The National Down Syndrome Project: Design and Implementation

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SYNOPSIS

Objective. The National Down Syndrome Project (NDSP), based at Emory University in Atlanta, Georgia, represents a multi-site, population-based, case-control study with two major aims: (1) to identify molecular and epidemiological factors contributing to chromosome nondisjunction and the consequent packaging of an extra chromosome into an egg or sperm, and (2) to identify risk factors for Down syndrome-associated birth defects.

Methods. The six national sites represent approximately 11% of U.S. births. Cases were newborns with Down syndrome (trisomy 21), and controls were infants without major birth defects randomly selected from the same birth populations. Biological samples were collected from case infants and their parents, and genetic markers were typed to determine the parental origin of chromosome 21 nondisjunction. Each site interviewed parents of case and control infants addressing pregnancy, medical and family history, occupation, and exposures. Sites collected medical information on case infants.

Results. The NDSP enrolled 907 infants as cases and 977 infants as controls (participation rates: 60.7% for cases; 56.9% for controls). Participation rates varied widely by site as did important demographic factors such as maternal age, race, and education. Nondisjunction during oogenesis accounted for 93.2% of the cases. Errors in spermatogenesis were found in 4.1%, and 2.7% were post-zygotic errors.

Conclusions. This exceptional compilation of questionnaire, clinical, and molecular data makes the NDSP a unique resource for ongoing studies of the etiology and phenotypic consequences of trisomy 21. The combined approach increases study power by defining subgroups of cases by the origin of nondisjunction. This report describes the design and successful implementation of the NDSP.

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Chromosome nondisjunction, the failure of chromosomes to segregate properly during meiosis, leads to aneuploid embryos with either a missing or an extra chromosome (monosomy or trisomy). Nondisjunction is extraordinarily common among humans and results in aneuploidy in an estimated 10%-35% of all conceptions.¹ This one type of chromosome error is a leading cause of pregnancy loss, mental retardation, and birth defects. Despite the clinical importance of aneuploidy, we are just beginning to understand the causes and associated risk factors for nondisjunction. Researchers have long recognized the importance of advanced maternal age, but the biological component of aging that increases the risk of nondisjunction remains unexplained. Similarly, investigators have yet to determine the biological mechanisms by which an extra chromosome causes the clinical phenotypes associated with specific aneuploidies such as Down syndrome (DS).

Down syndrome, caused by an extra chromosome 21 (trisomy 21), is the most intensively studied human aneuploid condition. The hallmarks of DS are mental retardation, hypotonia, and characteristic phenotypic features. Variable components include heart defects, digestive tract abnormalities, congenital cataracts, and leukemia. Of individuals with DS, 95% have an extra free-standing chromosome 21, caused, in most cases, by meiotic nondisjunction during the formation of either the egg or the sperm (standard trisomy 21). Approximately 4% are the result of a translocation involving chromosome 21, and the remainder are mosaics with a mixture of aneuploid and euploid cells.² Trisomy 21 is one of the few autosomal trisomies that survives to term, although approximately 50%-75% of all conceptuses with trisomy 21 are spontaneously aborted.^{3,4} The incidence of DS, approximately one in 600 to one in 1,000 live births,⁵⁻⁷ makes this syndrome a leading cause of mental retardation and birth defects.

Beginning in 1989, researchers at Emory University in Atlanta, in collaboration with the Metropolitan Atlanta Congenital Defects Program (MACDP)⁸ of the Centers for Disease Control and Prevention (CDC), established the Atlanta Down Syndrome Project (ADSP), a unique population-based case-control study of nondisjunction and the phenotypic consequences of trisomy 21. Infants identified as cases were live births with standard trisomy 21 or mosaic trisomy 21 occurring from 1989 to 1999 in the metropolitan Atlanta area. Infants identified as controls were live births without DS chosen from the same population. Trained study personnel administered questionnaires to each parent in the case and control groups, and collected blood samples from infants and their parents in the case group. The ADSP enrolled 308 case and 398 control families over the 11-year period, with participation rates of 76% and 69% respectively (unpublished data).

Compared to all previous studies of DS, the ADSP set an important design precedent. Using genetic markers along chromosome 21, ADSP personnel characterized those enrolled as cases with respect to the origin of the nondisjunction error as follows: maternal meiosis I (MI), maternal meiosis II (MII), paternal meiosis I (PI), paternal meiosis II (PII), and mitotic errors. This unique study design gave us greater precision when analyzing data from the parental questionnaires. For example, we included only cases of maternal origin when studying questions related to the mother.9-11 We considered that each error type (MI, MII, PI, PII, mitotic) might have different associated risk factors; thus, grouping by error type would increase the power of the study to identify these risks. Similarly, this approach could provide additional insight into the phenotype of DS. For example, would DS-associated birth defects vary by origin of the extra chromosome? This study design also allowed potential reporting and/or recall biases to be evaluated by including essentially two control groups. For example, with respect to topics covered in the maternal questionnaire, we could compare cases due to MI not only to controls but to cases resulting from MII errors.¹⁰

In 2000, we recognized the need for a larger sample size to address questions related to nondisjunction and the major birth defects associated with DS. To achieve this, we established the National Down Syndrome Project (NDSP) and increased our potential sample size from approximately 50 cases per year in Atlanta alone to 500 cases per year by adding five additional sites across the country. Together, the six sites represent populations with approximately 11% of all births in the United States. Each site has a population-based birth defects surveillance program that includes ascertainment of infants with DS in a specified geographical area.

In this article, we describe the methodological approach that we used for the NDSP, the largest DS study of its kind to date. We present our protocol and outline the challenges and successes that we encountered during the implementation of this multisite project.

METHODS

Study design

We designed the NDSP as a national, multisite, population-based, case-control study with headquarters in the Department of Human Genetics at Emory University

in Atlanta, Georgia. Table 1 contains a summary of the study design. We defined cases and controls on two levels: eligibility and recruitability. Eligibility described the ideal set of cases and controls that would fit the study criteria. For example, eligible cases would all be live births with standard trisomy 21 or mosaic trisomy 21. Recruitability then placed practical limits on which eligible cases and controls could be recruited. For example, because of limited resources, we could recruit only mothers who were fluent in either English or Spanish. Further, case families whose live-born child died or was put up for adoption were not recruitable because a biological sample could not be obtained from the case child. Even though our protocol did not require biological samples from controls, for the sake of comparability we extended these latter criteria to controls.

Each site was responsible for ascertaining, contacting, and enrolling its own cases and controls, administering parental questionnaires, and obtaining biological samples. Study personnel made intensive efforts to contact all eligible and recruitable families. They used commercial tracking services and street tracking where resources were available. Emory provided training and guidelines to each site including lay explanations of the components of the study and lists of frequently asked questions that study personnel might encounter when recruiting families. The NDSP produced all written materials in English and Spanish, and bilingual personnel were available to each site to communicate with Spanish-speaking families.

Sites initially contacted families with a letter of introduction explaining the aims of the NDSP. In a follow-up telephone call, study personnel invited the families to participate. At all sites the mother was considered the "gatekeeper." If she agreed to the study, the recruiter obtained her informed consent and administered the maternal questionnaire by telephone. The interviewer then made arrangements with each mother in the case group to obtain biological samples from her, her child, and the father of the child (if available). Sites reimbursed participating parents a nominal amount for their time and effort. The reimbursement procedure and amount varied based on site-specific Institutional Review Board (IRB) regulations. Reimbursement for completing the questionnaire was usually \$10 per parent for both case and control groups. Case families were also reimbursed for providing biological samples. The amounts varied by site and ranged from \$20 to \$40 for the nuclear family (child, mother, and father). The IRB at each site typically dictated whether reimbursement was to be provided with the introductory letter or after the questionnaires and biological sample collection were completed.

Participating sites

The combined annual birth population of all six sites was approximately 472,500 (Table 2). Before beginning active recruitment for the NDSP, each site obtained the necessary IRB approval. Recruitment for the NDSP occurred from 2000 to 2004 and the ascertainment periods varied by site (range: 2.5–3.75 years). Each site

	Cases	Controls
Eligibility	Standard trisomy 21 or mosaic trisomy 21 No translocations	No chromosome abnormality or major birth defect
	Live born during stud Mother resided in specified geograp	y period hic area at child's birth
Recruitability	Eligible as stated a Mother spoke English c Baby not deceased or	bove or Spanish adopted
Time frame to enroll	Target: six weeks to six months	after child's birth
Contact	Introductory letter to families/fol	low-up phone calls
Participants	Child, mother, father (if	available)
Consents	Telephone consent for questionnaire. Written	consent for biological samples.
Survey instruments	Mother's questionnaire (all mothers)—E Father's questionnaire (subset-see text)—	inglish or Spanish—paper -English or Spanish—paper
Medical records—mother	Selected records (se	e text)
Medical records—child	Yes	No
Biologicals	Child, parents Blood (GA), buccal cells (other sites)	No
Reimbursement	Varied by site (see text)	Varied by site (see text)

Table 1. National Down Syndrome Project design

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Site/geographic area/study period (birth years)	Birth population	Expected Down syndrome birthsª	Methods for selecting controls	Collaborating institutions
Arkansas statewide 10/1/2000–9/30/2003	35,000/year × 3 years	44/year × 3 years	birth hospitals	University of Arkansas for Medical Sciences, Arkansas Center for Birth Defects Research and Prevention, Arkansas Children's Hospital, Arkansas Reproductive Health Monitoring Systems
California 3 counties, 1/1/2001–6/30/2003	186,000/year × 2.5 years	233/year × 2.5 years	birth hospitals	California Birth Defects Monitoring Program, Public Health Institute
Georgia 5-county Atlanta area 1/1/2001–9/30/2004	50,000/year × 3.75 years	63/year × 3.75 years	birth hospitals 2001–2003; birth certificates 1/2004–9/2004	Department of Human Genetics, Emory University; Centers for Disease Control and Prevention
lowa statewide 2001–2003	38,000/year × 3 years	48/year × 3 years	birth certificates	University of Iowa, Registry for Congenital and Inherited Disorders
New Jersey statewide 1/1/2001–6/30/2004	114,500/year × 3.5 years	143/year × 3.5 years	birth certificates	New Jersey Department of Health and Senior Services; Special Child Health Services Registry; Eagleton Institute
New York 15 counties 10/1/2000–9/30/2003 Total	49,000/year × 3 years 1,419,250	61/year × 3 years 1,778	birth hospitals	New York State Department of Health Congenital Malformations Registry

Table 2. National Down Syndrome Project sites

^aBased on estimated rate of 1/800 live births per year. Expected number includes approximately 4% who would be ineligible for National Down Syndrome Program because the Down syndrome was due to a translocation.

ascertained cases and selected controls from a defined geographical area that ranged from three counties to the entire state. All participating sites were part of the National Birth Defects Prevention Network (NBDPN); a state-by-state description of their surveillance systems is available elsewhere.¹² The geographical area of California included in the NDSP was not the same as that included in the NBDPN.

Case identification/control selection

Case identification. Live born infants with standard trisomy 21 (47,XX,+21 or 47,XY,+21) or mosaic trisomy 21 were eligible. Additional chromosome abnormalities or variations did not disqualify an infant from being identified as a case. Infants with DS due to a translocation involving chromosome 21 were not eligible.

Control selection. NDSP sites selected infants for the control group randomly from among all infants born in the same study period and geographic area but without DS or other major birth defects (Table 1). Sites used either birth certificate data or hospital records for control selection (Table 2). We did not match on maternal age because one of the primary aims of the study was to investigate the relationship between meiotic nondisjunction and the age of the mother. Where

appropriate, we will control for maternal age in our analyses. Major birth defects that would exclude an infant as a control included those registered by the states as well as those eligible for the National Birth Defects Prevention Study (NBDPS) being conducted separately by CDC at all sites. Details of the state surveillance systems and the NBDPS are available in other publications.^{12,13} For the purposes of analyzing responses to the maternal questionnaire, our goal was to enroll an equal number of infants as cases and controls. Because our previous experience indicated a somewhat lower participation rate for control families, each site used its number of expected cases as a reference point and adjusted upward the number of controls to be identified.

Paternal cases and controls

Paternal cases. Our previous population-based studies had determined that at least 90% of the cases of standard trisomy 21 are due to an error in the egg and less than 10% are the result of a similar meiotic error during spermatogenesis.¹⁰ Because the overwhelming majority of cases are maternal in origin, we designed a protocol by which we could efficiently collect questionnaire data from fathers of paternal cases while

minimizing the number of fathers interviewed where the case was maternal in origin. Furthermore, the protocol had to ensure that the parental origin of the chromosome error was not revealed to the participating family. Once biological samples were collected from the family unit, molecular studies were conducted to determine parental origin of the chromosome error. If the error was paternal, and the father was available, Emory asked the site to recontact and interview the father. In addition, on a periodic basis, the sites were contacted to interview a father of a maternal case. At no time were the sites informed of the parental origin of any case. This ensured that parental origin was not released to the family.

Paternal controls. When determining the number needed in the control group for the upcoming year, each site specified a random subset in which the fathers would also be interviewed during the initial contact period. Because we anticipated so few cases would be of paternal origin, we wanted to achieve a 2:1 ratio of controls to cases for data analysis. Our previous experience had been that, of the control mothers who participated, only about half of those fathers also agreed to the study. Therefore, each site adjusted accordingly the number of control fathers to be interviewed.

Parental questionnaires

We developed parental questionnaires (Figure 1) based on ten years of experience with similar survey instruments in the ADSP. To ensure that personnel at all sites were administering the questionnaire accurately and uniformly, we implemented several quality control measures. First, Emory personnel prepared detailed annotations for each question in both maternal and paternal questionnaires. They required interviewers to review the annotations, become familiar with the questionnaires, and pass a test that consisted of satisfactorily administering a questionnaire to Emory personnel by telephone and submitting their completed test questionnaires for review. We found these tests to be crucial in identifying and correcting interviewer errors. In addition, interviewers from all sites attended annual project meetings to review and resolve discrepancies in recruitment and interviewing procedures.

Trained interviewers at each site administered the questionnaires by telephone or, in rare instances, inperson if the parents had no phone. The average length of time from birth of the index child until administration of the maternal questionnaire varied by site (Table 3). Sites mailed each completed questionnaire (excluding personal identifiers) to Emory soon after completion. Emory reviewed the questionnaire and contacted the site if deficiencies were noted. In order to document responses that mothers offered to specific questions about their reproductive histories, interviewers requested written permission from mothers in both the case and control groups to obtain pertinent medical records. Upon receipt of the signed medical record release form, the recruiter requested the appropriate records and forwarded the medical information to Emory after removing all personal identifiers.

Case infant medical records

The NDSP study design included a plan to link medical information about case infants to both the questionnaire and the molecular data. Linking with parental questionnaires will enable us to explore a number of important topics such as the occurrence of heart or gastrointestinal defects in relation to factors such as race, maternal age, family history, and environmental

Figure	1.	National	Down	Svnc	Irome	Proie	ect a	iuestionnai	ires—to	pics
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Mother®	Father ^b			
Demographics	Demographics			
Pregnancy history and index pregnancy	History of pregnancies fathered			
Medical history—mother ^a	Medical history—father ^b			
Family history—mother ^a and father ^b	Family history—father ^b			
Alcoholic beverages	Alcoholic beverages			
Smoking	Smoking			
Occupational history—mother ^a and father ^b	Occupation, sports, exercise, hobbies—father ^b			
Income	Income			
Menstrual history	Radiation exposure			
Birth control	Caffeine			
Time to administer: 30–40 minutes	Time to administer: 20 minutes			

^aMother of index infant ^bFather of index infant

Site	Days to enrollment® mean (range)	Maternal age ^ь at infant's birth mean (range)	Percent white ^c	Percent black ^c	Percent Hispanic ^c	Percent other ^c	Percent with ≥4 years high school ^d	Percent born in the U.S. ^d
Arkansas:								
Case	275 (5–886)	30.0 (15–46)	69.1	17.7	13.2	0.0	86.8	88.2
Control	342 (89–867)	26.3 (15–44)	69.3	21.3	8.0	1.4	85.3	93.3
Population ^e		25.2 (<14–>45)	70.9	20.1	6.9	2.1		
California:								
Case	388 (71–1,476)	33.0 (16–50)	13.9	3.4	77.8	4.9	60.5	29.5
Control	400 (85-1,449)	28.1 (14–45)	22.1	8.5	63.9	5.5	67.3	47.3
Population ^e		28.3 (<14–>45)	21.5	6.9	55.6	16.0		
Georgia:								
Case	182 (15–1,157)	33.4(16–45)	47.4	26.0	24.7	1.9	84.4	67.1
Control	185 (1–606)	28.9(14–40)	44.0	38.7	14.3	3.0	87.0	76.3
Population ^e		28.1(<14–>45)	37.1	38.1	18.8	6.0		
lowa:								
Case	355 (123–974)	31.8 (18–47)	94.5	0.0	5.5	0.0	97.3	96.0
Control	364 (145–1,016)	28.0 (18–40)	93.8	3.7	2.5	0.0	96.3	95.0
Population ^e		27.1 (<14–>45)	87.4	3.2	6.1	3.3		
New Jersey:								
Case	267 (90–770)	34.2 (16–49)	57.1	9.9	27.4	5.6	88.5	63.9
Control	35 (157–1,082)	29.9 (14–46)	57.9	15.5	18.5	8.1	93.3	72.4
Population ^e		29.2 (<14–>45)	52.5	15.9	21.9	9.7		
New York:								
Case	301 (118–1,003)	33.1 (14–45)	86.8	5.5	6.6	1.1	92.3	91.2
Control	274 (54–783)	29.2 (16–40)	73.6	11.0	12.1	3.3	89.9	84.3
Population ^e		29.1 (<14–>45)	63.9	11.4	15.6	9.1		

Table 3. National Down Syndrome Project: Selected characteristics of participating mothers and mothers in the birth population

^aNumber of days from infant's birth to questionnaire completion

^bIncludes all cases regardless of parental origin

^cSource: self-reported for cases/controls; birth certificates for population. Race and ethnicity distributions for enrolled families and population not comparable because population not restricted to English- and Spanish-speaking mothers.

^dSource: self-reported; information not available for the population.

^eBirth population at each geographic site.

exposures. Similarly, a link to the molecular data will facilitate our search for genes important in the DS phenotype.

Trained personnel at each site abstracted records from birth hospitals and tertiary facilities. The protocol required documentation of karyotypes for each case to ensure that the NDSP included only infants with standard trisomy 21 or mosaic trisomy 21 and excluded translocations. In addition, we made every effort to obtain information from the most definitive procedures available such as echocardiograms, cardiac catherizations, or operative reports for heart defects and surgical summaries for GI abnormalities. Sites recorded this information on forms designed for the purpose and sent the forms to Emory for review by a single, clinically-trained investigator prior to data entry.

Biological samples

Each site was responsible for obtaining biological samples on case infants and their parents. When the father was not available or not willing to participate, sites collected samples on the mother and child. The latter situation was not optimal, however, because the molecular analyses were less informative for origin of the extra chromosome 21 and recombination patterns.

The Georgia site obtained blood samples and used aliquots of these samples to extract DNA and establish lymphoblastoid cell lines. Study personnel trained in phlebotomy scheduled home visits to obtain written consent and draw the parental blood samples. They usually obtained the infant sample when blood was being drawn for clinical purposes such as thyroid function or pre-operative tests. Although this approach could mean waiting several weeks or months for the sample, it appealed to parents and was undoubtedly one of the main reasons for the high case participation rate at the Georgia site.

Because geographic areas were larger at the other five NDSP sites, the expense of blood collection was outside NIH budgetary constraints, and we were limited to collecting buccal samples for DNA. Each site mailed bar-coded buccal cell collection kits, consent forms, and instructions to participating families. Parents collected the cheek cell samples on themselves and their child using four buccal brushes per individual and returned the kits by regular mail. Sites stored the returned kits in freezers, batched them, and mailed them in cold-packs to Emory for DNA extraction and analysis.

DNA analysis

Emory laboratory staff extracted DNA from blood or buccal samples for genotyping chromosome 21-specific polymorphic markers. We chose markers based on their high degree of heterozygosity and placement on chromosome 21 (Figure 2). Later in the study when whole genome amplification became a potential, we added the step of storing an aliquot of the DNA sample prior to analyses.

We examined the genotyping data to detect Mendelian inconsistencies, non-paternity, and genotyping errors. Once these were resolved, we used an algorithm to identify origin of the nondisjunction error.

Algorithm for determining origin of the extra chromosome 21

In families for which we had samples from the child and both parents, we required that at least two chromosome 21 markers be informative to assign parent of origin of the extra chromosome. Once parent of origin was established, we used pericentromeric markers to determine the type of nondisjunction error (i.e., MI, MII, PI, PII, or mitotic). To do this, we used the closest informative marker within the predefined pericentromeric marker set (Figure 2). If parental heterozygosity was retained in the centromeric region in the trisomic offspring ("non-reduction") (Figure 3-i), we concluded that the error occurred during MI. If parental heterozygosity of the centromeric marker was reduced to homozygosity ("reduction") while heterozygosity of other, noncentromeric markers was retained, we declared the error to be meiosis II-type (Figure 3-ii). We considered the error mitotic if the informative markers were reduced to homozygosity along the entire length of the chromosome (Figure 3-iii).

If we received DNA from only the mother and





child, we considered the error maternal in origin if there were more than eight markers consistent with a maternal error (Figure 4-i). We considered the error paternal if at least two markers were inconsistent with a maternal error (Figure 4-ii).

In order to successfully "pass" the algorithm, the family could not have any discrepancies among markers that defined parental origin. A subset of samples failed to "pass" the algorithm due to a lack of DNA or genotype inconsistencies. In these situations, we requested a second sample. When non-paternity was suspected, we excluded the paternal sample from the Figure 3. Examples of scenarios that define the origin of the nondisjunction error. Hypothetical genotypes of three ordered markers are shown. The most centromeric marker is noted by an arrow. Reduction (R) or no reduction (N) to homozygosity for each marker in the offspring is noted. Samples from both parents and child are available: i) maternal MI error, ii) maternal MII error and iii) mitotic error.



analysis and repeated the algorithm using only data from the mother and child (Figure 4-i, 4-ii).

In addition to establishing the origin of the extra chromosome 21, we determined the recombination profile along the long arm of chromosome 21 based on the non-reduced/reduced status of each informative marker. A change of status from reduction to nonreduction (or vice versa) between adjacent informative markers indicated a recombination event (e.g., Figure 3-i, 3-ii).

Data management and security

For correspondence and data transfer between sites including questionnaires, samples, and clinical information, we used only ID numbers to identify individual participants. For Emory to monitor participation rates on an ongoing basis, the remote sites sent bimonthly electronic progress reports to Emory. NDSP personnel at Emory collected the hardcopy questionnaires and clinical data forms from all sites, sent these to a third party company for double entry and cross-checking, then uploaded the data into our primary data repository along with genotyping results from the Emory laboratory. The Emory site applied security at both the database and application levels to properly protect HIPAA-regulated data.

RESULTS

All NDSP sites completed their recruitment efforts by May 2005. Combining all sites, all years, we ascertained 1,673 eligible live born cases of trisomy 21 or mosaic trisomy 21. Of these, 1,494 families were recruitable and 907 were enrolled, giving an overall case participation rate of 60.7% and a range of 52.5%–74.8% among sites. Similarly, 1,767 controls were selected; of these, 1,716 were recruitable and 977 were enrolled, giving a participation rate of 56.9% (range: 43.2%–71.1%). Table 4 presents a breakdown of these numbers by site and includes details on the major reasons for non-enrollment.

Table 3 presents selected characteristics of the

Figure 4. Examples of scenarios that define the origin of the nondisjunction error. Hypothetical genotypes of three ordered markers are shown. The most centromeric marker is noted by an arrow. Reduction (R) or no reduction (N) to homozygosity for each marker in the offspring is noted. Samples from only mother and child are available: i) inferred maternal error—hypothetical genotypes of 3 of the required 8 informative markers are shown, ii) inferred paternal error.



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Site		Case	Control
Arkansas	Eligible	111	120
	Recruitable	98	117
	Lost	12	25
	Refused	17	17
	Enrolled ^a	68	75
	Participation rate ^b	69.4%	64.1%
California	Eligible	544	614
	Recruitable	509	604
	Lost	67	204
	Refused	112	139
	Enrolled ^a	267	261
	Participation rate ^b	52.5%	43.2%
Georgia	Eligible	228	251
	Recruitable	206	239
	Lost	7	29
	Refused	45	40
	Enrolled ^a	154	170
	Participation rate ^b	74.8%	71.1%
lowa	Eligible	143	153
	Recruitable	126	150
	Lost	6	19
	Refused	23	50
	Enrolled ^a	75	81
	Participation rate ^b	59.5%	54.0%
New Jersey	Eligible	480	472
	Recruitable	400	457
	Lost	17	108
	Refused	59	50
	Enrolled ^a	252	299
	Participation rate ^b	63.0%	65.4%
New York	Eligible	167	157
	Recruitable	155	149
	Lost	10	26
	Refused	33	32
	Enrolled ^a	91	91
	Participation rate ^b	58.7%	61.1%
Total	Eligible	1,673	1,767
	Recruitable	1,494	1,716
	Lost	119	411
	Refused	289	328
	Enrolled ^a	907	977
	Participation rate ^b	60.7%	56.9%

Table 4. National Down Syndrome Project:Enrollment and participation rates by site

 $^{\rm a}{\rm Enrolled}$ cases = mother's questionnaire and biological samples on child, mother +/– father.

Enrolled controls = mother's questionnaire

^bParticipation rate = enrolled/recruitable

enrolled case and control mothers as well as summary statistics for the birth populations represented in the NDSP. We included all enrolled case families in this table regardless of the parental origin of the extra chromosome 21. Thus, the age of the case mothers is an average that includes cases of maternal as well as paternal and mitotic origin. The mean age of case mothers (Table 3) is significantly increased over the means for both controls and the general population for all sites (p<0.01 for all sites). Importantly, the mean maternal age of NDSP controls closely matched that of birth mothers in the general population with the average differences ranging, by site, from 0.2 to 1.1 years (Table 3).

Race, ethnicity, education, and country of birth of the case and control mothers were self-reported in the maternal questionnaire, while we obtained race and ethnicity of the population from birth certificates. Furthermore, race and ethnicity data reported here for the population are not directly comparable to similar data for cases and controls because the population figures are not limited to English- or Spanish-speaking mothers. Clearly, the six NDSP sites varied widely in the racial makeup of mothers of cases, controls, and the birth population (Table 3). Similarly, among both cases and controls, there was inter-site variation in the education level of the mother and in the percentage of mothers who were born outside of the United States. All of these factors will need to be taken into account during data analysis.

The average time from birth of the case or control infant to completion of the maternal questionnaire was greater than our original target of six weeks to six months after birth, and the range was wide. However, there was generally good agreement between cases and controls within sites (Table 3). Only two sites, Arkansas (p=0.04) and New Jersey (p<0.0001), showed a significant difference in time to enrollment between cases and controls. The difference at the New Jersey site reflected their protocol for processing controls.

Table 5 contains summary information regarding the origin of the extra chromosome 21. Of all informative cases, 93.2% were the result of chromosome nondisjunction during meiosis in the maternal germ cells and, of these, 72.6% occurred during meiosis I. Only 4.1% occurred during meiosis in the sperm. The inability to determine the type of error in some maternal and paternal cases was due to insufficient informative markers or lack of a sample from the father. Only 2.7% of cases were categorized as mitotic in origin. There were no significant differences in this distribution by site (data not presented).

DISCUSSION

The total number of eligible case infants ascertained over all sites (1,673; Table 4) closely matches the number predicted at the start of the study. Based on the birth population at each site and a birth prevalence

Origin			nª	Proportion	Percent
Meiotic					
	Maternal (M)	Meiosis I (MI)	529	MI/(MI + MII) = 529/729	72.6
		Meiosis II (MII)	179	MII/(MI + MII) = 179/729	24.6
		Stage unknown	21		
		Subtotal	729	M/All = 729/782	93.2
	Paternal (P)	Meiosis I (PI)	13	PI/(PI + PII) = 13/32	40.6
		Meiosis II (PII)	18	PII/(PI + PII) = 18/32	56.3
		Stage unknown	1		
		Subtotal	32	P/AII = 32/782	4.1
Mitotic			21	Mitotics/All = 21/782	2.7
Total informative	e cases (all)		782		
Unknown ^b			125		
Total			907		

Table 5. National Down Syndrome Project: Origin of trisomy 21

 $a_n = all sites, all years$

 ${}^{\mathrm{b}}\mathrm{Unknown} = \mathrm{insufficient} \ \mathrm{biological} \ \mathrm{samples} \ \mathrm{or} \ \mathrm{DNA} \ \mathrm{markers} \ \mathrm{not} \ \mathrm{informative} \ \mathrm{for} \ \mathrm{origin}$

of 1/800, we estimated that the combined sites would ascertain approximately 1,778 infants with DS. After subtracting the 4% that would be due to a structural chromosome abnormality such as a translocation, we predicted that our eligible case sample would be approximately 1,707.

The primary aims of the NDSP required biological samples to determine origin of nondisjunction and patterns of recombination on chromosome 21. Therefore, for a case family to be counted as fully enrolled, the mother, in addition to completing a questionnaire, had to provide a biological sample on her child and herself with or without a sample from the father. Sites that collected buccal samples found that some mothers who completed the questionnaire did not return their sample kits (Table 4). This was true even after the mothers received reminder phone calls from the interviewers. Families in which the mother completed a questionnaire but never returned the samples were categorized as refusals when calculating participation rates. However, we can use data from these questionnaires to investigate epidemiological factors related to the clinical phenotype of DS. In addition, we can compare demographic information from these questionnaires with similar data from fully enrolled families to determine if our enrolled sample is representative. Because no samples were required from controls, full enrollment consisted only of completing the maternal questionnaire.

Participation rates for cases and controls represent the percentage of the eligible and recruitable families who were fully enrolled. For these determinations, we did not take the participation of the father into account. Participation rates varied by site for both cases and controls. We plan to identify the specific sites and years with the lowest participation rates and analyze the data with and without these. As expected, the participation rate for controls was somewhat lower than for cases. However, in designing the NDSP, we took into account the probability of a lower control participation rate when determining the number of controls to select. The result is that the numbers of enrolled cases (907) and controls (977) are very close to the 1:1 ratio we established as optimal for the goals of the study.

We were successful in assigning parent of origin in 86% of participating families, and for most of those we were also able to determine the type of error. We found that, similar to what was reported by our earlier study (ADSP) as well as by other investigators internationally, the overwhelming majority of cases are maternal in origin.^{10,14,15} Furthermore, these maternal cases again demonstrated the well-documented phenomenon of advanced maternal age. A major goal of our future analyses will be to examine both molecular and epidemiological factors that contribute to the maternal age effect. As documented in previous studies by our group and others,^{11,16} elevated maternal age was confined to NDSP cases of maternal origin. We did not observe an elevated maternal age when the small group of paternal cases was examined separately (data not shown).

Unfortunately, our ability to investigate risk factors associated with nondisjunction of paternal origin will be severely restricted by the small number of paternal cases. Over all sites, all years, we identified only 32 paternal cases. Only about half of the fathers in these cases agreed to provide answers to the paternal questionnaire. This, in combination with low participation rates at most sites for control fathers (18%–51%; data not shown), makes any attempt to study paternal nondisjunction problematic.

One limitation of the study is that we were not able to include pregnancies terminated because of a prenatal diagnosis of trisomy 21. Ascertaining terminations on a population basis and collecting biological samples from affected fetuses was not possible in the context of the multisite population-based study. Similarly, the NDSP did not include families of live born infants who died or were placed for adoption before recruitment. Another limitation was being restricted to collecting buccal samples instead of blood at the majority of sites. Blood samples have the advantage that the white blood cells can be transformed into long-lived lymphoblastoid cells, and these cell lines can be frozen for future use. With the genes on chromosome 21 just recently mapped and knowledge of their various functions only beginning to emerge, long-term availability of DNA samples from all NDSP families would have been invaluable. We highly recommend that funding agencies consider the feasibility of creating a central blood repository and supporting the collection of blood samples for long-term use in the study of DS.

In summary, the NDSP represents the largest populationbased study of DS to date in which the origin of the extra chromosome 21 was determined. Strengths of the NDSP include the fact that we collected this information in conjunction with extensive epidemiological and clinical data. This combined data set constitutes a major resource in efforts to understand the etiology of meiotic nondisjunction and the phenotypic consequences of trisomy 21. We will examine the effect of maternal age on the risk for maternal nondisjunction by meiotic stage of the chromosome error. As part of this effort we will explore factors related to ovarian ageing such as smoking and maternal hormone function. Our study of the DS phenotype will include a search for chromosome 21 genes important in the heart defects seen in DS and an investigation of how phenotype may be influenced by maternal health, family history, and demographics.

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