

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.

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 (A₉) allele ($\chi^2=8.31$, 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]

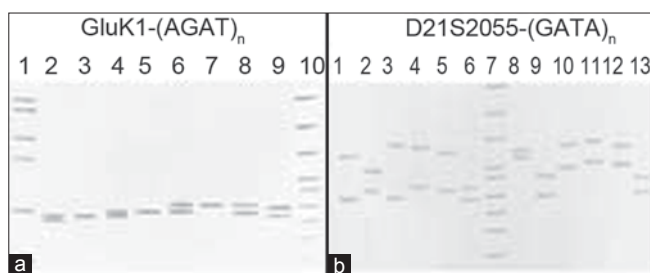


Figure 1(a,b): (a) Genotypes identified by PCR-based amplification of the GluK1-(AGAT)_n polymorphism. 'A' represents followed by polyacrylamide gel electrophoresis allele and subscript denotes number of repeats. Lane-1 ϕ X174DNA/*HaellI* digest; lane-2 A₈A₉; lane-3 A₉A₉; lane-4 A₉A₁₀; lane-5 A₁₀A₁₀; lane-6 A₁₀A₁₁; lane-7 A₁₁A₁₁; lane-8 A₉A₁₁; lane-9 A₈A₁₀; lane-10 ϕ X174DNA/*HinfI* digest. (b) Genotypes identified by PCR-based amplification of the D21S2055-(GATA)_n polymorphism. 'A' represents followed by polyacrylamide gel electrophoresis allele. Lane-1 A₁A₁₂; lane-2 A₃A₉; lane-3 A₁A₁₆; lane-4 A₅A₁₅; lane-5 A₃A₁₃; lane-6 A₁A₄; lane-7 ϕ X174DNA *HinfI* digest; lane-8 A₁₂A₁₄; lane-9 A₂A₇; lane-10 A₉A₁₈; lane-11 A₁₁A₁₉; lane-12 A₁₀A₁₇; lane-13 A₂A₆

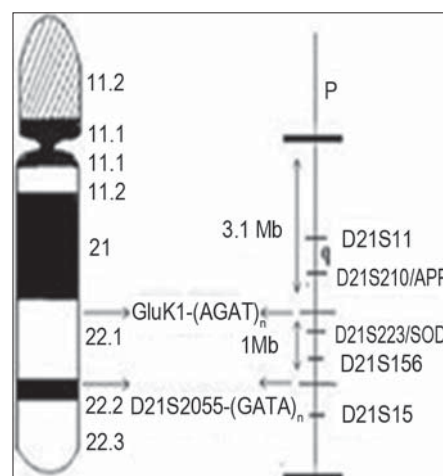
A multiple sequence alignment of genomic DNA sequences flanking each of the GluK1-(AGAT)_n and D21S2055-(GATA)_n polymorphisms followed by phylogenetic comparisons, by means of Neighbor-Joining tree construction, indicate that the sequence ~1 kb upstream and downstream of D21S2055-(GATA)_n is more genetically 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 mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-HCl (pH 8.8 at 25°C), 0.1% Triton X-100 (NEB), 1 mM MgSO₄, 200 μM dNTPs, and 0.2 U Taq DNA polymerase in 20 μl 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 (A₈) through eleven repeat (A₁₁) alleles. Each of these alleles was sequenced to confirm the number of repeats, and an electropherogram representative of the A₁₀ allele [Figure 1c] along with BLAST data is shown in Figure 1e. Although 420 chromosomes were genotyped for this polymorphism, the A₈A₈ homozygote for the GluK1-(AGAT)_n was not detected, indicating low frequency of the A₈ allele in the sample population. Allele frequencies show significant deviation from Hardy-Weinberg equilibrium proportions ($\chi^2=101.55$, $df=6$, $P=1\times 10^{-6}$, 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 (A₁) through nineteen repeat (A₁₉) alleles. Each of these alleles was sequenced to confirm the number of repeats, and an electropherogram representative of the A₁₂ 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 ($\chi^2=694.66$, $df=171$, $P=1\times 10^{-6}$, 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 ₈ A ₈	0	0.4535	0.4535	101.55	1×10 ⁻⁶
		A ₉	0.2071	A ₈ A ₉	6	4.1527	0.8217		
		A ₁₀	0.6762	A ₉ A ₉	26	8.9284	32.6418		
		A ₁₁	0.069	A ₈ A ₁₀	14	13.5561	0.0145		
				A ₉ A ₁₀	25	58.969	19.5678		
				A ₁₀ A ₁₀	117	95.9093	4.6379		
				A ₈ A ₁₁	0	1.3842	1.3842		
				A ₉ A 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 ₁ A ₁	3	1.1838	2.7866	694.655	1×10 ⁻⁶
		A ₂	0.069	A ₁ A ₂	1	2.2148	0.6663		
		A ₃	0.019	A ₂ A ₂	0	0.969	0.969		
		A ₄	0.05	A ₁ A ₃	0	0.611	0.611		
		A ₅	0.0357	A ₂ A ₃	0	0.221	0.221		
		A ₆	0.0167	A ₃ A ₃	1	0.0668	13.0311		
		A ₇	0.0286	A ₁ A ₄	1	1.6038	0.2273		
		A ₈	0.0238	A ₂ A ₄	2	1.4535	0.2055		
		A ₉	0.1048	A ₃ A ₄	0	0.401	0.401		
		A ₁₀	0.1333	A ₄ A ₄	2	0.5012	4.4821		
		A ₁₁	0.0595	A ₁ A ₅	2	1.1456	0.6373		
		A ₁₂	0.0619	A ₂ A ₅	0	1.0382	1.0382		
		A ₁₃	0.0762	A ₃ A ₅	0	0.2864	0.2864		
		A ₁₄	0.0571	A ₄ A ₅	1	0.7518	0.0819		
		A ₁₅	0.0714	A ₅ A ₅	1	0.2506	2.2411		
		A ₁₆	0.0476	A ₁ A ₆	0	0.5346	0.5346		
		A ₁₇	0.031	A ₂ A ₆	1	0.4845	0.5485		
		A ₁₈	0.0333	A ₃ A ₆	0	0.1337	0.1337		
		A ₁₉	0.0048	A ₄ A ₆	0	0.3508	0.3508		
		A ₅ A ₆	1	0.2506	2.2411				
		A ₆ A ₆	1	0.0501	18.0025				
		A ₁ A ₇	0	0.9165	0.9165				
		A ₂ A ₇	1	0.8305	0.0346				
		A ₃ A ₇	0	0.2291	0.2291				
		A ₄ A ₇	1	0.6014	0.2641				
		A ₅ A ₇	2	0.4296	5.7407				
		A ₆ A ₇	0	0.2005	0.2005				
		A ₇ A ₇	0	0.1575	0.1575				
		A ₁ A ₈	3	0.7637	6.5481				
		A ₂ A ₈	1	0.6921	0.137				
		A ₃ A ₈	0	0.1909	0.1909				
		A ₄ A ₈	1	0.5012	0.4964				
		A ₅ A ₈	0	0.358	0.358				
		A ₆ A ₈	0	0.1671	0.1671				
		A ₇ A ₈	0	0.2864	0.2864				
		A ₈ A ₈	0	0.1074	0.1074				
		A ₁ A ₉	1	3.3604	1.658				
		A ₂ A ₉	3	3.0453	0.0007				
		A ₃ A ₉	1	0.8401	0.0304				
		A ₄ A ₉	1	2.2053	0.6587				
		A ₅ A ₉	1	1.5752	0.21				
		A ₆ A ₉	2	0.7351	2.1766				
		A ₇ A ₉	0	1.2601	1.2601				
		A ₈ A ₉	1	1.0501	0.0024				
		A ₉ A ₉	9	2.2578	20.1341				
		A ₁ A ₁₀	4	4.2768	0.0179				
		A ₂ A ₁₀	6	3.8759	1.1641				
		A ₃ A ₁₀	0	1.0692	1.0692				
		A ₄ A ₁₀	3	2.8067	0.0133				
		A ₅ A ₁₀	2	2.0048	0				
		A ₆ A ₁₀	1	0.9356	0.0044				
		A ₇ A ₁₀	1	1.6038	0.2273				
		A ₈ A ₁₀	0	1.3365	1.3365				
		A ₉ A ₁₀	1	5.8807	4.0507				
		A ₁₀ A ₁₀	8	3.6754	5.0884				
		A ₁ A ₁₁	2	1.9093	0.0043				
		A ₂ A ₁₁	2	1.7303	0.042				
		A ₃ A ₁₁	1	0.4773	0.5723				
		A ₄ A ₁₁	0	1.253	1.253				
		A ₅ A ₁₁	2	0.895	1.3643				
		A ₆ A 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 ₇ A ₁₁	0	0.716	0.716		
				A ₈ A ₁₁	0	0.5967	0.5967		
				A ₉ A 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 ₁ A ₁₂	1	1.9857	0.4893		
				A ₂ A ₁₂	1	1.7995	0.3552		
				A ₃ A ₁₂	2	0.4964	4.5541		
				A ₄ A ₁₂	2	1.3031	0.3727		
				A ₅ A ₁₂	0	0.9308	0.9308		
				A ₆ A ₁₂	0	0.4344	0.4344		
				A ₇ A ₁₂	0	0.7446	0.7446		
				A ₈ A ₁₂	1	0.6205	0.2321		
				A ₉ A 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 ₁ A ₁₃	4	2.4439	0.9908		
				A ₂ A ₁₃	3	2.2148	0.2784		
				A ₃ A ₁₃	0	0.611	0.611		
				A ₄ A ₁₃	0	1.6038	1.6038		
				A ₅ A ₁₃	1	1.1456	0.0185		
				A ₆ A ₁₃	0	0.5346	0.5346		
				A ₇ A ₁₃	2	0.9165	1.2811		
				A ₈ A ₁₃	0	0.7637	0.7637		
				A ₉ A 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 ₁ A ₁₄	5	1.8329	5.4723		
				A ₂ A ₁₄	2	1.6611	0.0691		
				A ₃ A ₁₄	1	0.4582	0.6405		
				A ₄ A ₁₄	1	1.2029	0.0342		
				A ₅ A ₁₄	1	0.8592	0.0231		
				A ₆ A ₁₄	0	0.401	0.401		
				A ₇ A ₁₄	2	0.6874	2.5068		
				A ₈ A ₁₄	2	0.5728	3.5561		
				A ₉ A 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 ₁ A ₁₅	2	2.2912	0.037		
				A ₂ A ₁₅	5	2.0764	4.1166		
				A ₃ A ₁₅	0	0.5728	0.5728		
				A ₄ A ₁₅	2	1.5036	0.1639		
				A ₅ A ₁₅	0	1.074	1.074		
				A ₆ A ₁₅	0	0.5012	0.5012		
				A ₇ A ₁₅	0	0.8592	0.8592		
				A ₈ A ₁₅	1	0.716	0.1127		
				A ₉ A 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 ₁ A ₁₆	0	1.5274	1.5274		
				A ₂ A ₁₆	0	1.3842	1.3842		
				A ₃ A ₁₆	0	0.3819	0.3819		
				A ₄ A ₁₆	1	1.0024	0		
				A ₅ A ₁₆	0	0.716	0.716		
				A ₆ A ₁₆	0	0.3341	0.3341		
				A ₇ A ₁₆	2	0.5728	3.5561		
				A ₈ A 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 ₉ A 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 ₁ A ₁₇	0	0.9928	0.9928		
				A ₂ A ₁₇	0	0.8998	0.8998		
				A ₃ A ₁₇	0	0.2482	0.2482		
				A ₄ A ₁₇	1	0.6516	0.1863		
				A ₅ A ₁₇	0	0.4654	0.4654		
				A ₆ A ₁₇	0	0.2172	0.2172		
				A ₇ A ₁₇	1	0.3723	1.0582		
				A ₈ A ₁₇	0	0.3103	0.3103		
				A ₉ A 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 ₁ A ₁₈	0	1.0692	1.0692		
				A ₂ A ₁₈	1	0.969	0.001		
				A ₃ A ₁₈	1	0.2673	2.0084		
				A ₄ A ₁₈	0	0.7017	0.7017		
				A ₅ A ₁₈	0	0.5012	0.5012		
				A ₆ A ₁₈	0	0.2339	0.2339		
				A ₇ A ₁₈	0	0.401	0.401		
				A ₈ A ₁₈	0	0.3341	0.3341		
				A ₉ A 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 ₁ A ₁₉	0	0.1527	0.1527		
				A ₂ A ₁₉	0	0.1384	0.1384		
				A ₃ A ₁₉	0	0.0382	0.0382		
				A ₄ A ₁₉	0	0.1002	0.1002		
				A ₅ A ₁₉	0	0.0716	0.0716		
				A ₆ A ₁₉	0	0.0334	0.0334		
				A ₇ A ₁₉	0	0.0573	0.0573		
				A ₈ A ₁₉	0	0.0477	0.0477		
				A ₉ A 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

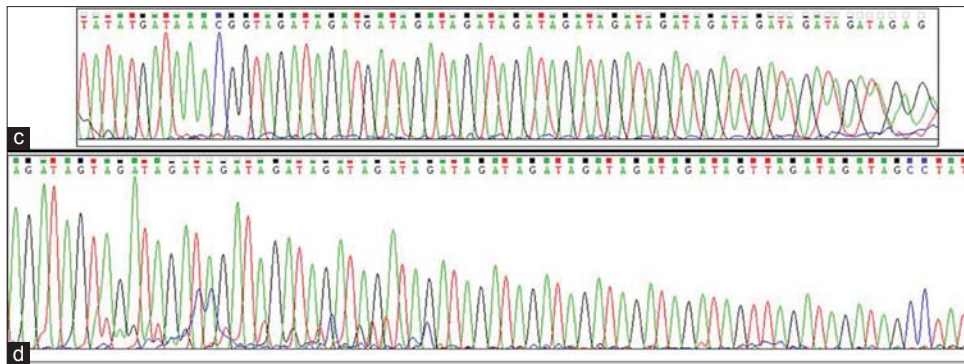


Figure 1: (c) An electropherogram showing the A₁₀ allele of GluK1-(AGAT)_n polymorphism. (d) An electropherogram showing the A₁₂ allele of D21S2055-(GATA)_n polymorphism

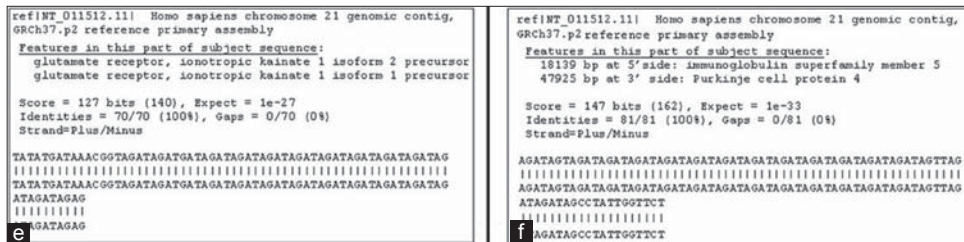


Figure 1: (e) The BLAST data of A₁₀ allele of GluK1-(AGAT)_n polymorphism. The data indicates % match and expect value of the given sequence. (f) The BLAST data of A₁₀ allele of D21S2055-(GATA)_n polymorphism. The data indicates % match and expect value of the given sequence

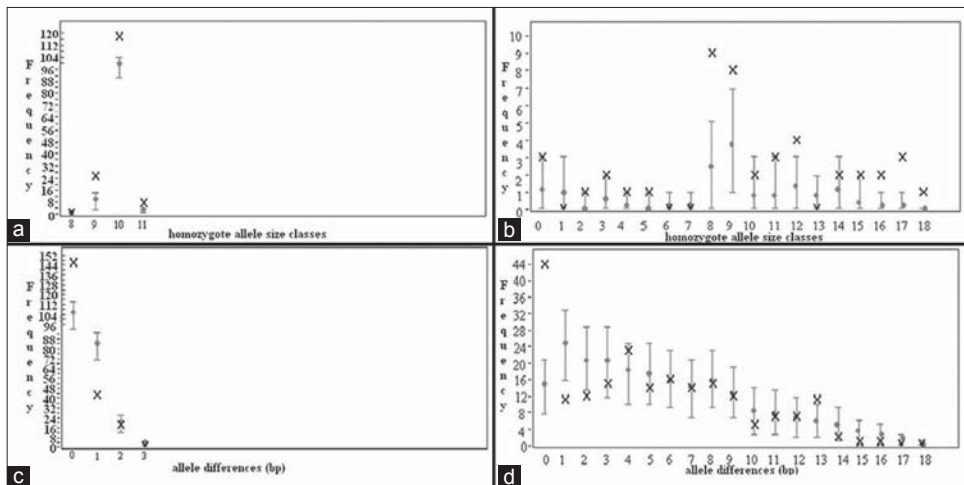


Figure 2: (a) The excess homozygote frequencies of allele classes for GluK1-(AGAT)_n polymorphism. 'X' indicates observed frequencies, '•' indicates expected frequencies. The significance is measured at 95% C.I. (b) The excess homozygote frequencies of allele classes for D21S2055-(GATA)_n polymorphism.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

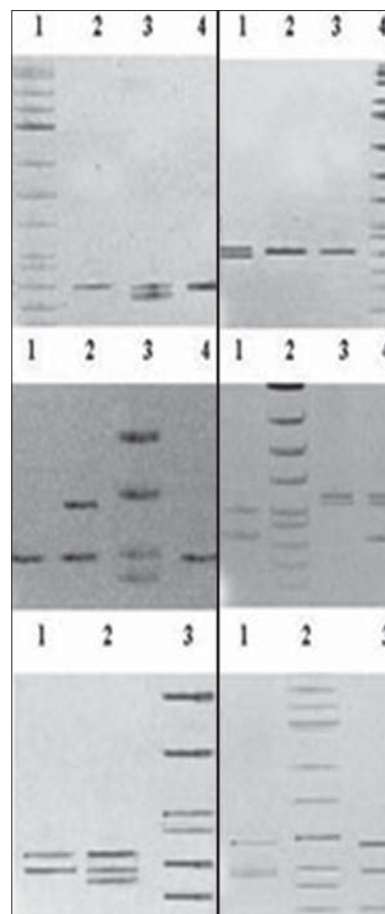
Table 2: Parent/stage of origin of non-disjunction in trios and duos genotyped for GluK1-(AGAT)_n

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 ₈ A 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 ₈ A ₁₀ (♂)	A ₁₀ A ₁₀ A ₁₀	M-I	1
A ₉ A ₉ (♀)	A ₁₀ A ₁₀ (♂)	A ₉ A ₉ A ₁₀	M-I	1
A ₁₀ A ₁₀ (♀)	A ₉ A ₉ (♂)	A ₉ A ₁₀ A ₁₀	M-I	1
A ₉ A ₁₀ (♀)	A ₁₀ A ₁₀ (♂)	A ₉ A ₁₀ A ₁₀	M-I	3
A ₉ A ₁₁ (♀)	Missing (♂)	A ₉ A ₉ A ₁₁	M-I	1
A ₁₀ A ₁₀ (♂)	A ₉ A 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 ₈ A ₁₀ (♀)	A ₁₀ A ₁₀ A ₁₀	M-I	1
A ₉ A ₉ (♂)	A ₉ A ₁₀ (♀)	A ₉ A ₉ A ₉	M-I	1
A ₉ A ₁₀ (♂)	A ₉ A ₉ (♀)	A ₉ A ₉ A ₁₀	M-I	1
A ₉ A ₁₀ (♂)	A ₁₀ A ₁₀ (♀)	A ₉ A ₁₀ A ₁₀	M-I	2
A ₁₁ A ₁₁ (♂)	A ₁₀ A ₁₀ (♀)	A ₁₀ A ₁₁ A ₁₁	M-I	1
A ₉ A ₁₀ (♀)	A ₁₀ A ₁₀ (♂)	A ₉ A ₉ A ₁₀	M-II	1
A ₉ A ₉ (♀)	A ₁₀ A ₁₀ (♂)	A ₉ A ₈ A ₁₀	M-II	1
A ₈ A ₁₀ (♀)	A ₈ A ₉ (♂)	A ₈ A ₁₀ A ₁₀	M-II	1
A ₉ A ₁₁ (♂)	A ₁₀ A ₁₀ (♀)	A ₉ A ₉ A 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 ₈ A ₁₀ (♀)	A ₁₀ A ₁₀ A ₁₀	M-II	1
A ₉ A ₁₀ (♀)	A ₁₀ A ₁₁ (♂)	A ₈ A ₁₀ A ₁₀	M-I/M-II	1
Missing (♀)	A ₁₀ A ₁₀ (♂)	A ₈ A ₁₀ A ₁₀ , A ₈ A ₈ A ₁₀	M-I/M-II	1
Missing (♂)	A ₉ A ₉ (♀)	A ₉ A ₉ A ₉	M-I/M-II	1
Missing (♂)	A ₁₀ A ₁₀ (♀)	A ₈ A ₁₀ A ₁₀	M-I/M-II	1
Missing (♂)	A ₁₀ A ₁₀ (♀)	A ₉ A ₁₀ A ₁₀ , A ₉ A ₉ A 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)

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



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

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

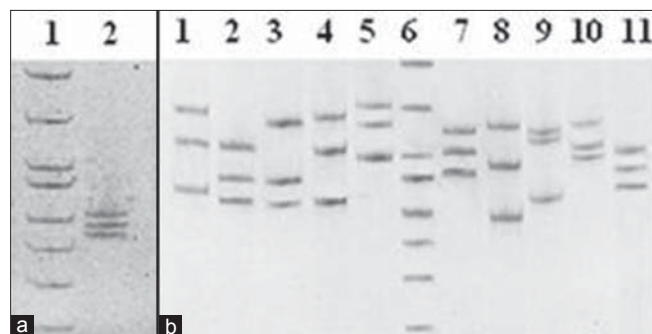


Figure 3: (a) Direct evidence of triallelic inheritance using GluK1-(AGAT)_n: lane 1: ϕ X174DNA/*Hinf*I digest marker, lane 2: A₈A₉A₁₁. (b) Direct evidence of triallelic inheritance using D21S2055-(GATA)_n: lane 1: A₄A₁₀A₁₆, lane 2: A₃A₅A₁₀, lane 3: A₃A₅A₁₅, lane 4: A₃A₁₁A₁₇, lane 5: A₁₀A₁₆A₁₉, lane 6: ϕ X174DNA/*Hinf*I digest marker, lane 7: A₉A₁₂A₁₆, lane 8: A₁A₉A₁₇, lane 9: A₃A₁₄A₁₆, lane 10: A₉A₁₁A₁₆, lane 11: A₃A₅A₁₀

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 ₃ A ₉ (♂)	A ₃ A ₁₀ A ₁₆	M-I	1
A ₁₀ A ₁₀ (♀)	A ₁ A ₁₁ (♂)	A ₁ A ₁₀ A ₁₀	M-I	1
A ₃ A ₁₀ (♀)	A ₉ A ₁₆ (♂)	A ₃ A ₁₀ A ₁₆	M-I	1
A ₂ A ₁₅ (♀)	A ₁₁ A ₁₂ (♂)	A ₂ A ₁₁ A ₁₅	M-I	1
A ₅ A ₁₃ (♀)	A ₁₀ A ₁₀ (♂)	A ₁ A ₁₀ A ₁₃	M-I	1
A ₃ A ₁₅ (♀)	A ₁ A ₅ (♂)	A ₃ A ₉ A ₁₅	M-I	1
A ₃ A ₉ (♀)	A ₁₃ A ₁₃ (♂)	A ₉ A ₉ A ₁₃	M-I	1
A ₃ A ₁₀ (♀)	A ₅ A ₇ (♂)	A ₃ A ₉ A ₁₀	M-I	1
Missing(♀)	A ₄ A ₄ (♂)	A ₄ A ₁₀ A ₁₀	M-I	1
A ₂ A ₁₀ (♀)	Missing(♂)	A ₂ A ₁₀ A ₁₄	M-I	1
Missing(♀)	A ₂ A ₁₆ (♂)	A ₁₀ A ₁₆ A ₁₉	M-I	1
A ₂ A ₁₈ (♀)	Missing(♂)	A ₂ A 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 ₂ A ₁₀ A ₁₆	M-I	1
A ₁₀ A ₁₅ (♀)	Missing(♂)	A ₂ A ₁₀ A ₁₅	M-I	1
A ₁ A ₁₁ (♂)	A ₁₁ A ₁₁ (♀)	A ₁ A ₁₁ A ₁₁	M-I	1
A ₃ A ₇ (♂)	A ₅ A ₉ (♀)	A ₃ A ₅ A ₇	M-I	1
A ₃ A ₉ (♂)	A ₉ A ₇ (♀)	A ₃ A ₉ A ₉	M-I	1
Missing(♂)	A ₁₀ A ₁₈ (♀)	A ₉ A ₁₈ A ₁₈	M-I	1
Missing(♂)	A ₉ A ₁₀ (♀)	A ₁₀ A ₁₀ A ₁₉	M-I	1
Missing(♂)	A ₉ A ₁₅ (♀)	A ₉ A ₁₂ A ₁₆	M-I	1
Missing(♂)	A ₉ A ₉ (♀)	A ₃ A ₉ A ₉	M-I	1
Missing(♂)	A ₂ A ₉ (♀)	A ₉ A ₉ A ₁₇	M-I	1
A ₁₀ A ₁₄ (♀)	A ₂ A ₁₀ (♂)	A ₂ A ₁₄ A ₁₄	M-II	1
A ₅ A ₁₄ (♀)	A ₁ A ₉ (♂)	A ₅ A ₁₄ A ₁₄	M-II	1
A ₉ A ₁₆ (♀)	A ₁₂ A ₁₅ (♂)	A ₉ A ₉ A ₁₅	M-II	1
A ₁ A ₄ (♀)	A ₁₅ A ₁₅ (♂)	A ₁ A ₁₅ A ₁₅	M-II	1
A ₁₆ A ₁₈ (♀)	A ₁₆ A ₁₆ (♂)	A ₁₆ A ₁₈ A ₁₈	M-II	1
A ₁ A ₁₀ (♀)	A ₁₈ A ₁₈ (♂)	A ₁ A ₁₈ A ₁₈	M-II	1
A ₄ A ₁₇ (♂)	A ₄ A ₄ (♀)	A ₄ A ₁₇ A ₁₇	M-II1	1
A ₂ A ₄ (♂)	A ₉ A ₁₈ (♀)	A ₂ A ₂ A ₉	M-II	1
A ₃ A ₁₆ (♂)	A ₁₀ A ₁₆ (♀)	A ₁₀ A ₁₆ A ₁₆	M-II	1
A ₁₀ A ₁₆ (♀)	A ₉ A ₁₆ (♂)			
A ₂ A ₁₄ (♂)	A ₁ A ₁₄ (♀)	A ₁₄ A ₁₄ A ₁₄	M-II	1
A ₁ A ₁₄ (♀)	A ₂ A ₁₄ (♂)			
A ₃ A ₉ (♀)	A ₁₇ A ₁₇ (♂)	A ₉ A ₉ A ₁₇	M-I/M-II	1
Missing(♀)	A ₁₀ A ₁₈ (♂)	A ₁₆ A ₁₆ A ₁₈	M-I/M-II	1
Missing(♀)	A ₂ A ₁₅ (♂)	A ₂ A ₂ A ₂	M-I/M-II	1
Missing(♀)	A ₁ A ₁₀ (♂)	A ₁ A ₄ A ₄	M-I/M-II	1
Missing(♀)	A ₁₈ A ₁₈ (♂)	A ₁ A ₁ A ₁₈	M-I/M-II	1
Missing(♂)	A ₁ A ₁ (♀)	A ₁ A ₁ A ₁₀ /A ₁₀ A ₁₀	M-I/M-II	1
Missing(♂)	A ₉ A ₉ (♀)	A ₉ A 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, the D21S2055-(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 informativeness governed by the differences in allele frequency. In our sample population, the A₁₀ allele for GluK1-(AGAT)_n is the major allele (0.675) [Supplementary Table 1a], whereas the A₉ (0.357) and A₁₀ (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, and therefore, useful for screening samples at low cost.

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