Original Article

Discerning non-disjunction in Down syndrome patients by means of GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ microsatellites on chromosome 21

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INTRODUCTION: Down syndrome (DS), the leading genetic cause of mental retardation, stems from non-disjunction of chromosome 21.
AIM: Our aim was to discern non-disjunction in DS patients by genotyping GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ microsatellites on chromosome 21 using a family-based study design.
MATERIALS AND METHODS: We have used a PCR and automated DNA sequencing followed by appropriate statistical analysis of genotype data for the present study
RESULTS AND DISCUSSION: We show that a high power of discrimination and a low probability of matching indicate that both markers may be used to distinguish between two unrelated individuals. That the D21S2055-$(GATA)_n$ allele distribution is evenly balanced, is indicated by a high power of exclusion [PE=0.280]. The estimated values of observed heterozygosity and polymorphism information content reveal that relative to GluK1-$(AGAT)_n$ [H_{obs}=0.286], the D21S2055-$(GATA)_n$ [H_{obs}=0.791] marker, is more informative. Though allele frequencies for both polymorphisms do not conform to Hardy-Weinberg equilibrium proportions, we were able to discern the parental origin of non-disjunction and also garnered evidence for triallelic (1:1:1) inheritance. The estimated proportion of meiosis-I to meiosis-II errors is 2:1 in maternal and 4:1 in paternal cases for GluK1-$(AGAT)_n$, whereas for D21S2055-$(GATA)_n$, the ratio is 2:1 in both maternal and paternal cases. Results underscore a need to systematically evaluate additional chromosome 21-specific markers in the context of non-disjunction DS.

Access this article online	
Quick Response Code:	Website: www.ijhg.com
	DOI: 10.4103/0971-6866.100769

Key words: Down syndrome, non-disjunction, short tandem repeat polymorphism, polymorphism information content, power of discrimination

Introduction

Non-disjunction of chromosome 21 results in Down syndrome (DS) that occurs in 1/700 live births and remains the leading genetic cause of mental retardation.[1-3] Several mechanisms have been proposed to explain the variable phenotype in DS. Increased dosage that arises from the presence of three, rather than two, copies of chromosome 21-specific genes may account for the observed phenotype.[2] Alternatively, trisomy may render the fetus increasingly susceptible to developmental instability.[4] It is also possible that varying allelic combinations exert variable penetrance.[5] Alternatively, non-disjunction in trisomic offspring may result in the reduction to homozygosity of a susceptibility allele inherited from a heterozygous parent.[6] Available evidence provides only partial support for each of these proposed mechanisms. There is, therefore, a need to systematically identify molecular markers on chromosome 21 that are informative with respect to non-disjunction in DS.

The accuracy of detection of the origin of non-disjunction has been increased by the use of polymorphic DNA markers.[7-9] Short tandem repeat (STR) polymorphisms (microsatellites) have been used previously to detect the origin of the non-disjoined chromosome 21 in DS,[10] for

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prenatal diagnosis of DS,[11-13] for detection of chromosomal rearrangement,[14] for detection of monozygotic twin discordance in DS[15] and for determining the parental origin of supernumerary chromosome in Robertsonian translocation.[16,17] Relative to dinucleotides, tetranucleotide repeat polymorphisms are deemed useful due to their increased stability during amplification in a polymerase chain reaction (PCR), high degree of specificity, and ease of genotyping.[18,19] Accordingly, we elected to study the intronic GluK1-$(AGAT)_n$and D21S2055-$(GATA)_n$ polymorphisms that are, respectively, 15.95 cM and 40.49 cM[20] away from the centromere on chromosome 21.

The GluK1-$(AGAT)_n$polymorphism (21q22.11)[20] is ~5cM telomeric to D21S210/APP and 3 cM centromeric to D21S223/SOD1[21] [Supplementary Figure 1]. A family-based study showed association of the nine repeat GluK1-$(AGAT)_9$ (A_9) allele (χ^2=8.31, df=1, *P*=0.004) with Juvenile Absence Epilepsy in the German population.[22] Given that epilepsy is co-morbid with DS in some cases,[23] it was of interest to genotype this polymorphism in our samples. The D21S2055-$(GATA)_n$ polymorphism (21q22.2) is flanked by genes coding for the Purkinje cell protein-4, the immunoglobulin superfamily-5-like protein,[20] [Supplementary Figure 1] and is stably propagated in Down syndrome patient-derived induced pluripotent stem cell lines.[24]

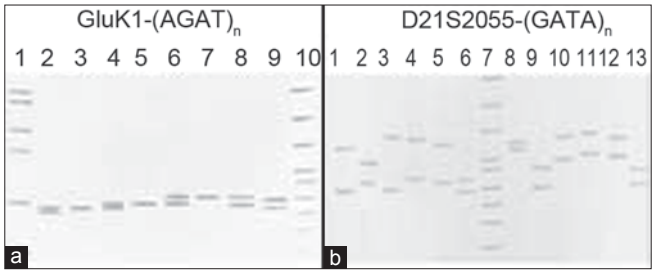


Figure 1(a,b): (a) Genotypes identified by PCR-based amplification of the GluK1-$(AGAT)_n$ polymorphism. 'A' represents followed by polyacrylamide gel electrophoresis allele and subscript denotes number of repeats. Lane-1 ϕ X174DNA/*Hae*III digest; lane-2 A_8A_9; lane-3 A_9A_9; lane-4 A_9A_{10}; lane-5 $A_{10}A_{10}$;lane-6 $A_{10}A_{11}$; lane-7 $A_{11}A_{11}$; lane-8 A_9A_{11}; lane-9 A_8A_{10}; lane-10 ϕ X174DNA/*Hinf*I digest. (b) Genotypes identified by PCR-based amplification of the D21S2055-$(GATA)_n$ polymorphism. 'A' represents followed by polyacrylamide gel electrophoresis allele. Lane-1 A_1A_{12}; lane-2 A_3A_9; lane-3 A_1A_{16}; lane-4 A_5A_{15}; lane-5 A_3A_{13}; lane-6 A_1A_4; lane-7 ϕ X174DNA *Hinf*I digest; lane-8 $A_{12}A_{14}$; lane-9 A_2A_7; lane-10 A_9A_{18}; lane-11 $A_{11}A_{19}$; lane-12 $A_{10}A_{17}$; lane-13 A_2A_6

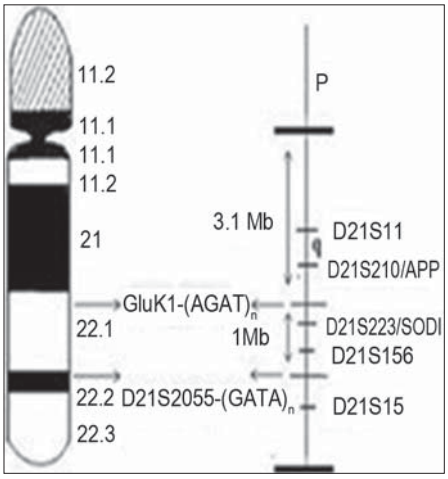
A multiple sequence alignment of genomic DNA sequences flanking each of the GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ polymorphisms followed by phylogenetic comparisons, by means of Neighbor-Joining tree construction, indicate that the sequence ~1 kb upstream and downstream of D21S2055-$(GATA)_n$ is more genetically diverse (0.009-0.314) than the sequence surrounding GluK1-$(AGAT)_n$ (0.009-0.038), and that these intronic sequences have evolved along phyletic lines (data not shown). Since increased genetic variation has the potential to yield markers of diagnostic value, we evaluated these polymorphisms with respect to population-specific parameters [allele frequencies, heterozygosity, power of discrimination (PD), power of exclusion (PE), matching probability (pM)] and ascertained utility of these polymorphisms in discerning parent-and stage-of-origin of non-disjunction in DS patient families.

Materials and Methods

Bio-informatics procedures

Sequence alignments

All the genomic DNA sequences were retrieved from the Ensembl database.[25] Intron-3 sequence of GluK1 (NT_011512.11) gene including GluK1-$(AGAT)_n$ and 1 kb upstream and downstream flanking sequence of D21S2055-$(GATA)_n$ STR polymorphisms of human chromosome 21 were aligned with the orthologous sequences from three non-human primates *viz*: *Pan troglodytes* [ENSPRTRG00000013825], *Pongo*



Supplementary Figure 1: Schematic representation of GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ on chromosome 21 showing physical distance between them

pygmaeus [ENSPPYG00000011323], *Macaca maculatta* [ENSMMUG00000004886] using Clustal X-v.1.83[26] under alignment parameters, with pair-wise gap penalties of 10.00 for gap opening and 0.20 for gap extension. The quality scores (Q-score), computed by the Clustal-X-v.1.83 software,[26] gives a measure for each nucleotide position in the alignment, and displays the scores as a histogram below the alignment pane. The individual Q-score was saved as a text file and was further analyzed by the Tune-clustal X-v.1.01[27] software to obtain the overall quality of the alignments.

Construction of Neighbor-Joining tree

A Neighbor-joining tree was constructed using Clustal X-v.1.83,[26] the number of bootstrap trials being set at 10000, the random number generator seed set at 111. All the sequences were rooted with *Macaca maculatta* as outgroup, and the phylogenetic tree was visualized with TreeView v.1.6.6 software.[28]

Subject ascertainment and diagnostic procedures

A total of 72 families (38 trios and 34 duos) were recruited from the out-patients Department of Manovikas Kendra. All patients fulfilled criteria for DS as per SMITH'S recognizable patterns of human malformation[29] and criteria for MR as per DSM-IV-TR.[30] The DS patient group had a mean age of 8.22±5.69 years and comprised 42 males and 30 females. Detailed demographic and clinical history was recorded by means of a structured questionnaire formation, and written informed consent was secured from all participants. A ~5 ml venous blood sample was collected from each participant for genetic analysis. The study had prior approval of the Institutional Human Ethics Committee of Manovikas.

Genotyping procedures

Genomic DNA was isolated from whole blood lymphocytes by the salting-out procedure.[31] PCR-based amplification of genomic DNA targets was carried out in the DNA Engine Thermal Cycler (MJ Research PTC-200). For the GluK1-$(AGAT)_n$ polymorphism, 5 pmol of each forward (5´-GCTAAATAGATATATGATAAACGG-3´) and reverse (5´-CTGGCAGTAAATGTCTATGAAAC-3´) primers[21] were used in reactions containing 100 ng of template DNA, 1-X Thermopol-II buffer (NEB) containing of 10 mMKCl, 10 mM $(NH_4)_2SO_4$, 20 mMTris-HCl (pH 8.8 at 25°C), 0.1% Triton X-100 (NEB), 1 mM $MgSO_4$, 200 µM dNTPs, and 0.2 U Taq DNA polymerase in 20µl reaction volume. The cycling conditions were as follows: Denaturation at 94°C for 2 min; 32 cycles of denaturation at 94°C for 1 min; annealing at 56°C for 1 min; elongation at 72°C for 1 min; a final elongation step at 72°C for 10 min.[21] For amplification of D21S2055-$(GATA)_n$, 10 pmol of forward (5'-TACAGTAAATCACTTGGTAGGAGA-3') and reverse primers (5'-AACAGAACCAATAGGCTATCTATC-3')[24] were used. The reaction conditions, as mentioned above, were used for PCR amplification. The cycling conditions were as follows: Denaturation at 95°C for 10 min followed by 30 cycles of denaturation at 95°C for 40 sec; annealing at 58°C for 40 sec; elongation at 72°C for 40 sec; final elongation step at 72°C for 7 min. Amplicons and size standards were resolved in 12% polyacrylamide gels containing 5% glycerol. Electrophoresis was carried out at 90V for ~14 h overnight. Size discrimination of bands was performed by means of Quantity One software (BioRad, CA). Parental origin of extra chromosome was determined by scoring the polymorphic allele when three different alleles are present in DS proband or by dosage analysis (2:1 or 1:2) when two different alleles were present in heterozygous form.[32] The signal intensity ratio (range: 1-2.33) cutoff for two allelic band ranges from 1.6 to 2.4 in ascertainment of 2:1 or 1:2 ratios.

Amplicon sequences were confirmed using Applied Biosystems 3130 Genetic analyzer and Big Dye, v 3.1 chemistry. The ~20ng of PCR product was purified using 0.5U Exonuclease II, 0.5U Shrimp Alkaline Phosphatase and 1X buffer after incubating at 37°C for 20 min followed by quenching the reaction at 80°C. The 1/16 dilution of Big Dye, 1X buffer and 3.75 pmole primer was used for cycle sequencing. Cycling conditions for D21S2055-$(GATA)_n$ was as follows: Initial denaturation at 95°C for 1 min followed by 25 cycles of 96°C for 10 sec, 55°C for 5 sec and 60°C for 4 min. For GluK1-$(AGAT)_n$,a two step cycle [i.e. 96°C for 10 sec followed by 60°C for 4 min] was used as the T_m of the primer was 59.5°C. The sequence analysis was performed using Sequencing Analysis Software, v 5.2 (Applied Biosystems) and Chromas v 2.33.

Statistical analysis

Polymorphism Information Content,[33] probability of matching, power of discrimination, power of exclusion, expected heterozygosity, and observed heterozygosity were calculated as previously described.[34,35] Analysis of allele and genotype frequency, heterozygosity, tests for Hardy-Weinberg equilibrium, fixation index was performed using the Popgene version 1.31 software program. Statistical tests (mean, standard deviation) were performed using SigmaPlot 10 software (Cranes Software International Limited, India). The image J v.1.43 software program was used for densitometric analysis. The null allele calculation was performed with Micro-Checker v.2.2.3 software program.[36]

Results

As shown in Figure 1a, the GluK1-$(AGAT)_n$ polymorphism manifests with 4 alleles and 8 genotypes. The PCR amplicons were in the size range of 110 bp to 122 bp corresponding to eight repeat (A_8) through eleven repeat (A_{11}) alleles. Each of these alleles was sequenced to confirm the number of repeats, and an electropherogram representative of the A_{10} allele [Figure 1c] along with BLAST data is shown in Figure 1e. Although 420 chromosomes were genotyped for this polymorphism, the A_8A_8 homozygote for the GluK1-$(AGAT)_n$ was not detected, indicating low frequency of the A_8 allele in the sample population. Allele frequencies show significant deviation from Hardy-Weinberg equilibrium proportions (χ^2=101.55, df=6, P=1×10^{-6}, Supplementary Table 1a).

The D21S2055-$(GATA)_n$ polymorphism manifests with 19 alleles and 101 genotypes, of which 12 representative genotypes are shown in Figure 1b. The PCR amplicons were in the size range of 116 bp to 188 bp corresponding to the single repeat (A_1) through nineteen repeat (A_{19}) alleles. Each of these alleles was sequenced to confirm the number of repeats, and an electropherogram representative of the A_{12} allele [Figure 1d] along with BLAST data is shown in Figure 1f. Since many of the D21S2055-$(GATA)_n$ polymorphic alleles are present at very low frequencies in the sample populations, it was not possible to score all genotypes. Allele frequencies show significant deviation from the Hardy-Weinberg equilibrium proportions (χ^2=694.66, df=171, P=1×10^{-6}, Suppl. Table 1b).

Since deviation from Hardy-Weinberg equilibrium may be due to non-panmixia or genotyping errors, the data was tested for presence of null alleles, allelic dropout, and scoring errors due to stuttering effects as reported previously.[36-40] We found that the observed homozygous class is in excess of that which is expected, for both GluK1-$(AGAT)_n$ [Figure 2a] and D21S2055-$(GATA)_n$ [Figure 2b] genotype data. Figure 2c and 2d show the frequencies of genotypes categorized by the size difference between the alleles. In both cases, the observed and expected frequencies appear to coalesce when the allele size difference is > 8bp. However, we

Table 1: Evaluation of population characteristics of polymorphic alleles at the GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ loci

Parameters	GluK1-$(AGAT)_n$	D21S2055-$(GATA)_n$
Matching probability (pM)	0.023	0.010
Power of discrimination (PD)	0.977	0.990
Power of exclusion (PE)	0.029	0.280
Observation heterozygosity (Ho)	0.286	0.791
Expected heterozygosity (He)	0.494	0.931
Wright's fixation index (Fst)	0.420	0.149
Wright's fixation index (Fis)	0.005	0.009
Polymorphism information content (PIC)	0.449	0.925

Data pertain to analysis of genotype data from 210 individuals as described in method section

Supplementary Table 1a: Determination of Hardy-Weinberg equilibrium proportion in GluK1-$(AGAT)_n$

Polymorphism	No. of Individuals	Alleles	Frequency	Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	χ^2	P(df=6)
GluK1-$(AGAT)_n$	210	A_8	0.0476	A_8A_8	0	0.4535	0.4535	101.55	1×10^{-6}
		A_9	0.2071	A_8A_9	6	4.1527	0.8217		
		A_{10}	0.6762	A_9A_9	26	8.9284	32.6418		
		A_{11}	0.069	A_8A_{10}	14	13.5561	0.0145		
				A_9A_{10}	25	58.969	19.5678		
				$A_{10}A_{10}$	117	95.9093	4.6379		
				A_8A_{11}	0	1.3842	1.3842		
				A_9A_{11}	4	6.0215	0.6786		
				$A_{10}A_{11}$	11	19.6563	3.8121		
				$A_{11}A_{11}$	7	0.969	37.5379		

did not detect any size difference class of 12bp for GluK1-$(AGAT)_n$ [Figure 2c] and of>68 bp for D21S2055-$(GATA)_n$ [Figure 2d]. Null alleles may be present given the general excess of homozygotes for most allele size

Supplementary Table 1b: Determination of Hardy-Weinberg equilibrium proportion in D21S2055-$(GATA)_n$

Polymorphism	No. of individuals	Alleles	Frequency	Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	χ^2	*P* (df=171)
D21S2055-$(GATA)_n$	210	A_1	0.0762	A_1A_1	3	1.1838	2.7866	694.655	1×10^{-6}
		A_2	0.069	A_1A_2	1	2.2148	0.6663		
		A_3	0.019	A_2A_2	0	0.969	0.969		
		A_4	0.05	A_1A_3	0	0.611	0.611		
		A_5	0.0357	A_2A_3	0	0.221	0.221		
		A_6	0.0167	A_3A_3	1	0.0668	13.0311		
		A_7	0.0286	A_1A_4	1	1.6038	0.2273		
		A_8	0.0238	A_2A_4	2	1.4535	0.2055		
		A_9	0.1048	A_3A_4	0	0.401	0.401		
		A_{10}	0.1333	A_4A_4	2	0.5012	4.4821		
		A_{11}	0.0595	A_1A_5	2	1.1456	0.6373		
		A_{12}	0.0619	A_2A_5	0	1.0382	1.0382		
		A_{13}	0.0762	A_3A_5	0	0.2864	0.2864		
		A_{14}	0.0571	A_4A_5	1	0.7518	0.0819		
		A_{15}	0.0714	A_5A_5	1	0.2506	2.2411		
		A_{16}	0.0476	A_1A_6	0	0.5346	0.5346		
		A_{17}	0.031	A_2A_6	1	0.4845	0.5485		
		A_{18}	0.0333	A_3A_6	0	0.1337	0.1337		
		A_{19}	0.0048	A_4A_6	0	0.3508	0.3508		
				A_5A_6	1	0.2506	2.2411		
				A_6A_6	1	0.0501	18.0025		
				A_1A_7	0	0.9165	0.9165		
				A_2A_7	1	0.8305	0.0346		
				A_3A_7	0	0.2291	0.2291		
				A_4A_7	1	0.6014	0.2641		
				A_5A_7	2	0.4296	5.7407		
				A_6A_7	0	0.2005	0.2005		
				A_7A_7	0	0.1575	0.1575		
				A_1A_8	3	0.7637	6.5481		
				A_2A_8	1	0.6921	0.137		
				A_3A_8	0	0.1909	0.1909		
				A_4A_8	1	0.5012	0.4964		
				A_5A_8	0	0.358	0.358		
				A_6A_8	0	0.1671	0.1671		
				A_7A_8	0	0.2864	0.2864		
				A_8A_8	0	0.1074	0.1074		
				A_1A_9	1	3.3604	1.658		
				A_2A_9	3	3.0453	0.0007		
				A_3A_9	1	0.8401	0.0304		
				A_4A_9	1	2.2053	0.6587		
				A_5A_9	1	1.5752	0.21		
				A_6A_9	2	0.7351	2.1766		
				A_7A_9	0	1.2601	1.2601		
				A_8A_9	1	1.0501	0.0024		
				A_9A_9	9	2.2578	20.1341		
				A_1A_{10}	4	4.2768	0.0179		
				A_2A_{10}	6	3.8759	1.1641		
				A_3A_{10}	0	1.0692	1.0692		
				A_4A_{10}	3	2.8067	0.0133		
				A_5A_{10}	2	2.0048	0		
				A_6A_{10}	1	0.9356	0.0044		
				A_7A_{10}	1	1.6038	0.2273		
				A_8A_{10}	0	1.3365	1.3365		
				A_9A_{10}	1	5.8807	4.0507		
				$A_{10}A_{10}$	8	3.6754	5.0884		
				A_1A_{11}	2	1.9093	0.0043		
				A_2A_{11}	2	1.7303	0.042		
				A_3A_{11}	1	0.4773	0.5723		
				A_4A_{11}	0	1.253	1.253		
				A_5A_{11}	2	0.895	1.3643		
				A_6A_{11}	0	0.4177	0.4177		

Supplementary Table 1b: Continued

Polymorphism	No. of individuals	Alleles	Frequency	Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	χ^2	*P* (df=171)
				A_7A_{11}	0	0.716	0.716		
				A_8A_{11}	0	0.5967	0.5967		
				A_9A_{11}	2	2.6253	0.1489		
				$A_{10}A_{11}$	0	3.3413	3.3413		
				$A_{11}A_{11}$	2	0.716	2.3027		
				A_1A_{12}	1	1.9857	0.4893		
				A_2A_{12}	1	1.7995	0.3552		
				A_3A_{12}	2	0.4964	4.5541		
				A_4A_{12}	2	1.3031	0.3727		
				A_5A_{12}	0	0.9308	0.9308		
				A_6A_{12}	0	0.4344	0.4344		
				A_7A_{12}	0	0.7446	0.7446		
				A_8A_{12}	1	0.6205	0.2321		
				A_9A_{12}	1	2.7303	1.0966		
				$A_{10}A_{12}$	2	3.4749	0.626		
				$A_{11}A_{12}$	2	1.5513	0.1298		
				$A_{12}A_{12}$	3	0.7757	6.3787		
				A_1A_{13}	4	2.4439	0.9908		
				A_2A_{13}	3	2.2148	0.2784		
				A_3A_{13}	0	0.611	0.611		
				A_4A_{13}	0	1.6038	1.6038		
				A_5A_{13}	1	1.1456	0.0185		
				A_6A_{13}	0	0.5346	0.5346		
				A_7A_{13}	2	0.9165	1.2811		
				A_8A_{13}	0	0.7637	0.7637		
				A_9A_{13}	2	3.3604	0.5507		
				$A_{10}A_{13}$	4	4.2768	0.0179		
				$A_{11}A_{13}$	0	1.9093	1.9093		
				$A_{12}A_{13}$	3	1.9857	0.5181		
				$A_{13}A_{13}$	4	1.1838	6.6999		
				A_1A_{14}	5	1.8329	5.4723		
				A_2A_{14}	2	1.6611	0.0691		
				A_3A_{14}	1	0.4582	0.6405		
				A_4A_{14}	1	1.2029	0.0342		
				A_5A_{14}	1	0.8592	0.0231		
				A_6A_{14}	0	0.401	0.401		
				A_7A_{14}	2	0.6874	2.5068		
				A_8A_{14}	2	0.5728	3.5561		
				A_9A_{14}	3	2.5203	0.0913		
				$A_{10}A_{14}$	4	3.2076	0.1957		
				$A_{11}A_{14}$	2	1.432	0.2253		
				$A_{12}A_{14}$	0	1.4893	1.4893		
				$A_{13}A_{14}$	1	1.8329	0.3785		
				$A_{14}A_{14}$	0	0.6587	0.6587		
				A_1A_{15}	2	2.2912	0.037		
				A_2A_{15}	5	2.0764	4.1166		
				A_3A_{15}	0	0.5728	0.5728		
				A_4A_{15}	2	1.5036	0.1639		
				A_5A_{15}	0	1.074	1.074		
				A_6A_{15}	0	0.5012	0.5012		
				A_7A_{15}	0	0.8592	0.8592		
				A_8A_{15}	1	0.716	0.1127		
				A_9A_{15}	2	3.1504	0.4201		
				$A_{10}A_{15}$	5	4.0095	0.2447		
				$A_{11}A_{15}$	5	1.79	5.7566		
				$A_{12}A_{15}$	2	1.8616	0.0103		
				$A_{13}A_{15}$	1	2.2912	0.7276		
				$A_{14}A_{15}$	0	1.7184	1.7184		
				$A_{15}A_{15}$	2	1.0382	0.8911		
				A_1A_{16}	0	1.5274	1.5274		
				A_2A_{16}	0	1.3842	1.3842		
				A_3A_{16}	0	0.3819	0.3819		
				A_4A_{16}	1	1.0024	0		
				A_5A_{16}	0	0.716	0.716		
				A_6A_{16}	0	0.3341	0.3341		
				A_7A_{16}	2	0.5728	3.5561		
				A_8A_{16}	0	0.4773	0.4773		

Supplementary Table 1b: Continued

Polymorphism	No. of individuals	Alleles	Frequency	Genotype	Observed (O)	Expected (E)	$(O-E)^2/E$	χ^2	*P* (df=171)
				A_9A_{16}	3	2.1002	0.3855		
				$A_{10}A_{16}$	2	2.673	0.1695		
				$A_{11}A_{16}$	2	1.1933	0.5453		
				$A_{12}A_{16}$	3	1.2411	2.493		
				$A_{13}A_{16}$	1	1.5274	0.1821		
				$A_{14}A_{16}$	0	1.1456	1.1456		
				$A_{15}A_{16}$	0	1.432	1.432		
				$A_{16}A_{16}$	2	0.4535	5.2745		
				A_1A_{17}	0	0.9928	0.9928		
				A_2A_{17}	0	0.8998	0.8998		
				A_3A_{17}	0	0.2482	0.2482		
				A_4A_{17}	1	0.6516	0.1863		
				A_5A_{17}	0	0.4654	0.4654		
				A_6A_{17}	0	0.2172	0.2172		
				A_7A_{17}	1	0.3723	1.0582		
				A_8A_{17}	0	0.3103	0.3103		
				A_9A_{17}	1	1.3652	0.0977		
				$A_{10}A_{17}$	2	1.7375	0.0397		
				$A_{11}A_{17}$	1	0.7757	0.0649		
				$A_{12}A_{17}$	0	0.8067	0.8067		
				$A_{13}A_{17}$	2	0.9928	1.0217		
				$A_{14}A_{17}$	0	0.7446	0.7446		
				$A_{15}A_{17}$	1	0.9308	0.0051		
				$A_{16}A_{17}$	0	0.6205	0.6205		
				$A_{17}A_{17}$	2	0.1862	17.6733		
				A_1A_{18}	0	1.0692	1.0692		
				A_2A_{18}	1	0.969	0.001		
				A_3A_{18}	1	0.2673	2.0084		
				A_4A_{18}	0	0.7017	0.7017		
				A_5A_{18}	0	0.5012	0.5012		
				A_6A_{18}	0	0.2339	0.2339		
				A_7A_{18}	0	0.401	0.401		
				A_8A_{18}	0	0.3341	0.3341		
				A_9A_{18}	1	1.4702	0.1504		
				$A_{10}A_{18}$	3	1.8711	0.6811		
				$A_{11}A_{18}$	0	0.8353	0.8353		
				$A_{12}A_{18}$	0	0.8687	0.8687		
				$A_{13}A_{18}$	0	1.0692	1.0692		
				$A_{14}A_{18}$	0	0.8019	0.8019		
				$A_{15}A_{18}$	0	1.0024	1.0024		
				$A_{16}A_{18}$	2	0.6683	2.654		
				$A_{17}A_{18}$	0	0.4344	0.4344		
				$A_{18}A_{18}$	3	0.2172	35.6567		
				A_1A_{19}	0	0.1527	0.1527		
				A_2A_{19}	0	0.1384	0.1384		
				A_3A_{19}	0	0.0382	0.0382		
				A_4A_{19}	0	0.1002	0.1002		
				A_5A_{19}	0	0.0716	0.0716		
				A_6A_{19}	0	0.0334	0.0334		
				A_7A_{19}	0	0.0573	0.0573		
				A_8A_{19}	0	0.0477	0.0477		
				A_9A_{19}	0	0.21	0.21		
				$A_{10}A_{19}$	0	0.2673	0.2673		
				$A_{11}A_{19}$	0	0.1193	0.1193		
				$A_{12}A_{19}$	0	0.1241	0.1241		
				$A_{13}A_{19}$	0	0.1527	0.1527		
				$A_{14}A_{19}$	0	0.1146	0.1146		
				$A_{15}A_{19}$	0	0.1432	0.1432		
				$A_{16}A_{19}$	0	0.0955	0.0955		
				$A_{17}A_{19}$	0	0.0621	0.0621		
				$A_{18}A_{19}$	0	0.0668	0.0668		
				$A_{19}A_{19}$	1	0.0024	417.002		

classes. This is unlikely, however, since sequencing of individual amplicons did not reveal any mutations. The observed deviation of allele frequencies from HWE is most likely due to small sample size. The adjusted allele frequencies for both loci are given in Supplementary Table 2a and 2b.There is no evidence for either scoring

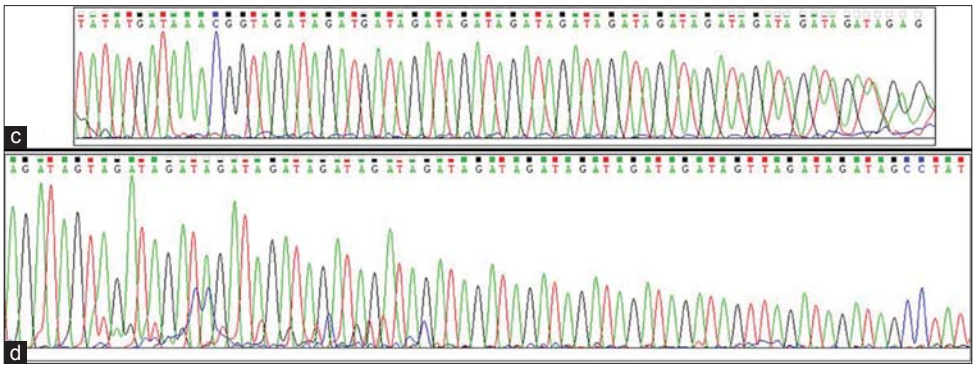


Figure 1: (c) An electropherogram showing the A_{10} allele of GluK1-$(AGAT)_n$ polymorphism. (d) An electropherogram showing the A_{12} allele of D21S2055-$(GATA)_n$ polymorphism

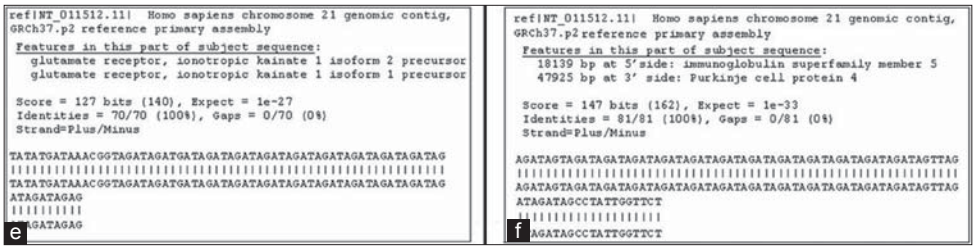


Figure 1: (e) The BLAST data of A_{10} allele of GluK1-$(AGAT)_n$ polymorphism. The data indicates % match and expect value of the given sequence. (f) The BLAST data of A_{10} allele of D21S2055-$(GATA)_n$ polymorphism. The data indicates % match and expect value of the given sequence

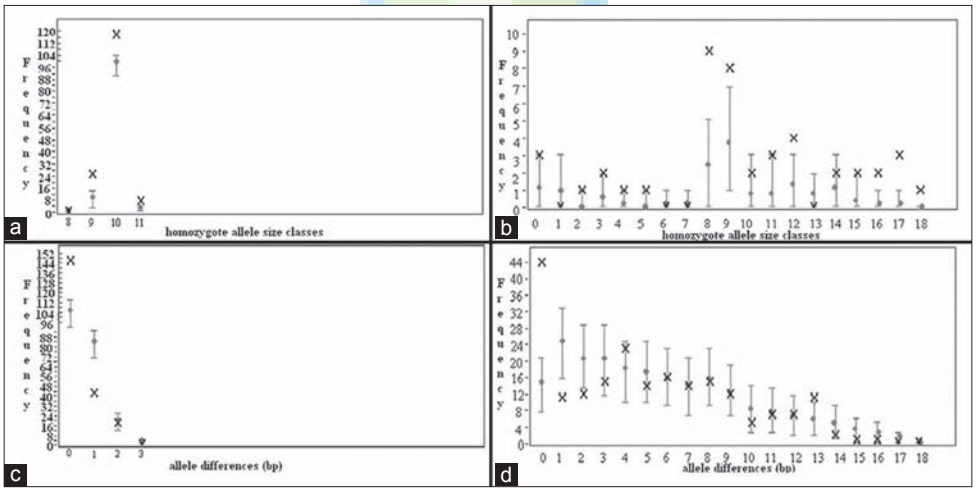


Figure 2: (a) The excess homozygote frequencies of allele classes for GluK1-$(AGAT)_n$ polymorphism. 'X' indicates observed frequencies, '' indicates expected frequencies. The significance is measured at 95% C.I. (b) The excess homozygote frequencies of allele classes for D21S2055-$(GATA)_n$ polymorphism. 'X' indicates observed frequencies, '' indicates expected frequencies. The significance is measured at 95% C.I. (c) The frequencies of allele size difference (bp) is indicated for GluK1-$(AGAT)_n$. 'X' indicates observed frequencies, '' indicates expected frequencies. The significance is measured at 95% C.I. (d) The frequencies of allele size difference (bp) is indicated for D21S2055-$(GATA)_n$. 'X' indicates observed frequencies, '' indicates expected frequencies. The significance is measured at 95% C.I.

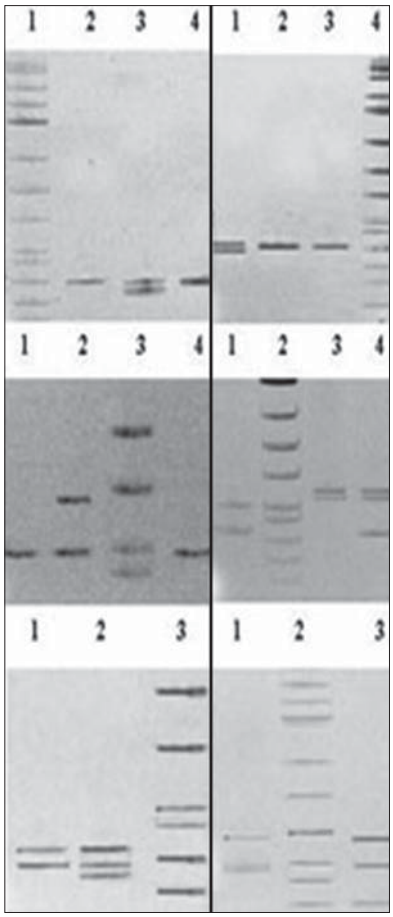
error due to stuttering effects[40] or large allele drop-out.[39]

Table 1 shows sample population-specific data for the GluK1-$(AGAT)_n$ and D21S2055-$(GATA)_n$ polymorphisms. A high power of discrimination (PD) and a low probability of matching (pM) indicate that both marker polymorphisms may be used to distinguish between two unrelated individuals. The relatively higher power of exclusion (PE) [Table 1] reveals that D21S2055-$(GATA)_n$ allele distribution

Table 2: Parent/stage of origin of non-disjunction in trios and duos genotyped for GluK1-(AGAT)$_n$

NDJP	CDJP	Offspring genotype	Stage of origin	#trio/ duo
$A_{10}A_{10}$ (♀)	$A_{10}A_{11}$ (♂)	$A_{10}A_{10}A_{10}$	M-I	1
$A_{10}A_{10}$ (♀)	A_8A_{10} (♂)	$A_{10}A_{10}A_{10}$	M-I	1
$A_{10}A_{10}$ (♀)	$A_{10}A_9$ (♂)	$A_{10}A_{10}A_{10}$	M-I	1
Missing (♀)	A_8A_{10} (♂)	$A_{10}A_{10}A_{10}$	M-I	1
A_9A_9 (♀)	$A_{10}A_{10}$ (♂)	$A_9A_9A_{10}$	M-I	1
$A_{10}A_{10}$ (♀)	A_9A_9 (♂)	$A_9A_{10}A_{10}$	M-I	1
A_8A_{10} (♀)	$A_{10}A_{10}$ (♂)	$A_8A_{10}A_{10}$	M-I	3
A_9A_{11} (♀)	Missing (♂)	$A_8A_9A_{11}$	M-I	1
$A_{10}A_{10}$ (♂)	A_9A_{10} (♀)	$A_{10}A_{10}A_{10}$	M-I	3
$A_{10}A_{10}$ (♂)	$A_{10}A_{11}$ (♀)	$A_{10}A_{10}A_{10}$	M-I	1
Missing (♂)	$A_{10}A_{11}$ (♀)	$A_{10}A_{10}A_{10}$	M-I	1
Missing (♂)	A_8A_{10} (♀)	$A_{10}A_{10}A_{10}$	M-I	1
A_9A_9 (♂)	A_9A_{10} (♀)	$A_9A_9A_9$	M-I	1
A_9A_{10} (♂)	A_9A_9 (♀)	$A_9A_9A_{10}$	M-I	1
A_9A_{10} (♂)	$A_{10}A_{10}$ (♀)	$A_9A_{10}A_{10}$	M-I	2
$A_{11}A_{11}$ (♂)	$A_{10}A_{10}$ (♀)	$A_{10}A_{11}A_{11}$	M-I	1
A_9A_{10} (♀)	$A_{10}A_{10}$ (♂)	$A_9A_9A_{10}$	M-II	1
A_8A_9 (♀)	$A_{10}A_{10}$ (♂)	$A_8A_8A_{10}$	M-II	1
A_8A_{10} (♀)	A_8A_9 (♂)	$A_8A_{10}A_{10}$	M-II	1
A_9A_{11} (♂)	$A_{10}A_{10}$ (♀)	$A_9A_9A_{10}$	M-II	1
$A_{10}A_{11}$ (♀/♂)	$A_{10}A_{11}$ (♂/♀)	$A_{10}A_{10}A_{10}$	M-II	1
$A_{10}A_{11}$ (♂)	A_8A_{10} (♀)	$A_{10}A_{10}A_{10}$	M-II	1
A_8A_{10} (♀)	$A_{10}A_{11}$ (♂)			
Missing(♀)	$A_{10}A_{10}$ (♂)	$A_8A_{10}A_{10}$/ $A_8A_8A_{10}$	M-I/M-II	1
Missing (♂)	A_9A_9 (♀)	$A_8A_8A_9$	M-I/M-II	1
Missing (♂)	$A_{10}A_{10}$ (♀)	$A_8A_{10}A_{10}$	M-I/M-II	1
Missing (♂)	$A_{10}A_{10}$ (♀)	$A_9A_{10}A_{10}$/$A_9A_9A_{10}$	M-I/M-II	2

The proband genotype is recombinant of the correctly disjoining parental (CDJP) genotype, the non-disjoining parent (NDJP) could be discerned based on proband genotype. Meiosis-I (M-I) and Meiosis-II (M-II)



Supplementary Figure 2: Non-disjunction in DS trios and duos genotyped for GluK1-(AGAT)$_n$ and D21S2055-(GATA)$_n$ polymorphisms

is evenly balanced. Interestingly, the high values of expected heterozygosity (*He*) and polymorphism information content (PIC) show that, relative to GluK1-(AGAT)$_n$, the D21S2055-(GATA)$_n$ marker is more informative. Positive F_{is} values [Table 1] suggest heterozygote deficiency, and low global F_{st} values [Table 1] indicate reduced genetic differentiation within the sample population.

We reasoned that a large size difference between individual alleles may increase instability during meiotic recombination and tested the possibility by genotyping these polymorphisms in DS cases. Resolution of allelic combinations reveals triallelic (1:1:1) genotypes as indicated in Figure 3a and 3b. The difference in allele size ranges from 4 bp (lane 2: Figure 3a) to 64 bp (lane-8: Figure 3b). However, the majority of trisomic cases (52/72) give rise to the 2:1, a genotypic pattern, perhaps due to the reduced heterozygosity at these loci among parents. We ascertained the parent-and stage-of-origin of non-disjunction by genotyping these polymorphisms in DS patient families [Supplementary Figure 2a-e], and a detailed analysis of meiosis-I and meiosis-II errors for both polymorphisms are presented in Tables 2 and 3.

Genotyping of GluK1-(AGAT)$_n$yields 32 informative families [Table 2], of which 11 families contain one heterozygous parent, whereas the proband and other parent was homozygous. In such families, the heterozygous parent was assumed to be informative and the correctly disjoining parent (CDJP) while the other parent was deemed to be the non-disjoining parent (NDJP) in whom the error occurred in meiosis-I [Table 2]. Analysis of 9 other DS families reveals that one parent and the proband are both heterozygous. In such families, the homozygous parent is informative (CDJP) as the offspring genotype is recombinant; the heterozygous parent is, therefore, NDJP [Table 2]. In 2 trios, the probands are heterozygous, whereas both parents are either homozygous or heterozygous; NDJP was discerned based on the proband genotype [Table 2].

As shown in Table 2, there were 11 cases each of

Supplementary Table 2a: Adjusted allele frequencies of GluK1-$(AGAT)_n$ polymorphism

Class	Obs. allele freq.	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
8	0.0476	0.0488	0.0352	0.0411	0.0397
9	0.2024	0.1548	0.1494	0.1748	0.1689
10	0.681	0.5528	0.5027	0.5883	0.5682
11	0.069	0.0538	0.051	0.0596	0.0576

Supplementary Table 2b: Adjusted allele frequencies of D21S2055-$(GATA)_n$polymorphism

Class	Obs. allele freq.	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
1	0.0762	0.0716	0.0701	0.0707	0.0692
2	0.069	0.0716	0.0635	0.0641	0.0627
3	0.019	0.0168	0.0175	0.0177	0.0173
4	0.05	0.0463	0.046	0.0464	0.0454
5	0.0357	0.0339	0.0328	0.0332	0.0324
6	0.0167	0.0144	0.0153	0.0155	0.0151
7	0.0286	0.029	0.0263	0.0265	0.026
8	0.0238	0.0241	0.0219	0.0221	0.0216
9	0.1048	0.0871	0.0963	0.0972	0.0952
10	0.1333	0.1217	0.1226	0.1238	0.1211
11	0.0595	0.0563	0.0547	0.0553	0.0541
12	0.0619	0.0563	0.0569	0.0575	0.0562
13	0.0762	0.0691	0.0701	0.0707	0.0692
14	0.0571	0.0589	0.0525	0.053	0.0519
15	0.0714	0.0691	0.0657	0.0663	0.0649
16	0.0476	0.0438	0.0438	0.0442	0.0433
17	0.031	0.0265	0.0285	0.0287	0.0281
18	0.0333	0.0265	0.0306	0.0309	0.0303
19	0.0048	0.0024	0.0044	0.0044	0.0043

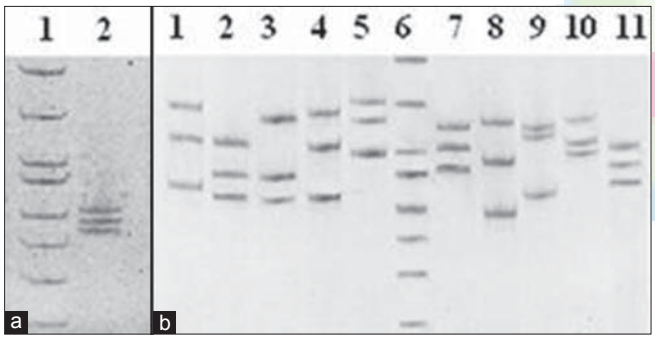


Figure 3: (a) Direct evidence of triallelic inheritance using GluK1-$(AGAT)_n$: lane 1: ϕ X174DNA/*Hinf*I digest marker, lane 2: $A_8A_9A_{11}$. (b) Direct evidence of triallelic inheritance using D21S2055-$(GATA)_n$: lane 1: $A_4A_{10}A_{16}$, lane 2: $A_3A_5A_{10}$, lane 3: $A_3A_5A_{15}$, lane 4: $A_3A_{11}A_{17}$, lane 5: $A_{10}A_{16}A_{19}$, lane 6: ϕ X174DNA/*Hinf*I digest marker, lane 7: $A_9A_{12}A_{16}$, lane 8: $A_1A_9A_{17}$, lane 9: $A_3A_{14}A_{16}$, lane 10: $A_9A_{11}A_{16}$, lane 11: $A_3A_5A_{10}$

non-disjunction in paternal meiosis-I and 10 cases in maternal meiosis-I. Furthermore, we discerned six cases where non-disjunction occurred in meiosis-II [Table 2]. The stage of origin of non-disjunction could not be ascertained unequivocally in 5 families [Table 2] although the parent of origin was clearly discerned. The estimated ratio of meiosis-I to meiosis-II errors arising from allelic non-disjunction of GluK1-$(AGAT)_n$ is 2:1 in maternal cases and 4:1 in paternal cases.

We excluded 32 families out of 72 as being non-informative for the D21S2055-$(GATA)_n$ marker. As shown in Table 3, 40 families were informative, out of which, 23 families possess offspring genotypes that were recombinant with respect to parental genotypes; this helped in the assignment of the CDJP and NDJP in each case. There were 8 cases where the non-disjunction originated in paternal meiosis-I and 15 cases where the error originated in maternal meiosis-I. Furthermore, we discerned 10 cases where non-disjunction occurred in meiosis-II [Table 3]. The stage of origin of non-disjunction could not be ascertained unequivocally in 7 families [Table 3] although the parent of origin was clearly discerned. The estimated ratio of meiosis-I to meiosis-II errors arising from allelic non-disjunction of D21S2055-$(GATA)_n$ is 2:1 in both maternal and paternal cases.

Systematic analysis of genotyping data indicates that 53/72 patient families were informative for at least one marker, and the parent of origin of non-disjunction may be discerned unequivocally in each of these cases. Among all families, 24/72 DS families were informative for both markers, 36/72 families were informative for either one of the two markers, and 12/72 families were

Table 3: Parent/stage of origin of non-disjunction in trios and duos genotyped for D21S2055-(GATA)$_n$

NDJP	CDJP	Offspring genotype	Stage of origin	#trio/ duo
$A_{10}A_{16}$(♀)	A_3A_9(♂)	$A_3A_{10}A_{16}$	M-I	1
$A_{10}A_{10}$(♀)	A_1A_{11}(♂)	$A_1A_{10}A_{10}$	M-I	1
A_3A_{10} (♀)	A_9A_{16} (♂)	$A_3A_{10}A_{16}$	M-I	1
A_2A_{15} (♀)	$A_{11}A_{12}$ (♂)	$A_2A_{11}A_{15}$	M-I	1
A_1A_{13} (♀)	$A_{10}A_{10}$ (♂)	$A_1A_{10}A_{13}$	M-I	1
A_3A_{15} (♀)	A_1A_5 (♂)	$A_3A_5A_{15}$	M-I	1
A_9A_9 (♀)	$A_{13}A_{13}$ (♂)	$A_9A_9A_{13}$	M-I	1
A_3A_{10} (♀)	A_5A_7 (♂)	$A_3A_5A_{10}$	M-I	1
Missing (♀)	A_4A_4 (♂)	$A_4A_6A_{10}$	M-I	1
A_2A_{10} (♀)	Missing (♂)	$A_2A_{10}A_{14}$	M-I	1
Missing (♀)	A_2A_{16} (♂)	$A_{10}A_{16}A_{19}$	M-I	1
A_2A_{18} (♀)	Missing (♂)	$A_2A_{10}A_{18}$	M-I	1
$A_{10}A_{16}$ (♀)	Missing (♂)	$A_{10}A_{11}A_{16}$	M-I	1
$A_{10}A_{16}$ (♀)	Missing (♂)	$A_2A_{10}A_{16}$	M-I	1
$A_{10}A_{15}$ (♀)	Missing (♂)	$A_2A_{10}A_{15}$	M-I	1
A_1A_{11} (♂)	$A_{11}A_{11}$ (♀)	$A_1A_{11}A_{11}$	M-I	1
A_3A_7 (♂)	A_5A_9 (♀)	$A_3A_5A_7$	M-I	1
A_9A_9 (♂)	A_9A_{17} (♀)	$A_9A_9A_9$	M-I	1
Missing (♂)	$A_{10}A_{18}$ (♀)	$A_9A_{18}A_{18}$	M-I	1
Missing (♂)	A_9A_{10} (♀)	$A_{10}A_{10}A_{19}$M-I	1	
Missing (♂)	A_9A_{15} (♀)	$A_9A_{12}A_{16}$	M-I	1
Missing (♂)	A_9A_9 (♀)	$A_3A_9A_9$	M-I	1
Missing (♂)	A_2A_9 (♀)	$A_9A_9A_{17}$	M-I	1
$A_{10}A_{14}$ (♀)	A_2A_{10} (♂)	$A_2A_{14}A_{14}$	M-II	1
A_8A_{14} (♀)	A_1A_8 (♂)	$A_8A_{14}A_{14}$	M-II	1
A_9A_{16} (♀)	$A_{12}A_{15}$ (♂)	$A_9A_9A_{15}$	M-II	1
A_1A_4 (♀)	$A_{15}A_{15}$ (♂)	$A_1A_1A_{15}$	M-II	1
$A_{16}A_{18}$ (♀)	$A_{16}A_{16}$ (♂)	$A_{16}A_{18}A_{18}$	M-II	1
A_4A_{10} (♀)	$A_{18}A_{18}$ (♂)	$A_4A_4A_{18}$	M-II	1
A_4A_{17} (♂)	A_4A_4 (♀)	$A_4A_{17}A_{17}$	M-II1	
A_2A_4 (♂)	A_9A_{18} (♀)	$A_2A_2A_9$	M-II	1
A_9A_{16} (♂)	$A_{10}A_{16}$ (♀)	$A_{10}A_{16}A_{16}$	M-II	1
$A_{10}A_{16}$ (♀)	A_9A_{16} (♂)			
A_2A_{14} (♂)	A_1A_{14} (♀)	$A_{14}A_{14}A_{14}$	M-II	1
A_1A_{14} (♀)	A_2A_{14} (♂)			
A_9A_9 (♀)	$A_{17}A_{17}$ (♂)	$A_9A_9A_{17}$	M-I/M-II	1
Missing (♀)	$A_{10}A_{18}$ (♂)	$A_{16}A_{16}A_{18}$	M-I/M-II	1
Missing (♀)	A_2A_{15} (♂)	$A_2A_2A_2$	M-I/M-II	1
Missing (♀)	A_1A_{10} (♂)	$A_1A_4A_4$	M-I/M-II	1
Missing (♀)	$A_{18}A_{18}$ (♂)	$A_1A_1A_{18}$	M-I/M-II	1
Missing (♂)	A_1A_1 (♀)	$A_1A_1A_{10}/A_1A_{10}A_{10}$	M-I/M-II	1
Missing (♂)	A_9A_9 (♀)	$A_9A_{11}A_{11}$	M-I/M-II	1

Based on proband genotype that is recombinant of correctly disjoining parent's (CDJP) genotype, the non-disjoining parent (NDJP) could be discerned. Meiosis-I (M-I) and Meiosis-II (M-II)

non-informative for both markers. Among 24 informative families, 15 families show same parent of origin, out of which, 9 families show same parent-and stage-of-origin of non-disjunction that provides internal validation for the observations reported in this study.

Discussion

The study presents evidence for allelic non-disjunction at the GluK1-intron 3-(AGAT)$_n$ and D21S2055-(GATA)$_n$ STR loci on chromosome 21. Relative to GluK1-(AGAT)$_n$, theD21S2055-(GATA)$_n$ marker is more informative due to its higher power of discrimination, probability of matching, observed heterozygosity, polymorphism information content, and power of exclusion. A triallelic pattern (1:1:1) of inheritance is observed in 19/72 DS cases for the D21S2055 marker, whereas a similar pattern is observable in 1/72 cases when genotyped for the GluK1-(AGAT)$_n$ marker. The parent-and stage-of origin of non-disjunction is traced in 51/72 families using the polymorphism D21S2055 marker polymorphism but only in 34/72 families when considering the GluK1-(AGAT)$_n$ polymorphism. The ratio of non-disjunction errors in MI: MII are estimated as 2:1 for GluK1-(AGAT)$_n$ of maternal origin, and 4:1 when they are of paternal origin, and for D21S2055-(GATA)$_n$, the ratio is 2:1 for both parents. The elevated MI ratio in this case may be biased by small sample size.

Marker informativenessis governed by the differences in allele frequency. In our sample population, the A_{10} allele for GluK1-(AGAT)$_n$ is the major allele (0.675) [Supplementary Table 1a], whereas the A_9 (0.357) and A_{10} (0.414) alleles are the major alleles in German samples.[41] A search of the ALFRED database[42] revealed population-specific difference in allele frequencies for also D21S2055-(GATA)$_n$. Allele frequencies for both polymorphisms show significant deviation from Hardy-Weinberg equilibrium proportions [Supplementary Table 1a and b]. While analysis of our data [Figure 2a-d] suggests the presence of null alleles,[36] they have been ruled out as follows: We have genotyped both parent samples prior to testing of DS cases, quantified band intensities, and also by direct sequencing of PCR amplicons. Given that both marker polymorphisms are located in intronic regions, the potential impact of null alleles, if any, on gene product function remains currently unknown.

The advent of quantitative fluorescent (QF)-PCR[12] and real time PCR[43] ease the detection of trisomy; they are, however, expensive methods. The limitation of PCR-based gel electrophoresis is that it relies on a semi-quantitative estimation of allele pattern [1:2 or 2:1] in DS cases, but it is cheaper, andtherefore, useful for screening samples at low cost.

Acknowledgment

We thank all patient families and volunteers for their support

of our research. Debarati Ghosh is a recipient of Senior Research Fellowship from CSIR [9/840(0007)/2010 EMR-I dated 09-03-2010]. Krishnadas Nandagopal is a grantee of the Dept of Science and technology-SERC [SR/SO/HS-59/2003]. We thank Dr. Kunal Ray and Mr. Saswata Mukherjee of the Indian Institute of Chemical Biology, Kolkata 700032 for their assistance in sequencing some of the DNA samples.

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Cite this article as: Debarati G, Swagata S, Anindita C, Krishnadas N. Discerning non-disjunction in down syndrome patients by means of gluk1-$(agat)_n$ and d21s2055-$(gata)_n$ microsatellites on chromosome 21. Indian J Hum Genet 2012;18:204-16.

Source of Support: Work was supported with financial aid from DST-SERC, Govt of India, vide letter no. SR/SO/HS-59/2003 grant to Dr. K.Nandagopal and the fellowship grant from CSIR, Govt of India, vide letter no. 9/840(007)/2010 EMR-I dated 09-03-2010 to Debarati Ghosh as Senior Research Fellow,
Conflict of Interest: None declared.