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ORIGINAL ARTICLE

Altered incidence of meiotic errors and Down syndrome birth under extreme low socioeconomic exposure in the Sundarban area of India

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Abstract We conducted a survey to analyze the genetic epidemiology of trisomy 21 Down syndrome births in the Sundarban delta region of India. In this region, inhabitants are chiefly from marginalized poor tribal communities and have lived in extremely low socioeconomic condition for several generations. Microsatellite genotyping revealed an meiosis I/meiosis II ratio that is different from the previous reports on the Down syndrome populations from other parts of the world. Analyses of distribution of achiasmate nondisjunction at maternal meiosis I in interaction with different maternal age groups (young, middle, and old) revealed a very concordant pattern to that of urban and semi-urban Down syndrome cases previously studied by our group. However, the frequency of achiasmate meiosis is much lower, which suggests that extreme low socioeconomic exposure imparts risk of chromosomal nondisjunction even when the maternal chromosomes 21 engage in proper chiasma formation at prophase I of oogenesis.

Keywords Down syndrome · Maternal age · Nondisjunction · Socioeconomic exposure · Recombination · Sundarban population

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Introduction

Down syndrome (DS) represents the most frequent genetic form of intellectual disability and live-born aneuploidy in humans. The overwhelming majority of this syndrome is caused by trisomy 21 genetic condition that originates with nondisjunction (NDJ), i.e., nonseparation of chromosome 21 (Ch21) either at parental gametogenesis (meiotic NDJ) or at an early phase of embryonic development (postzygotic NDJ). Previously, DS samples from several different ethnic populations have been studied and for all of them the meiosis I (MI) stage of oogenesis has been identified as the most vulnerable phase for NDJ errors to occur (Sherman et al. 2007). Advanced maternal age at conception and altered patterns of recombination of the Ch21 are the two well-documented maternal risk factors for Ch21 NDJ (Lamb et al. 2005a, b; Sherman et al. 2007; Oliver et al. 2008; Ghosh et al. 2009). In particular, absence of chiasma formation and a single telomeric chiasma have been identified as risk factors for maternal MI NDJ, whereas a pericentromeric single chiasma has been reported as risk for meiosis II (MII) errors.

The set of environmental and behavioral risk factors to which women are exposed have also been implicated in the etiology of trisomy 21 birth (Chen et al. 1999; Kaufman 1983; Padmanabhan et al. 2004; Yang et al. 1999; Martínez-Frías et al. 2001). Moreover, recent epidemiological studies by our group (Ghosh et al. 2011) on liveborn DS infants from Kolkata, India, revealed interactions among maternal age, recombination pattern of Ch21, and the mother's behavioral risk factors. We hypothesized that the risk environment in the oocyte for NDJ of Ch21 is extremely complicated and arises from complex, multidimensional interactions among genetic and environmental predisposing factors.

With regard to the association between low socioeconomic status (SES) and DS, Torf and Christianson (2003) conducted epidemiological studies on DS samples from the

USA and reported that low SES of mothers significantly increases their risk for a recognized pregnancy with DS. They included 997 clinically recognized DS cases and considered four parameters indicative of low SES in their study, namely mother's low education, father's lower occupation, father's low education, and maternal grandfather's lower occupation. They analyzed the association for all four factors separately as well as in conjunction and found that there was a twofold increase in the risk of DS birth when all the four factors were present together. Later, the authors extended the study and evaluated the cumulative effect of adverse socioeconomic factors on maternal meiotic errors, i.e., MI NDJ or MII NDJ (Christianson et al. 2004). The results demonstrated a strong association of all the selected low SES variables with maternal MII NDJ. The authors argued that the lifetime exposures associated with low SES may generate some adverse effect at the time of oocyte maturation.

In the present study, we have conducted an epidemiological survey and subsequent molecular analyses on DS samples from the Sundarban area, a lower Gangetic delta of West Bengal, India. This area has been declared a world heritage site by the United Nations Educational, Scientific and Cultural Organization for its unique geographical and ecological attributes. The population of the Sundarban area has remained in extreme low socioeconomic ambience for several generations. The population is highly marginalized and suffers from acute poverty. Ecologically, the area is predominately mangrove forest and is inundated periodically with saline water from rivers, creeks, and estuaries. The livelihoods of both men and women include fishing, farming, honey collection, timber collection, and boating. The women suffer from malnutrition and poor health condition and often remain submerged for extended periods in the saline water of rivers and creeks for the purpose of catching shrimp larvae. We deemed this population very unique for evaluating the low SES as risk factor for DS birth. We compared the results of the present analysis with those of our previously published report on DS samples from urban and semi-urban areas of West Bengal (Ghosh et al. 2010) to contrast the etiology of DS births between the two populations exposed to entirely different ecological and socioeconomic environments.

Materials and methods

Population sample

We conducted an epidemiological survey in the villages of the Sundarban area and collected blood from consenting DS family trios (DS child and parents) from January 2006 through December 2011. Additionally, we collected buccal epithelium for those who donated blood samples of volume 1 ml or less. The sample population consists chiefly of those enlisted as "schedule castes" and "schedule tribes" by the Government of India. A total of 279 families carrying DS child participated and gave their consent for using their donated tissue samples in the molecular study. Women (mothers of the DS baby) were interviewed very privately, in person, only after receiving their consents. A preprinted, extensive set of questions was used for each family to collect the detailed family history, information about their lifestyle, and other relevant epidemiological details. We used the SES variables described by Christianson et al. (2004), with the addition of some new criteria (detail given in Table 1). All the participants were selected randomly. All families were below poverty level and exhibited very low values for all selected SES variables.

Cytogenetic screening

To ascertain the cytogenetic etiology of DS according to karyotype, we analyzed at least 40 G-banded metaphase plates for the trios (DS, mother, and father) of participating families. Parental karyotyping was done to exclude the chance of hidden mosaicism. All participating cases were free trisomy 21. We did not find any de novo translocations or isochromosome cases among the DS samples.

Molecular analysis

We isolated DNA from the residual blood samples left after karyotyping and from buccal epithelium where blood samples were exhausted in karyotyping. A set of 21 microsatellite markers specific for the Ch21 long arm (centromere-D21S2053-D21S1884-D21S214-D21S1257-D21S1914-D21S265-D21S210-D21S1270-D21S226-D21S1908-D21S224-D21S167-D21S1412-D21S2055-D21S168-D21S212-D21S1260-D21S1411-D21S1890-D21S1903-D21S1446-telomere) was used to determine the parental origin of supernumerary the Ch21 in the DS child through PCR. The genotype data from the same set of markers was used in recombination scoring. Genotyping a subset of five pericentromeric makers (D21S1431-D21S1904-D21S192-D21S1432-D21S11) helped us to interpret the meiotic stage of error occurrence, i.e., either MI or MII. We inferred MI error when the parental heterozygosity of these markers was retained in the trisomic child (i.e., the marker was "nonreduced") and MII error when parental heterozygosity was "reduced" to homozygosity. We estimated recombination using a linkage analysis approach. Briefly, a recombination event was scored when we observed a transition of two successive markers from nonreduction to reduction or vice versa. The details of the method have been described elsewhere (Feingold et al. 2000). For age-related recombination analysis, we stratified the women into three age categories according to their age at conception; young (28- years and younger), middle (29–34 years), and old (35 years

Table 1 Distribution of socio-economic variables in Down syndrome bearing rural and urban families

Socioeconomic variables		Frequency (%)		*p* value of chi-square test
		Rural DS sample *N*=228	Urban DS sample[a] *N*=169	
Family income	≥20,000 Rupees	2.2	37.27	<0.0001
	>5,000-19,999 Rupees	9.21	42.01	
	≤5,000 Rupees	88.59	20.71	
Mother's education	Illiterate	75.43	7.1	<0.0001
	Less than high school	14.91	23.07	
	High school and more	9.64	75.73	
Father's education	Illiterate	82.89	3.55	<0.0001
	Less than high school	9.64	18.34	
	High school and more	7.45	78.1	
Fathers occupation	Unemployed	13.6	4.14	<0.0001
	Farmer/labor	77.63	5.32	
	Service	1.75	66.86	
	Others	7.01	23.66	
Grandfather's occupation	Unemployed	18.85	2.95	<0.0001
	Farmer/labor	78.94	8.87	
	Service	0.87	78.1	
	Others	1.31	10.05	
No. of times major meal available to women	Only once per day	73.24	10.05	<0.0001
	Twice per day	26.76	89.94	
Folate supplement taken by women at periconceptional period	Ever	11.4	78.1	<0.0001
	Never	88.6	21.89	

[a] DS sample from urban and semi-urban population reported in Ghosh et al. (2010)

and older) and scored the number of detectable chiasma for each women in each age group.

We used Fisher's exact test to compare various MI/MII ratios, chi-square tests for various SES variables, *t* tests for testing the differences in maternal age at conception, and linear regression for evaluating interaction between maternal age and amount of recombination. In all these statistical analyses, we compared the Sundarban DS sample with the DS sample from urban and semi-urban areas of West Bengal.

Results

We observed a surprisingly lower value for MI/MII error ratio (Table 2) for maternally originating cases as compared to other published data (Allen et al. 2009; Oliver et al. 2008; Ghosh et al. 2009). This observation suggests higher frequency of MII errors among these poor, tribal women who have a child with DS. Nearly 41 % of total maternally originated cases exhibited MII error and the ratio differs significantly (p=0.005) from the MI/MII ratio estimated for DS samples from the urban and semi-urban economically stable population as published in our previous report (Ghosh et al. 2010; Table 2). This MI/MII ratio also differs significantly (p=0.02) from the published US data (MI/MII=74.7:25.3 %; Sherman et al. 2007). All of the SES variables we measured show substantial differences between this Sundarban DS population and the urban DS samples from Kolkata and adjoining areas (Table 1). The estimated mean maternal age at conception of the DS child in this tribal population is also lower than in our urban sample [24.92±-7.1 years (mean±SD) vs. 27.62±5.61 years; p=0.0007 for the MI error category; 26.63±4.2 years vs. 29.02±2.32 years; p=0.001 for the MII error category].

Another important finding of the present study is the less frequent detectable achiasmate or nonrecombinant meiosis among the women with MI errors than in our previously reported DS cases from the urban Bengali population. We recorded 0.33, 0.55, and 0.12 as the observed frequencies for "zero recombinant", "single recombinant", and "double recombinant" cases, respectively, of all the reported maternal MI NDJ cases in the Sundarban sample. In other words, approximately 33 % of all the reported maternal MI cases (45 out of 135 combining all age categories) exhibit no detectable recombination event. This percentage differs significantly (p<0.0001) from the recorded achiasmate frequency of 73 % among maternal MI errors from the urban and semi-urban areas of West Bengal (Ghosh et al. 2010). The estimated frequencies of products of tetrads in the MI

Table 2 Parent of origin and meiotic outcome of nondisjunction error of chromosome 21 with mean parental age at conception of Down syndrome child in the rural and urban families

Parent of origin	Meiotic outcome group	DS sample from poor rural population				DS sample from urban and semi-urban population as reported in Ghosh et al. (2010)			
		Sample size	Percent	Maternal age in year (mean±SD)	Paternal age in year (mean±SD)	Sample size	Percent	Maternal age in year (mean±SD)	Paternal age in year (mean±SD)
Maternal	MI[a]	135	59.21	24.92±7.1	32.91±4.25	131	77.51	27.62±5.61	33.82±6.77
	MII[b]	93	40.78	26.63±4.2	34.22±3.1	38	22.49	29.02±2.32	34.04±5.21
	Stage unknown	10				8			
	Subtotal	238	88.15			177	88.06		
Paternal	MI	10	43.46	25.02±3.4	31.07±4.6	8	47.06	24.57±3.11	30.26±4.12
	MII	13	56.52	28.44±6.12	35.02±28	9	52.94	27.01±5.03	34.33±4.14
	Stage unknown	3				3			
	Subtotal	26	9.63			20	9.95		
Mitotic		6	2.23			4	1.99		
Origin unknown		9				11			

[a] Meiosis I nondisjunction

[b] Meiosis II nondisjunction

NDJ group were 0.67, 0.27, and 0.06 for non-exchange, single-exchange, and double-exchange chromosomes, respectively. The average number of detectable exchanges in the maternal MI NDJ group is 0.78 in this rural population, which is much higher than we observed in the urban DS population (~0.22) as estimated in our previous study (Ghosh et al. 2009). It is also greater than the average of 0.43 reported in a US population (Oliver et al. 2008, 2012).

The age-stratified distribution of achiasmate maternal MI errors, which can be used to examine interaction between amount of recombination and maternal age, exhibits gradual linear decrease in the frequency of achiasmate meiosis with advancing age. We scored 42, 35, and 12 % with no detectable crossover among the young, middle, and older age groups, respectively. The trend is statistically significant (r=−0.96; p=0.04) by linear regression. This pattern of interaction between maternal age and amount of recombination in MI error groups is concordant with our previous report for urban and semi-urban population of West Bengal as well as with the US DS population (Oliver et al. 2008). Pairwise comparison by chi-square test revealed statistically significant difference (p=0.0001) between the young and older age groups.

Discussion

The present study revealed striking new information regarding the etiology of DS birth in the poor and marginalized Sundarban (India) community. The population resides in a unique ecological niche and has been exposed to extreme low socioeconomic conditions for several generations. The result of our present study is surprising since it suggests that extreme low socioeconomic exposure may alter the usual pattern (more common MI and few MII errors) of Ch 21 NDJ associated with DS birth. Moreover, we showed that achiasmate meiosis is less frequent in maternal MI NDJ in this population than in others that have been studied previously. Chiasmata usually stabilize the homologous chromosome pair on the meiotic spindle and balance the pull from opposite poles in order to ensure correct chromosome segregation. Failure of chiasma formation between homologous pair causes random chromosome movement towards the poles, leading to NDJ (Cheung et al. 2007). For Ch21, absence of chiasma and subsequent nonrecombination have been identified as major risks for NDJ at the MI phase of maternal meiosis (Lamb et al. 2005a, b; Sherman et al. 2007; Oliver et al. 2008; Ghosh et al. 2009). In this regard, the results of the present study are absolutely new. However, we should be cautious not to over interpret the result for two reasons. First, our sample size is small and, second, the number of STR markers that we used previously in studying the urban DS population was smaller than the maker set used in the present study. We cannot rule out the possibility that we might have failed to record some exchanges on Ch21 in the urban population with only a few STR markers in our previous study (Ghosh et al. 2010). Nevertheless, comparison with the US sample studied with almost the same set of markers (Oliver et al. 2008) as the present study suggests that absence of recombination may not be the major risk of maternal MI NDJ of Ch21 in the Sundarban population.

We found a trend of gradual decrease of proportion of non-recombinant MI errors from younger to older women (Fig. 1) and the trend is very concordant with that of the previous reports (Oliver et al. 2008; Ghosh et al. 2009, 2010). We describe this risk as "maternal age independent" because the two variables (maternal age and frequency of nonrecombinant events) exhibit completely reverse pattern of gradual change. The low MI/MII ratio supports the hypothesis proposed by Christianson et al. (2004) and suggests that low SES may impart risk of NDJ at the final stage of oocyte maturation just prior to ovulation when the oocyte completes second meiotic division. At the earlier phase of oogenesis (when MI error occurs), the nutritional or any other deficiency due to low SES may have some effect but, with age, the adverse effect cannot be tolerated further. Moreover, the comparatively lower mean age of conception of DS fetuses among these poor women suggests that even at younger chronological age (which is otherwise considered a "low risk" age for DS pregnancy), the women are at risk of NDJ. Despite the incidence of frequent DS birth to younger women, we did not find any history of recurrence of DS birth in any particular family. The birth of DS infants to the younger mother suggests that low SES might impart an "age-independent" risk of NDJ.

At this point, it is hard to ascertain whether the effect of SES is of maternal or grand maternal in origin since the families have been inhabitants of this delta area for several generations. Moreover, identification of a variable as the specific risk for MII or MI NDJ is also difficult. The risk may arise due to deficiency of some important nutrients like folic acid or the depressed expression of some important genetic components involved in chromosome segregation system. Another intuitive epidemiological risk factor is the practice of shrimp larvae collection by women during which they remain exposed to extremely saline water (they remain submerged for hours) contaminated with oil spills from mechanized boats (very common mode of transportation in Sundarban delta area). Elaborate epidemiological survey and subsequent experimental validation are needed to confirm this prediction. We are pursuing studies to explore other risks factors for Ch21 NDJ in this poor, rural tribal population with larger sample size. The list includes altered positioning of chiasma on nondisjoined Ch21 in interaction with maternal age, genetic polymorphisms, and the environmental or habitual factors that may predispose the women to NDJ. We hope to find additional information in the near future that will help us to understand the etiology of DS birth more decisively.

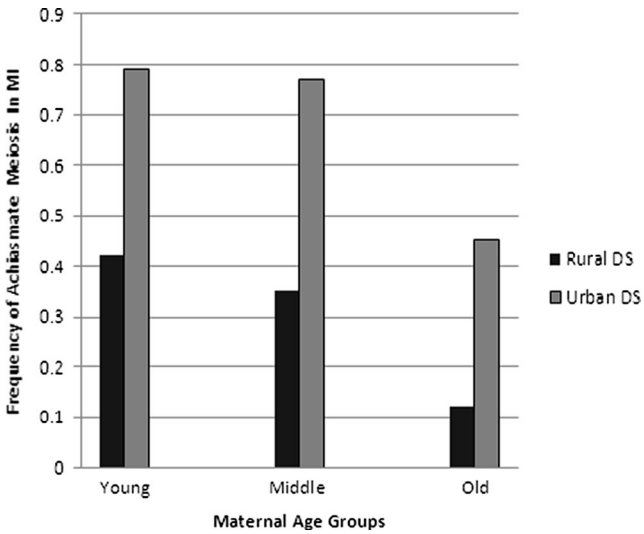

Fig. 1 Distribution of achiasmate meiosis I events among the rural and urban women stratified by their age at conception (young, ≤28 years; middle, 29–34 years; old, ≥35 years) of Down syndrome child. The figure shows gradual decease in achiasmate meiosis I errors with age suggesting that achiasmate meiosis is maternal-age-independent risk; but the poor, rural women experience less achiasmate meiosis than the urban women, which suggests that the former group is at the risk of nondisjunction under extreme low socioeconomic conditions even when the chromosome experiences chiasma formation

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Compliance with ethics guidelines The experiments with human subjects described in the manuscript comply with the current laws of India as well as the principles outlined in the Declaration of Helsinki. The ethical approval for these works was received from the institutional ethics committee of West Bengal University of Technology, Kolkata.

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