Clinical and cytogenetic analysis of terminal 22q13.3 deletion in two patients with ring chromosome 22

Samira Ismail[b,e], Alaa K. Kamel[a,e], Engy A. Ashaat[b,e], Amal M. Mohamed[a,e], Maha S. Zaki[b], Eman H. A. Aboul-Ezz[d,e], Saida A. Hammad[a,e], Inas S. M. Sayed[c,e], Mona O. El Ruby[b,e]

Departments of [a]Human Cytogenetics, [b]Clinical Genetics, [c]Oro-Dental Genetics, Human Genetics and Genome Research Division, [d]Basic Dental Sciences Department, Oral and Dental Research Division, [e]Center of Excellence For Human Genetics, National Research Centre, Giza, Egypt

Correspondence to Engy A. Ashaat, PhD, Clinical Genetics Department, Center of Excellence for Human Genetics, National, Research Centre (NRC), P.O. Box: 12622 Cairo, Egypt
Tel: +20 100 148 4157; Fax: (+202) 33370931
e-mail: nogy80@hotmail.com

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Background
The 22q13.3 deletion syndrome is a rare autosomal aberration with a wide phenotypic spectrum. Overall, 20% of the cases result from ring chromosome 22 [r(22)] or unbalanced translocation disrupting the 22q13 region. Up to 2013, only 60 cases with r(22) have been reported.

Patients and methods
Two unrelated Egyptian female patients of consanguineous parents presented with profound developmental delay, absent speech, microcephaly, seizures, and autistic behavior and were subjected to comprehensive clinical and orodental examination and imaging studies. Conventional cytogenetic analysis was done for the patients and their parents, and fluorescence in-situ hybridization analysis was performed for both patients.

Results
Karyotyping showed a r(22) abnormality in the two patients and normal chromosomes in the parents. Fluorescence in-situ hybridization revealed deletion of 22q13.3 and 22q subtelomere in both patients.

Conclusion
Our patients add to the previously reported rare cases of r(22). 22q13.3 terminal deletion should be considered in cases of intellectual disability and delayed speech associated with seizures and autistic behavior, even in consanguineous mating.

Keywords:
global developmental delay, microcephaly, orodental abnormalities, ring chromosome 22

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Introduction

The 22q13.3 deletion syndrome, also known as Phelan–McDermid syndrome, is due to simple terminal deletion of the long arm of chromosome 22 in 80% of cases (Maitz *et al.*, 2008). The remaining 20% result from ring chromosome 22 r(22) (Phelan *et al.*, 2001; Jeffries *et al.*, 2005) or unbalanced translocation disrupting the 22q13 region (Jamsheer *et al.*, 2008; Toruner *et al.*, 2009). The 22q13.3 deletion syndrome is a developmental disorder with variable features, including neonatal hypotonia, developmental delay, severely delayed speech, accelerated growth, autistic behavior, and minor dysmorphic features (Durand *et al.*, 2007). The deleted size varies from 100 kb to 95 Mb (Anderlid *et al.*, 2002; Wilson *et al.*, 2003). *SHANK3*, *ACR*, and *RABL2B* genes are located at the smallest deletion region, and the largest deletions may include more than 90 genes (Bonaglia *et al.*, 2006; Dhar *et al.*, 2010).

r(22), was first described by Weleber *et al.* (1968), and subsequently, ~60 cases were reported (Hannachi *et al.*, 2013). Ring chromosome results from breakage and fusion of both chromosome arms to form a ring with loss of terminal short and long arm sequences. Ring 22 is usually a de novo abnormality occurring very early in the development of the embryo with minimal recurrence risk. Rarely, it may be inherited from a parent (Jeffries *et al.*, 2005; Jobanputra *et al.*, 2009; Hannachi *et al.*, 2013; Laura *et al.*, 2018).

Based on observed cases, r(22) has appeared to affect females more frequently than males. The phenotypic abnormalities of r(22) syndrome are variable but are usually more severe than simple terminal 22q13.3 deletions (De Mas *et al.*, 2002; Lam *et al.*, 2006; Dhar *et al.*, 2010).

The consistent clinical manifestations in patients with r(22) are severe speech disability, growth retardation, microcephaly, and hypotonia. Some dysmorphic manifestations have been reported such as epicanthus,

large and dysplastic ears, long eyelashes, full eyebrows, and hypertelorism. Syndactyly of second and third toes, unsteady gait, hyperactivity, aggressive behavior, autistic behavior, seizures, or abnormal EEG have been also reported (Mahajan *et al.*, 2012).

Herein, we report on two unrelated female patients with r(22) exhibiting profound psychomotor delay and intractable seizures.

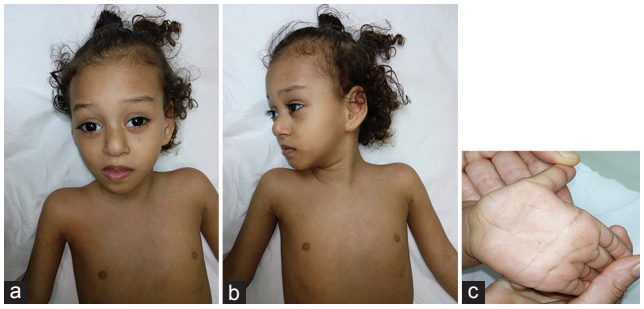
Clinical reports

Patient 1

A 2-year-old female child was referred to the multiple congenital anomalies clinic of Center of Excellence for Human Genetics, the National Research Centre, complaining of respiratory distress, cyanosis, convulsions, and delayed motor and mental milestones. The pregnancy history was uneventful. She had a low birth weight (1.750 kg), and cyanosis was noted at birth. Pedigree analysis showed remote consanguinity of parents with no history of similarly affected family members. On clinical examination, she had minor dysmorphic features in the form of long face, long eyelashes, blue sclera, left epicanthic fold, asymmetry of ears, and short neck (Fig. 1a–c). Moreover, there were bilateral hypoplastic thenar and hypothenar muscles of the hands. Anthropometric measurements showed normal weight and length. However, head circumference was 45 cm (–2.2 SD).

Follow-up re-examination was done at the age of 3.5 years; the child had constipation and sleep disorder, in addition to developing intractable seizures. Anthropometric reevaluation showed microcephaly (head circumference: 46 cm; –2.9 SD). Height and weight were within normal range. On neurological examination, the patient was hypotonic with hyporeflexia. Evaluation of psychomotor development using Arabic version of Portage program showed that her developmental age was 1.6 months, whereas developmental quotient was 4%. Autistic behavior was noted in the form of hand flapping and lacking eye contact and social skills. Childhood Autism Rating Scale was applied and showed severe autism. Cardiac evaluation was performed by echocardiography and revealed trivial aortic regurgitation and mitral regurgitation with mild mitral valve prolapsed. EEG was normal. MRI on the brain showed cortical brain atrophy, bilateral deep sylvian fissures, dilatation of the lateral ventricles, and thin corpus callosum. Orodental examination showed malar hypoplasia, long philtrum, long uvula, prominent median palatine raphe and attrition, thick everted lower lip, prognathism, malocclusion, and macroglossia.

Figure 1

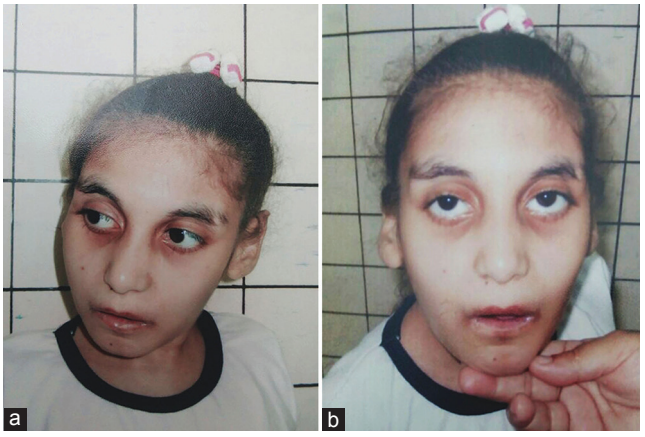


(a–c) Patient 1 showing some dysmorphic features (long face, long eyelashes, thin straight eyebrows, blue sclera, left epicanthic fold, large asymmetric ears, bulbous nose and short neck, and bilateral hypoplastic thenar and hypothenar muscles of hands). Note also, widely spaced nipples.

Patient 2

A 16-year-old female patient was referred to us complaining of convulsions and renal problems. The pregnancy history was uneventful. Pedigree analysis showed first-degree consanguinity of the parents with no history of similarly affected family members. On clinical examination, she had dysmorphic features in the form of long face, long eyelashes, large ears, and short neck (Fig. 2a and b). Anthropometric measurements showed low weight (19.5 kg; –3.7 SD), marked short stature (110; –8.7 SD), and microcephaly (head circumference was 49 cm; –4.6 SD). The patient had profound intellectual disability, with no development of speech or walking skills; the neurological examination revealed hypotonia. Autistic repetitive behavior was not evident on clinical evaluation; however, she had a history of repetitive behavior in early childhood. Social adaptation was evaluated using Vineland Social Maturity Scale showing a profound delay.

Figure 2



(a, b) Patient 1 showing some dysmorphic features (long face, long eyelashes, large asymmetric ears, thick lateral side of eyebrows, bulbous long nose, and short neck).

Orodental examination showed malar hypoplasia, long philtrum, thin upper lip, malocclusion, high arched palate, thick alveolar ridges, fissured lip, macroglossia, and prognathism.

The patient was subjected to EEG and revealed moderate generalized cerebral dysfunction. Computed tomography of the brain showed cortical brain atrophy, and echocardiography showed mitral regurgitation. Pelvic ultrasonography showed multiple cysts in the left kidney, and bilateral hydronephrosis.

Patients and methods

Cytogenetic analysis

Conventional cytogenetic analysis was performed for both patients and their parents. Metaphase chromosome preparations were obtained from PHA-stimulated lymphocyte cultures according to standard procedures, and stained using GTG banding technique at a 500-band level. Fifty metaphases were analyzed for each patient to detect possible mosaicism. Karyotype description followed the ISCN guidelines (ISCN, 2016).

Fluorescence in-situ hybridization

Fluorescence in-situ hybridization (FISH) was performed on metaphase spreads prepared from lymphocyte cultures using standard procedures following the manufacturer's instructions. Probes used were LSI TUPLE1 (HIRA) (22q11) spectrum orange SO/LSI ARSA (22q13.3) spectrum green SG and TelVysion 22q Spectrum Orange SO, specific for 22q subtelomere (Vysis Abbott Molecular Inc., Des Plaines, IL., USA).

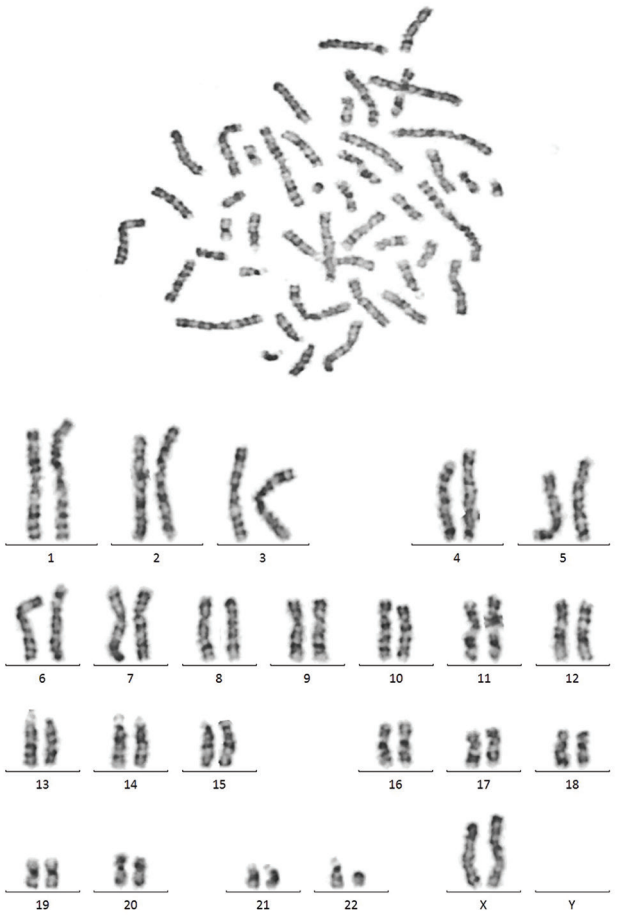
Informed written consents were obtained from parents of the patients, and the study was approved by the Ethics Committee at the National Research Center.

Results

Detailed chromosomal analysis of the metaphase spreads of both patients showed a nonmosaic r(22): 46, XX, r(22) (Figs. 3 and 4). Karyotypes of the parents of both patients were normal.

FISH analysis revealed a deletion of both 22q13.3 locus and 22q subtelomere in the ring chromosome in the two patients with normal of 22q11 locus (Figures 5 and 6). The karyotype was thus re-designated as 46, XX, r(22). ish r(22) (TUPLE1+, ARSA−, 22qter−).

Figure 3



A metaphase spread and karyotype of patient 1 showing 46, XX, r(22). r(22), ring chromosome 22.

Discussion

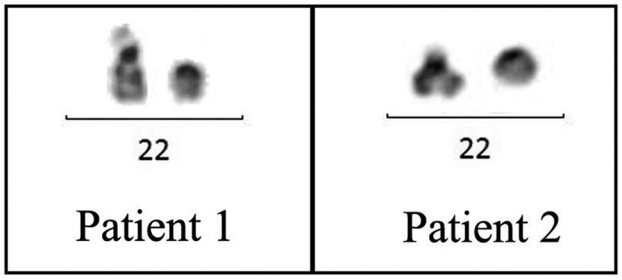
Our two patients with a de novo r(22) presented with severe psychomotor retardation and intractable seizures. FISH analysis revealed a terminal deletion disrupting the 22q13.3 region with no detected mosaicism in blood cells in both cases.

Patient 1 exhibited normal weight and height, but microcephaly was evident at the age of 3.5 years, whereas patient 2 exhibited global growth retardation. Both patients also shared mild dysmorphic features, orodental anomalies, hypotonia, absent speech, autistic behavior, structural brain abnormalities, and mitral valve regurgitation.

Phelan *et al.* (2001) compared the clinical data of 24 published patients with 22q13.3 deletion syndrome with 37 new patients. They found that all of them showed marked global mental and motor developmental delay, absent speech, and severe hypotonia, which are consistent manifestations in 22q13.3 deletion syndrome.

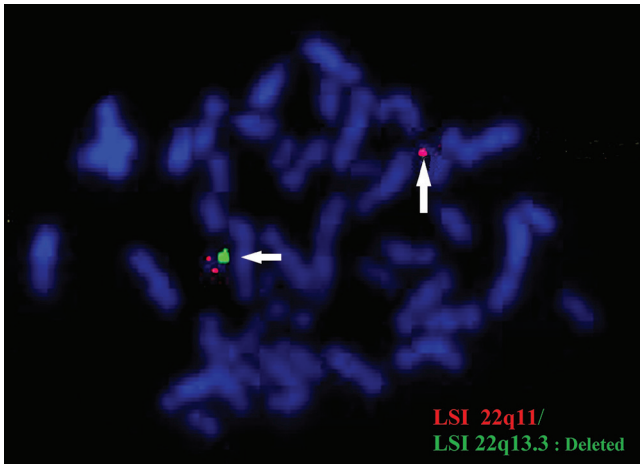
A review of 17 patients with r(22) revealed phenotypic similarities with cases of simple 22q13

Figure 4



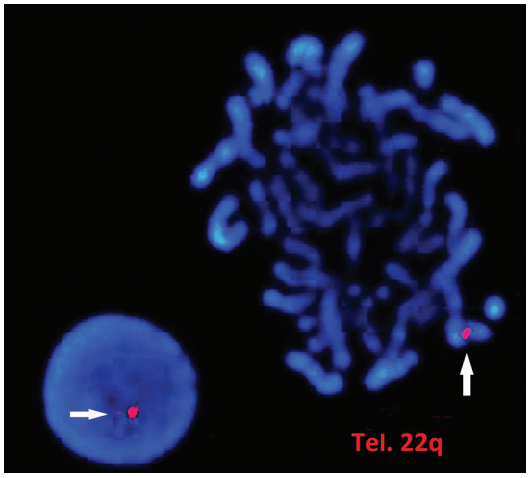
A G-banded partial karyotype of r(22) in patient 1 and 2. r(22), ring chromosome 22.

Figure 5



FISH on a metaphase spread using LSI 22q11(SO)/LSI 22q13.3(SG) showing two signals for 22q11 and a deleted 22q13.3 locus in the r(22) of patient 1. FISH, fluorescence in-situ hybridization. r(22), ring chromosome 22.

Figure 6



FISH using subtelomere 22q specific probe (SO) showing deletion of 22qter of the ring in patient 2. FISH, fluorescence in-situ hybridization.

deletion syndrome of same ages and deletion size (Luciani *et al.*, 2003). However, patients with r(22) usually have growth failure instead of speeding up or normal growth as in 22q13.3 deletion syndrome (Hunter *et al.*, 1977; Jeffries *et al.*, 2005; Dhar *et al.*, 2010). This could be owing to a larger deletion size in r(22) cases (Dhar *et al.*, 2010). Moreover, Luciani *et al.* (2003) supposed that the increased rate of cell death in r(22) patients caused by difficulties in sister chromatid separation at cell division is responsible for growth failure.

A study conducted by Jeffries *et al.* (2005) included 35 patients with r(22) and had shown that the amount of material lost varied between 0.15 and 21% of the total chromosome length (67 kb to 10.2 Mb); all patients showed deletion of *SHANK3* gene. This gene is a member of a gene family that codes for Shank proteins of the postsynaptic densities of excitatory synapses. They act as a scaffold that supports the connections between neurons and are involved in the synapse formation and maturation of dendritic spines. *SHANK3* is hypothesized to be a major causative gene of the neurological symptoms of 22q13 deletion syndrome (Wilson *et al.*, 2003). A recurrent breakpoint within the *SHANK3* gene was identified in 22q13.3 deletion suggesting a deletion hotspot (Anderlid *et al.*, 2002; Bonaglia *et al.*, 2006; Dhar *et al.*, 2010). However, Wilson *et al.* (2008) reported two patients with interstitial deletion of the 22q13 region and intact *SHANK3* suggesting that haploinsufficiency for other genes on 22q13 could have a major role on cognitive and language development. Genes proximal to *SHANK3* gene, including *PLXNB2*, *PANX2*, *ALG12*, and *MLC1*, were implicated in the neurological abnormalities, either directly or by regulating *SHANK3* gene expression (Hannachi *et al.*, 2013).

Our two patients shared intractable seizures, associated with structural brain anomalies in the form of cortical brain atrophy in both patients, in addition to bilateral deep sylvian fissures, asymmetric dilatation of lateral ventricles, and thin corpus callosum in patient 1.

Seizures or abnormal EEG have been reported in r(22) deletion (De Mas *et al.*, 2002) and in 22q13.3 deletion in ~40% of cases (Soorya *et al.*, 2013; Dhar *et al.*, 2010). In addition, structural brain abnormalities have been reported in up to 75% of patients with simple 22q13.3 deletion syndrome in the form of nonspecific white matter changes, hypoplasia of the corpus callosum, ventricular dilatation, and arachnoid cysts (Nesslinger *et al.*, 1994; Manning *et al.*, 2004; Dhar *et al.*, 2010; Soorya *et al.*, 2013).

Bruxism has been also described in 22q13.3 deletion syndrome, which was manifested in patient 1 as attrition (Cusmano-Ozog *et al.*, 2007).

The present patients also shared early repetitive stereotyped movements and absent eye contact and social skills in addition to severe delayed speech, all are among diagnostic criteria of autism spectrum disorders (ASDs).

The association between autism and 22q13.3 deletion syndrome was first reported in a 14-year-old girl by Goizet *et al.*) 2000). Accumulating data support the potential implication of *SHANK3* gene deletions or mutations in the development of ASD-type behaviors (Moessner *et al.*, 2007).

In their study on an ascertained sample of 32 patients with *SHANK3* deficiency, Soorya *et al.* (2013) emphasized the effect of *SHANK3* gene deletion or mutation on the severity of intellectual, motor, and speech impairments and in ASD and highlighted the importance of ASD in 22q13.3 deletion patients.

Owing to the high prevalence of ASD in 22q13.3 deletions, further investigation of the role of *SHANK3* gene and its potential overlap with other known genetic causes in ASD should be done (Pfaender *et al.*, 2017; Richards *et al.*, 2017). Kolevzon *et al.* (2014) suggested that all individuals diagnosed with 22q13.3 deletions should undergo ASD assessment.

Renal anomalies were reported in ~25% of 22q13.3 deletion patients, including cystic kidneys, dysplastic kidney, renal stones, and ureteric reflux. Patient 2 presented with renal problems and showed multiple cysts of the left kidney with bilateral hydronephrosis. It is recommended for all 22q13.3 children to perform a renal ultrasound for early diagnosis of renal abnormalities before development of symptoms (Phelan and McDermid, 2012).

Congenital heart defects were reported in association with r(22) and in more than 25% of 22q13.3 deletion patients, most commonly in the form of atrial septal defect, tricuspid valve regurgitation, patent ductus arteriosus, and pulmonary return (Nesslinger *et al.*, 1994; Manning *et al.*, 2004; Dhar *et al.*, 2010; Soorya *et al.*, 2013). Both present patients had congenital heart defects mainly affecting the mitral valve, being more severe in patient 1.

Both present patients exhibited similar dysmorphic features in the form of long face, long eyelashes, bulbous long nose, long philtrum, thick lateral side of eyebrows, large asymmetric ears, short neck, and orodental abnormalities. Patient 2 also showed a high arched palate, which was previously reported (Funderburk *et al.*, 1979; Kolevzon *et al.*, 2014).

The most common oral and facial abnormalities reported in 22q13.3 deletion syndrome include epicanthal folds, periorbital fullness, long eyelashes, bulbous nose, long face, pointed chin, ear anomalies, large fleshy hands, hypoplastic or dysplastic nails, and high arched palate. Other less common manifestations such as malocclusion or widely spaced teeth, micrognathia, malar hypoplasia, flat midface, and occasionally cleft palate and bruxism were also reported (Barajas-Barajas *et al.*, 2004; Cusmano-Ozog *et al.*, 2007; Kolevzon *et al.*, 2014).

Our patients also developed gastrointestinal problems in the form of gastro-oesophageal reflux, constipation, and vomiting, which were reported among the gastrointestinal symptomatology of 22q13.3 deletion syndrome (Phelan and McDermid, 2012; Soorya *et al.*, 2013).

The phenotypic heterogeneity of our patients and other reported r(22) patients could be attributed to several factors including the mitotic instability of the ring chromosome with varying levels of mosaicism which may have different tissue distribution. The size of terminal genetic material with loss or presence of the minimal critical region involving *SHANK3* gene and/or implication of other genes proximal to it and the unmasking of recessive alleles on the normal chromosome 22 by the terminal deletion, all have their role on the phenotypic expression (Hannachi *et al.*, 2013; Soorya *et al.*, 2013; Sarasua *et al.*, 2014).

Even though, positive consanguinity, developmental delay, microcephaly, and intractable seizures favored the initial suspicion of an autosomal recessive disorder rather than a chromosomal aberration, the diagnosis of simple 22q13.3 deletions or r(22) must be considered when intellectual disability is associated with delayed speech, seizures, hypotonia, and ASD.

Our report adds two patients with r(22) exhibiting severe manifestations of 22q13.3 deletion syndrome to the rare cases previously reported.

The recent introduction of high throughput genome analysis technologies such as array CGH and next generation sequencing will allow for more precise detection of 22q13 deletion size, *SHANK3* gene deletion or mutations, and involvement of other genes which will help more accurate genotype/phenotype correlation in cases of ASD, intellectual disability, and intractable seizures.

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Conflicts of interest
There are no conflicts of interest.

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