

## Subtelomeric rearrangements in Indian children with idiopathic intellectual disability/developmental delay: Frequency estimation & clinical correlation using fluorescence *in situ* hybridization (FISH)

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**Background & objectives:** Subtelomeres are prone to deleterious rearrangements owing to their proximity to unique sequences on the one end and telomeric repetitive sequences, which increase their tendency to recombine, on the other end. These subtelomeric rearrangements resulting in segmental aneuploidy are reported to contribute to the aetiology of idiopathic intellectual disability/developmental delay (ID/DD). We undertook this study to estimate the frequency of subtelomeric rearrangements in children with ID/DD.

**Methods:** One hundred and twenty seven children with idiopathic ID/DD were tested for subtelomeric rearrangements using karyotyping and FISH. Blood samples were cultured, harvested, fixed and GTG-banded using the standard protocols.

**Results:** Rearrangements involving the subtelomeres were observed in 7.8 per cent of the tested samples. Detection of rearrangements visible at the resolution of the karyotype constituted 2.3 per cent, while those rearrangements detected only with FISH constituted 5.5 per cent. Five deletions and five unbalanced translocations were detected. Analysis of parental samples wherever possible was informative regarding the inheritance of the rearrangement.

**Interpretation & conclusions:** The frequency of subtelomeric rearrangements observed in this study was within the reported range of 0-35 per cent. All abnormal genotypes were clinically correlated. Further analysis with array technologies presents a future prospect. Our results suggest the need to test individuals with ID/DD for subtelomeric rearrangements using sensitive methods such as FISH.

**Key words** Chromosomal - cytogenetics - developmental delay - fluorescence *in situ* hybridization - subtelomeric rearrangements

Intellectual disability (ID) is prevalent in 2-3 per cent of the population worldwide. While the aetiology for ID is well-established in about half of the affected individuals, it remains idiopathic in the rest<sup>1</sup>. Of the various aetiological factors, genetic causes are reported in a significant proportion; about 9.5 per cent of the affected individuals have microscopically visible chromosomal abnormalities<sup>2</sup>. The frequency of subtelomeric chromosomal rearrangements in ID averages around six per cent<sup>3</sup>; however, the range of frequencies reported varies between 0 and 35 per cent<sup>4-9</sup>. It is suggested that the large variation in the reported frequencies of subtelomeric alterations may be attributed to factors such as patient selection criteria, sample size, ethnicity, method of detection, polymorphic variants and chance<sup>10</sup>.

The similarity of telomeric repetitive sequences among non-homologous chromosomes increases the probability of a recombination event. Such an event may involve the proximal neurodevelopmental genes in a deleterious rearrangement, causing ID and associated developmental delay (DD)<sup>11</sup>. Thus, the subtelomeric regions are likely candidates to test in an affected individual. The acquisition of genotype data from large population screens has become simple with the advent of novel molecular cytogenetic methods; however, the overlap in the clinical features of individuals with subtelomeric rearrangements makes the diagnosis uncertain. Hence, to characterize newer syndromes associated with ID, a definitive genotype-phenotype correlation is necessary. Therefore, in this study, we aimed to estimate the frequency of sub-microscopic rearrangements in the subtelomeric regions in children with idiopathic ID/DD using fluorescence *in situ* hybridization (FISH) and to find a relation between the genotype and phenotype.

### Material & Methods

The experimental study was conducted between February 2012 and January 2013. One hundred and twenty seven children (7 months to 16 yr old) were enrolled for this study from August 2011 to September 2012 from the paediatric out-patient clinics (n = 92) of Sri Ramachandra University, Porur, Amrita Institute of Medical Sciences, Kochi, and Kanchi Kamakoti Childs Trust Hospital, Chennai and schools for special children (n = 35), namely, Vidya Sudha, Vasantham and Madhuram Narayanan Centre for Exceptional Children, Chennai, Tamil Naidu, India, after obtaining informed consent from the parent/caregiver. These children were recruited based on the clinical evaluation of ID/DD with at least one of the

following criteria: (i) facial (>2) and/or extra-facial (>1) dysmorphism (such as, but not limited to, microcephaly, hypertelorism, clinodactyly and hypospadias); (ii) pre and/or post-natal developmental defects (such as, but not limited to, seizures, feeding difficulties and speech delay); and (iii) a positive family history for unexplained ID/related conditions (a relative with ID/DD or seizures).

Patients with a clinical history suggestive of a common aetiology or with aneuploidies detected on karyotyping were excluded from the study. A brief description of the patients is provided in Table I. The study was approved by the Institutional Ethics Committee. All experimental work was carried out in the department of Human Genetics, Sri Ramachandra University.

**Metaphase chromosome analysis:** Lymphocytes separated from peripheral blood (2 ml) were cultured, harvested and fixed using standardized protocols described previously<sup>12</sup>. Metaphase chromosome preparations were G-banded using trypsin, and a minimum resolution of 550 bphs was considered for analysis. Karyotypes were re-analysed retrospectively to elucidate the rearrangements detected with FISH.

**Fluorescence in situ hybridization:** FISH was performed for all the 127 samples, as per the manufacturer's instruction with slight modifications for standardization, on chromosome preparations using the VysisToTelVysion™ Multi-Color DNA

**Table I.** Stratification of children based on inclusion criteria

Category	Proportion of subjects falling in the particular category (%)
Total number of subjects	127
Age (group)	
Seven months to 10 yr	83.5
10-16 yr	16.5
ID/DD	
Mild-moderate ID	11
Severe ID	12.6
DD	76.4
Dysmorphic features	61.4
Pre-natal abnormalities	9.4
Growth abnormalities	45.7
Family history of ID/seizures	10.2
ID, intellectual disability; DD, developmental delay	

FISH probe mixtures (Abbott Laboratories, USA) targeting the subtelomeric regions of all chromosomes, except the acrocentric 'p' arms. At least three slides were used for each sample, with 15 marked regions for each of the 15 probe mixes. The probes were co-denatured with target metaphase chromosomes and hybridized overnight using Hybrite™ (Abbott Vysis, USA). Excess unbound probes were removed by post-hybridization washing, and slides were counterstained with DAPI (4',6-diamidino-2-phenylindole). Quality of the FISH experiment was evaluated based on mitotic index, chromosome morphology and signal intensities. Analysis was considered conclusive if the first 10 metaphases were concordant. In cases of discordance, the number of metaphases analysed was increased to 20. FISH was repeated for mixes showing the absence or excess of signals or indicating translocations, and the analyses were compared between two cytogeneticists. The images were documented using the CytoVision software, version 3.1 (Leica Biosystems, Germany). Where available, parental samples were also tested using FISH and GTG banding to determine whether the abnormality was inherited.

### Results

The metaphase chromosomes were successfully prepared from all blood samples collected ( $n = 127$ ); analyses of G-banded metaphases showed a karyotype of 46,XY ( $n = 90$ ) for normal males and 46,XX ( $n = 33$ ) for normal females. Of the 127 children, 123 (97%) showed a normal karyotype and the remaining four showed an abnormal karyotype. Three abnormalities involved a deletion on chromosome 18 (patients 1, 7 and 8, Figs 1-3) and one involved an inversion on chromosome 17 (patient 6, Fig. 4).



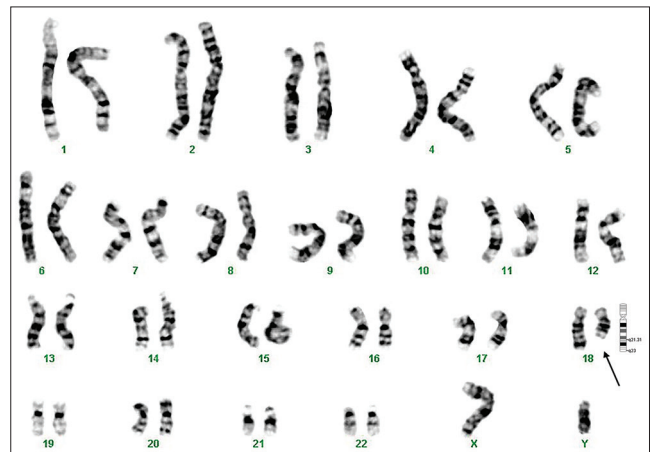
**Fig. 1.** GTG-banded karyotype image of patient 1. The arrow points to the deletion on the 'p' arm of chromosome 18.

FISH was performed to detect the subtelomeric alterations in all the patients. FISH revealed sub-microscopic rearrangements in 5.5 per cent ( $n = 7$ ) and confirmed terminal deletions in those (2.3%) showing an abnormal result on GTG banding ( $n = 3$ ). The overall results showed that while the frequency of abnormalities using GTG banding alone was 2.3 per cent, FISH as a stand-alone technique yielded a value of 7.8 per cent.

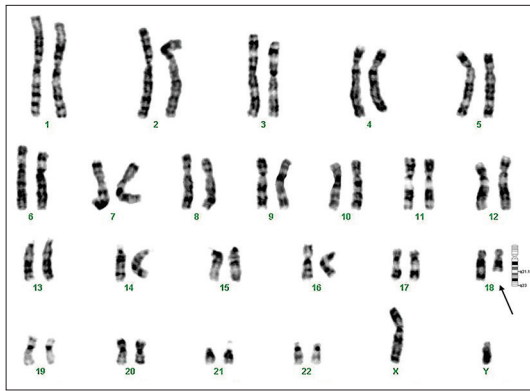
The clinical description and chromosomal abnormalities of the patients with subtelomeric rearrangements are described in Table II. Overall, five children showed deletions and five showed an unbalanced translocation, probably inherited from a parent who was a balanced translocation carrier. The deletions observed were 18p-, 18q- (patient 7, Fig. 5) and 17p- (patient 6, Fig. 6) and 4p-. The unbalanced translocations were 7q-3q+ (patient 2, Fig. 7a and b), Yq-Yp+, 1q-5p+, 4p-8p+, 6p-8p+. Parental samples were available for four children, two of whom showed an apparently balanced translocation: mother of patient 2 showed a balanced translocation between 3q and 7q (Fig. 8a and b) and the father of patient 9 showed a balanced translocation between 4p and 8p. The parents of the other two patients showed no chromosomal abnormality, indicating a *de novo* deletion in the child. The karyotype and FISH results are presented in Table III. In addition, subtle deletions in two patients were apparent on re-analysis of the karyotypes, with the knowledge of the FISH results.

### Discussion

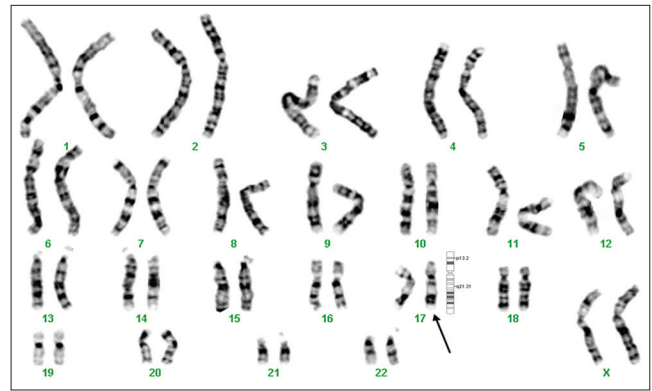
The subtelomeric regions are recombination hotspots, whose rearrangements may lead to segmental



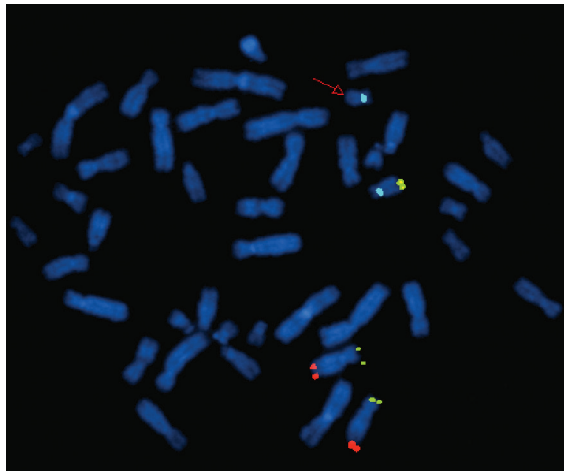
**Fig. 2.** GTG-banded karyotype image of patient 7. The arrow points to the deletion on the 'q' arm of chromosome 18.



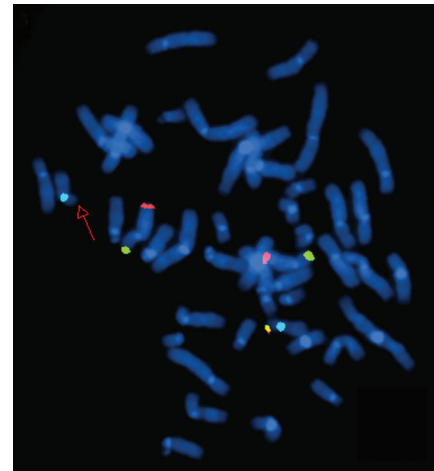
**Fig. 3.** GTG-banded karyotype image of patient 8. The arrow points to the deletion on the 'q' arm of chromosome 18.



**Fig. 4.** GTG-banded karyotype image of patient 6. The arrow points to the pericentric inversion on chromosome 17.



**Fig. 5.** Fluorescence *in situ* hybridization image of metaphase plates of patient 7 - Mix 12 with probes for telomeres of chromosome 12p (green), 12q (red), 18q (yellow) and control region, centromere 18 (aqua). The red arrow points to the absence of yellow signal on one of the chromosome 18s, indicating a deletion of 18qter.



**Fig. 6.** Fluorescence *in situ* hybridization image of metaphase plates of patient 6 - Mix 8 with probes for telomeres of chromosome 8p (green), 8q (red), 17p (yellow) and control region, centromere 17 (aqua). The red arrow points to the absence of yellow signal on one of the chromosome 17s, indicating a deletion of 17pter.

aneusomy and haploinsufficiency of genes in the flanking regions. With at least one-third of the total human genes being expressed in the brain<sup>13</sup>, the probability of a subtelomeric rearrangement resulting in a neurodevelopmental disorder is extremely high. Continual research in this area has led to the characterization of distinct syndromes associated with subtelomeric rearrangements.

The abnormalities described in our study included 18p-, 7q-3q+, Yq-Yp+, 1q-5p+, 4p-, 17p-, 18q-, 4p-8p+ and 6p-8p+. Some of these rearrangements have been reported previously; however, the phenotypes and levels of severity are variable. About 10 per cent of cases with 18p- have been reported to have brain malformations as part of the holoprosencephaly

spectrum, and in general, the deletions reported on 18p are microscopically visible<sup>14</sup>. 3q29 duplication and 7q36 deletion syndromes have been reported either as the sole abnormality or with the involvement of another chromosome. A three generation family in which five members had mild-to-moderate ID and minor dysmorphic features associated with an interstitial micro-duplication of chromosome 3q29 has been reported<sup>15</sup>. The 7q36.1-qter region has been reported to comprise two genes: the *HLXB9* gene involved with Currarino syndrome (sacral dysgenesis, anorectal atresia and a presacral mass) and the sonic hedgehog gene involved with holoprosencephaly<sup>16,17</sup>. The *SHOX* gene on Yp11.3 is reported to be involved in growth retardation and cause short stature, however, may not affect intelligence<sup>18</sup>. Joyce *et al*

**Table II.** Chromosomal and clinical description of patients with subtelomeric rearrangements

Patient	Age (yr)	Sex	Result	Clinical description	Observation relative to published reports
1	6	Female	del 18p	Short stature Triangular face, low posterior hairline, upslanting eyes, hypertelorism, strabismus, stammering Poor scholastic performance Positive family history of ID	No holoprosencephaly
2	1	Male	dup 3q; del 7q	Antenatal IUGR Global DD, seizures, hypocalcaemia Bilateral convergent squint, bilateral low-set ears, right epicanthic fold, narrow palpebral fissures, microstomia, micrognathia Penile hypospadias, right undescended testis	No sacral dysgenesis or holoprosencephaly Genital abnormality may be attributed to 3qter duplication
3	2	Male	dup Yp; del Yq	DD and delayed speech Closed anterior fontanelle, bilateral convergent squint, epicanthal fold, hypertelorism, periorbital puffiness Repetitive movements of the right upper limb, brain MRI: possible demyelination Positive family history of seizures	Association of der (Y) with ID uncertain
4	2	Male	del 1q; dup 5p	DD Bilateral inferior iris coloboma, nystagmus, hypertelorism, bilateral medial deviation of eyes, short columella, low-set ears, high-arched palate, tapering fingers, short toes Hypertonia, brain MRI: two well-defined cystic lesions, mild white matter volume loss in parieto-occipital region, markedly hypoplastic corpus callosum	Contiguous gene deletion on 1q44 likely cause of clinical symptoms, 5p involvement unclear
5	5	Male	del 4p	Severe ID and global DD, 2 episodes of febrile seizures, limited movement, excessive startling, speech difficulties Telecanthus, upslanted eyes, overhanging nasal tip, micrognathia, bilateral low-set flared pinna, hypoplastic alae nasi Bilateral 5 <sup>th</sup> digit clinodactyly with pesplanovalgus, index finger with ulnar deviations	Deletion breakpoint at 4p16.3 Involvement of WHSCR uncertain
6	8	Female	del 17p	Delayed milestones, mild ID, suspected Treacher Collins phenotype Micrognathia, retrognathia, arched eyebrows, prominent forehead, low-set ears, hypertelorism, mild hearing loss Overriding of left 5 <sup>th</sup> toe over the 4 <sup>th</sup> Surgical intervention for mandibular reconstruction, cleft palate, and ear malformations	No lissencephaly Parents negative for deletion Likely variant

*Contd....*

Patient	Age (yr)	Sex	Result	Clinical description	Observation relative to published reports
7	1	Male	del 18q	Global DD Severe microcephaly, brachycephaly, closed anterior fontanelle, hypertelorism, low-set dysplastic pinna with prominent antihelix, upslanted eyes, left divergent squint, short columella, severe anteverted nares, short philtrum, thin upper and lower lips, carp-shaped mouth with bilateral downward deviation Long fingers with mild camptodactyly, widely placed hypoplastic nipples, dorsal oedema on both feet, bilateral prominent heels, rocker-bottom feet, extensive Mongolian blue spots on back and legs, hypotonia, absence of crease on both thumbs, bilateral single palmar crease Tonic seizures of the left upper and lower limbs associated with left eye closure at six months, brain MRI: partial agenesis of the corpus callosum	Some features consistent with de Grouchy syndrome  Variable phenotype compared to deletion from 18q21.1
8	1	Male	del 18q	Global DD, head bobbing movements and limited speech Severe microcephaly, hypertelorism, bilateral ptosis, arched eyebrows, left epicanthal fold, upturned nose, microretrognathia, bilateral low-set posteriorly rotated ears Bilateral single palmar crease, bilateral ulnar deviation of index finger, partial cutaneous syndactyly between second and third fingers bilaterally	Some features consistent with de Grouchy syndrome  Variable phenotype compared to deletion from 18q21.3
9	1	Male	del 4p; dup 8p	Antenatal IUGR Global DD, on speech and occupational therapy Mother had previous spontaneous abortion at 48 days	Mild features compared to WHS phenotype
10	1	Male	del 6p; dup 8p	Antenatal polyhydramnios, mild ventriculomegaly, persistent right umbilical vein at ninth month Plagiocephaly, low-set ears, short philtrum, retrognathia, mildly prominent eyes, hypertelorism, short anteverted nostrils, widely spaced nipples Echocardiogram showed a small ostium secundum. Brain ultrasound revealed mild prominence of lateral and third ventricles and a cavum septum pellucidum	Features may be attributed to both deletion and duplication

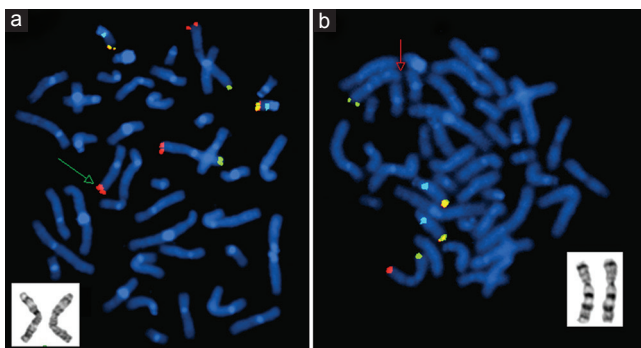
ID, intellectual disability; DD, developmental delay; IUGR, intrauterine growth restriction; MRI, magnetic resonance imaging; WHS, Wolf-Hirschhorn syndrome; WHSCR, Wolf-Hirschhorn syndrome critical region

had reported a complex chromosomal rearrangement with nine breakpoints involving five chromosomes including the Y chromosome in a boy with mild ID, DD, short stature and microcephaly<sup>19</sup>. The deletion on 1q44 is thought to encompass multiple genes in the

region, and thus, the manifestations are a result of the contiguous gene deletion and no specific genes have been implicated. Duplication of 5p-p14.3 has been reported in two sisters with ID, obesity, mandibular prognathism with eye and skin anomalies<sup>20,21</sup>. The

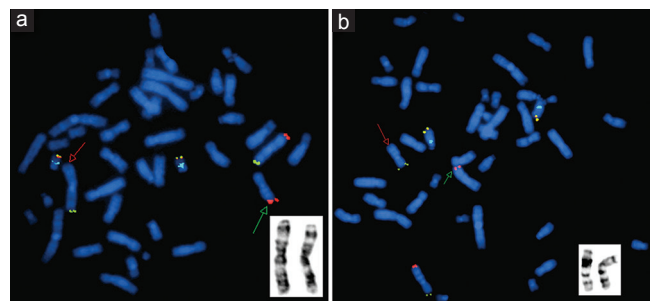
**Table III.** Karyotype and fluorescence *in situ* hybridization (FISH) results of patients with subtelomeric rearrangements

Patient	Results		Inheritance
	Karyotype	FISH	
1	46, XX, del (18p)	ish del(18)(pter-)(D18S552-)	Unknown
2	46, XY	ish der(7)t(3;7)(qter+,qter-) (D3S4560+,STS2000H-)mat	Mother ish t(3;7)(D3S4560-, VIJyRM2000+; VIJyRM2000-, D3S4560+)
3	46, XY	ish der(Y)(pter+,qter-)(DXYS129+,Z43206-)	Unknown
4	46, XY	ish der(1)t(1;5)(qter-,pter+)(D1S3738-,C84c11/ T3+)	Unknown
5	46, XY	ish del(4)(pter-)(GS10K2/T7-)	Unknown
6	46, XX, inv (17) (?p13.2:?q21.3)	ish del(17)(pter-)(282M15/SP6-)	<i>de novo</i>
7	46, XY, del (18)(q21.3→qter)	ish del(18)(qter-)(D18S1390-)	<i>de novo</i>
8	46, XY, del (18)(q21.1→qter)	ish del(18)(qter-)(D18S1390-)	Unknown
9	46, XY, der (4) t (4;8) pat	ish der(4)t(4;8)(pter-,qter+)(GS10K2/ T7-,D8S504+)pat	Father: ish t(4;8)(GS10K2/ T7-,D8S504+;D8S504-,GS10K2/T7+)
10	46, XY	ish der(6)t(6;8)(pter-,qter+)(6PTEL48-D8S504+)	Unknown



**Fig. 7.** Fluorescence *in situ* hybridization image of metaphase plates of patient 2 - **(a)** Mix 3 with probes for telomeres of chromosome 3p (green), 3q (red), and 22q (yellow) and control region, 22q11 (aqua). The green arrow points to the presence of an additional red signal on one of the chromosome 7s, indicating a derivative 7 with duplicated 3qter. GTG-banded partial karyotype in the inset shows both homologues of chromosome 3. **(b)** Mix 7 with probes for telomeres of chromosome 7p (green), 7q (red), and 14q (yellow) and control region, 14q11.2 (aqua). The red arrow points to the absence of red signal on one of the chromosome 7s, indicating a deletion of 7qter. GTG-banded partial karyotype in the inset shows both homologues of chromosome 7.

Wolf-Hirschhorn syndrome (WHS) critical region is located in 4p16.3. Deletions with a size <3.5 Mb have been described in the mild variant of WHS, also known as Pitt-Rogers-Danks syndrome, with the critical deletion region narrowed down to 165 kb at 4p16.3. The loss of multiple genes on 4p such as *WHSC1*, *LETMI* and *MSX1*, which are thought to play significant roles in early development, is reported to cause the typical features of the syndrome<sup>14</sup>.



**Fig. 8.** Fluorescence *in situ* hybridization image of metaphase plates of the mother of patient 2 - **(a)** Mix 3 with probes for telomeres of chromosome 3p (green), 3q (red), and 22q (yellow) and control region, 22q11 (aqua). The red arrow points to the absence of red signal on chromosome 3qter, and green arrow points to the presence of red signal on chromosome 7qter, indicating t(3;7). GTG-banded partial karyotype in the inset shows both homologues of chromosome 3. **(b)** Mix 7 with probes for telomeres of chromosome 7p (green), 7q (red), and 14q (yellow) and control region, 14q11.2 (aqua). The red arrow points to the absence of red signal on chromosome 7qter, and the green arrow points to the presence of red signal on chromosome 3qter, indicating an apparently balanced reciprocal t(3;7). GTG-banded partial karyotype in the inset shows both homologues of chromosome 7.

Terminal deletions of 17p have been known to result in a distinctive phenotype, Miller-Dieker syndrome, a contiguous gene syndrome. However, phenotypically normal subjects with telomeric 17p deletions up to 600 kb have also been reported<sup>22,23</sup>. Since the patient in our study did not show classical features of Miller-Dieker syndrome or isolated lissencephaly, the clinical significance of this *de novo* deletion was indeterminate. The breakpoints as well as clinical presentation for

18q deletion syndrome vary greatly among reports, suggesting that there are no hotspots. However, various critical regions have been identified, including a 4.3-Mb region within 18q22.3-q23 that is responsible for the typical 18q deletion phenotype<sup>24</sup>. Monosomy 4p with trisomy 8p results in a WHS phenotype. However, since the extent of the deletion is minimal, the features are atypical. The deletion of region 6p up to 6p24 has been implicated in various syndromes. Patients with 6p deletion were characterized with DD and hypotonia, with an abnormal skull shape<sup>25</sup>. The significance of the presence of three copies of 8p23, however, is not known. Inverted duplications of the region proximal to 8p23.3, without involvement of the subtelomeric region, have been reported with clinical abnormalities<sup>26</sup>. The significance of trisomy 8p in our patients remained unclear; the loss of gene-rich regions on 4p and 6p as well as the excess of genes on 8p could be responsible for the observed phenotype.

The frequency of subtelomeric rearrangements in individuals with ID ranges from 0 to 35 per cent<sup>4-9</sup>. The lack of concordance on the reported frequencies among studies may be due to various factors. Patient inclusion criteria vary among studies and that is known to influence the observed rearrangement frequency. The largest study so far on 11688 subjects with ID reported an abnormality rate of only three per cent<sup>5</sup>. The low frequency observed in their study was attributed to the study population being unselected. Our study employed less stringent inclusion criteria that did not adhere to the de Vries *et al* checklist<sup>27</sup>. However, the frequency observed in our study was within the range of published reports.

The presence of polymorphic variants among subtelomeric rearrangements has been reported in healthy control groups or in the families of affected subjects in a few studies<sup>4,5</sup>. It has also been reported that the subtelomeres of certain chromosomes such as 2q and 10q may be polymorphic<sup>5,28</sup>. Ravnan *et al*<sup>5</sup> described partial deletions of the 10q telomere as deduced by their diminished fluorescent intensity in 14 subjects as variants, some of them familial. In our experience, FISH analysis of metaphases from eight patients showed reduced fluorescence intensity in one of the 10q telomeres. However, since the test is not quantitative and as parental samples for these subjects were unavailable, it remained unsubstantiated to classify them as polymorphic variants.

It is not known whether subtelomeric rearrangement frequencies differ among various ethnic groups.. Our study reports the frequency in a sample of the Indian

population, from which data are limited. A previous report from India did not detect any subtelomeric rearrangement using multiplex ligation-dependant probe amplification in a study population of 122 individuals<sup>1</sup>.

The association of the phenotypes with the deduced genotypes depends on further characterization of the regions involved in the rearrangements at higher resolution. An assessment of the whole genome may be the best possible approach; however, it is not feasible in every case. Technologies such as array CGH have revolutionised the field of molecular cytogenetics, however, these still require standardization, validation and cost optimization.

In conclusion, our study reiterates the necessity to screen patients with unexplained ID/DD for subtelomeric rearrangements, which may be missed in routine cytogenetic testing, using a sensitive technique such as FISH. When the clinical presentation warrants a genetic diagnosis, screening the genomic hotspots at high resolution is recommended.

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**Conflicts of Interest:** None.

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