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SNaPshot Assay in Quantitative Detection of Allelic Nondisjunction in Down Syndrome

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Aim: We wished to identify markers associated with allelic nondisjunction in nuclear families with Down syndrome (DS) offspring. Since the *GRIK1* and *GARS-AIRS-GART* genes, mapping to chromosome 21q22.1, may be informative in this regard, we genotyped four single-nucleotide polymorphisms [30952599(A/G) rs363484; 30924733(A/G) rs363506; 34901423(A/G) rs2834235; 34877070(A/G) rs7283354] present in these genes using the SNaPshot™ assay protocol. *Results:* We have reported 30952599(A/G)-rs363484 to be monomorphic in our sample population. Genotyping revealed 35/65 families to be informative for 34877070(A/G)-rs7283354 (GARS-AIRS-GART), whereas only 25/65 and 11/65 are informative for 34901423(A/G)-rs2834235 (GARS-AIRS-GART) and 30924733(A/G)-rs363506 (GRIK1) polymorphisms, respectively. The parent- and stage-of-origin of nondisjunction could be traced in 48/65 families using at least one polymorphic marker. A single trio provided internal validation for assignment of the parent- and stage-of-origin of nondisjunction whereby the nondisjoining alleles were independently identified as G-rs363506, G-rs2834235, and G-rs7283354, respectively. An enhanced ratio of meiosis-I to meiosis-II errors during maternal or paternal meioses accounts for allelic nondisjunction. *Conclusions:* The SNaPshot assay is quantitative and permits multiplexing for detection of allelic nondisjunction. Inclusion of additional informative chromosome 21-specific markers may aid rapid aneuploidy detection, screening, and prenatal counseling of parents at risk of having babies with DS.

Introduction

Down syndrome (DS), arising from nondisjunction of chromosome 21, affects ~1/700 live births (Antonarakis *et al.*, 2004; Wiseman *et al.*, 2009) and is the leading genetic cause of mental retardation in children (Dierssen *et al.*, 2009; Korbel *et al.*, 2009; Gardiner *et al.*, 2010). Conventional approaches to detect trisomy rely on karyotyping that requires ~2–3 weeks but fails to detect aneuploidy in less than 5 Mb sequence (Oitmaa *et al.*, 2010), thereby precluding diagnosis of partial or segmental trisomy. Alternatives to karyotyping are now provided by rapid aneuploidy detection technologies like interphase fluorescence *in situ* hybridization (Leclercq *et al.*, 2008), quantitative fluorescent polymerase chain reaction (PCR) (Cirigliano *et al.*, 2009; Hills *et al.*, 2010), multiplex ligation-dependent probe amplification (Hochstenbach *et al.*, 2005; Kooper *et al.*, 2008; Van Opstal *et al.*, 2009), and T21-arrayed primer extension-2 (APEX-2) assays (Oitmaa *et al.*, 2010). The present study describes the quantitative detection of allelic nondisjunction in DS by means of a multiplex SNaPshot™ assay (Markridakis and Reichardt, 2001).

The SNaPshot assay is based on incorporation of a single-fluorescent ddNTP using an extension primer that abuts one nucleotide 5′ (upstream) of a given single-nucleotide polymorphism (SNP) followed by automated DNA sequencing analysis (Markridakis and Reichardt, 2001). This approach has been used for the quantitative detection of loss of heterozygosity in tumors (Bujalkova *et al.*, 2008), colorectal cancer (Goranova *et al.*, 2009), and mitochondrial DNA heteroplasmy (Vallone *et al.*, 2004) where the amount of DNA applied is directly proportional to the amount of amplified product and there is equal incorporation of ddNTPs in heterozygous samples (Markridakis and Reichardt, 2001). The assay has been used to simultaneously and accurately genotype multiple known single-nucleotide polymorphic markers (Markridakis and Reichardt, 2001). Accordingly, we used the multiplex SNaPshot assay protocol to ascertain the parent- and stage-of-origin of nondisjunction in DS patient families.

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Previous studies from our laboratory have shown that the *GRIK1* and *GARS-AIRS-GART* genes, located in a critical region of chromosome 21q22.1 (Patterson *et al*., 1981; Hard *et al*., 1986; Gregor *et al*., 1994), are informative with respect to DS (Ghosh *et al*., 2009; Banerjee *et al*., 2012). Therefore, we genotyped four SNPs [30952599(A/G) rs363484; 30924733(A/G) rs363506; 34901423(A/G) rs2834235; 34877070(A/G) rs7283354] that map to these genes and manifest with moderate heterozygosity (0.398 [rs363506]; 0.490 [rs2834235]; 0.429 [rs7283354]) (www.ncbi.nlm.nih/gov/projects/SNP/) using the SNapShot assay. The rs363484 polymorphism is located between the RNA editing site and editing complementary region in intron-13 of the kainate receptor subunit *GRIK1* and may influence pre-mRNA editing (Barbon and Barlati, 2000). The rs363506 variant in intron-17 of *GRIK1* abolishes binding sites for splice factors (nhRNP-F and nhRNP-H) that are part of the complex, which enhances alternative splicing in mouse neuronal tissues (Chou *et al*., 1999). The rs2834235 and rs7283354 SNPs are present within intron-7 and intron-20, respectively, of the *GARS-AIRS-GART* gene that is involved in *de novo* purine biosynthesis (Aimi *et al*., 1990) and is overexpressed in postmortem DS cerebellum (Brodsky *et al*., 1997). The functional importance of these polymorphisms is currently unknown. We have demonstrated the utility of the SNaPshot assay in ascertainment of the parent- and stage-of-origin of nondisjunction.

Materials and Methods

Bio-informatic procedures

The information regarding rs363484, rs363506, rs2834235, and rs7283354 SNPs and their flanking sequences were obtained from the dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP/). The Splicing Rainbow software program (www.ebi.ac.uk/asd-srv/wb.cgi?method=8) was used to detect splice factor binding sites flanking the SNPs. The primer-3 algorithm (Rozen and Skaletsky, 2000; http://frodo.wi.mit.edu/primer3/primer3_www.cgi) was used for designing PCR primers. The internal primers for single-base extension (SBE) were designed manually so that they end just 5′ of the SNP to be genotyped (Table 1). The multiplex genotyping of SNPs was facilitated by addition of a nonspecific $[GATC]_n$ stretch to the 5′ end of the primers thereby varying the length of primers (Table 1) (Lindblad-Toh *et al*., 2000).

Subject ascertainment and diagnostic procedures

The inclusion and exclusion criterion for DS was as per DSM-IV TR 2000 (American Psychiatric Association, 2000) and SMITH'S Recognizable patterns of human malformation (Jones, 2006). A thorough clinical and psychological evaluation was carried out by collaborating psychologists and psychiatrists at the outpatient department of Manovikas Kendra. A total of 65 DS patient families (35 trios and 30 duos) were recruited for the study after obtaining written informed consent. Detailed socio-demographic and clinical history were obtained by means of a structured questionnaire. An ~5-mL blood sample was collected from each participant. The study protocol was previously approved by the Human Ethics Committee of Manovikas Kendra.

Genotyping procedures

Genomic DNA from whole-blood lymphocytes was isolated by the salting-out procedure of Miller *et al.* (1988). PCR-

Table 1. Single-Base Extension Primers for SNaPShot Multiplex Genotyping Assay

Markers	*Single-base extension primers*	*Primer size (bp)*	*DNA strand direction*
rs363484 (A→G)	**5′-[GACTGACTGACTGACT]**CCCCATATACTACTTTACCCATGT-3′	40	Forward
rs363506 (A→G)	**5′-[GACTGACTGACTGACTGACTGACT]**ACCTGGTTGAGTTAGCATGG-3′	52	Forward
rs2834235 (A→G)	**5′-[GACTGACTGACTGACTGACTGACTGACTGACT]**TGAAAATCATAAAGCTAAAATACCT-3′	61	Forward
rs7283354 (A→G)	**5′-[GACTGACTGACTGACTGACTGACTGACTGACTGACTGACT]**CCACCTTCCTGTCACCATGA-3′	72	Forward

As described in **Materials and Methods**, nonspecific GACT tailing at the 5′ end of each primer (shown in bold) was employed to vary fragment size and improve resolution in the multiplex assay.

based amplification of genomic DNA targets was carried out in the DNA Engine Thermal Cycler (MJ Research PTC-200). For the rs363484 polymorphism of the *GRIK1* gene, 5 pmol of each forward (F-5′-GGAAGGAGCAGGGGTCTC-3′) and reverse (R-5′-AATAGTTGAAGAAAGTGGGAAAATC-3′) primers were used in reactions containing 100 ng of template DNA, 1-X Thermopol-II buffer (New England Biolabs) containing 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8 at 25°C), 0.1% Triton X-100 (New England Biolabs), 1 mM $MgSO_4$, 200 μM dNTPs, and 0.2 U Taq DNA polymerase in 20 μL reaction volume. The cycling conditions were as follows: denaturation at 95°C for 10 min; 30 cycles of denaturation at 95°C for 40 s; annealing at 60°C for 40 s; elongation at 72°C for 40 s; a final elongation step at 72°C for 10 min. For the rs363506 polymorphism of the *GRIK1* gene, 5 pmol of each forward (F-5′-AAACACACCTTCAACTCTCTTATTCA-3′) and reverse (R-5′-CTGGGGAAACGGGCAAG-3′) primers were used in reactions and cycling condition as mentioned above. The amplification of rs7283354 and rs2834235 polymorphisms was performed using 10 pmol of forward and reverse primers (rs2834235: F-5′-ACAGAAGGGGATGATGAGGA-3′, R-5′-TCGGGAAGTTATTTTTGGCTA-3′) and (rs7283354: F-5′-TGCCAAACACAGAAATGAGG-3′, R-5′-GCTTGAACTCCCCACCTTC-3′, respectively). The reaction and PCR cycle conditions were similar as described previously except the concentration of $MgSO_4$ was 1.5 mM.

Multiplex genotyping by minisequencing

Exonuclease-I (Exo-I; Applied Biosystems) and shrimp alkaline phosphatase (SAP; Applied Biosystems) enzymes were used to remove the unincorporated dNTPs in 10 μL reaction volume containing 0.5 U Exo-I and 0.5 U SAP and initial incubation for 60 min at 37°C followed by 15 min incubation at 75°C for enzyme denaturation. The minisequencing reaction was performed with 2 μL SNaPshot multiplex minisequencing kit reaction mix (Applied Biosystems), 3 μL pooled PCR product, 1 μL pooled SBE primers, and sterile autoclaved Milli-Q water up to 4 μL. The thermal cycling conditions for SBE were as follows: 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The enzymatic purification of SBE product was performed with 1 U SAP followed by incubation at 37°C for 60 min and 75°C for 15 min in order to remove the unincorporated, degraded fluorescent ddNTPs. The samples were prepared for electrophoresis by adding 0.5 μL of purified SBE products with 9 μL Hi-Di formamide and 0.5 μL GS120Liz internal size standard and were electrophoresed in an ABI prism 3130 Genetic Analyzer (Applied Biosystems), under the SNP1_POP5 default module. The standard dye set (E5) used for genotyping includes dR6G (ddATP; emits green fluorescence), R110 (ddGTP; emits blue fluorescence), dTAMRA (ddCTP; emits yellow fluorescence), and dROX (ddATP: emits red fluorescence) (Applied Biosystems). The GeneMapper 3.5 software (Applied Biosystems) was used to analyze the electropherograms.

Statistical analysis

The fluorescent peak height intensities were quantitated by ABI GeneMapper Generic software (Applied Biosystems). The signal intensity ratio for disomic heterozygous controls ranges from 1.02 to 1.6 and the cutoff for ascertainment of 1:1

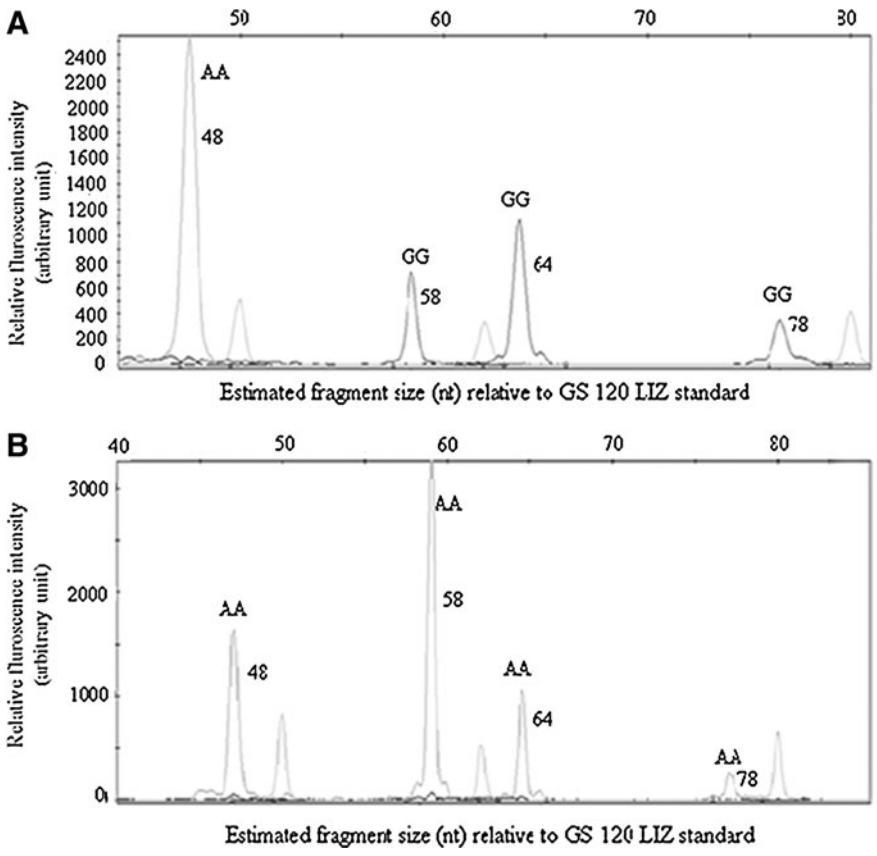
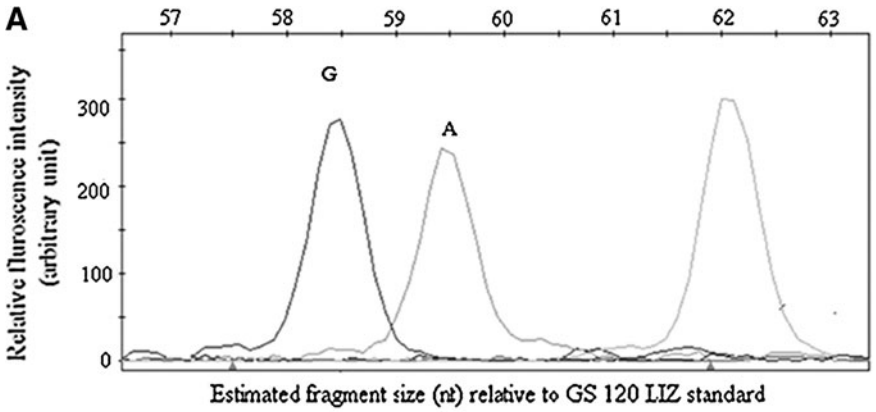


FIG. 1. Multiplexed SNaPshot™ assay-based genotyping of rs363484, rs36506, rs2834235, and rs7283354 markers. **(A)** The peak at 48 nt denotes the AA genotype for rs36484, which is nonpolymorphic. The peaks at 58, 64, and 78 nt denote the GG homozygotes for rs36506, rs2834235, and rs7283354, respectively. **(B)** The peak at 48 nt denotes the AA genotype for rs36484, which is nonpolymorphic. The peaks at 58, 64, and 78 denote the AA homozygotes for rs36506, rs2834235, and rs7283354, respectively.



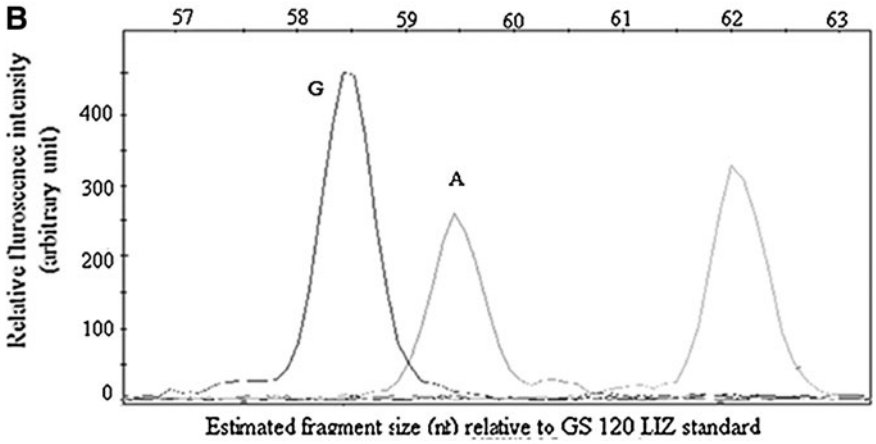
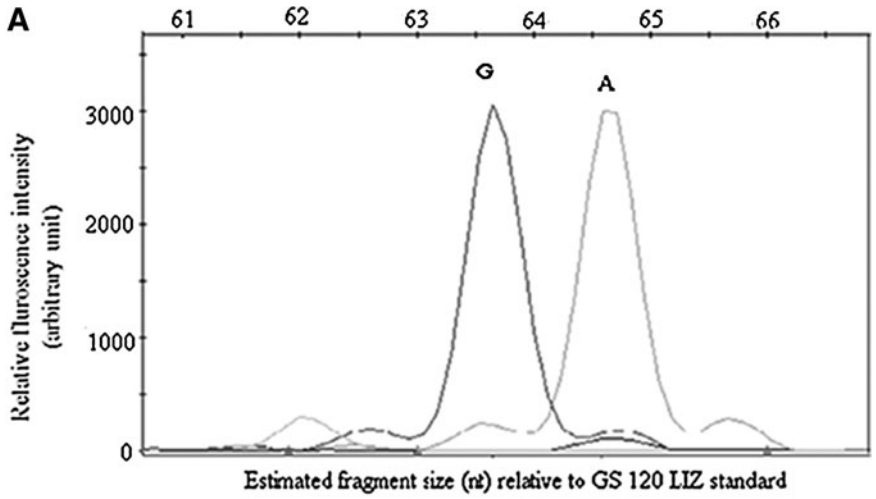


FIG. 2. **(A)** Genotyping of DS offspring based on parental heterozygous genotypes for rs363506. **(B)** Shows a GGA (2:1) genotype of rs363506 for the DS offspring based on ratiometric evaluation of peak height intensities as described in **Materials and Methods.** The 2:1 fluorescence intensity ratio derives from peak height values being 450 (for G allele) and 262 (for A allele), respectively, in the above case. DS, Down syndrome.



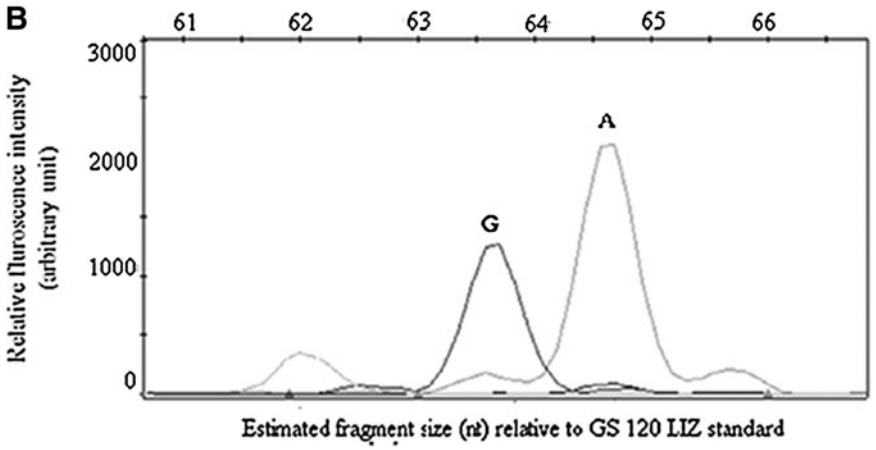


FIG. 3. **(A)** Genotyping of DS offspring based on parental heterozygous genotypes for rs2834235. **(B)** Shows a GAA (1:2) genotype of rs2834235 for the DS offspring based on ratiometric evaluation of peak height intensities as described in **Materials and Methods.** The 1:2 fluorescence intensity ratio derived from peak height values are 1261 (for G allele) and 2106 (for A allele), respectively, in the above case.

was set at 1 to 1.5. The signal intensity ratio for trisomic heterozygotes ranges from 1.54 to 2.45 and the cutoff for ascertainment of 2:1 or 1:2 was set at 1.54 to 2.45, respectively.

Results

Since all the polymorphisms under study consisted of a transition from adenine to guanine, the green peaks in Figure 1A and B denote the A allele and the blue peaks in Figure 1A depict the G allele. The homozygous genotypes for all the major alleles of all the polymorphisms (rs363506, rs2834235, and rs7283354) are shown in Figure 1A, whereas the homozygous genotypes for all the minor alleles of these polymorphisms are shown in Figure 1B. The rs363484 locus is nonpolymorphic and was thus excluded from further analysis. The fragment sizes, as indicated in Figure 1, are different from those expected (Table 1). This is due to mobility difference of the fluorophores used to detect each of the bases (Hurst *et al.*, 2009). However, the probes are sufficiently spaced such that minor differences in mobility do not impair interpretation of the resulting electropherograms. As shown in Figure 1A and B, the fragments are well resolved.

Using genotype data of heterozygous parents as internal disomic controls, the nondisjoining allele in DS offspring was determined by quantitation of peak height intensities in the genotype data obtained from trisomic, heterozygous DS offspring (Figs. 2A, B, 3A, B, and 4A, B). The ratio of peak height intensity for disomic heterozygous parents was considered to be 1:1, whereas that of trisomic heterozygous DS patients was either 2:1 or 1:2 (Table 2A–C). Of 21 disomic parental heterozygous genotypes analyzed in this study, only one (father in family F7; rs2834235 polymorphism genotype; Table 2B) exhibited significant deviation of peak height intensity ratio from the expected 1:1, the reason for which is presently unknown. This individual, however, is neither trisomic nor mosaic as inferred by examining other molecular genetic markers (data not shown).

A total of 35 DS trios were genotyped for the rs363506 polymorphism and 10 families were informative (Table 3A). The remaining 25 trios were considered noninformative as their genotypes were all homozygous and thus excluded from the further analysis. Of the informative families, seven showed one parent and proband to be homozygous and the other parent was heterozygous (Table 3A). The homozygous

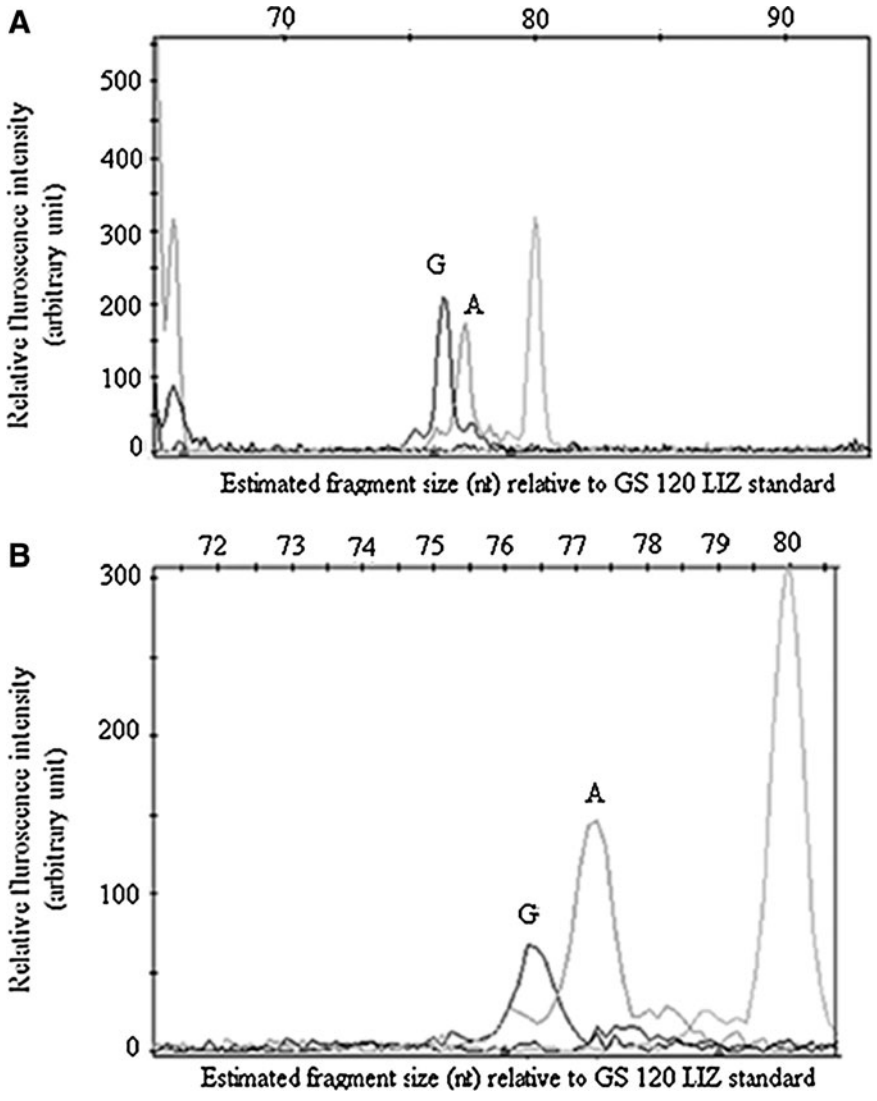


FIG. 4. **(A)** Genotyping of DS offspring based on parental heterozygous genotypes for rs728354. **(B)** Shows a GAA (1:2) genotype of rs7283354 for the DS offspring based on ratiometric evaluation of peak height intensities as described in **Materials and Methods.** The 1:2 fluorescence intensity ratio derived from peak height values are 70 (for G allele) and 150 (for A allele), respectively, in the above case.

parent is the nondisjoining parent (NDJP) in whom the error occurred in meiosis-I (Table 3A). In two other families, one parent and the proband were heterozygous, whereas the other parent was homozygous. The heterozygous parent is the NDJP, whereas the homozygous parent is the correctly disjoining parent (CDJP). Since parental heterozygosity of the NDJP is retained in the offspring, the error must have originated in meiosis-I. Furthermore, in a single trio, both parents were heterozygous and the proband was homozygous. In this family, the parent of origin could not be determined unequivocally; the origin of nondisjunction is meiosis-II (Table 3A).

Of 30 duos studied, a single family was informative, in which the proband genotype was a recombinant of the available parental genotype. This parent is the CDJP. The missing parental genotype was deemed informative in whom the error originated in meiosis-I (Table 3A).

Overall, the G allele manifested as the nondisjoining allele in all DS patients and allelic nondisjunction arises in maternal meiosis-I (three cases), maternal meiosis-II (one case), paternal meiosis-I (seven cases), and paternal meiosis-II (one case).

A total of 35 trios were genotyped for the rs2834235 polymorphism of which 21 trios were noninformative. Among 14 informative trios, there were four trios having one parent and offspring of homozygous genotype and in those families, the heterozygous parent was the CDJP, whereas the other parent is the NDJP in whom the error originated in meiosis-I (Table 3B). There were five trios with one parent and proband of heterozygous genotype and the other parent was homozygous. The heterozygous parent was the NDJP; as the parental heterozygosity is retained in the offspring, the nondisjunction error is in meiosis-I (Table 3B). In the remaining four trios, both sets of parents were heterozygous, whereas the proband was either heterozygous or homozygous. In those families, the stage of origin was considered as meiosis-II as the parental heterozygosity of the CDJP was reduced to homozygosity in the DS offspring. However, parent of origin could not be

Table 2A. Genotyping of Down Syndrome Offspring Based on Available Parental Genotypes in Duos and Trios

Polymorphism	*Family*	*Sample*	*Genotype*	*Fluorescence intensity (arbitrary unit)*		*Fluorescence intensity ratio (G/A)*
rs363506	F1	Father	GA	280	250	1:1
		Proband	GGA	450	262	2:1
	F2	Mother	GA	330	240	1:1
		Proband	GGA	3483	2248	2:1
	F3	Proband	GGA	650	370	2:1

The genotypes were ascertained based on ratiometric evaluation of peak height intensity as described in **Materials and Methods.**

Table 2B. Genotyping of Down Syndrome Offspring Based on Available Parental Genotypes in Duos and Trios

Polymorphism	*Family*	*Sample*	*Genotype*	*Fluorescence intensity (arbitrary unit)*		*Fluorescence intensity ratio (G/A)*
rs2834235	F1	Father	GA	1021	746	1:1
		Proband	GAA	379	827	1:2
	F4	Mother	GA	415	307	1:1
		Proband	GGA	460	262	2:1
	F5	Mother	GA	1069	825	1:1
		Proband	GGA	1170	528	2:1
	F6	Mother	GA	598	635	1:1
		Proband	GGA	628	303	2:1
	F7	Father	GA	1064	658	1:1
		Mother	GA	676	535	1:1
		Proband	GAA	893	1540	1:2
	F8	Proband	GGA	1997	1234	2:1
	F9	Mother	GA	1252	1290	1:1
		Proband	GAA	100	180	1:2
	F10	Mother	GA	608	520	1:1
		Proband	GGA	530	260	2:1
	F11	Mother	GA	528	570	1:1
		Proband	GGA	582	331	2:1
	F12	Proband	GAA	2159	3501	1:2
	F13	Proband	GAA	200	370	1:2
	F14	Mother	GA	3010	3003	1:1
		Proband	GAA	1261	2106	1:2
	F15	Father	GA	2446	1758	1:1
		Proband	GGA	2779	1624	2:1
	F15	Proband	GAA	1085	1767	1:2
	F17	Father	GA	2397	3131	1:1
		Proband	GGA	4214	2658	2:1

The genotypes were ascertained based on ratiometric evaluation of peak height intensity as described in **Materials and Methods.**

Table 2C. Genotyping of Down Syndrome Offspring Based on Available Parental Genotypes in Duos and Trios

Polymorphism	*Family*	*Sample*	*Genotype*	*Fluorescence intensity (arbitrary unit)*		*Fluorescence intensity ratio (G/A)*
rs7283354	F18	Proband	GGA	99	60	2:1
	F19	Father	GA	260	244	1:1
		Proband	GGA	454	264	2:1
	F20	Father	GA	76	68	1:1
		Mother	GA	140	170	1:1
		Proband	GGA	127	62	2:1
	F9	Mother	GA	210	180	1:1
		Proband	GAA	70	150	1:2
	F10	Mother	GA	104	84	1:1
		Proband	GGA	100	62	2:1
	F21	Proband	GGA	156	68	2:1
	F22	Proband	GGA	249	108	2:1
	F23	Proband	GGA	402	171	2:1
	F24	Proband	GAA	38	66	1:2
	F25	Proband	GGA	378	166	2:1
	F26	Proband	GGA	211	113	2:1
	F27	Proband	GAA	64	157	1:2
	F28	Proband	GAA	68	147	1:2
	F15	Proband	GA	293	240	1:1
	F29	Father	GGA	786	327	2:1
		Proband	GGA	269	136	2:1
	F30	Father	GA	330	250	1:1
		proband	GGA	423	185	2:1

The genotypes were ascertained based on ratiometric evaluation of peak height intensity as described in **Materials and Methods.**

traced as both parents were heterozygous (Table 3B). A single trio had both parents with homozygous and proband with heterozygous genotype. In that family, the parent and stage of origin of nondisjunction were discerned based on the proband genotype (Table 3B).

Out of a total 30 duos, 11 were informative (Table 3B). There were six duos in which the proband genotype is a recombinant of the available parental genotype (Table 3B). In these duos, the missing parental genotype derives from the NDJP. In the remaining five duos, proband genotypes were nonrecombinant of the available parental genotype. Thus, the available parent is the NDJP and as parental heterozygosity is retained in the offspring, the origin of nondisjunction error is in meiosis-I (Table 3B).

The results indicated that the nondisjoining allele was G in 49 DS patients and A in 16 DS patients (Table 3B) with the nondisjunction error occurring in maternal meiosis-I (11 cases), maternal meiosis-II (four cases), paternal meiosis-I (10 cases), and paternal meiosis-II (four cases).

Genotyping of 35 trios for the rs7283354 revealed 17 families to be informative (Table 3C). In 10 of these families, one parent and proband are homozygous; the homozygous parent was the NDJP in whom the error occurred during meiosis-I. Of the remaining seven families, two trios contain one parent and proband with heterozygous genotype, in which the heterozygous parent is the NDJP and homozygous parent was the CDJP (Table 3C). Furthermore, if the parental heterozygosity is retained in the offspring, origin of nondisjunction is in meiosis-I, whereas if the parental heterozygosity is reduced to homozygosity in the offspring, the nondisjunction error is in meiosis-II (Table 3C). When both parents and proband showed heterozygous genotype, the stage of origin of nondisjunction was ascertained from the proband genotype (Table 3C). There were three trios with parental genotypes being heterozygous, but the proband was homozygous (Table 3C). In these families, nondisjunction occurred in meiosis-II, but the parent of origin could not be ascertained (Table 3C). When both parents were homozygous and the proband was heterozygous, the NDJP was assigned based on the proband genotype and the error originated in meiosis-I (Table 3C).

From a total of 30 duos genotyped for rs7283354, we excluded 12 families, as they were noninformative (Table 3C). In 15 out of 18 informative families, the proband genotypes were recombinant of the available parental genotype. In these families, the available parent was the CDJP and missing parent was assumed to be the NDJP (Table 3C). In the remaining three duos, the available parent and proband both were heterozygous. The available parent was the CDJP where the error originated in meiosis-I (Table 3C). The analyses indicated that the G allele is involved in nondisjunction in 51 cases, whereas A allele occurred in 14 cases. The error occurred in maternal meiosis-I (11 cases), maternal meiosis-II (three cases), paternal meiosis-I (21 cases), and paternal meiosis-II (four cases) (Table 3C).

Pooled data analysis

There were 48 families in which parent/stage of origin could be determined from at least one marker. Considering any two out of three markers, 15 families were found with same parent and stage of origin of nondisjunction. Among these, two families showed the same parent and stage of origin of nondisjunction with respect to rs363506 and rs2834235 markers, 11 families showed the same parent and stage of origin of nondisjunction with respect to rs2834235 and rs7283354. Furthermore, three families showed the same

Table 3A. Parent and Stage of Origin of Nondisjoining Allele(s) in Down Syndrome Patient Families Genotyped for rs363506 Polymorphism of *GRIK*1 Gene

NDJP genotype	*CDJP genotype*	*Child genotype*	*Marker status*	*Stage of origin*	*No. of trios (T)/duos (D)*
GG (♀)	GA (♂)	GGG	N	M-I	2
GG (♂)	GA (♀)	GGG	N	M-I	5
GA (♂)	GG (♀)	GGA	N	M-I	1
GA (♀)	GG (♂)	GGA	N	M-I	1
GA (♂/♀)	GA (♀/♂)	GGG	R	M-II	1
Missing(♂)	GG (♀)	GGA	N	M-I	1

NDJP, nondisjoining parent; CDJP, correctly disjoining parent; R, marker status reduced to homozygosity; N, marker status nonreduced to homozygosity; M-I, meiosis-I; M, meiosis-II.

Table 3B. Parent and Stage of Origin of Nondisjoining Allele(s) in Down Syndrome Patient Families Genotyped for rs2834235 Polymorphism of *GARS-AIRS-GART* Gene

NDJP genotype	*CDJP genotype*	*Child genotype*	*Marker status*	*Stage of origin*	*No. of trios (T)/duos (D)*
GG (♀)	GA (♂)	GGG	N	M-I	1
AA (♀)	GA (♂)	AAA	N	M-I	1
GG (♂)	GA (♀)	GGG	N	M-I	2
GA (♀)	GG (♂)	GGA	N	M-I	3
GA (♂)	AA (♀)	GAA	N	M-I	1
GA (♀)	AA (♂)	GAA	N	M-I	1
GG (♀)	AA (♂)	GGA	N	M-I	1
GA (♂/♀)	GA (♀/♂)	GAA	R	M-II	1
GA (♂/♀)	GA (♀/♂)	GGG	R	M-II	2
GA (♂/♀)	GA (♀/♂)	AAA	R	M-II	1
Missing (♂)	GA (♀)	AAA	N	M-I	3
Missing (♂)	GG (♀)	GAA	N	M-I	1
Missing (♂)	AA (♀)	GAA	N	M-I	1
Missing (♀)	GG (♂)	GAA	N	M-I	1
GA (♀)	Missing (♂)	GGA	N	M-I	2
GA (♂)	Missing (♀)	GGA	N	M-I	2
GA (♀)	Missing (♂)	GAA	N	M-I	1

Table 3C. Parent and Stage of Origin of Nondisjoining Allele(s) in Down Syndrome Patient Families Genotyped for rs7283354 Polymorphism of GARS-AIRS-GART

NDJP genotype	*CDJP genotype*	*Child genotype*	*Marker status*	*Stage of origin*	*No. of trios (T)/duos (D)*
GG (♀)	GA (♂)	GGG	N	M-I	5
GG (♂)	GA (♀)	GGG	N	M-I	4
AA (♂)	GA (♀)	AAA	N	M-I	1
GA (♀)	AA (♂)	GAA	N	M-I	1
GG (♀)	AA (♂)	GGA	N	M-I	1
GA (♂/♀)	GA (♀/♂)	GGA	N	M-I	1
GA (♂)	AA (♀)	GGA	R	M-II	1
GA (♂/♀)	GA (♀/♂)	GGG	R	M-II	2
GA (♂/♀)	GA (♀/♂)	AAA	R	M-II	1
Missing (♂)	GA (♀)	AAA	N	M-I	4
Missing (♂)	GA (♀)	GGG	N	M-I	2
Missing (♂)	GG (♀)	GGA	N	M-I	3
Missing (♂)	GG (♀)	GAA	N	M-I	1
Missing (♂)	AA (♀)	GAA	N	M-I	1
Missing (♂)	AA (♀)	GGA	N	M-I	2
Missing (♀)	GG (♂)	GGA	N	M-I	1
Missing (♀)	AA (♂)	GAA	N	M-I	1
GA (♀)	Missing (♂)	GGA	N	M -I	1
GA (♂)	Missing (♀)	GGA	N	M-I	2

parent and stage of origin of nondisjunction for rs363506 and rs7283354. There was a single family that showed the same parent and stage of origin with respect to all three polymorphisms (data not shown). These observations provided internal validation for our findings.

Discussion

Using the SNaPshot assay, we present evidence for allelic nondisjunction at rs363506 in the *GRIK1* gene and rs2834235 and rs7283354 in the *GARS-AIRS-GART* gene that are important candidates for genetic studies on DS-related nondisjunction. This is a first report in the field from India. Due to the low heterozygosity value estimated for these markers, the distribution of allele frequencies was not in Hardy–Weinberg equilibrium (data not shown) and thus precluded prediction of a robust risk haplotype. However, our sample included one informative trio, in which the nondisjoining alleles were independently identified as G-rs363506, G-rs2834235, and G-7283354, respectively.

We confirmed the elevated maternal meiosis-I to meiosis-II error ratios as reported earlier by other investigators (Sherman *et al.*, 1991; Ramírez *et al.*, 2007). Although we observed increased paternal meiosis-I errors in our sample, in contrast to previous reports of increased nondisjunction in paternal meiosis-II (Savage *et al.*, 1998; Oliver *et al.*, 2009), it is important to note that chromosome 21, which has one chaisma, is generally more prone to nondisjunction in normal males (Soares *et al.*, 2001).

The SNaPshot technique is a useful approach in detecting parent- and stage-of-origin of nondisjunction and underscores the need for characterizing additional markers. This may help in rapid aneuploidy detection, screening, and prenatal counseling of parents who are at risk of having children with DS. The advantages of this method are as follows: (1) sample turnaround time is ~72–96 h; (2) fetal cell culturing is not required; (3) the setup is flexible, can be multiplexed, and can be easily customized to extend it to other loci.

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Author Disclosure Statement

The authors report no conflict of interest regarding any financial relationship with industry. Authors disclose no financial arrangement with a company whose product is pertinent to the manuscript or with a company making a competing product. The authors alone are responsible for the content and writing of the article.

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