A diagnostic clinical genetic study of craniofacial dysmorphism

H.M. Farag,[1] S.M. Kotb,[1] G.A. Sweify,[2] R.K. Fawzy[3] and S.R. Ismail[1]

دراسة تشخيصية سريرية وراثية لتشوهات الوجه والقحف (الجمجمة)

حنان محمد الهادي فرج وسامية مرسي قطب وجمال السويفي وراوية خليل فوزي وسوزان رشدي إسماعيل

خلاصة: أجري تقييم تشخيصي لتشوهات الوجه والقحف (الجمجمة) إما وحدها أو كجزء من متلازمة وراثية في خمسة وعشرين مريضاً (ثمان من الإناث وسبعة عشر من الذكور)، تراوحت أعمارهم من شهرين إلى 47 سنة. وقد أجري لهم فحص جيني كامل، ودراسة شجرة النسب، فضلاً عن تسجيل القياسات البشرية/الانثروبومترية والفحوص الشعاعية. وقد تضمنت الدراسات الخلوية التهجين الموضعي الوامض عندما لزم ذلك. ولقد تبيَّن إجمالاً أن خمسة عشر من المرضى كانت لديهم شذوذات صبغية. فكانت لدى خمسة مرضى تركيبات صبغية غير متوازنة بينما وجدت واسمات صبغية لدى ستة منهم. وكان ثلاثة من المرضى إيجابيين لاختبار التهجين الموضعي الوامض، لإصابتهم بمتلازمة وليام. وكان مريض واحد إيجابياً لمتلازمة برادر - ويللي. وكانت هناك اضطرابات وحيدة المنشأ في عشرة من المرضى. وتم تشخيص خمسة بأن لديهم متلازمات التحام التدارز الباكر. ويستنتج الباحثون أن دراسة الملامح البسيطة تفيد في تشخيص التشوهات الوراثية.

ABSTRACT A diagnostic evaluation of craniofacial anomalies, either isolated or as part of a genetic syndrome was conducted on 25 patients (8 females, 17 males), age range 2 months to 47 years. Complete genetic examination, pedigree analysis, anthropometric measurements and radiological studies were carried out. Cytogenetic studies included fluorescence in situ hybridization (FISH) when indicated. In all, 15 patients had chromosomal abnormalities. Five patients had unbalanced chromosome rearrangements and six had chromosome markers. Three patients were FISH-positive for William syndrome and one was positive for Prader–Willi syndrome. Ten patients had monogenic disorders. Five were diagnosed as craniosynostosis syndromes. We conclude that minor features are useful for making a diagnosis of congenital anomalies.

Etude génétique, clinique et diagnostique de la dysmorphie craniofaciale

RESUME Une évaluation diagnostique des anomalies craniofaciales, isolées ou associées à un syndrome génétique, a été effectuée chez 25 patients (8 femmes, 17 hommes) dont l'âge était compris entre 0,17 et 47 ans. On a procédé à un examen génétique complet, une analyse de l'arbre généalogique, des mesures anthropométriques et des études radiologiques. Les études cytogénétiques comprenaient l'hybridation *in situ* par la fluorescence (FISH) lorsque celle-ci était indiquée. Sur toutes les études, 15 patients avaient des anomalies chromosomiques. Cinq patients présentaient des déséquilibres dans les réarrangements chromosomiques et six avaient des marqueurs chromosomiques. Trois patients avaient une hybridation (FISH) positive pour le syndrome de Williams-Beuren et un était positif pour le syndrome de Prader-Labhart-Willi. Dix patients avaient des maladies monogéniques. Cinq ont été diagnostiquées comme craniostéroses associées à un syndrome. Nous concluons que des éléments mineurs sont utiles dans le diagnostic des anomalies congénitales.

[1]Human Genetics Department; [3]Radiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt.
[2]Maxillofacial Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.
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Introduction

Approximately 3% of all infants are born with a congenital anomaly, including anomalies of the craniofacial structures [*1*]. The latter may be isolated, or be part of a malformation syndrome indicating a more generalized alteration of embryonic development. Many malformation syndromes have a characteristic facial appearance that may be diagnostic, or an important clue to diagnosis, in a large number of cases. Minor anomalies of the craniofacial structures also serve as diagnostic aids for many malformation syndromes because of their common occurrence [*2*]. A minor anomaly or combination of anomalies may indicate isolated abnormal morphogenesis, a familial trait, or be a manifestation of a major systemic disorder [*3*].

The present study aimed to make a diagnostic evaluation of craniofacial anomalies, either isolated or as part of a genetic syndrome, and to highlight the importance of using minor features, as well as very apparent ones, to obtain accurate diagnoses — a prerequisite for effective genetic counselling and prevention.

Patients and methods

The study was carried out on 25 patients (8 females, 17 males) with craniofacial anomalies. Patients were selected from the Human Genetics Clinic at the Medical Research Institute, Alexandria University, Egypt (17 patients) and, through a joint study programme, from the Genetics Clinic, University of South Florida, USA (8 patients). Ages ranged from 2 months to 47 years. Detailed genetic histories, pedigree analysis, clinical genetic examinations with emphasis on both major and minor craniofacial features, anthropometric measurements [*4*], chromosomal analysis [*5*], radiological examination, ultrasonography and brain computed tomography (CT) scan (when indicated) were carried out for all patients. Fluorescence in situ hybridization (FISH) — a recent technique that has the ability to identify specific chromosomes with labelled probes — was used in some cases [*6*]. Other investigations were performed when needed. These included ophthalmological evaluation, electrocardiogram of the heart, metabolic screening, audiometry and TORCH titres.

Results and discussion

Patients were classified into two groups, depending on the diagnostic clinical criteria in syndromic cases, and the results of cytogenetic studies in those with chromosomal abnormalities (Table 1). The cases studied were numbered 1–25. Of the 25, 15 had chromosomal abnormalities (Group I). The distribution of cases according to their chromosomal abnormalities is summarized in Table 2. Group II consisted of 10 cases of monogenic diseases.

Chromosomal abnormalities are a significant cause of birth defects and craniofacial disorders. Marker chromosomes are known to be heterogeneous with respect to size and composition [*7*]. In this study, six cases were diagnosed cytogenetically with chromosome markers. The presence of the marker was found in 35% of cells in case 1 and in 66% in case 2. It was present in all cells in cases 5 and 6. In case 3, FISH testing revealed inv,dup(15), which accounts for 40% of chromosome markers. It is identified in mentally handicapped cases who are non-syndromic with minor dysmorphic

Table 1 **Distribution of patients with craniofacial dysmorphism**

Chromosomal disorders	Group I No.	Monogenic disorders	Group II No.
Chromosome marker	6	Craniosynostosis isolated	1
Prader–Willi syndrome	1	Craniosynostosis syndromes	4
William syndrome	3	Greig cephalopolysyndactyly	1
46,XY,der(5),t(5;7)(p15q32)pat	1	Treacher Collins syndrome	1
46,XY,del(5p)	1	Holoprosencephaly	1
46,XY,7q+	1	Cleidocranial dysplasia	1
46,XY,t(q;13)(q11p11)mat	1	Mandibuloacral dysplasia	1
46,XX,rec(8;18)(q24q21)	1		
Total	15		10

Table 2 **Distribution of patients with craniofacial dysmorphism due to chromosomal defects**

Case No.	Age	Sex	Karyotype	FISH	Diagnosis
1	10 y 3 m	M	46,XY/47 XY,+mar	–	Chromosome marker
2	1 y 2 m	M	46,XY/47 XY,+mar	–	Chromosome marker
3	1 y 1 m	F	46,XX,+mar	inv dup (15)	Chromosome marker
4	2 y 11 m	M	46,XY,+mar	tetrasomy 18	Chromosome marker
5	3 y	M	46,XY,+mar	–	Chromosome marker
6	6 m	M	46,XY,+mar	–	Chromosome marker
7	5 y 4 m	M	46,XY	+ve	Prader–Willi syndrome
8	47 y	F	46,XX	+ve	William syndrome
9	5 y 10 m	F	46,XX	+ve	William syndrome
10	7 y 8 m	M	46,XY	–ve	William syndrome
11	4 y 6 m	M	46,XY,der(5), t(5;7)(p15q32) pat	+ve	Unbalanced translocation
12	10 m	M	46,XY,del(5p)	–	del 5p
13	1 y 1 m	M	46,XY,7q+	–	7q+
14	2 m	M	46,XY,der(9) t(9;13)(q11p11) mat	–	Unbalanced translocation
15	2 y	F	46,XX,rec(8;18) (q24q21)	–	Unbalanced translocation

y = years m = months M = male F = female
FISH = fluorescence in situ hybridization

features [*8*]. This agrees with the present study. The marker chromosome in case 4 was i(18p) again revealed by FISH. Patients with i(18p) demonstrate a recognizable phenotype [*9*]. Case 4 had hypotelorism and short palpebral fissures. The karyotype of the father in case 5 showed the presence of the marker in 30% of cells. Transmission of the marker from one generation to another has been observed in both sexes, and variability in clinical findings between affected family members may be found [*10*]. Some of the chromosome markers are associated with physical and mental handicap, while others seem to have no recognizable phenotypic effect [*11*]. Case 1 had trigonocephaly and up-slanting palpebral fissures, while case 2 had attention deficit, dolichocephaly and long face. Case 5 showed squint and epicanthus. Mild microcephaly and short stature were found in case 6. The risk of phenotypic abnormality is correlated with whether the marker is *de novo* or familial; the risk being higher for *de novo*.

Deletion of the paternal chromosome 15(q11q13) accounts for 70%–80% of cases of Prader-Willi syndrome (PWS). Absence of the same region in the maternally transmitted chromosome leads to Angelman syndrome. This is an example of genetic imprinting [*12*]. Case 7 had the clinical features of PWS (obesity, hypotonia, almond-shaped eyes and crypto-orchidism). FISH studies confirmed the diagnosis. Recent studies employing FISH indicate that both inherited and sporadic cases of William syndrome (WS) are caused by deletion in one elastin allele located in chromosome 7(q11 23) [*13*] This agrees with our study, as cases 8 and 9 had positive FISH for WS. Case 10 had a negative result. This does not rule out the diagnosis, however, as in 4%–9% of WS patients the deletions are not identified. The three cases had the diagnostic facial features of WS (periorbital fullness, downslanting palpebral fissure, malar hypoplasia and wide mouth). Stellate iris pattern was detected in cases 8 and 9. It is a useful diagnostic clue in infants [*14*].

Unbalanced chromosomal rearrangement 46,XY,der(5),t(5;7)(p15q23)pat, resulting in monosomy 5p15 and trisomy 7q32ter was found in case 11. These findings were confirmed by FISH study (Figure 1). The patient had multiple congenital abnormalities of trisomy 7q (frontal bossing, epicanthus and various skeletal anomalies). He presented only a few of the facial features of del(5p), despite the fact that the short arm of chromosome 5 was almost deleted. This may be due to phenotypic heterogeneity seen in del(5p) individuals [*15*]. These features were evident in case 12, who had low birth weight, round face, hypertelorism, metopic ridge and short nose.

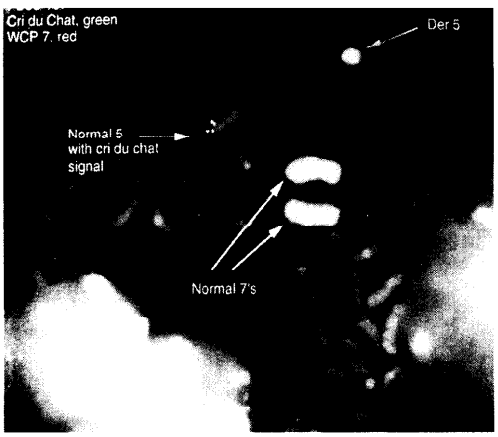

Figure 1 **Fluorescence in situ hybridization (FISH) in case 11 showing derivative 5 with a +ve signal for chromosome 7 paint probe**

This karyotype shows del(5p). This was *de novo*, as the parents' karyotypes were normal.

The karyotype of case 13 showed 46,XY,7q+. The parents refused to undergo chromosomal analysis. The child had microcephaly, up-slanting palpebral fissure, peaked nose and microretrognathia. He had another sibling with similar features. This supports the presence of a balanced translocation carrier parent.

Case 14 was diagnosed clinically as monosomy 9p. The karyotype was 46,XY,der(9),t(9;13)(p11q11)mat (Figure 2). The maternal grandfather was also a balanced translocation carrier. The patient had monosomy 9p and trisomy 13, although he did not show the features of trisomy 13. The break point in this patient was 9p11. This is different from the common sites of monosomy 9p (9p24 and 9p22) [*16*] without translocation. *De novo* translocation was found in case 15. She had coarse facial features (hypertelorism, dolichocephaly, down-slanting palpebral fissures, upturned nose, hypertrichosis and marked gum hypertrophy). The karyotype showed 46,XX,rec(8;18)(q24q21). Unbalanced translocation could be associated with different phenotypes according to the missed part.

Craniosynostosis is etiologically and pathologically heterogeneous. Premature sutural closure may occur in isolation, as in case 16 (she had metopic and coronal suture synostosis), or it can occur as part of a genetic syndrome [*13*]. Isolated craniosynostosis is sporadic with multifactorial inheritance. Case 17 had partial closure of the coronal suture and broad thumbs and big toes. He was diagnosed as Pfeiffer syndrome, designated by Temtamy [*17*] as a distinct entity. This case fits into type I Pfeiffer syndrome [*18*]. Autosomal dominant inheritance, as well as sporadic cases have been seen in type I, while sporadic cases occur only in types II and III [*13*].

Case 18 was diagnosed as Crouzon syndrome (brachycephaly, mid-face hypoplasia, down-slanting palpebral fissures and proptosis which was confirmed by skull radiographs). Evidence of paternal age effect in new mutation has been reported [*19*]. The paternal age in this patient was 37 years. The patient's older brother, case 19, had some features of Crouzon syndrome

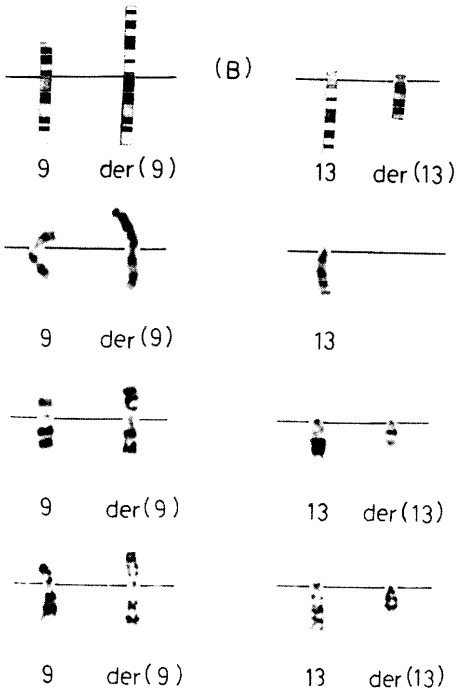

Figure 2 **Partial karyotype of case 14 showing der(9) and karyotype of patient's mother and maternal grandfather showing reciprocal translocation between chromosomes 9 and 13**

and early closure of the sagittal suture. Crouzon syndrome is inherited as an autosomal dominant disorder with variable expression [*13*]. The presence of two affected siblings in this study with phenotypically normal parents raises the possibility of a recessive form of craniosynostosis affecting the coronal suture (case 18) and sagittal suture (case 19). Case 20 was diagnosed as Carpenter syndrome, recognized as a distinct genetic entity by Temtamy [*20*]. He had preaxial polydactyly of the toes and closure of the coronal suture. Carpenter syndrome clearly has autosomal recessive inheritance. The parents of this patient were second cousins.

Case 21 was diagnosed as Greig cephalopolysyndactyly. He had turricephaly, deep-set eyes, a short broad nose, long philtrum and preaxial polydactyly. There was no hypertelorism, which is a feature of the syndrome. This agrees with Merlob et al. [*21*]. The syndrome has an autosomal dominant mode of inheritance [*13*]. Fresh mutation was suggested in this patient.

Case 22 had the characteristic facial appearance of Treacher Collins syndrome (asymmetric, small dysplastic ear, downslanting palpebral fissures, bilateral lower eyelid colobomata, mid-face hypoplasia, micrognathia and macrosomatia). This syndrome has been well documented as an autosomal dominant disorder related to the mutant gene occurring in the craniofacial complex [*22*], with high penetrance and marked variability in expression.

Premaxillary agenesis is characterized by a median pseudocleft, agenesis of the nasal bones and primary palate and ocular hypotelorism [*23*]. These features were present in case 23. The patient had a female sibling with the same condition who died shortly after birth. A CT scan showed microcephaly with small brain but normal structure, suggesting it to be a case of lobar holoprosencephaly. Recurrence of the condition in two siblings indicates autosomal recessive inheritance.

Cleidocranial dysplasia is an autosomal dominant bone dysplasia which is fully penetrant with variable expressivity [*24*]. Case 24 had typical craniofacial and skeletal features of cleidocranial dysplasia. X-ray confirmed these findings. The patient had delayed skull mineralization, absent clavicles, butterfly vertebrae, stippled femoral epiphyses and wide symphysis pubis. Germ line mosaicism or autosomal recessive inheritance are probable in case 24, as there was a history of a similarly affected female sibling. The fact that the parents were non-consanguineous does not support this assumption, but neither does it rule it out.

Case 25 demonstrated the main diagnostic criteria for mandibuloacral dysplasia (sparse light-coloured hair, pointed nose, dental malocclusion and joint stiffness). X-ray showed hypoplastic mandible, clavicles and acrodysplasia. The occurrence of this condition in siblings strongly suggests autosomal recessive inheritance [*25*]. Positive parental consanguinity in case 25 is in accordance with this type of inheritance.

Conclusion

Craniofacial dysmorphism was a frequent presenting sign and/or condition in patients attending the clinics. The distinctive facial appearance was a clue to diagnosis in many cases. In some patients minor anomalies served as diagnostic aids. Chromosomal analysis was important in every case (even with mild dysmorphism). Abnormalities were shown up by G-banding, and by FISH studies. This proved to be a rapid and efficient method.

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