0.3	-	-	0
4	0.8	-	-	0
5	0.1	-	-	0
6	0.3	-	-	0
7	0.9	-	-	0
8	0.8	-	-	0
9	0.7	0.1	-	0
10	0.5	-	-	0
11	0.1	-	-	0
12	0.2	-	-	0
13	1.1	0.3	0.005	2.8
14	1.0	-	-	0
15	1.7	-	-	0
16	7.5	-	-	0
17	0.1	-	-	0
18	1.1	1.1	0.01	5.4
19	-	-	-	0
20	0.6	-	-	0
21	2.3	1.3	0.13	23.8
227	2.7	0.2	-	0
Double Trisomy	0.8	-	-	0

[16]*Assuming 15% spontaneous abortion and 1% stillbirth of clinically recognized pregnancies

Parental Origin

It is possible to determine the parent in which the nondisjunctional event occurred through the use of Menedelian inheritance patterns of genes, cytogenetic heteromorphisms, or molecular polymorphisms. A cytogenetic heteromorphism is a stable heritable alteration in size and shape of heterochromatic regions of certain chromosomes. Cytogenetic heteromorphisms rely upon an individual's ability to interpret subtle differences at the limit of resolution of the light microscope. A molecular

polymorphism is a heritable DNA sequence that is highly variable in the population. Molecular polymorphisms are a less subjective and easier method of tracing paternal origin of non-disjunction. Examples of molecular polymorphisms include restriction fragment length polymorphisms, very numerous tandem repeat polymorphisms (VNTRs) detected with a Southern Blots, and GT repeat polymorphisms amplified with polymerase chain reaction.

Origin of Error

It is also possible to determine the cell division of non-disjunction through the use of molecular polymorphisms. The non-disjunctional error resulting in a trisomy can occur in either the ovum, the sperm, or in early postzygotic division. The error can occur during a premeiotic mitotic division of the oogonia or spermatagonia, during the first or second meiotic divisions of the oocyte or spermatocye, or during early division of the zygote with a postzygotic mitotic (PZM) error. A premeiotic mitotic division cannot be differentiated from a meiotic error because the additional chromosome will be paired and segregated at future meiotic divisions. The cell division of error can be assigned by determining the pattern of polymorphisms along the parents' and trisomy's non-disjoined chromosome. A polymorphism is reduced when the non-disjunctional event results in an individual that is homozygous for a single polymorphism. Figure one illustrates the difference between a meiosis I and meiosis II error (MI and MII). If non-disjunction occurs during meiosis, the trisomic conception will receive either two copies of the same chromosome from a single parent (meiosis II non-disjunction) or two copies of different chromosomes from a single parent (meiosis I non-disjunction). Centromeric and distal polymorphisms distinguish whether the error occurred during meiosis or PZM.

Centromeric markers prevent misclassfication of MI and MII errors because of crossing over events which occur in distal regions of the chromosome. In order to differentiate between MI and MII errors, it is necessary to have a centromeric polymorphism that is heterozygous in the parent whose chromosome is duplicated in the trisomy. For both a PZM error and a MII error the polymorphism is reduced to homozygosity (Table 2). For a MI error the polymorphisms is non-reduced. In order to distinguish a PZM error from a MII error it is necessary to look at polymorphisms that are distal to the centromere. During a PZM error all polymorphisms will be reduced, for a MII error the distal polymorphisms will be non-reduced.

Figure 1: Meiosis and Nondisjunction

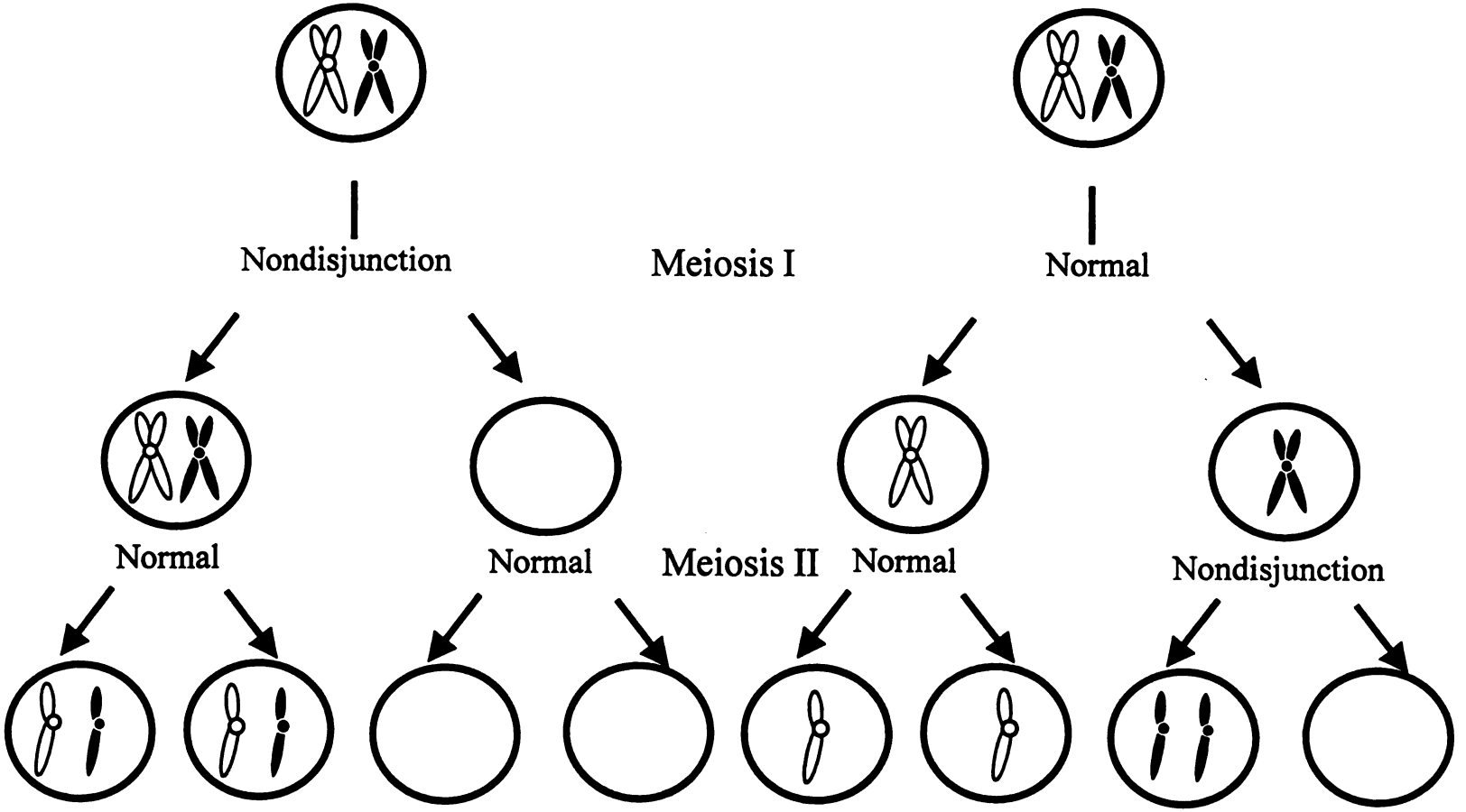


Table 2: Cell Division of Nondisjunctional Error

Cell Division	Status of polymorphism		
	Centromeric	Proximal	Distal
MI			
Nullichiasmate	Nonreduced	Nonreduced	Nonreduced
Chiasmate	Nonreduced	Nonreduced	Reduced
MII	Reduced	Reduced	Nonreduced
PZM	Reduced	Reduced	Reduced

Etiology

Extrinsic risk factors for chromosomal trisomy that have been previously investigated include: exposure to ionizing radiation, oral contraceptive use, fertility drug use, alcohol use, caffeine use, and cigarette smoking[17]. In addition, there has been a focus on intrinsic factors such as thyroid autoimmunity, decreased parental HLA heterogeneity, persistent nucleolar associations, and cytogenetic heteromorphism[16]. The only clear and consistent risk factor for chromosomal trisomy is advanced maternal age. One major problem with previous etiologic studies is that they failed to separate maternal, paternal, MI and MII errors. Each of these different errors may be triggered by different intrinsic or extrinsic risk factors.

Much of what is known about trisomy comes from one large epidemiologic study[18]. This study sampled women from hospitals in New York City and Honolulu. The New York City cases were selected at hospital admission for spontaneous abortion or fetal death from April 1974 to May 1984. Prior to 1981, patients were sampled from three Manhattan hospitals. From 1981 to 1984 patients were sampled from only one hospital. The total sample consists of 2,587 karyotyped cases with known maternal age. The New York City sample has eight cases of inherited trisomy, 2,312 women with one

karyotyped abortion, 125 women with two karyotyped abortions, and seventeen women with three karyotyped abortions.

The Honolulu data set sampled cases from one hospital from April 1976 to May 1985. A total of 2,921 karyotyped samples with known maternal age were available. The Honolulu sample consists of 21 cases with inherited translocations, 2,594 women with only one karyotyped abortion, 148 women with two karyotyped abortions, and 10 women with three karyotyped abortions. Multiple authors have analyzed data from these two samples. The results from these studies will be presented and discussed in later sections.

Maternal Age

In 1933, twenty-six years before the chromosomal basis of the disease was known, Penrose discovered an association between advanced maternal age and an increase in the risk for having a child with DS[19]. In 1983, Hook *et al* published population frequencies of DS by maternal age category based on data from prenatal cytogenetics studies. They calculated regression-smoothed maternal age-specific rates of DS abnormalities and multiplied them by a fetal selection coefficient to adjust for the excess likelihood of loss of cytogenetically abnormal fetuses. The result was estimated maternal age-specific rates of DS in live-born infants. These rates apply to women whose only risk factor is advanced maternal age. Hook *et al.* found that the risk for DS increases moderately in young mothers and much steeper after age 30 (Table 3). These rates were calculated prior to the implementation of prenatal screening.

Table 3: The risk for Down syndrome by maternal age and stage of pregnancy.

Maternal Age (years)	Incidence		
	At CVS (9-11 weeks)	At Amniocentesis (16 weeks)	At birth
15-19	-	-	1/1250
20-24	-	-	1/1400
25-29	-	-	1/1100
30	-	-	1/900
31	-	-	1/900
32	-	-	1/750
33	-	1/420	1/625
34	-	1/325	1/500
35	1/240	1/250	1/350
36	1/175	1/200	1/275
37	1/130	1/150	1/225
38	1/100	1/120	1/175
39	1/75	1/100	1/140
40	1/60	1/75	1/100
41	1/40	1/60	1/85
42	1/30	1/45	1/65
43	1/25	1/35	1/50
44	1/20	1/30	1/40
45 and over	1/10	1/20	1/25

[20]

Penrose and others have concluded the increased risk of trisomy with age can be separated into two components. One component increases in a linear fashion with chronologic maternal age. The second component of the age effect increases in a curvilinear with maternal age. Based on spontaneous abortion studies from the Honolulu and New York data increased maternal age is a risk for trisomy for all autosomes[21-24]. However, the effect of maternal age varies by chromosome being more pronounced for small chromosomes and less pronounced for larger ones. In addition, for chromosomes 16 and 2, the maternal age effect is strictly linear. Two trisomy studies of the cell division of origin and the parent of origin found a maternal age effect for both maternal MI and maternal MII errors[25, 26].

Models for the maternal age effect

There are three popular models that have been suggested to explain the maternal age effect. They are the "relaxed selection," "older egg," and "production line," hypotheses.

The "relaxed selection" model suggests that the age-dependent increase in trisomy is due to a decreased likelihood of aborting a trisomy and not an increased frequency of trisomy at conception[27]. This model has little support in the literature. It predicts a maternal age effect regardless of parental origin or stage of error. However, a study of trisomy 21 in 1992 showed that the increase in maternal age effect was present in cases of maternal not paternal origin[26]. Another molecular study of trisomy 21 found that errors of mitotic origin showed no increase in maternal age effect regardless of parent of origin[28]. In general, the increase in maternal age effect is restricted to cases involving maternal meiotic non-disjunction[29]. Also, the miscarriage frequency of trisomic fetuses increases with maternal age and the miscarriage frequency of fetuses with other types of chromosome imbalance shows no relation to maternal age[30].

The 'older egg' theory suggests that the maternal age effect is related to a declining quality of oocyte pool. This hypothesis is supported by the fact that DS cases resulting from translocated chromosomes do not show a maternal age effect[31, 32]. According to this hypothesis, factors that affect the availability of oocytes should affect the risk of trisomy. For example, data that show that unilateral ovarectomy is a risk factor for trisomy support the 'older egg' hypothesis[33]. If the oocyte pool is reduced due to unilateral ovarectomy, then the risk of a nondisjoined oocyte becoming fertilized increases. If reproductive age is viewed as a continuum from menarche through

menopause, then early onset of menarche and menopause may be indicators of an increased risk for chromosomal trisomy. A retrospective case-control study in 1995 hypothesized that a woman who has a child with trisomy 21 at younger than 30 years of age would be more likely to undergo premature menopause (menopause at less than 35 years of age)[34]. In addition, they suggested that women over 30 who had a child with trisomy 21 would be closer to menopause than an age matched control that had a normal child. They analyzed data from interviews. They found no cases of premature menopause among 35 women who had delivered trisomy 21 children under 30 years of age. Also, they found no difference between the mean age of menopause among 106 case and control women over 35. This study did not support the predictions of the 'older egg' hypothesis.

The "production line" hypothesis postulates that those oocytes produced last in fetal life would form fewer chiasmata, making nondisjunction more likely[35]. They would ovulate later in adult reproductive life than those oocytes produced earlier in fetal development. The literature indicates that reduced recombination may play a role in nondisjunction. Three investigators have reported decreases in recombination in the non-disjoined chromosomes through the use of DNA polymorphic markers along the chromosome [36-38]. Sherman found that older mothers had fewer recombinational events in the non-disjoined chromosome than younger mothers. According to Sherman's work, reduced recombination seems to play an important role in trisomy 21 non-disjunction especially for young mothers. Currently the "production line" hypothesis has the most support in the literature.

Paternal Age

Paternal age has been extensively studied as a risk factor for chromosomal trisomy. In 1977, Stene et al reported an increased risk of DS in fathers over the age of 55[39]. The result was supported by similar findings of Matsunaga et al. (1978) and Erickson and Bjerkdal (1981)[40, 41]. In addition, Stene et al. in 1981 found an increased risk of trisomy 21 in fathers over age 41 based on amniocentesis data[42]. Other studies have found no link between DS and increased paternal age [43-50]. All of these studies did not separate maternal, paternal, MI and MII errors. This is important because, one would not expect to find a paternal age effect among maternal errors. At this point in time the weight of evidence suggests that paternal age is not an important factor for chromosomal trisomy, and only a small proportion of trisomy nondisjunctional errors are paternal in origin. It is necessary to wait for larger numbers of cases that have been identified via DNA polymorphisms to be paternal errors before any conclusions can be made as to the contribution of paternal age.

Recurrence Studies

Studies that quote recurrence risks hint that trisomy may not be a purely random event (Table 4). A number of studies have looked at data from live births. Initial studies by Oster and Carter found that hospitalized DS patients had a higher than expected number of siblings with DS (compared to population data)[51, 52]. They did not exclude translocation cases from their analysis. Stene reanalyzed this data in 1970 excluding translocation cases. This analysis found an increased recurrence risk for mothers under 30, and the population risk for mothers over 30. Richards repeated this finding in 1977 in sibships of institutionalized DS patients[53]. Data from trisomy 18 and trisomy 13 was

collected by Baty in 1994[54]. Among families in a support group for trisomy 18 and 13 the risk for recurrence of trisomy 18 or 13 among siblings was not increased. This study found a recurrence risk of 0.55% (95% CI 0-1.63%) but had limited power to find a difference due to small sample size.

Mikkelsen and Stene looked at data from multiple European Centers and found that mothers below 25 had a recurrence risk significantly greater than the population risk[55]. They did not indicate reason for amniocentesis among these women or separate out translocation cases. Daniel looked at amniocentesis data from women who had an amniocentesis performed because of a previous child with DS[56]. They found an overall recurrence rate of 1% but did not specify age-specific rates.

Caron in 1999 looked at amniocentesis data for women referred for amniocentesis due to advanced maternal age[57]. They found a risk of recurrence of trisomy of 1.3% for women under 35 and 4.8% for mothers 35 or older. This recurrence risk is one and a half times the population risk for women under 35 and over twice the population risk for women 35 or older.

The strengths of this study were its comparisons to several reference groups. In addition, the authors looked at other chromosome abnormalities than trisomy. The sample came from tissues over a long period of time. The weaknesses include that the population was sampled at hospital admission. Therefore, the population may over-represent trisomies which present with later fetal loss and under-represent trisomies which present with loss earlier in pregnancy. Also, the authors didn't separate out the maternal, paternal MI, and MII errors. Therefore, this study does not show if one specific type of non-disjunction could be genetic.

Other studies have looked at data from spontaneous abortions. In initial studies, a total of 87 women with two karyotyped spontaneous abortions were looked at[58-60] [61]. Among women who had a trisomic abortion the second abortuses tended to have a trisomic karyotype as well. However there were problems with this data. Women with their first trisomic abortion are on average older than women with their first normal abortion and older at their second karyotyped abortion. This leads to an apparent increase in the rate of trisomy when the comparison group is women whose first abortion had a non-trisomic karyotype. Also, women with a previous chromosomally normal spontaneous abortion have a lower rate of trisomic abortions than do unselected women and do not make a good comparison group.

These problems were addressed by a study by Warburton et al., in 1987[62]. They looked for an association between the karyotype of a previous spontaneous abortion with the karyotype of subsequent spontaneous abortions in the New York City and Honolulu data. The data were analyzed by city and combined. The authors performed an unconditional and a conditional maximum-likelihood logistic regression analysis. They adjusted for the potential confounders of maternal age, payment status in the New York City sample (private versus public facilities), prior abortions, and location (New York City versus Honolulu) in the combined analysis. The authors used all women with only a single karyotyped abortion irrespective of reproductive history as the reference group. They repeated their analysis using two other reference groups: (1) women who were primigravida at the time of the first abortion and (2) women who had a prior term delivery but no prior spontaneous or induced abortions. The authors were concerned that their first reference group may have over-represented women at high risk for spontaneous

abortion who are known to have an increased rate of chromosomally normal abortions. All three analyses yielded similar results.

The authors defined the 'index abortion' as the second karyotyped abortion for women with two karyotyped abortions and the only karyotyped abortion for all other women. In the adjusted analysis, the odds of trisomy at the index abortion among women with a previous trisomic abortion were similar to those among women without a previous karyotyped abortion. The combined estimation for the odds of trisomy at the index abortion relative to prior trisomy abortion was 1.3 (95% confidence interval 0.7 to 2.1).

The authors performed separate analyses for women under thirty and for women greater than or equal to 30 years of age. This analysis did not show an increased risk for women in the younger age category. The adjusted odds ratios were 1.3 (95% confidence interval 0.4 to 4.5) for the under thirty women and 1.2 (95% confidence interval 0.7 to 2.1) for the women who were thirty and older. Since the risk of trisomy increases with age, the authors had small numbers of women (n=17) in the under 30 group and limited power to find a 20-30% increase in risk. The results indicate that karyotype of spontaneous abortion is not a good predictor for future trisomy. The authors suggested possible explanations for the disconcordant findings as compared to live birth and amniocentesis data. The authors suggested that they may have found no association because trisomy proneness could be confined to certain trisomies or only women under 30. With an effect so restricted, this study would not have had the power to find an association. A second possibility they proposed was that the increased recurrence rate among live births and amniocentesis data is due to parental mosiacism. Thus trisomies which were compatible with survival would appear to have an increased recurrence risk

among livebirths. The current literature does not rule out either of these two possibilities. In addition due to the rarity of trisomy it is difficult to find studies with enough power to answer these questions.

Table 4: Population based studies which analyze the risk of trisomy recurrence.

Study	Population	Design	Major Findings	Notes
Oster, 1953[a]	Siblings of DS patients	Cross-sectional	higher than expected number earlier and later born sibs	Did not exclude translocation cases
Carter & Evans, 1961[b]	Siblings of DS patients	Cross-sectional	Significantly more than expected younger & older sibs for mothers under 35	Did not exclude translocation cases
Stene, 1970[c]	Reanalysis of Oster, Carter& Evans data		Increased risk for mothers under 30, population risk for mothers 30 and over	Excluded translocated cases
Richards, 1977[d]	Recurrence of DS in sibships institutionalized DS patients	Cross-sectional Questionnaire Data	Among mothers under 30 the laterborn sibs of DS patients had a recurrence rate of 2-3%	Translocation cases not separated in analysis
Baty, 1994[e]	Families in a support group for trisomy 18& 13 children	Cross-sectional Questionnaire Data	0.55% sibling recurrence risk for trisomy 18 or trisomy 13	

Table 4 Continued: Population based studies which analyze the risk of trisomy recurrence.

Study	Population	Design	Major Findings	Notes
Repeated Abortus Data[f]	Pairs of repeated abortions Boue & Boue- 43 pairs Alberman - 23 pairs Lauritsen - 7 pairs Kajii - 18 pairs	Cross-sectional	Hinted that if the 1st abortus was trisomic, the 2nd abortus tended to be trisomic	
Warbuton, 1987[g]	Spontaneous abortion at hospital admission	Prospective	No increase risk for recurrent trisomic abortion	
Mikkelsen, 1978[h]	Multicenter Study	Cross-sectional Amnio Data	Mothers below 25 recurrence for DS significantly greater than the population risk	Excluded translocation Cases
Daniel, 1982[i]	Women referred for prenatal diagnosis due to previous DS child	Cross-sectional Amnio Data	Overall 1% recurrence rate	Age-specific recurrence rates not given
Caron, 1999[j]	Amnio Data for woman referred due to advanced maternal age	Cross-sectional	Recurrence following a prior child or fetus with a trisomy 1.3% for women under 35 and 4.8% for mothers 35 or older	

Data from: a -[51] b-[52] c-[63] d-[53] e-[54] f-[58-61] g-[62] h-[55] i-[56] j-[57]

Trisomy 21-parental origin

Trisomy 21 is the most common trisomy at birth. Chromosome 21 has heteromorphisms located at the centromere and close to the centromere on the short arm. Very little recombination occurs on the short arm so it is possible to extrapolate the origin of the extra chromosome. Early studies looked at over 1500 families using chromosome heteromorphisms. Problems with this method included: the subjective evaluation of size and staining intensity of bands, large numbers of uninformative families, and the heteromorphisms were located on only one side of the centromere so crossovers between the centromere and the short arm went undetected. Based on heteromorphism studies, the observed level of paternal non-disjunction ranged from 0-57%.

It became clear that the estimates of non-disjunction from chromosome heteromorphisms were not reliable. However, two pieces of valuable information were learned. First, most of trisomy 21 occurs from non-disjunction events occurring at maternal MI. Second, some paternal and MI, paternal MII and maternal MII errors occur. DNA polymorphisms that have been identified near the centromere on chromosome 21suggest that 91% of the nondisjunctional errors leading to a trisomy 21 conceptus are maternal in origin[26, 38].

Trisomy 21-stage of error

Among the 500 maternal errors that have been classified, 75% occur during meiosis I, 22% during meiosis II, and 3% during PZM (Table 4)[64]. Among 30 paternal errors classified, 50% occurred during meiosis II, 23% during meiosis I, and 27% during PZM.

Table 5: Proportion of meiotic errors for trisomies 21, 18, 16, and 13.*

	Trisomy 21 Proportion[a]	Trisomy 18 Proportion[b]	Trisomy 13 Proportion[c]	Trisomy 16 Proportion[d]
Cell Division				
Paternal MI	13%	0	12%	0%
Paternal MII	7%	0	4%	0%
Maternal MI	68%	29%	68%	100%
Maternal MII	13%	62%	16%	0%

*PZM errors occur less than 5% of the time for all trisomies

a-[25]sample size = 500

b [65]sample size = 63

c-[66]sample size = 30

d-[67]sample size = 62

Trisomy 21-effect of recombination

In 1987, Warren showed that the level of recombination was reduced along the trisomic chromosome among trisomy 21 cases[36]. Sherman repeated this result in 1991[38]. The unit of genetic map distance is the Morgan. The Morgan is defined as the length of choromsomal segment which on average undergoes one exchange per individual chromatid strand. Sherman found that the average genetic map at maternal MI is 39 centimorgans for a trisomic 21 versus 72 centimorgans for a normal 21. In other words, mothers of children with trisomy 21 experience far fewer recombinational events on chromosome 21 than mothers with non-trisomic children

Trisomy 18-Epidemiology

Trisomy 18 is the second most common autosomal trisomy among live births. There is a strong association with maternal age[21]. Recent molecular studies of live births and abortus tissue indicate that 87-95 % of trisomy 18 occurs as a result of a maternal error[65, Kupke, 1989 #176, Nothen, 1993 #177].

There are no polymorphic centromere markers available for chromosome 18. Therefore, the stage of cell division for nondisjunction must be determined through the use of pericentromeric markers. There may be some abnormal recombination among trisomy 18 cases. Fisher found that one third of maternal MII errors were associated with absence of recombination. The rest appeared to be normal[68].

Trisomy 13-Epidemiology

Trisomy 13 is compatible with survival to term. In 1987, Jacobs et al. presented data on the trisomy 13 cases from their Honolulu sample[69]. Trisomy 13 was the fourth most common trisomy in their sample. The mean maternal age for the non-translocation trisomies in the Honolulu sample was significantly greater than that for the whole study population ($t=3.14$, $p<0.05$).

By using both cytogenetic and molecular techniques, Hassold et al., analyzed the parent and cell division of error in 30 cases of trisomy 13 from their Honolulu sample[66]. They were able to determine the parent in which the error occurred in 20 cases with 17 (85%) being maternal and three (15%) being paternal in origin (Table 3). The most common mechanism of origin was maternal MI non-disjunction that accounted for 68% of cases. A trend towards increased maternal age was seen for the maternal MI and MII errors but not for the paternal errors. This suggests that increased maternal age is a risk factor for trisomy 13. The authors were unable to determine if recombination was reduced or enhanced.

Trisomy 16-Epidemiology:

Trisomy 16 is the most common trisomy in humans. It occurs in over 1% of clinically recognized conceptions[64]. Trisomy 16 conceptuses rarely survive to term.

The risk of trisomy 16 increases linearly with maternal age[24, 70]. Parental origin has been determined in 62 trisomy 16 cases[67]. In all cases the additional chromosome was maternal in nature. The stage of error was studied in 58 trisomy 16 conceptuses. A single centromeric marker was informative in 54 cases and all were due to a maternal MI error. Preliminary data suggests that trisomy 16 is associated with a reduction in recombination. In addition, this reduction is restricted to pericentromeric regions with the distal portions having normal amounts of recombination.

Table 6: Summary of trisomies 21, 18, 13, and 16 information

	Trisomy 21	Trisomy 18	Trisomy 13	Trisomy 16
Syndrome	Down syndrome	Edward syndrome	Patau syndrome	None
Frequency Rank	Most common at birth	Second most common at birth	Third most common at birth	Most common during pregnancy
Recombination	Reduced	Reduced/ Normal	?	Normal
Maternal Age	Curvilinear & Linear Effects	?	?	Linear Effect Only

Summary of Part One

- It has been 40 years since the discovery of the first human trisomy.
- Trisomy contributes significantly to pregnancy loss.
- There is a large variation in the frequency of different types of chromosomal trisomy sampled at different times in pregnancy.
- Through the use of molecular markers, it is possible to determine the parent in which the nondisjunctional event occurred and the cell division of error.
- Maternal age is the only well documented risk factor for chromosomal trisomy.

CHAPTER 2: APOLIPOPROTEIN E

Background

Apolipoprotein E (ApoE) is a 299 amino acid plasma glycoprotein involved in cholesterol transport and metabolism. ApoE is synthesized mainly in the liver but also in small amounts in most organs including the brain and ovaries. Three different alleles give rise to the three most common isoforms: E2, E3, and E4. The ε3 allele is the most common form among whites with an allele frequency of 78.5% while ε4 and ε2 have allele frequencies of 13.5% and 8% respectively[71]. The frequency of the ε4 allele varies among population and has been found to be higher in particular African (~20-40%) [72], Finnish (~20%) [73, 74], and Swedish (~20%)[75] populations, and lower among several Asian populations (~8%)[76]. ApoE genotype can be determined by polymerase chain reaction (PCR), restriction enzyme digestion, and gel electrophoresis. The three isoforms have variations in sequence that results in differing locations of *Hha I* restriction sites. Each digested DNA sequence results in a unique restriction fragment pattern (see Figure 1). ApoE genotype has been investigated as a risk factor for numerous health conditions including longevity, cholesterol level, cardiovascular disease, stroke, recovery from head trauma, presence of gallstones, hip fractures among the elderly, and retinitis pigmentosa.

Figure 2: Hha I Restriction pattern of different ApoE genotypes

	4/4	3/4	3/3	2/4	2/3	2/2
91 bp		—	—	—	—	—
83 bp				—	—	—
72 bp	—	—		—		
48 bp	—	—	—	—	—	
35 bp	—	—	—	—	—	

bp=base pairs

Longevity Studies

In a study of 325 French centenarians, the ε4 frequency was decreased to 5.8% compared to 12.1% among controls and the ε2 frequency was elevated to 12.8 % among the centenarians compared to 6.8% among controls[77]. Similar findings were seen among 179 centenarians and 95 nonagenarians in Finland[78, 79], in healthy Swedes over 60 years old[75], among American females[80], and Asian and Italian subjects[81-83]. The limitation of these case-control studies is that they do not tell us why ε2 carriers more frequently survive to very old ages and ε4 carriers do not. Unlike ApoE, common polymorphisms in other genes involved in lipoprotein metabolism, thrombosis, or homocysteine metabolism have not been consistently associated with longevity[84-86].

Cholesterol Levels, Cardiovascular Disease, and Stroke

In addition to being associated with longevity, ε2 is associated with decreased levels of total cholesterol and low density lipoprotein (LDL), and ε4 is associated with increased levels of total cholesterol and LDL. Alleles ε2 and ε4 are also associated with

increased and decreased plasma ApoE levels, respectively. A 1996 meta-analysis of nine studies found that ε4 was associated with a mild increased risk for coronary heart disease (CHD) (odds ratio =1.26 versus reference ε3)[87]. On the other hand, a 3.5 year prospective study of 1067 elderly Finns failed to find an association between the ε4 allele and CHD[88]. A five-year study of 666 elderly Finnish men found a twofold increase in the ε4 allele frequency among those who died from CHD[88].

A 1999 meta-analysis looked at the association of ε4 with cerebrovascular disease or stroke among nine published studies. The ε4 allele was associated with an increased risk with an odds ratio of 1.68 compared with ε3. In summary, ε4 is associated with a more atherogenic lipoprotein profile and moderately increased risk for CHD and stroke.

Other Associations

ApoE genotype has been investigated as a risk factor for recovery from head trauma, presence of gallstones, hip fractures among the elderly, and retinitis pigmentosa. In two studies of head trauma, ε4 was a negative risk factor for recovery [89, 90] and ε4 is associated with an increased and ε2 with decreased prevalence of gallstones in women[91, 92]. In addition, ε4 may be a risk factor for injury in the elderly. In a 7-year longitudinal study of 1750 women over 65 ε4 carriers had higher rates of bone loss and were at increased risk to have hip fractures[93]. Finally, homozygosity for ε2 or ε4 has been associated with having retinitis pigmentosa[94, 95].

Link to Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia after age 40. Prevalence increases from 0.3% in 60 to 69 age category up to 10.8% after age 80 [96].

Differential diagnosis is made at autopsy. AD is characterized by the presence of neurofibrillary tangles composed of hypophosphorylated tau in the neurons of the cerebral cortex and hippocampus along with the deposits of β–amyloid within senile plaques and cerebral blood vessels. Clinically, patients experience a slow progressive loss of memory and cognitive abilities. There is a genetic predisposition to AD demonstrated by an increased prevalence in first degree relatives of AD subjects.

Other than age, ApoE genotype is the strongest established risk factor for AD. According to a meta-analysis in 1997, compared to ε3/ε3 subjects the odds for having AD among whites is 3.2 for ε3/ε4 subjects and 14.9 for ε4/ε4 subjects[97]. On the other hand, the ε2 allele is protective. Among whites the odds for having AD is 0.6 among the ε2/ε3 and ε2/ε2 carriers as compared to ε3/ε3 genotypes[97]. ApoE ε4 is also associated with the severity of AD. Compared with non-ε4 carriers, AD subjects with an ε4 allele have an increased number of senile plaques, increased brain β-amyloid levels, decreased entorhinal cortex volume, decreased choline acetyltransferase activity, and increased neuronal degeneration in the basal nucleus[98]. In a study of newly diagnosed AD patients ε4/ε4 patients had the most rapid decline of cognition, while ε2 carries had the slowest rate[99]. The mechanism by which the different allelic forms of ApoE affect the pathology of AD is not understood although it may have to do with differential binding to the proteins of the neurofibrillary tangles.

Apolipoprotein E, Alzheimer's Disease, and Down Syndrome

AD and DS have been linked together in several ways. First, adults over 40 with DS are more likely to develop symptoms of AD and have the same neuropathological

lesions[100]. The similarity of brain lesions could suggest that the underling pathogenic pathways leading to AD and DS may have some features in common and perhaps could be caused by the same genetic risk factors.

Epidemiologic data also supports the idea of shared etiologic or pathogenic factors for DS and AD. In one study, women who had a DS child before the age of 35 were at an increased risk of developing AD[101]. Furthermore, it has been shown that among first-degree relatives, there is an increased prevalence of AD in families with DS relatives [102, 103] and the prevalence of DS is higher than expected among the relatives of AD patients.[104, 105]. Other studies, while not statistically significant, have results that point towards a higher rate of DS in the families of AD patients[106, 107]. There has been some suggestion that mothers that give birth before 19 years of age are at an increased risk for having a DS child and for AD.

A third line of evidence that supports a link between AD and DS is clinical evidence. Fingerprint dermatoglyphic patterns observed in AD patients are similar to DS patients. AD patients much like DS patients have an increased frequency of ulnar loops on fingertips, Simian creases on the palms, palmar hypothenar patterns, and large distal loops in the hallucal region. This similarity may be restricted to early onset AD patients [108, 109]. These clinical similarities suggest that common genetic factors influence the developmental processes in DS and AD.

The evidence for a genetic risk factor for AD linked to chromosome 21 has been varied[105]. An initial study linked chromosome 21 to familial AD in four AD families. In addition, at the same time it was detected that the gene for β-amyloid precursor protein (APP) maps to chromosome 21. This supported the theory that APP was one of the genes

for AD, and the AD-like symptoms of DS patients were explained by the extra dose of the APP gene. However, other studies did not find an association between chromosome 21 and AD. When divided into early-onset and late-onset cases of AD, the association, although not universal, was strongest with early-onset cases. The interest in the association between chromosome 21 and AD later decreased after sequencing of the APP exons in AD affected individuals revealed that mutations in APP were very rare and explain only 1-3% of familial AD cases.

Other evidence suggests that the similarities between AD and DS are not due solely to over- expression of the APP gene located on chromosome 21. Although APP is over-expressed in some tissues from DS patents, substantial variability exists in the β-amyloid deposition within DS patients from the same age groups[105]. Not all DS patients develop AD-type dementia although β-amyloid deposits are found in the brain at autopsy. In addition AD-type neuropathology is detectable in the brains of DS patients by age 35 while the average of onset of clinical dementia is between 51 and 54 years (range 39-69years). These findings suggest other factors may be contributing to the severity and timing of β-amyloid deposition and that the accumulation of β-amyloid is not enough to develop AD-type dementia.

A study by Avramopoulos in 1996 proposed that since AD and DS have many similarities and ApoE ε4 is associated with AD, perhaps ApoE ε4 was also associated with DS[110]. The authors found that ApoE ε4 was significantly more common among young mothers of DS children. This correlation was specific to MII errors. They theorized that ApoE ε4 may predispose an individual to chromosome non-disjunction and potentially to trisomy 21 mosaicism and AD. Individuals with AD have been found to

have increased numbers of cells trisomic for chromosome 21 in their circulation[111]. In 1996, Potter suggested that the correlation is specific to MII because MII most closely resembles mitosis[112]. During MII and mitosis, centromeres divide and separate and correct chromosome segregation depends on maintaining a balanced bi-directional tension on each pair of kinetochores.

Summary of Part Two:

- ApoE is a glycoprotein involved in cholesterol transport and metabolism.
- ApoE genotype has been associated with many health conditions including, but not limited to, longevity, cholesterol level, coronary heart disease, stroke, gallstones, hip fractures among the elderly, retinitis pigmentosa, and AD.
- Age and ApoE genotype are the strongest established important risk factors for the development of AD.
- AD and DS have similar pathologic, clinical, and epidemiological findings which support the existence of a underlying genetic link.
- AD and DS have been associated in a recent study with Apolipoprotein E ε4.

CHAPTER 3: LESSONS FROM A FEASIBILITY STUDY: *An Investigation of Apolipoprotein E as an hereditary risk factor for non-disjunction and Alzheimer's disease.*

Rationale and Specific Aims

There are two main reasons to study a potential link between ApoE ε4 and chromosomal trisomy. The first is to increase the understanding of the mechanisms leading to trisomy and the potential for preventive measures. The second reason to is to look for risk factors to provide more precise genetic screening and counseling regarding the risks of trisomy in offspring. We hypothesized that the genetic factor(s) which have been shown to link AD and DS actually link AD and MII non-disjunction in general. Therefore, the association with ApoE e4 should apply to all types of chromosomal trisomy. Previous studies have failed to combine epidemiologic data about family history of disease, ApoE genotype, and data about non-disjunctional stage of error to describe the risk factors for trisomy. We propose that the information provided by ApoE genotype and family history of AD could be used in a general population to augment present screening protocols for trisomy.

To answer these two questions we would need to build on the previous research by Schupf and Avramopoulos who found associations between AD and DS. We would expand the ApoE hypothesis to MII non-disjuntion in general in a study that would incorporate the following specific hypotheses and aims:

> Hypothesis 1 : The prevalence of AD is increased in trisomic families.
> Specific Aim 1: We will compare the prevalence of AD in the families of women with a history of trisomy (cases) and with no history of trisomy (controls).

Hypothesis 2: ApoE ε4 is more prevalent in young mothers of trisomy pregnancies than in controls.
Specific Aim 2: We will compare the ε4 gene frequency in case mothers and controls under 35.

Hypothesis 3: The association with the ApoE ε4 allele is specific to maternal MII errors.
Specific Aim 3: We will compare the frequency of the ApoE ε4 allele in each group (Mat MI & MII, Pat MI & MII)

Feasibility Study Goals

Prior to launching a large-scale study we chose to do a feasibility study with the following goals:

1) Develop an interview which could be used to collect demographic and health information from case (trisomy positive pregnancy) and control (trisomy negative pregnancy) women

2) Field test a sample collection protocol and laboratory assay that could identify Apolipoprotein E genotype

3) Test the potential of using the MSU Prenatal Screening Program as a population of cases and controls.

4) Identify potential strengths and weaknesses with our case-control study design

Study Design

Our feasibility work was a case-control study. For this initial study, a case-control design best suits our hypothesis because our exposure variable is a genetic risk factor and trisomy is a rare disease. Our case definition was women who: 1)had experienced a karyotype confirmed trisomic pregnancy and 2)were identified by the MSU Genetics program during the years 1995 to 1997. Our controls were matched to cases on ethnicity, and frequency matched on age category. We chose to match by ethnicity to control for potential confounding and maternal age in order to have sufficient age-specific strata for our analysis. We did not have any non-Caucasian controls in our

feasibility study. Controls consisted of women who had not experienced a trisomic pregnancy and were ascertained by the MSU Prenatal Screening Program during the years 1995 to 1997. Third variables that we planned on including in our analysis were: parity, trisomy type, parent of error, and cell division of error. Parity is important since the more pregnancies a woman has, the more opportunities she has to have a trisomic pregnancy. In addition, higher parity was found to be a risk factor for DS in a recent study[113]. The authors found a 15% higher risk for DS with both age and parity considered above the age related risk. However, they did not take into consideration that higher parity is associated with a negative attitude about termination.

Study Sample

Potential cases for the Trisomy Project were identified through three sources; the MSU Prenatal Screening Program, the MSU Cytogenetics Laboratory, and the MSU Genetics Clinics (see figure three).

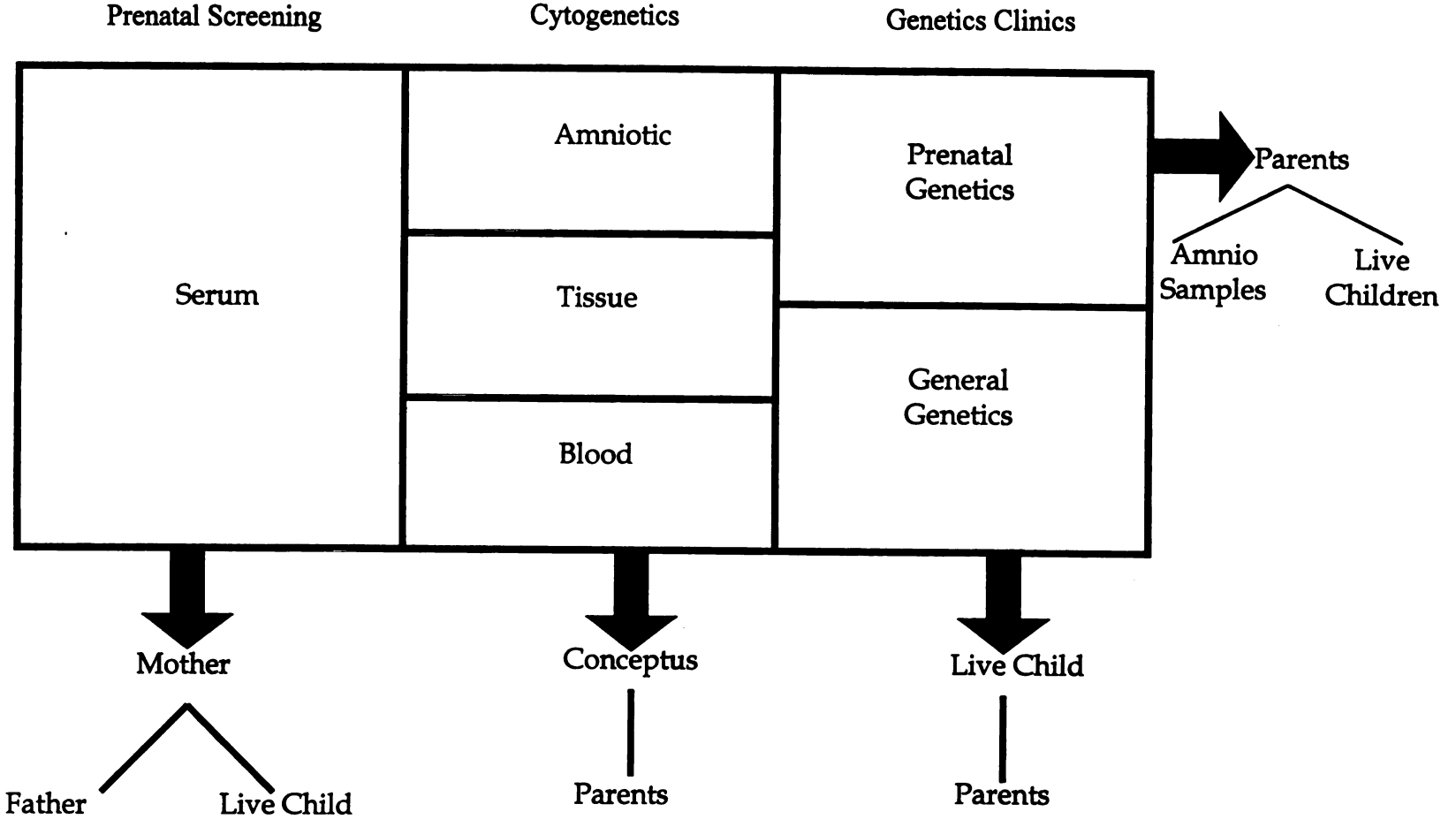
Our case population from the MSU Prenatal Screening Program consisted of mothers who were screened for maternal alpha fetoprotein (AFP) during the years 1995 to 1997 and who indicated a history of a prior trisomic pregnancy on the test requisition form, had a positive screen for trisomy which was not a false positive, or a negative screen which was later found upon follow up to be a false negative. For these cases, the mother's, father's, and (if living) child's DNA had to be self-sampled and mailed to MSU laboratories.

The MSU Cytogenetics Laboratory case population consisted of abnormal pregnancy material sent to the laboratory for testing during the years 1995 to 1997. For these cases, the fetal/child DNA had already been collected by the lab, and the parent

DNA samples were self-sampled and delivered by mail. Three types of biologic samples were available through the MSU Cytogenetics Laboratory, amniocentesis fluid, abortus tissue, and peripheral blood. Amniocentesis is performed primarily for reasons of advanced maternal age, prior trisomic pregnancies, other family history of chromosomal abnormalities, unusual findings on ultrasound, or elevated AFP or DS risk or triple test. Blood is drawn from a live birth to perform chromosome analysis to rule out the diagnosis of chromosomal trisomy if a child's features are suggestive of trisomy. Karyotyping is performed on abortus tissue primarily when there is a history of multiple spontaneous abortions. Compared with our other sources for cases and controls, the MSU Cytogenetics population is the least representative of the general population of pregnant women.

The MSU Genetics Clinics' cases consisted of families who came to MSU for prenatal or informational counseling during the years 1995 to 1997. For these cases, DNA from the mother, father, and fetus/child was collected at the time of counseling. The majority of these patients overlapped with the MSU Cytogenetics program.

Figure 3: Origin of Cases



The controls were selected from the MSU Prenatal Screening database during the years 1995 to 1997. Of the populations we used for case ascertainment, the Prenatal Screening Program most closely represents the general population of pregnant women. Controls were frequency matched to cases based on maternal age at their estimated date of confinement (EDC). Case ages were calculated at the time of karyotype analysis for abortus tissue, at EDC for amniocentesis samples, and at delivery of child for livebirth cases. Maternal age was divided into six categories: below 19, 20 to 24, 25 to 29, 30 to 34, 35 to 39, and above 40. The ethnicity and age of the case mothers was unknown until after the interview for cases from MSU Cytogenetics and MSU Genetics' clinics. The ethnicity and age of the mother was known prior to interviewing for the control mothers.

Protocol for Contact of Cases and Controls

Case women were selected for participation in chronological order beginning with cases ascertained in 1995. One of three letters was generated for each woman based on the method of ascertainment. Letters were mailed to the most recent address available. Control women were randomly selected from all women screened during the years 1995 to 1997 by the MSU Prenatal Screening database and their letters were mailed to the address on the laboratory requisition form.

Letters sent to eligible women stated that "we are conducting a study on the causes of chromosomal abnormalities." They were informed that at the time of the interview we would be requesting a DNA sample. No mention of Alzheimer's disease or our hypothesis was included in the letters. Women were given a letter to return which had two options: to request not to be in the study, or to inform us of their new telephone

number. For the case women we listed the most recent telephone number available. For the control women, we used listed telephone numbers available at www.switchboard.com. Women who wished to participate in the study and for whom we had updated telephone information were not required to return the letter. We later included an option of returning the letter with an indication of preferred times to be contacted for the interview. We also included a 1-800 phone number for them to call to update a telephone number or to ask questions. The letter instructed women that we would be calling them in two weeks.

Interviewing Methods

Four different interviewers were used. Interviewers were undergraduate students in their final year of the MSU zoology bachelors in science four-year program. Interviewers were not masked to the hypothesis and practiced administering the interview with volunteer women not in our study population.

Two weeks after mailing the letter to our study women, our interviewers began contacting all women who had not refused to participate. Our twelve-page interview was identical for cases and controls and took approximately forty-five minutes to administer. Each woman was asked at the beginning of the interview if she had a pregnancy with a chromosomal abnormality. Control women who stated that they did have a pregnancy with a chromosomal abnormality were allowed to become part of the case population. We allowed this cross-over because misclassification of women into case and control categories would cause us to calculate the genetic risk incorrectly.

Interview Content

The interview was broken down into five sections (see appendix). Section I collected basic demographic information about age, race, education, and occupation. Section II collected health information about the woman's biological mother, father, maternal grandparents, and paternal grandparents. We included any conditions that have been associated with Apolipoprotein E in the literature, as well as conditions that may be associated with premature aging. We asked about age and cause of death for each family member as well as a list of medical conditions that included high blood pressure, stroke, heart attack, high cholesterol, diabetes, thyroid disease, Parkinson's disease, Alzheimer's disease or senile dementia, and premature graying. For the female relatives we asked about age of menopause. In Section II we recorded information about family history of chromosomal abnormalities including trisomy. Finally, we asked about history and Alzheimer's disease or dementia among biological great grandparents.

Section III asked the woman to answer the same list of health questions for herself with the addition of a question about what age menstruation began and if she ever had one ovary removed. Section IV collected details about reproductive background including history of fertility problems, use of hormonal birth control methods, and pregnancies with chromosomal abnormalities.

DNA Sample Collection

Following the interview, we requested a DNA sample from the mother, father, and, if living, trisomic child. When a subject agreed to donate a sample, a collection kit was sent through the mail. Each kit included two cytology brushes for each participant, an informed consent sheet, an instruction sheet for collecting the samples, a postage paid return envelope, and the 1-800 number to call with any questions. Participants were

instructed that the results of the testing would be confidential and not available to them. Due to limited resources, women who did not return their collection kits were contacted a maximum of one time to remind them about the study. The reason for not returning the collection kits was not recorded. Anecdotally a number of women noted that they were too busy.

APOE Laboratory Assay Methods

Participants were instructed to collect cheek brush samples by "vigorously rubbing" a sterile cytology brush against the inside of each cheek. Upon receiving the samples, the brushes were prepped immediately or stored at 4°C for up to two days. The two brushes were placed into a single tube containing 400µls of 50mM NaOH. The tubes was heated to 95°C for ten minutes and immediately placed on ice for ten minutes. The brushes were discarded and 40µls of Tris base pH 8.0 was added to each tube and the samples were mixed. The prepared DNA was stored at -20°C.

DNA was prepared from cultures of abortus tissue and cultured amniocytes following a standard with Gentra® DNA kit reagents. Two coverslips were used for amniocentesis cultures and one flask was used for abortus tissue cultures. Coverslips/flasks were rinsed in phosphate buffered saline. The cultured cells were trypsinized and transferred into a 10-ml tube. The cells were centrifuged at 2.5 K for ten minutes. The supernatant was discarded and the cell pellet was transferred to a 1.5 ml microfuge tube with 300 µl Cell Lysis Solution. After pipetting the solution up and down a few times, 12 µl 1 M DTT and 3 µl 10 mg/ml proteinase K were added to each tube. The cells were incubated at 55°C overnight in a water bath. After cooling to room temperature, 200 µl of Protein Precipitation Solution was added and the mixture was

vortexed vigorously for 30 seconds. The mixture was iced for five minutes and microfuged at 12K RPM for three minutes. The supernatant was transferred to a new tube and 300 µl isopropanol was added. The tubes were mixed by inversion and microfuged at 12K RPM for one minute. The supernatant was discarded and the pellet was dried. The dried pellet was dissolved in 250µl 50 mM NaOH. The mixture was heated to 95°C for 10 minutes and 25µl of 1M Tris pH 8.0 was added to each tube. The prepared DNA was stored at -20°C.

White cells were isolated from blood samples within one month of the initial draw date. One hundred microliters of blood was added to 500µl of cell lysis buffer. The solution was vortexed and microfuged at 12K RPM for 30 seconds. The supernatant was discarded and 100µl red cell lysis buffer was added to the pellet. The solution was vortexed and heated at 95°C for 10 minutes. The solution was placed on ice for 10 minutes and 10µl 1 M Tris pH 8.0 was added. The prepared DNA was stored at -20°C.

The DNA was amplified by polymerase chain reaction in a DNA Thermal Cycler (Perkin Elmer Cetus model 9600) using oligonucleotide primers. The forward primer sequence was 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and the reverse primer sequence was 5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'. Each amplification reaction contained: 5µl prepared DNA, 25pmol of each primer, 2.5mmol magnesium chloride, 10% dimethyl sulfoxide, 0.5mmol dinucleotide triphosphates, Perkin Elmer 10x PCR buffer, and 0.625 units Taq polymerase in a final volume of 25 µl. Each amplification reaction was subjected to an initial denaturing period of 95°C for 5 minutes. The samples were amplified for 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 70°C for 30 seconds. The products were subjected to a final extension period of five

minutes at 72°C. Following the amplification, the products were digested with 10 U of Hha I at 37°C for at least three hours. The digested products were separated on a 12% non-denaturing acrylamide gel at 30 mA current for two hours. The resulting gel was stained in ethidium bromide and viewed on an ultraviolet light box. The separated bands were photographed with a Polaroid camera.

Results Goal 1

To develop an interview which could be used to collect demographic and health information about cases and controls

A total of 26 Caucasian case women and 56 Caucasian control women were interviewed (Table 6). The frequency matching by age was not as close as desired. Three case women of non-Caucasian ethnic backgrounds were interviewed. Due to the small number of non-Caucasian case women in our feasibility sample, we did not attempt to find frequency matched age controls for these women. The majority of cases came from the MSU Cytogenetic Laboratory (Table 7).

Table 7: Number of Caucasian woman interviewed by age category

Mother's Age	19 & Under	20-24	25-29	30-34	35-39	40& Over	Total
Cases	2	1	6	6	6	5	26
	(7%)	(3%)	(21%)	(21%)	(21%)	(17%)	
Controls	5	4	8	17	13	9	56
	(8%)	(7%)	(13%)	(28%)	(21%)	(15%)	

Table 8: Ascertainment of Caucasian Cases

Method of Ascertainment	Number of Cases	DNA Available
Cytogenetics	21	Fetus and Parents
Follow up of Positive AFP	5	Living Child and Parents
Genetic clinics	2	Living Child and Parents
Prior pregnancy indicated on AFP Test Requisition	0	Living Child and Parents

Our hypothesis that the prevalence of AD is increased in trisomic families in a larger study hinges on the collection of reliable data about AD among case and control family members. The presence of AD is a censored variable because some family members will die before they have the opportunity to express the disease characteristics. In order to properly analyze this censored variable, it is necessary to have data about age of death for family members. Therefore, one of the important results of our feasibility study is the analysis of the quality of data that was collected for age of death. Table 8 shows the number of women who reported that a relative had died and were able to report an estimated age of death. The majority of women were able to report an age of death for the relatives that we asked about (84%). Woman reported information about their parents more completely than about their grandparents. One hundred percent of women who stated that their parent had died were able to estimate the age of death compared to 83% of women who stated that their grandparent had died. The number of women in each category is too small to test whether maternal age is correlated with knowledge about age of parents or grandparents.

The amount of information that women are able to share about a diagnosis of Alzheimer's disease among their relatives is also key to our hypothesis (Table 9). Table 9 presents data for the women who responded " I don't know" to the question about a diagnosis of AD. Once again, the women were more able to report information about their parents than their grandparents. Fifteen percent of women were unable to report about AD among their parents compared to 40% percent of women who were able to report about AD among their grandparents. None of the women reported that they had experienced symptoms of dementia themselves. The information about great-

grandparents cannot be compared to the information about parents and grandparents because the question was asked in a different way.

Table 9: Number of Women Able to Estimate Relatives' Age of Death Compared to the Number of Women who stated that the relative had died

	Mother's Age	19 & Under	20-24	25-29	30-34	35-39	40 & Above
Mom	Case	-	-	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
	Control	-	-	-	1/1 (100%)	1/1 (100%)	3/3 (100%)
Dad	**Case**	**-**	**-**	**1/1 (100%)**	**1/1 (100%)**	**4/4 (100%)**	**-**
	Control	**-**	**-**	**-**	**3/3 (100%)**	**4/4 (100%)**	**2/2 (100%)**
Mother's Mother	Case	-	-	2/3 (67%)	4/4 (100%)	3/5 (60%)	5/5 (100%)
	Control	2/2 (100%)	-	3/4 (75%)	10/12 (83%) 1=d.k.	11/12 (92%)	8/8 (100%)
Mother's Father	**Case**	**1/1 (100%) 3=d.k.**	**1/1 (100%)**	**3/4 (75%)**	**5/6 (83%)**	**4/6 (67%)**	**5/5 (100%)**
	Control	**3/3 (100%)**	**1/3 (33%)**	**5/6 (83%)**	**10/11 (91%) 3=d.k.**	**11/12 (92%)**	**8/9 (89%)**
Father's Mother	Case	-	1/1 (100%)	4/5 (80%)	6/6 (100%)	2/3 (67%) 1=d.k	4/5 (80%)
	Control	0/2 (0%)	-	4/5 (80%)	12/14 (86%) 3=d.k.	11/11 (100%) 1=d.k.	7/8 (88%)
Father's Father	**Case**	**0/1 (0%)**	**0/1 (0%)**	**2/5 (40%)**	**5/5 (100%)**	**2/5 (40%) 1=d.k.**	**4/5 (80%)**
	Control	**3/4 (75%)**	**0/1 (0%)**	**6/7 (86%)**	**11/14 (79%) 3=d.k.**	**9/10 (90%) 2=d.k.**	**7/8 (88%) 1=d.k.**

d.k.= Woman does not know if relative is still living

Table 10: Number of Women Unable to Report about Alzheimer's Disease Among Their Parents and Grandparents

	Mother's Age	19 & Under	20-24	25-29	30-34	35-39	40 & Above
Mom	Case	-	-	-	-	-	-
	Control	-	-	-	-	-	1/9 (11%)
Dad	Case	-	-	2/6 (33%)	-	1/6 (17%)	-
	Control	1/5 (20%)	-	-	2/17 (12%)	1/13 (8%)	1/9 (11%)
Mother's Mother	Case	1/2 (50%)	-	1/6 (17%)	1/6 (17%)	4/6 (67%)	1/5 (20%)
	Control	-	-	1/8 (12%)	3/17 (18%)	2/13 (15%)	8/9 (89%)
Mother's Father	Case	2/5 (40%)	-	3/6 (50%)	2/6 (33%)	4/6 (67%)	1/5 (20%)
	Control	1/5 (20%)	1/4 (25%)	3/8 (38%)	8/17 (47%)	4/13 (31%)	4/9 (44%)
Father's Mother	Case	-	1/1 (100%)	3/6 (50%)	3/6 (50%)	3/6 (50%)	1/5 (20%)
	Control	2/5 (40%)	1/4 (25%)	1/8 (12%)	7/17 (41%)	3/13 (23%)	3/9 (33%)
Father's Father	Case	1/5 (20%)	1/1 (100%)	3/6 (50%)	3/6 (50%)	5/6 (83%)	3/5 (60%)
	Control	2/5 (40%)	3/4 (75%)	3/8 (38%)	8/17 (47%)	6/13 (46%)	6/9 (67%)

Results Goal 2

Field test a sample collection protocol and laboratory assay which could identify Apolipoprotein E genotype

A total of 24 DNA buccal swab collection kits were mailed out to case women. Two case women declined to give a DNA sample after the telephone interview and

before their kit had been sent. Two case women declined to give a DNA sample upon receiving their kit in the mail. Fourteen kits were returned from case women for a return rate of 64% (14/22). Fifty-five DNA collection kits were mailed out to control women. One control woman declined to give a DNA sample after completing the interview. Twenty collection kits were returned for a return rate of 37% (20/54) for the control women.

Four of the 14 samples collected from the case mothers failed to amplify under our PCR conditions. An additional extraction procedure using phenol was attempted to improve the quality of DNA. This attempt was unsuccessful, 4/4 did not amplify after the additional extraction procedure.

Gene frequencies in our feasibility sample are presented in table 10. Though our feasibility study was not designed to test the hypothesis linking trisomy to the ApoE e4 allele, we did calculate the sample size that would be required to test the hypothesis in a larger study. In order to detect a two-fold difference in the ε4 allele frequency (30% vs 15% as reported in Avramopoulos) between women under 30 with maternal MII trisomy and women under 30 with no trisomy at an alpha equal to 0.05 with 80% power, 86 case women under thirty with maternal MII errors would be needed and 344 control women over 30 would be needed. The total population that would be needed would depend on the percentage of errors that are maternal MII in nature. The gene frequencies we found using our laboratory assay among the case and control women for ε2, ε3,and ε4 are presented in table 10.

Table 11: Gene Frequencies for Caucasian Mothers

	ε2	ε3	ε4
Case Mothers	0.10	0.80	0.10
Control Mothers	0.11	0.78	0.11

Results Goal 3

To test the feasibility of our study using the MSU prenatal screening program as a population of cases and controls.

During our two years of case ascertainment we were able to identify 255 women who fit our case definition and could potentially have been included in our feasibility study. In order to test our hypothesis 86 case women under thirty would be needed. The rate of maternal MII errors ranges for different chromosomes. For example, the published rate for trisomy 16 is 0%, trisomy 21 is 13% and trisomy 18 is 62%. The total number of cases needed would depend upon the average rate of meiosis II non-disjunction in our case population. The majority of our potential cases came through the Cytogenetic laboratory (Table 12). Our potential cases include examples of trisomy 4, 6, 7, 9, 13, 15, 16, 17, 18, 21, and 22. Our control population contained enough women to randomly sample and still have a 4:1 ratio.

Table 12: Ascertainment of Potential Cases

Ascertainment Method	Number of Potential Cases
Cytogenetics laboratory	233
AFP Test - Positive Screen	81
AFP – Prior History on Test Requistion	5
AFP – Follow-up Negative Screen	1
Genetics Clinic	17
Prenatal Clinic	4
TOTAL	341

Cases in our study were identified in a retrospective manner. We attempted to contact by mail 57 cases for our study. One case woman refused to be in the study by a

postcard, 3 woman refused over the telephone, and we never made contact with 24 potential case woman. Women were classified as never made contact if we mailed a letter to an address but we were unable to contact a person at a telephone number due to a missing or non-valid phone number. We could not verify that the address that we mailed the letter to was valid, therefore we do not know if the woman ever received an invitation into the study. Twenty-nine case women were interviewed for an enrollment of 51% and a direct refusal rate of 7%.

We attempted to contact by mail 128 controls. Nine women refused to be in the study by post card, 9 women refused to be in the study over the phone, and 61 women were interviewed. Our enrollment rate for controls was 48% and our direct refusal rate was 14%. The control women in the youngest two age groups were the most difficult to contact (Table 12).

Table 13: Control Contact Results by Age Groups

Mother's Age	19 & below	20-24	25-29	30-34	35-39	40 & above
Never Made Contact	35 (81%)	19 (79%)	13 (50%)	7 (23%)	17 (57%)	14 (54%)
Refusal	3 (7%)	1 (4%)	5 (19%)	6 (20%)	0 (0%)	3 (12%)
Interviewed	5 (12%)	4 (16%)	8 (31%)	17 (57%)	13 (43%)	9 (35%)
Total	43	24	26	30	30	26

Results Goal 4

Identify potential strengths and weaknesses with our case-control study design

Our methodology would need a number of improvements in order to repeat this study on a larger scale. First, we spent a large amount of resources on finding women. A number of women had changed addresses and telephone numbers. We attempted to contact women by mail up to two years after the index pregnancy. One way to resolve

this problem would be to contact women and enroll them on a prospective basis, but this would greatly increase the timeframe of the study. The most mobile and difficult group of women to locate were the youngest age category (under 20 years). Unfortunately, the younger women are crucial to support our hypothesis. In the future, extra recruiting resources would have to be spent on targeting these women. Our controls were more labor intensive to contact than our cases. The primary reason was that we did not have their telephone numbers recorded in the database. The MSU Prenatal Screening Program has subsequently started collecting and recording the telephone numbers of women they screen. This addition could increase our enrollment rates that were very low overall.

Once we were able to contact women on the telephone, we had high participation rates for the interview. In addition, the majority of women 94% stated they were willing to donate a DNA sample. However, 64% of the case women and 37% of the control women mailed back their collection kits. Maybe improvements in our strategies to re-contact women would improve our collection kit retrieval rates. It is anticipated that allocating more personnel time and resources towards this process would assist. The case women may have felt more motivated to complete their participation in the study because of their personal experience with a trisomic pregnancy. Unfortunately, the return of the DNA kit was crucial to two of our three specific aims. In the future we could restrict the interview to women who are willing to donate a sample first in order to save on resources.

Some of the returned DNA samples failed to amplify. This could be due to delays in mailing samples. Some women indicated that they had let their sample sit before mailing it. We could modify the instructions with the DNA collection kit to suggest that

women mail their sample immediately after collecting it. A second option is to pilot other non-invasive DNA collection methods. Thirdly, we could call and go collect the DNA sample in-person. Due to our limited resources, we were unable to perform the DNA microsatellite analysis that could be used to identify the cell division and parent of error. Development of protocols for each individual chromosome is a difficult and time-consuming process. In a larger study, it may be more cost-efficient to contract an outside individual to analyze cell division and parent of error.

A few other minor improvements could be made to our study protocols. We could attempt to frequency match the cases and controls on interviewer so that each interviewer interviews the same percentage of cases and controls in each age category. Also, we could set limits on the number of calls made to an individual woman and ask that women identify people in their household that we can leave messages with regarding the study. Identifying a household contact person allows us to leave messages without violating an individual's confidentiality while still assisting us with our follow-up data collection calls.

Table 14: Summary of suggested changes in Protocol

Methodological Difficulty	Suggestive Corrective Action(s)
Difficulty in finding participants	Contact on a prospective basis
Low numbers of young women interviewed	Target young women
Low DNA kit return rate	1)Offer money 2)Only interview patients with samples 3)Go to home and collect sample
DNA sample failure	1)Suggest immediate mailing 2)Pilot other non-invasive sample collection methods 3)Go to home and collect sample
Limited resources for microsatellite analysis	Contract outside individual to analyze cell division and parent of error.
Cases and Controls not matched on interviewer	Match on interviewer
Interviewers not blinded to hypotheses	Blind interviewers to hypotheses
Numerous calls made to few women	Limit number of calls made to individual
Unable to leave telephone message with household members	Get women's permission on consent form to speak with household members

Our methodology had a number of strengths. Our study population had an excess number of women to sample from. We found that our letter sent out to women initially was successful at recruiting women into the study. Both the 1-800 telephone number and the returned letter were used by women as ways of contacting us to update us on their telephone number. In general, we found that the notification by mail two weeks prior to telephoning allowed women time to contact us by telephone or mail if they wished to decline participation. Also, the letter adequately introduced the study and motivated women to participate. Women were familiar with study when we telephoned. We had a low refusal rate for the interview that suggests that this format is very acceptable to our study population. The non-invasive method of DNA collection was easily exchanged through the mail and successfully used with young children.

Table 15: Summary of Methodology Strengths

- Study population
- Initial recruitment letter
- 1-800 phone number
- Letter for women to return
- Two week waiting period
- Non-invasive method of DNA collection
- Telephone interview format

Discussion

The number of participants in our study is small which limits our ability to make any strong conclusions about data trends. The majority of women reported information about age at death of relatives (84%). In addition women were better at reporting information about their parents (100%) than their grandparents (83%). Since we were relying on self-reported data, we would need to validate this information by getting death certificates to comment on its accuracy.

There was a large amount of missing data for the AD questions (36%), especially for the grandparents (40%) as compared to the parents (15%). One possibility is that the women who do not know this information about their grandparents have the greatest age gap differences between their grandparents and them. If we collected information on this age gap (via birth date of the mother and grandparents or estimated age difference) we could test this hypothesis. An additional dilemma is that younger women are at the center of our hypothesis, but their parents could be too young to reach the peak AD age. A study could expand the definition of AD to include symptoms of AD when the clinical diagnosis was unknown to the interviewee. One possible symptomatic definition would be " Did your relative ever experience a slow progressive loss of memory, cognitive

abilities, and functioning on intellectual tasks?" This definition could include non-AD conditions resulting from other causes of dementia. In order to keep the less reliable symptomatic diagnosis separate from the physician diagnosed cases, we could classify cases into categories of definite – self-report of physician diagnosed AD confirmed by medical record, probable – self-report of AD unable to be confirmed by medical record, and suspicious – report of AD-like symptoms.

Some the buccal brush DNA samples that were collected failed to amplify. Once again these samples were crucial to our hypothesis. A recent study by Garcia-Closas found that a single mouthwash sample collection resulted in higher yields and better quality DNA than two cytobrush samples (in press). This sample collection procedure is feasible for the adults in our study. However, the young children in our study would not be able to follow the mouthwash sample collection protocol. The most feasible solution for the children is to continue to use the buccal brush collection procedure with an increased emphasis in our instruction materials to participants on timely return of the samples. In addition, modifying our extraction techniques or number of PCR cycles for the buccal samples may be necessary.

Enrollment rates were 51 % for cases and 48% for controls of the women we sent a mailing. It is difficult to classify the number of women who were lost to study because we have no way of knowing what percentage of women actually got the letter that we sent them. Women in the youngest age category were most difficult to contact. Our contact rates could be improved if women were contacted on a more prospective basis. In addition, the recent addition of collecting women's telephone number by MSU

Prenatal Screening Program could assist in the contact of controls. Motivation for involvement in the study was not recorded.

We would have liked to match our controls to cases based on ethnicity and age. For this feasibility study we were only able to include Caucasian controls. In a larger study it would be feasible to sample controls from different ethnic background and match on ethnicity as we would have liked. In addition, our recruitment strategies did not efficiently recruit case and control women into the corresponding age categories. Based on this and other problems this feasibility study aided in determining resources needed for a larger study. Specifically resources would need to be included to spend time re-contacting women for sample collection, over-sampling the youngest population, and refining the laboratory collection techniques.

Major Feasibility Study Accomplishments

There were four major accomplishments made by our feasibility study. We identified a collection of potential cases (Table 12), archived trisomy DNA samples, refined our interview and laboratory instruments through field-testing, and developed and debugged a Microsoft-Access database capable of storing our interview data.

APPENDIX

Appendix: Interview Content

SECTION I

The first set of questions ask some background information.

1. **What is your date of birth?**

_____	_____	19____
Month	Day	Year

2. **What is your race or ethnic background?**

White/Caucasian 1
Black/African-American.....2
Asian..... 3
Hispanic..... 4
Other (specify)..... 5

3. **What is the highest grade you have finished in school?**

Elementary	1	2	3	4	5	6	7	8
High School	9	10	11	12				
College	13	14	15	16				
Post College	17+							
No formal schooling	0							
GED								

4. **What is your usual occupation?** *(include home maker, student)*

_____

SECTION II

Now, I would like to ask you some questions about your family. Please answer the questions in this section as they relate to your biological relatives. If you are unsure of your answer to any question, please feel free to respond "I don't know".

First your biological parents:

5. **Is your (biological) mother alive?**
   Yes___ No___ Don't know___
   If Yes or Don't know, go to question 8.
   If No, go to question 6.

6. **At what age did she die?** ____

7. **What was the cause of her death?** _____

8. **Now, I would like to read you a list of medical conditions. Please indicate if your mother has/had been physician diagnosed with any of the following conditions. Respond with yes, no, or I don't know.**

a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
   if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
   if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
   Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
   ..... Yes___ No___ Don't know___

9. **At approximately what age did she reach menopause?** _____ Don't Know_____

If known, **Was this the result of a hysterectomy?** Yes_____ No_____ Don't Know_____

10. **Is your (biological) father alive?**
Yes___ No___ Don't know___
If Yes or Don't know, go to question 13.
If No, go to question 11.

11. **At what age did he die?** ____

12. **What was the cause of death?** _____

13. **I will again read you the same list of medical conditions. Please indicate if your father has/had been diagnosed with any of the following:**

a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
..... Yes___ No___ Don't know___

14. **Now I would like to ask you about your biological grandparents, starting with your mother's parents.**
Is your mother's mother alive?
Yes___ No___ Don't know___

15. *If yes*, **How old is she?** ____
If no, **At what age did she die?** ____

16. **What was the cause of her death?** _____

17. **Has/Had she been diagnosed with any of the following conditions:**

a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
..... Yes___ No___ Don't know___

18. **At approximately what age did she reach menopause**_____ Don't know_____

If known, **Was this the result of a hysterectomy?** Yes_____ No_____ Don't know_____

19. **Now your maternal grandfather:**
Is your mother's father alive?
Yes___ No___ Don't know___

20. *If yes,* **How old is he?** _____
If no, **At what age did he die?** ____

21. **What was the cause of his death?** _____

22. **Has/Had he been diagnosed with any of the following conditions:**

a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
..... Yes___ No___ Don't know___

23. **Now I will ask you about your father's parents:**
Is your father's mother alive?
Yes___ No___ Don't know___

24. *If Yes,* **How old is she?** ____
If No, **At what age did she die?** ____

25. **What was the cause of her death?** _____

26. **Has/Had she been diagnosed with any of the following conditions:**
a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
..... Yes___ No___ Don't know___

27. **At approximately what age did she reach menopause?** _____ Don't know_____

Was this the result of a hysterectomy? Yes___ No___ Don't know____

28. **Now your paternal grandfather. Is your father's father alive?**
Yes___ No___ Don't know___

29. *If yes,* **How old is he?** ____
If no, **At what age did he die?** ____

30. **What was the cause of his death?** _____

31. **Does/Did he have any of the following conditions:**

a. **High blood pressure** ..... Yes___ No___ Don't know___
b. **Stroke** ..... Yes___ No___ Don't know___
c. **Heart attack** ..... Yes___ No___ Don't know___
d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___
e. **Diabetes** ..... Yes___ No___ Don't know___
if Yes: **Age of onset?** _____yrs Don't know_____
f. **Thyroid disease** ..... Yes___ No___ Don't know___
if Yes: **Overactive?**___**Underactive?**___ Don't know___
g. **Parkinson's disease** ..... Yes___ No___ Don't know___
h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___
i.**Cancer** .....Yes___ No___ Don't know___
Kind_____
j. **Premature graying (which is a significant amount of gray hair before age 25)**
..... Yes___ No___ Don't know___

32. **Now I'm going to ask you about other members of your family; your brothers, sisters, cousins, uncles, aunts, nieces and nephews. Is there anyone you know of in your biological family who has had a child or a pregnancy with:**

a.**Trisomy 21 or Down syndrome**.....Yes___ No___ Don't know___
b.**Trisomy 18 or Edward syndrome**.....Yes___ No___ Don't know___
c.**Trisomy 13 or Patau syndrome**.....Yes___ No___ Don't know___
d. **Trisomy 16**.....Yes___ No___ Don't know___
e. **Any other chromosomal trisomy**.....Yes___ No___ Don't know___
f. **Another chromosomal abnormality**.....Yes___No___Don't know___

If No or Don't know, go to Question 34.
If Yes go to Question 33.

33. **Please tell me how that person (s) with the chromosome abnormality is (was) related to you?**

_____

34. **Do you know of any twins in your biological family?** Yes____ No____
If No, go to Question 37.
If Yes, go to Question 35 .

35. **Identical Twins**____ **Fraternal/unlike Twins**____ Don't know____

36. **Please tell me how they are related to you**_____

37. Did any of your biological great grandparents develop Alzheimer's disease or dementia?

Yes___ No___ Don't know_____

If No, go to question 39.

If Yes, go to Question 38.

38. How was that great grandparent related you?_____

SECTION III

39. **Now I would like to ask some questions about your own health.**

When you were not pregnant, have you ever been treated for, or been told you have, any of the following:

a. **High blood pressure** ..... Yes___ No___ Don't know___

b. **Stroke** ..... Yes___ No___ Don't know___

c. **Heart attack** ..... Yes___ No___ Don't know___

d. **High cholesterol level (over 240)** ..... Yes___ No___ Don't know___

e. **Diabetes** ..... Yes___ No___ Don't know___

if Yes: **Age of onset?** _____yrs Don't know_____

f. **Thyroid disease** ..... Yes___ No___ Don't know___

if Yes: **Overactive?**___**Underactive?**___ Don't know___

g. **Parkinson's disease** ..... Yes___ No___ Don't know___

h. **Alzheimer's disease or senile dementia**..Yes___No___ Don't know___

i.**Cancer** .....Yes___ No___ Don't know___

Kind_____

j. **Premature graying (which is a significant amount of gray hair before age 25)**

..... Yes___ No___ Don't know___

40. **At what age did you begin menstruation?** _____

41. **Have you reached menopause?** Yes___ No___

If No, go to Question 42.

If Yes,. **At what age did your periods stop?** _____

Was this the result of hysterectomy?____

42. **Prior to your trisomic pregnancy, have you had surgery to remove either of your ovaries?**

Yes____ No____ Both____

If Yes, **Why?** _____

When? _____

SECTION IV

Now I would like to ask you some questions about your pregnancies.

43. **How many times have you been pregnant including any losses?**

1 2 3 4 5 6 7 8 9 10 11 12

44. **When you were trying to get pregnant, did it (ever) take more than three months?**

Yes___ No___ Doesn't apply___

If No or Doesn't apply, go to Question 46.

If Yes, go to Question 44.

45. **More than six months?**

Yes___ No___

If No, go to Question 46.

If Yes, go to Question 45.

46. **Have you ever had a physician prescribe medication to help you get pregnant?**

Yes___ No___

47. **Prior to your trisomic pregnancy, Have you ever used a hormonal contraceptive method, including birth control pills, Depo Provera, and Norplant?** Yes _____ No _____

If yes, **At what age did you begin this method? _____**

For how many years (total) ? _____

48. **Were any of your pregnancies twins or triplets?** Yes___ No___

If No, go to Question 52.

If Yes, go to Question 49.

49. **Which pregnancies?** __ 1 2 3 4 5 6 7 8 9 10 11 12

50. **Please tell me if the twins/ triplets were identical (alike) or fraternal (unlike)**

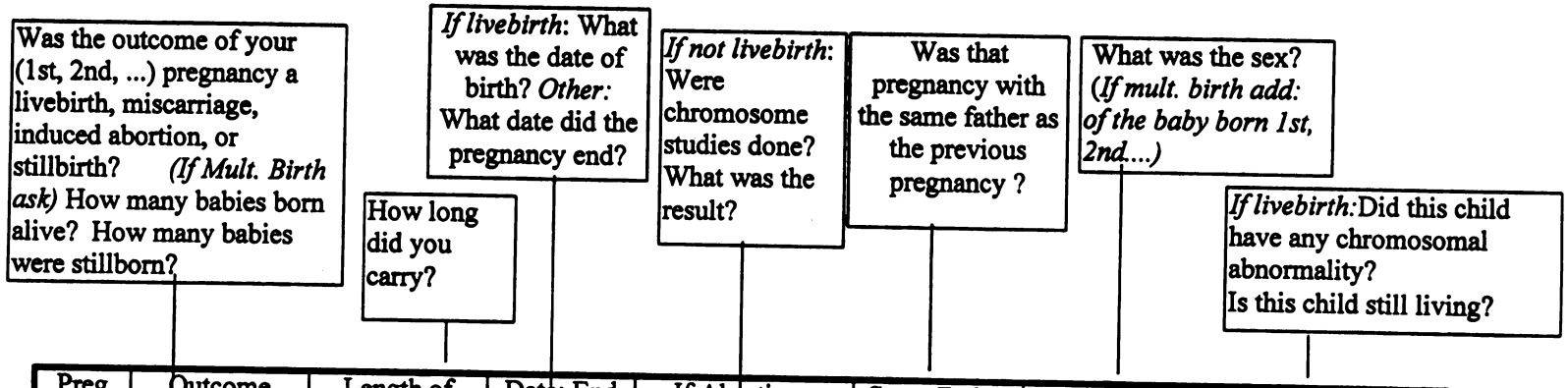
Pregnancy____ Identical___ Fraternal___ Different sex___ Same sex___ Don't know___
Pregnancy____ Identical___ Fraternal___ Different sex___ Same sex___ Don't know___
Pregnancy____ Identical___ Fraternal___ Different sex___ Same sex___ Don't know___

51. **Were you having treatment for infertility at the time you conceived these twins/triplets?**

Yes___ No___

52. **Now I would like to ask you some specific questions about your pregnancy history.**

Beginning with your first pregnancy....(read questions off table)



Preg #	Outcome	Length of Carry	Date: End of Pregnancy	If Abortion.. C'some studies	Same Father	Gender	C'some Abnormality Child still living
		_____weeks _____months		yes no result_____		M F	Ch.Ab. Living Yes Yes No No Ch.Ab._____
		_____weeks _____months		yes no result_____	yes no unknown	M F	Ch.Ab. Living Yes Yes No No Ch.Ab._____
		_____weeks _____months		yes no result_____	yes no unknown	M F	Ch.Ab. Living Yes Yes No No Ch.Ab._____

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