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Cryptic Rearrangements in Idiopathic Intellectual Disability Diagnosed by Molecular Cytogenetic Analysis

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KEYWORDS Dysmorphisms. FISH. Karyotype. Subtelomeric Anomalies. Telomeres.

ABSTRACT With the development of molecular cytogenetic techniques, it is possible to identify cryptic rearrangements involving the end of chromosomes. Subtelomeric chromosomal rearrangements represent a significant cause of idiopathic intellectual disability accounting for 6-10% of moderate to severe cases and 0.5% in individuals with mild intellectual disability. We investigated 50 patients with severe intellectual disability combined with a dysmorphic features and normal 400-550 band karyotype for unbalanced subtelomeric rearrangements by using fluorescence in situ hybridization with probes mapping to forty one telomeric-specific regions. Nine positive cases (18%) were found. Six were de novo deletions (1p, 2q, 6p, 9q, 10q, 22q) and one wasis de novo duplication (10q) .Two unbalanced translocation (a der(3)t(3p; 2q) and a der(3)t(3p; Xq)) were inherited from the balanced mothers. Our study supportsed the hypothesis that subtelomeric rearrangements are a significant cause of idiopathic intellectual disability. The clinical features of patients with subtelomeric abnormalities and the candidate genes proposed inside each region will help to better delineate the phenotype- genotype correlation.

INTRODUCTION

Intellectual disability (ID) occurs in 1-3% of general population (Hunter et al. 2000), ID is defined as a significant impairment of both cognitive (IQ<70) and social adaptive functions, with on set before 18 years of age. 25%-50% of moderate to severe ID is resulted from genetic etiology (Shaffer 2005). Segmental aneusomy due to subtle structural chromosome abnormalities is an important cause of intellectual disability. Conventional cytogenetic analysis is a routine test for ID and it detects a frequency of microscopic chromosomal aberrations ranged from 9% to 36% (Schreppers-Tijdink et al. 1988). However, karyotype cannot detect cryptic subtelomeric rearrangements. Segmental aneusomy due to subtle structural chromosome abnormalities is an important cause of intellectual disability. Conventional cytogenetic analysis using karyotype cannot detect these cryptic subtelomeric rearrangements. The limited resolution of these methods has been overcome with the resolution of molecular cytogeneticscytogenetic analysis, especially with the advent of fluorescence in situ hybridization and comparative genomic hybridization which has enhanced the ability to detect submicroscopic rearrangements smaller than 3 Mb (Xiang et al. 2010) and has resulted in detection of submicroscopic rearrangements in approximately 6% of ID patients (Knight et al. 1999).. Subtelomeric regions are usually enriched for genes-rich and are more susceptible to aberrant rearrangements than other chromosomal regions rearrangements in these regions would

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involve many genes (Hila et al. 2010). Subtelomeric anomalies are widely associated as leading to ID or congenital malformations, although the exact origin and effect relationship has not been well defined (De Vries et al. 2005). The clinical implications are probably determined by the location and the size of the rearrangement, including the numbers and the function of the genes involved (Shaw-Smith et al. 2004). These regions are likely to contain undiscovered genes associated with ID. Molecular cytogenetic analysis helps to determine the critical regions and novel candidate genes for ID.

Many of these subtelomeric anomalies are now recognized as clinically recognizable syndromes such as 1p36, 3p- and 22qter syndromes (Battaglia et al. 2008; Fernandez et al. 2008; Ye Wu et al. 2010). Deletion 1p36 is associated with growth retardation, epilepsy, visual problems, facial dysmorphism with large anterior fontanelle, asymmetrical and low set dysplastic ears, deep set eyes, depressed nasal bridge, pointed chin, and clinodactyly of 5th finger (Riegel et al. 1999; Ortigosa et al. 2011). Another anomaly wasis discovered in patients with submicroscopic chromosome 22qter deletions in which, in addition to the hypotonia and intellectual disability, absence of speech and autism (De Vries et al. 2000; Dhar et al. 2010).

Deletion of distal short arm of chromosome 3 is another anomaly which correlates with specific phenotype. It has been associated with low birth weight, microcephaly, mental and growth retardation, triongonocephally, hypotonia, ptosis, telecanthus, downward slanting palpepral fissures, and micrognathia (Fernandez et al. 2008).

Previous reports have estimated an abnormality rate of 6%, with a range of 2-30% because of different inclusion criteria. Clinical criteria are proposed to improve preselecting of mentally retarded patients for subtelomeric screening are the five item checklist of De Vries et al (De Vries et al. 2001), that includes family history of Mental Retardation (MR), prenatal and post natal growth retardation, two or more facial dysmorphic features and/or congenital malformations.

We report in this paper, the subtelomeric rearrangements in a series of 50 patients with normal standard karyotype, selected on the basis of severe intellectual disability, dysmorphic features, family history and/or congenital malformations. Subtelomeric aberrations were identified and possible candidate genes are proposed.

MATERIALS AND METHODS

Patients

Among three hundred mentally retarded patients referred to the Cytogenetic and biology of reproduction department, fifty consecutive patients evaluated by the same medical geneticist (E.H) have beenwere submitted to telomeric analysis. Selection was done according to the clinical criteria described by De Vries et al (De Vries et al. 2001) including moderate to severe MR, two facial dysmorphisms at least, hand and foot anomalies, and/or congenital malformations, and/or family history. The mean age was 8 years (range: 3-18) (Table 1). All patients had unexplained MR without ethological diagnosis after through clinical evaluations, with moderate to severe MR (QI<55, Psychiatric diagnosis was done with DSMIV (diagnostic and statistical Manual of Mental disorders, APA, 1994) and intellectual diagnosis with EDEI-R and PM47 tests), exclusion of prenatal brain injury, no history of toxication, central nervous system infection and crania trauma, normal karyotype, no evidence of inherited metabolic disorder or specific neurodegenerative disorders by brain imaging and urinary metabolic screening, negative for mutations in the FMR1 gene for boy and negative for typical clinical features of syndromes.

The study protocol was approved by the institutional review board at the Farhat Hached University Teaching Hospital and the Medical Ethics Committee of the Tunisian Network on Intellectual disability.

Cytogenetic Analysis

Conventional R-banding

Chromosomal analysis was performed according to standard procedures. Peripheral blood lymphocytes were cultured in Roswell Park Memorial Institute medium 1640 (RPMI, Gibco®, Grand Island, NY, USA) enriched with 20% fetal calf serum, L-glutamine, antibiotics (penicillin and streptomycin) and antibodies (Phytohemmaglutinine). The cells were cultured for 72 hours in a humidified environment

Table 1: Main clinical findings and FISH results in the 9 patients with subtelomeric rearrangements.

Patients	*Age*	*Sex*	*Clinical features*	*Abnormality and origin*
P1	6	M	Severe intellectual disability, prenatal and postnatal growth retardation, microcephaly, seizures with abnormal EEG, hypotonia, a prominent forehead, straight eyebrows, deep-set eyes, Large anterior fontanel, strabismus, bilateral epicanthic folds, Midface hpoplasia, short philtrum, high-arched palate, broad nasal root, pointed chin, low set ears, brachydactyly, clinodactyly of thumb and short fingers and toes.	46, XX. ish del(1)(p36.3)dn
P2	5	F	Severe intellectual disability, hyperactivity, prenatal and postnatal growth retardation , round face, frontal bossing, broad nasal bridge, long eyebrows, hypertelorism, epicanthic fold, anteverted nostrils, long prominent philtrum, low-set ears, small nose, short neck, short fingers and toes, brachydactyly, axial hypotonia and brittle hair.	46,XX. ish del(2)(q37.2)dn
P3	6	F	Family history of severe intellectual disability, microcephaly, postnatal growth retardation, high forehead, low-set ears, hypertelorism, ptosis, broad nasal bridge, long philtrum, short nose, long fingers with bilateral clinodactyly of the 5th finger, spina bifida occulta.	46,XX.ish der(3)t(3;2)(p26;q37.2)mat
P4	3	M	Family history of severe intellectual disability, prenatal and postnatal growth retardation, microcephaly , hypotonia, myoclonic seizures and frequent respiratory and urinary tract infections, bitemporal narrowing, prominent metopic suture, bilateral epicanthus, broad nasal bridge, a thin and short pointed nose, short philtrum, small mouth, round cheeks, pointed chin, shaped ears, short neck, bilateral ectopic testis, abnormal, hypoplastic callosum.	46,XY. ish der(3)t(X,3)(q27.3;p26.3)mat
P5	5	M	Severe intellectual disability, hypertelorism, abnormal helix, low-set ears, sloping forehead, profound neurosensory deafness, poor visual contact, hypoplasia of the middle level, interventricular communication (CIV).	46, XY. ish del(6)(p25.2)dn
P6	7	M	Severe intellectual disability, obesity, macrocephaly, brachycephaly, flat nasal bridge, epicanthic folds, long philtrum, small noise, low set-ears, ambiguous genitalia.	46,XY. ish del(9)(q34.3)dn
P7	5	M	Family history of severe intellectual disability, postnatal growth retardation, microcephaly, flat profile, hypertelorism, rounded nose tip, small mouth, thin long, upper lip, micrognathia, low set small ears, overfolded helix, prominent eat lobe, short neck, autistic features.	46,XY. ish del(10)(q26.2)dn
P8	11	F	Family history of severe intellectual disability, postnatal growth retardation, microcephaly, epileptic seizures, failure to thrive, triangular face, low set hair, long eyelashes, hypertelorism, ptosis, large mouth, flat nasal bridge, bulbous nose, long philtrum, prominent upper lip, high arched palate, low set ears, malformed bones, short fingers, clinodactily of 5th finger.	46,XY. ish dup(10)(q26.2)dn
P9	11	M	Family history of severe intellectual disability, postnatal growth retardation, neonatal hypotonia, microcephaly, frontal bossing, low set and posteriorlly angulated ears, hypertelorism, large mouth, bulbous nose, short philtrum, high arched palate, absent speech, fine appearance of the corpus callosum, bilateral ventricular dilatation, autistic feature.	46, XY.ish del (22)(q13.2)dn

with 5% CO_2 in 37°C incubator until harvest. For the 72 hours culture, the sample wassamples were incubated with Colcemid solution (final concentration 0.05µg/ml) for 45 minutes. After the harvesting, the cells were exposed to hypotonic solution (0.075mol/L KCl) and fixed with methanol/acetic acid (3: 1).

The slides were prepared and stained using the R-bands (Reverse-bands) technique on peripheral blood lymphocyte cultures. A minimum of 50 metaphases were analyzed from each sample and karyograms were prepared using the Applied imaging CytoVision Automated Karyotyping System®. Chromosomal abnormalities have been reported in accordance with the current international standard nomenclature (Shaffer et al. 2009).

Fluorescence in situ Hybridization

A FISH protocol with a complete set of probes has beenwas applied. The TOTEVysion™ Multicolor DNA Probe Mixtures (Vysis®, Downers Grove, Illinois, USA), which involves the use of the same set of a different combination of probes with different colors. This permitted

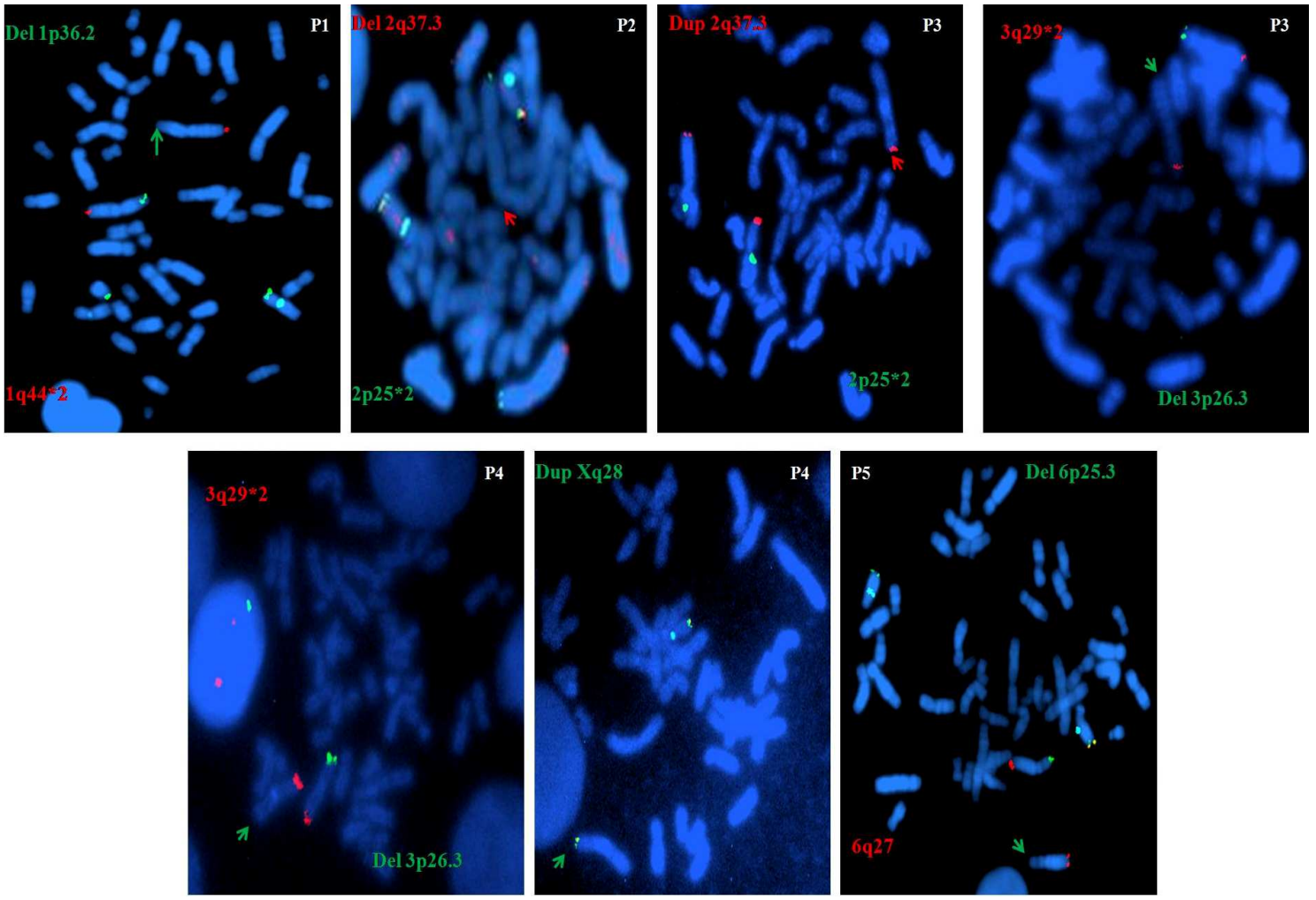

Fig. 1. Partial metaphases representative of the subtelomeric rearrangements detected in this study. Green signals indicated short arms, red signals indicate long arms. P1[del (1p36.3)], P2[del(2q37.2)], P3[del(3p26.3), dup(2q37.2)], P4[del(3p26.3), dup(Xq28)], P5[del(6p25.3)].

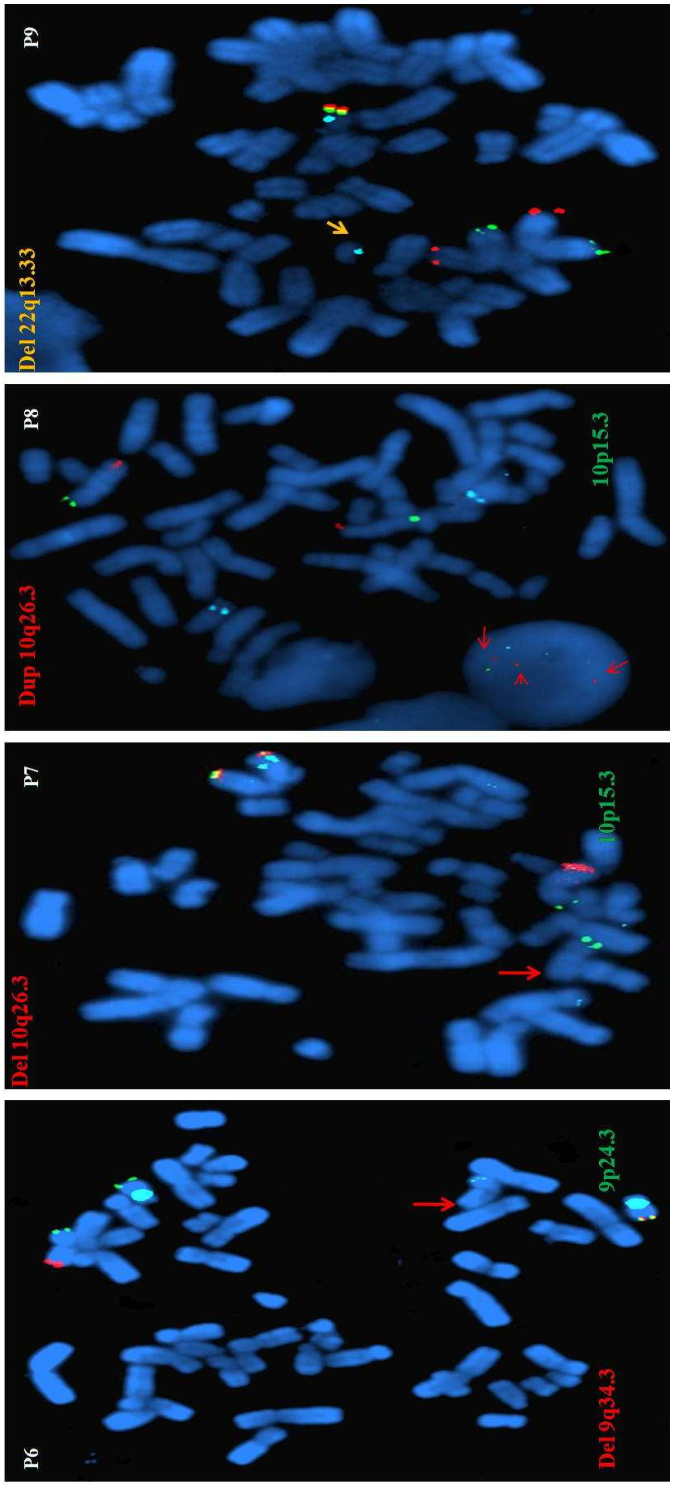

Fig. 2. FISH analysis of metaphases of P6[del(9p24.3)], P7[del(10q26.3)], P8[dup(10q26.3)], P9[del(22q13.33)].

the evaluation of 41 telomeric regions, excluding the short arm of the acrocentric chromosomes (13, 14, 15, 21 and 22). The probes size ranged from 60 Kb to 191 Kb, and each contained a locus estimated to be within 300 Kb of the end of the chromosome.

In order to hybridize all mixtures, we use a minimum of three slides with five scribed target areas. We followed the standard hybridization method which consisted of 2xSSC aging of slide, 70% formamide denaturation of slide, dehydration through an ethanol series of 70%, 85% and 100% for one minute each, denaturing the probes mixture followinged by hybridization and post-hybridization washing by 2xSSC and NP40 (Vysis®).

Slides arewere then air-dried and mounted using DAPI (4', 6'-diamino-2-phenylindole) in Vectashield (Vectorlabs). Finally, slides are cover slipped and are ready to view.

The hybridized chromosome spreads has been viwed by using were viewed using a fluorescent microscope equipped with appropriate filters and cytovision FISH system image capture software wars (Zeiss Axioskop 2 plus). Slides arewere scored simply by the number of telomeric signals for each metaphase. For each target area, twenty hybridized metaphases were analyzed and scored.

RESULTS

Among the 50 investigated patients, 9 (18%) were found havingto have subtelomeric rearrangements in this study. Six were de novo deletions (1p, 2q, 6p, 9q, 10q, 22q) and one is de novo duplication (10q) and two unbalanced translocations a der(3)t(3p;2q) and a der(3)t(3p;Xq) which have been inherited from a maternal balanced reciprocal translocations mothers (Fig.1 and Fig.2). The distribution of positive cases with respect to age group, sex, degree of intellectual disability, presence of facial dysmorphic features and/or congenital anomalies, prenatal/postnatal growth retardation, family history of MR and origin of the rearrangement areis showed in Table 1.

DISCUSSION

Immediately adjacent to the (TTAGGG)n tandem repeats, there are repetitive DNA regions in tandem and subtelomeric regions which may extend for several hundred of kilobases (Kb) that wasare the subject of our study (Flint et al. 1997). These regions have shown interest in certain diseases and in particular intellectual disability. Indeed, several studies that using molecular cytogenetic methods have proved the involvement of subtelomeric sequences in children with intellectual disability (Baker et al. 2002; Anderlid et al. 2002a; Ravnan et al. 2005). The subtelomeric regions are gene-rich so alterations in these regions predicted to be more likely to result in abnormal phenotypes (Ballif et al. 2004; Hila et al. 2010).

Repetitive DNA sequences at telomeres can be a major cause of mismatch during meiosis and thus facilitate chromosomal rearrangements, such as deletions, duplications and translocations (Ghaffari et al. 1998). Since the study of telomeres is accessible, and because of their high concentration of genes, several teams estimated the frequency of telomere abnormalities in children with intellectual disability and for whom no further investigation washad allowed a diagnosis (Riegel et al. 2001; Baker et al. 2002; Ye Wu et al. 2010).

In our study, we identified 9 cryptic telomeric rearrangements on 50 families using telomeric FISH. We obtained an incidence of 18%. These results are higher than some series in the literature: Anderlid et al. (2002a): 13.6% (6 / 44), Knight et al. (1999): 7.4% (21/284), Riegel et al. (2001): 5.1% (13/254) and Baker et al. (2002): 4.1% (8 / 197). The number of incidence areis highly variable from one series to another. Several criteria are responsible for this variability. The criteria of selection of our patients wcriterion of selection of our patients wasere accorded to previous studies in order to increase the occurrence of cryptic telomeric abnormalities. In fact, we have considered the degree of intellectual disability (mild, moderate or severe) and only patients with severe mental retardation associated to dysmorphic disorder and / or congenital malformations and with normal karyotype analysis have beenwere investigated. Indeed, Knight et al. (1999) concluded that the research for cryptic abnormalities in children with mild intellectual disability had little interest with an incidence of 0.5 %. However in moderate to severe intellectual disability the incidence of subtelomeric abnormalities was 7.4% (Knight et al. 1999). Also, Baker et al. (2002) studied separately the children with a single delay (incidence: 1.9%) and those with

Table 2: Comparison of the phenotypic features of 9 patients (P1, P2, P3, P4, P5, P6, P7, P8 and P9) with reports from the literature.

CF in monosomy 1p36 [1] *Cranio-facial dismorphism*	*P1*	*CF in monosomy 2q37* [2] *Cranio-facial dismorphism*	*P2*	*CF in monosomy 3p26* [3] *Cranio-facial dismorphism*	*P3*	*P4*	*CF in monosomy 6p25* [4] *Cranio-facial dismorphism*	*P5*
Microcephaly	+	Macrocephaly/Microcephaly	-	Microcephaly	+	+	Sloping/Broad forehead	+
Growth retardation	+	IUGR	+	Short stature - postnatal	+	+	Hypertelorism	+
Large anterior fontanel	-	Brachycephaly	-	Low hairline - back	+	+	Down-slanting palpebral fissures	-
Straight eyebrows	+	Frontal bossing	+	Hypertelorism	+	-	Midface hypoplasia	+
Deep-set eyes	+	Deep set eyes	-	Ptosis	+	-	Micrognathia	-
Broad nasal root	+	Pointed chin	-	Short/small nose	+	+	Abnormal ears	+
Midface hpoplasia	+	Micrognathia	-	Broad nasal bridge	+	+	***Neurological features***	
Pointed chin	+	Short/small nose	+	Long philtrum /Short philtrum	+	+	Deafness - neurosensory	+
Low-set ears	+	Broad/bulbous nasal bridge	+	ear abnormality	+	+	Brain abnormality	-
Limb abnormalities	+	Anteverted nostrils	+	Short neck	+	+	***Cardiovascular problems***	+
Neurological features		Long philtrum	+	Limb abnormalities	+	-		
Developmental delay	+	Low set ears	+	***Neurological features***				
Language defects	-	Short neck	+	Developmental delay	+	+		
Hypotonia	+	Limb abnormalities	+	Language defects	+	+		
Seizures	+	Hair anomalies	+	Hypotonia	-	+		
Brain abnormality	-	***Neurological features***		Seizures	-	+		
Eye defects	-	Developmental delay	+	Brain abnormality	+	+		
Hearing loss	-	Speech delay/defect	-	Genital abnormaliy	-	+		
Cardiovascular problems		Hypotonia	+	***Cardiovascular problems***	-	-		
Cardiomopathie	-	Cerebral malformations	-					
Structural heart defects	-	Behaviour disorder/hyperactivity/psychosis			+			
		Seizures of any type	-					
		Cardiovascular problems	-					

CF in monosomy 9q34 [5] *Cranio-facial dismorphism*	*P6*	*CF in monosomy 10q26* [6] *Cranio-facial dismorphism*	*P7*	*CF in trisomy 10q26* [7] *Cranio-facial dismorphism*	*P8*	*CF in monosomy 22q13* [8] *Cranio-facial dismorphism*	*P9*
Small for gestational age	-	Short stature - postnatal	+	Short stature - postnatal	+	Microcephaly	+
Macrocephaly	+	Low hairline - back	-	Microcephaly	+	Epicanthal folds	-
Generalised obesity	+	Microcephaly	+	Low hairline - front	+	Dolicocephaly	-
Brachycephaly	+	Flat occiput	-	Abnormal face	+	Large/ dsplasic ears	+
High forehead	-	Hypertelorism	+	Ptosis	+	ptosis	-
Hypertelorism	-	Deep set eyes	-	Flat nasal bridge	+	Limb abnormalities	+
Flat nasal bridge	+	Beaked nose	-	Long philtrum	+	***Neurological features***	
Pointed chin	-	Thin lips	+	High arched palate	+	Developmental delay	+
Prognathism	-	Low set ears	+	Low set ears	+	Autistic behavior	+
Epicanthic folds	+	Helix absent /abnormal	+	Limb abnormalities	+	Delayed/ absent speech	+
Short/small nose	+	Short neck	+	***Neurological features***		Neonatal hypotonia	+
Short philtrum	-	***Neurological features***		Developmental delay	+	Accelerated growth	+
Low set ears	+	Developmental delay	+	Hypotonia	-	Brain abnormality	+
Neurological features		Autism	+	Brain abnormality	-	***Cardiovascular problems***	
Developmental delay	+	hypotonia	-	***Cardiovascular problems***	-	Structural heart defects	+
Language defects	-	Brain abnormality	-	***Bones malformations***	+		

Table 2: Contd.....

CF in monosomy 9q34 [5] *Cranio-facial dismorphism*	*P6*	*CF in monosomy 10q26* [6] *Cranio-facial dismorphism*	*P7*	*CF in trisomy 10q26* [7] *Cranio-facial dismorphism*	*P8*	*CF in monosomy 22q13* [8] *Cranio-facial dismorphism*	*P9*
Hypotonia	-	***Cardiovascular problems***					
Brain abnormality	-	Structural heart defects	-				
Cardiovascular problems		***Ambiguous genitalia***	-				
Cardiomopathie	-						
Structural heart defects	-						
Visceral anomalies	-						
Genital anomalies	-						

(CF: Common features)

[1] : Slavotinck and Shaffer 1999; Knight-Jones et al. 2000; Heildest et al. 2003 a; Heildest et al. 2003 b; Ballif et al. 2004; Redon et al. 2005; Battaglia et al. 2008 ; Gajecka et al. 2010; Rosenfeld et al. 2010.

[2] : Wilson et al. 1995; Bonaglia et al. 2000 ; Syrrou et al. 2002; Casas et al. 2004; Roberts et al. 2004; Kitsiou-Tzeli et al. 2007; Jones et al. 2011.

[3]: Mowrey et al. 1993; Phipps et al. 1994; Cargile et al. 2002; Dijkhuizen et al. 2006; Verloes et al. 2006; Malmgren et al. 2007; Fernandez et al. 2008; Shuib et al. 2008; Pohjola et al. 2010.

[4]: Mirzayans et al. 2000; Baruch and Erickson 2001 ; Saleem et al. 2001 ; Grosso et al. 2002; Maclean et al. 2005 ; Caluseriu et al. 2006; Aldinger et al. 2009; Tumer and Bach-Holm 2009.

[5]: Cormier-Daine et al. 2003; Steward et al. 2004; Harada et al. 2004; Stewart et al. 2004; Yatsenko et al. 2004; Kleefstra et al. 2005; Kleefstra et al. 2006 a; Kleefstra et al. 2006 b.

[6]: Irving et al. 2003; Kehrer-Sawatzki et al. 2005; Tanabe et al. 2006; Miller et al. 2009; Yatsenko et al. 2009.

[7]: Petek et al. 2001; Aglan et al. 2002; Migliori et al. 2002 ; Carer et al. 2010 ; van Bon et al. 2010.

[8]: Praphanphoj et al. 2000; De Vries BB et al. 2000; Bonaglia et al. 2001; Phelan et al. 2001 ; Anderlid et al. 2002b; Wilson et al. 2003; Manning et al. 2004; Koolen et al. 2005; Lindquist et al. 2005; Bonaglia et al. 2006; Cusmano-Ozog et al. 2007; Durand et al. 2007; Philippe et al. 2008; Wilson et al. 2008; Delahaye et al. 2009 ; Sykes et al. 2009; Chen et al. 2010; Dhar et al. 2010; Phelan and Betancur 2011.

intellectual disability associated with dysmorphism and malformations (incidence: 4.1%) (Baker et al. 2002). Therefore, by following all these criteria associated to a careful genetic counseling, we had the opportunity to better target the population at risk.

The ID and the dysmorphic features are the principal clinical signs of redundant patients with a subtelomeric imbalance (Table 2). Often, subtelomeric abnormalities are not associated with a characteristic phenotype. But, sometimes a specific phenotype canould be defined from molecular similarities, as monosomy 1p36 (Battaglia et al. 2008) or the terminal 22q deletion (Dhar et al. 2010) and the syndrome 3p- (Fernandez et al. 2008) (Table 2).

Deletion Syndrome 1p36

The 1p36 deletion was diagnosed in several patients with concordant phenotypes and has been identified a new syndrome of intellectual disability (Battaglia et al. 2008). This syndrome is characterized by contiguous gene with different degrees of intellectual disability, growth retardation, microcephaly, seizures with abnormal EEG, hypotonia, large anterior fontanelle, straight eyebrowsdysplastic ears, deep set eyes, basal nasal root, midface hypoplasia, pointed chin, low-set ears, limb abnormalities and neurological and cardiovascular problems fifth finger clinodactyly (Slavotinck and Shaffer 1999; Knight-Jones et al. 2000; Heildest et al. 2003 a; Heildest et al. 2003 b; Ballif et al. 2004; Redon et al. 2005; Battaglia et al. 2008 ; Gajecka et al. 2010; Rosenfeld et al. 2010Knight et al. 2000) (Table 2). In 1p36 syndrome many candidate genes have been identified associated with dysmorphisms and epilepsy. *MMP23* and *SKI* genes hadve been proposed to be responsible for a large, late-closing anterior fontanel (Battaglia et al. 2008; Rosenfeld et al. 2010) and deletion of *KLHL17* and *GABRD* genes had beenare associated with the epilepsy phenotype (Pasiorkowski et al. 2011; Rosenfeld et al. 2010). In our case (P1), the consistent finding with the deletion 1p36 is severe ID, prenatal and postnatal growth retardation , microcephaly, seizures with abnormal EEG, a prominent forehead, deep-set eyes, strabismus, bilateral epicanthic folds, short philtrum, high-arched palate, brachydactyly, clinodactyly of thumb and short fingers and toes.

Deletion Syndrome 22q13.3

22q13.3 deletion syndrome was first described by Watt et al. in 1985 (Watt et al. 1985) and it is phenotypically similar to an Angelman syndrome, mainly associated with intellectual disability, hypotonia, microcephalydevelopmental delay, autistic features and especially an absence of language (Precht et al . 1998). Cranio-facial dismorphism was characterized by epicanthal folds, large ears, pointed chin and a dolichocephaly, ptosis and limb abnormalities. This syndrome was usually associated with brain and heart congenital malformations may also be present in this deletion (Praphanphoj et al. 2000; De Vries BB et al. 2000; Bonaglia et al. 2001; Phelan et al. 2001 ; Anderlid et al. 2002b; Wilson et al. 2003; Manning et al. 2004; Koolen et al. 2005; Lindquist et al. 2005; Bonaglia et al. 2006; Cusmano-Ozog et al. 2007; Durand et al. 2007; Philippe et al. 2008; Wilson et al. 2008; Delahaye et al. 2009 ; Sykes et al. 2009; Chen et al. 2010; Dhar et al. 2010; Phelan and Betancur 2011Knight et al. 2000) (Table 2). The patient (P9) in our series showed major signs described above, including hypotonia, autism and the lack of language. Several teams have worked on the characterization at the molecular level the breakpoints of subtelomeric 22q13.3 microdeletion by molecular cytogenetic analysis (Anderlid et al. 2002b; Dhar et al. 2010; Phillipe et al. 2008). Anderlid et al. (2002b) reported the critical area in 22q13.3 to be 100 Kb containing three known genes *SHANK3*, *ACR* and *RABL2B* (Anderlid et al. 2002b). *SHANK3* is a candidate gene expressed in the cerebral cortex and cerebellum, encoding a scaffolding protein involved in the postsynaptic density of excitatory synapses, deletion of these genes is reported in individuals with autism (Marshall et al. 2008; Sykes et al. 2009). So, the 22q ter deletion canould explain the neurological status of our patient.

3p- Syndrome: del (3) (p25-p26)

The phenotype of the 3p- syndrome is characterized by prenatal and postnatal growth retardation, microcephaly, profound intellectual disability, hypotonia, facial dysmorphism with low hairline – back, hypertelorism, ptosis, short and small nose, broad nasal bridge, long philtrum or short philtrum, ear abnormality, short neckl and

imb abnormalities and congenital malformations (Mowrey et al. 1993; Phipps et al. 1994; Cargile et al. 2002; Dijkhuizen et al. 2006; Verloes et al. 2006; Malmgren et al. 2007; Fernandez et al. 2008; Shuib et al. 2008; Pohjola et al. 2010 Mowrey et al. 1993; Phipps et al. 1994; Cargile et al. 2002; Dijkhuizen et al. 2006; Verloes et al. 2006; Malmgren et al. 2007; Fernandez et al. 2008; Mowrey et al. 1993; Phipps et al. 1994; Cargile et al. 2002; Dijkhuizen et al. 2006; Verloes et al. 2006; Malmgren et al. 2007; Fernandez et al. 2008; Shuib et al. 2008; Pohjola et al. 2010 ; Shuib et al. 2008; Pohjola et al. 2010Phillipe et al. 2008) (Table 2). The majority of reported deletions are de novo and some are inherited in an unbalanced translocation of one parent, as the case of our two patients, a girl (P3) with a deletion 3pter associated with a duplication 2qter inherited by a maternal balanced reciprocal translocation (46, XX, t (3; 2) (p26; q37.2) mat) and a boy (P4) with an inherited chromosome 3 derivative of a maternal balanced reciprocal translocation (46, X, t (X, 3) (q27.3; p26.3). The size of a terminal 3p deletion and the association to another partial trisomy greatly influences the phenotype of the patient, as the case of our two patients, the girl presented a phenotype of 3p- syndrome but for the boy hiser phenotype is similar to the functional disomy Xq27qter. Among the genes in chromosome 3pter, *CALL*, *CNTN6*, *CNTN4*, *LRRN1* and *CRBN* are particularly interesting, they are 3p neurodevelopmental genes (Shuib et al. 2009). While duplications involving the *MECP2* gene in Xq28 wasere the most frequent reported microduplications associated with intellectual disability and seizure, suggesting that the *MECP2* gene is the most important dosage-sensitive gene responsible for the abnormal phenotype in functional Xq disomy syndrome (Breman et al. 2011).

9q34 Terminal Deletion

Microscopically visible distal 9q deletions are associated with intra uterine growth retardation (IUGR), macrocephaly, generalised obesity , brachycephaly, high forehead, hypertelorism, flat nasal bridge, pointed chin, prognathism, epicanthic folds, short/small nose, short philtrum, low set ears, developmental delay, language defects, hypotonia, brain abnormality, cardiomopathie, structural heart defects, visceral anomalies and genital anomalies with craniofacial dysmorphisms, hypotonia, obesity, microcephaly and speech delay (Cormier-Daine et al. 2003; Steward et al. 2004; Harada et al. 2004; Stewart et al. 2004; Yatsenko et al. 2004; Kleefstra et al. 2005; Kleefstra et al. 2006 a; Kleefstra et al. 2006 bStewart et al. 2004) (Table 2).Yatsenko et al. (2004) reported ten patients with 3 to 0.8 Mb of terminal deletion 9q34. This area contains 9 genes and 2 are known to be expressed in human brain. Among them, *EHMT1* and *CACNA1B* might be candidate genes (Yatsenko et al. 2004). Kleefstra et al. (2006 a) refined the critical area to 100 Kb in 9q34, which contains one gene, *EHMT1*. The deletion and mutation in this gene caused the same phenotype of 9q34 (Kleefstra et al. 2006 b). In our study, we found one deletion in 9q34 in Patient (P6), a 7-year-old boy, presented with severe ID, brachycephaly, flat nasal bridge, epicanthic folds, long philtrum and low set-ears, ambiguous genitalia.

6q Terminal Deletion

A deletion in 6p25.2 was detected in P (5). This aberration is reported to be present with intellectual disabilityID, neurosensory deafness, congenital heart disease, prenatal and postnatal growth retardation, ophthalmologic defects, and kidney anomalies and dismorphism with sloping/broad forehead, hypertelorism, down-slanting palpebral fissures, midface, hypoplasia, micrognathia and abnormal ears (Mirzayans et al. 2000; Baruch and Erickson 2001 ; Saleem et al. 2001 ; Grosso et al. 2002; Maclean et al. 2005; Caluseriu et al. 2006; Aldinger et al. 2009; Tumer and Bach-Holm 2009Maclean et al. 2005) (Table 2). In this region, *FOXC1* and *FKHL7* might be the candidate genes because they it areis involved in early kidney, eyes, heart and cerebral developments and may play a role in the phenotype of patients with 6p25.2 deletion (Mirzayans et al. 2000; Aldinger et al. 2009; Tumer and Bach-Holm 2009).

2q37 Terminal Deletion

Submicroscopic subtelomeric 2qter deletion was reported by many authors (Wilson et al. 1995; Bonaglia et al. 2000 ; Syrrou et al. 2002; Casas et al. 2004; Roberts et al. 2004; Kitsiou-Tzeli et al. 2007; Jones et al. 2011), it is usually

associated macrocephaly/microcephaly, IUGR, brachycephaly, frontal bossing, deep set eyes, pointed chin, micrognathia, short/small nose, broad/bulbous nasal bridge, anteverted nostrils, long, philtrum, low set ears, short neck, limb abnormalities, hair anomalies, developmental delay, speech delay/defect, hypotonia, cerebral malformations, behaviour disorder, hyperactivity, psychosis, with mild intellectual disability, short stature, round face, brachymesophalangism and epilepsy and congenital heart defect (Table 2)(Wilson at al. 1995; Ghaffari et al. 1998). Also, the 2qter deletion was reported in several patients having a phenotypically normal parent with a similar deletion suggestive of a familial polymorphism in the database of genomic variations (http://projects.tcag.ca/variation/) (Clarksonet al. 2002). We identified one patient (P2) with a 2q37.3 deletion de *novo*. So, it can be causative of the phenotype. The terminal region of the long arm of chromosome 2 contains many genes. *HDAC4* might be a candidate gene, it is a histone deacetylase that regulates genes important in bone, muscle, neurological and cardiac development and the its haploinsufficiency results in brachydactyly ID syndrome (Williams at al. 2010).

10q Terminal Deletion and Duplication

We found both deletion (P7) and duplication (P8) in the terminal region of 10q. Terminal deletions and duplications of this region are rare. the common clinical features shared by patients with 10qter deletions include short postnatal stature, low hairline back, microcephaly, flat occiput, hypertelorism, deep set eyes, beaked nose, thin lips, low set ears, helix absent or abnormal, short neck, developmental delay, autism, hypotonia, brain abnormality, structural heart defects and ambiguous genitalia ID, prenatal and postnatal growth retardation, microcephaly, genital anomalies in males associated to cardiac and renal anomalies (Irving et al. 2003; Kehrer-Sawatzki et al. 2005; Tanabe et al. 2006; Miller et al. 2009; Yatsenko et al. 2009keher-sawatzki et al. 2005) (Table 2). The distal trisomy 10qter wasis associated with mild to severe ID, short postnatal stature, microcephaly, low hairline front , abnormal face, ptosis, flat nasal bridge, long philtrum, high arched palate, low set ears, limb abnormalities, hypotonia, brain abnormality cardiovascular problems and bones malformations growth retardation, hypotonia, round flat face, short nose, low-set ears, short neck and kyphoscoliosis (Petek et al. 2001; Aglan et al. 2002; Migliori et al. 2002 ; Carer et al. 2010 ; van Bon et al. 2010Migliori et al. 2002). Our patients shared the clinical features described above. Partial deletion or duplication of the 10q subtelomere are most likely a common polymorphism, much like the common polymorphism previously described for the 2q telomere region (Wong et al. 2005). However, parental analysis is recommended to establish genotype-phenotype correlations in abnormalities of 10qter, in this study the rearrangements are de novo and the commercial assay “ToTelVysion” was used to avoid detection of this polymorphism.

In our study the most frequent signs in fifty selected patients are the profound intellectual disability (100%), dysmorphism (100%), congenital malformations (35%), growth retardation small size (28%) and hypotonia (25%). Microcephaly that was extremely frequent in other series wasis in contrary increased in our patients with a frequency of 2% (De Vries et al. 2001).

The importance of cryptic subtelomeric chromosomal anomalies as a cause of idiopathic intellectual disability is now well recognized with higher occurrence in moderate to severe intellectual disability. Many factors likely influence the incidence of positive findings such as the technique of chromosome preparation and banding, the inclusion criteria and the methodology of recruitment. The genetic counseling for families with a segregation of cryptic translocation wasis needed to better reveal the cryptic subtelomeric abnormalities. Fortunately, a rapid prenatal FISH test could be offered to the parents and their foetus.

CONCLUSION AND RECOMMENDATIONS

This study reports the detection of submicroscopic subtelomeric aberrations in Tunisian patients with intellectual disability for the first time. Subtelomeric rearrangements were found in 18%. Although benign subtelomeric variations exist (Clarksonet al. 2002; Wong et al. 2005), most de novo subtelomeric aberrations are considered pathogenic. Further observations of a large number of patients with similar ab-

normalities may lead to the recognition of specific phenotypes , and will be helpful in the clinical etiologic diagnosis of ID. Moreover fine mapping of aberrations in gene-enriched subtelomeric regions will provides essential tools for localizing and identifying new candidate genes associated with ID.

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