7 Kleczkowska A, Fryns JP, D'Hondt F, Jaeken J, Van den Berghe H. Partial duplication 8p due to interstitial duplication: inv dup(8)(p21.1→p22). Further delineation of the phenotype from birth to adulthood. *Ann Genet* 1987;**30**:47-51.
8 Feldman GL, Weiss L, Phelan MC, Schroer RJ, Van Dyke DL. Inverted duplication of 8p: ten new patients and review of the literature. *Am J Med Genet* 1993;**47**:482-6.
9 Floridia G, Piantanida M, Minelli A, *et al*. The same molecular mechanism at the maternal meiosis I produces mono- and dicentric 8p duplications. *Am J Hum Genet* 1996;**58**:785-96.
10 Hutchinson R, Wilson M, Voullaire L. Distal 8p deletion (8p23.1→8pter): a common deletion? *J Med Genet 1992*;**29**:407-11.
11 Pettenati MJ, Rao N, Johnson C, *et al*. Molecular cytogenetic analysis of a familial 8p23.1 deletion associated with minimal dysmorphic features, seizures, and mild mental retardation. *Hum Genet* 1992;**89**:602-6.
12 Wu BL, Schneider GH, Sabatino DE, Bozovic LZ, Cao B, Korf BR. Distal 8p deletion (8)(p23.1): an easily missed chromosomal abnormality that may be associated with congenital heart defect and mental retardation. *Am J Med Genet* 1996;**62**:77-83.
13 Dhooge C, Van Roy N, Craen M, Speleman F. Direct transmission of a tandem duplication in the short arm of chromosome 8. *Clin Genet* 1994;**45**:36-9.
14 Engelen JJ, de Die-Smulders CE, Sijstermans JM, Meers LE, Albrechts JC, Hamers AJ. Familial partial trisomy 8p without dysmorphic features and only mild mental retardation. *J Med Genet* 1995;**32**:792-5.
15 Barber JC, Joyce CA, Collinson MN, *et al*. Duplication of 8p23.1: a cytogenetic anomaly with no established clinical significance. *J Med Genet* 1998;**35**:491-6.
16 Krasikov N, Lamb AN, Vetrano LA, *et al*. Benign variant 8p23.1? *Am J Hum Genet Suppl* 1993;**53**:A568.
17 Williams L, Larkins S, Roberts E, Davison EV. Two further cases of variation in band 8p23.1. Not always a benign variant? *J Med Genet* 1996; **33**(suppl 1):A3.020.
18 Vocero-Akbani A, Helms C, Wang JC, *et al*. Mapping human telomere regions with YAC and P1 clones: chromosome-specific markers for 27 telomeres including 149 STSs and 24 polymorphisms for 14 proterminal regions. *Genomics* 1996;**36**:492-506.
19 Joyce C, Collinson M, Barber J. Validation of a subtelomeric probe and its amplification in cytogenetic duplications of 8p with no detectable phenotypic effect. *J Med Genet* 1996;**33**(suppl 1):A3.014.
20 Gosden CM, Davidson C, Robertson M. Lymphocyte culture. In: Rooney DE, Czepulkowski BH, eds. *Human cytogenetics: a practical approach*. Oxford, IRL Press, 1992:31-54.

J Med Genet 1999;**36**:422–424

Cloverleaf skull anomaly and de novo trisomy 4p

EDITOR—Cloverleaf skull deformity (CS, Kleeblattschaedel, MIM 148800) is a severe form of craniosynostosis rarely associated with chromosomal aberrations.[1 2] Recently we observed a newborn male presenting with multiple congenital anomalies including a cloverleaf skull and a de novo partial 4p trisomy. He was a 12 day old male, born at 35 weeks of gestation to healthy, non-consanguineous parents. Respiratory distress was present at birth. At 12 days, his weight was 2650 g (5th centile), length 45 cm (<5th centile), and head circumference 30.5 cm (<<5th centile). On clinical evaluation, multiple congenital anomalies were observed, including cloverleaf skull, orbital hypoplasia with proptosis, hypertelorism, right iris coloboma, depressed nasal bridge, anteverted nostrils, low set ears, wide superior alveolar ridge, and pointed chin. Furthermore, inverted nipples, camptodactyly of the hands, club feet, overlapping toes, shawl scrotum, cryptorchidism, and generalised hypertonia were noted. Skeletal *x* rays showed vertebral anomalies, including hypoplasia of the 5th cervical vertebra, the presence of hemivertebrae of the lumbar spine, and eleven ribs bilaterally. Echocardiography showed a mild atrial septal defect and PDA. Cranial 3D CT scan (fig 1) showed protruding temporal bones and fusion of the coronal, lambdoidal, and temporoparietal sutures with temporoparietal bone ridges. The craniosynostosis partially spared the sagittal and metopic sutures. MRI showed asymmetrically enlarged temporal horns and a hypoplastic corpus callosum. Renal scan was normal; the testes were found by ultrasound inside the inguinal canal bilaterally. EEG was characterised by mild brain electric hypoactivity. Visual evoked potentials were delayed. At 6 months of age, the patient died of cardiac and respiratory failure.

Standard R banding of the patient's chromosomes disclosed the presence of supernumerary chromosomal bands on 2q. This segment was later identified as part of chromosome 4 by FISH, carried out according to Pinkel *et al*[3] and using a whole chromosome 4 painting library (Oncor) (fig 2, left). Prometaphase RHG banded chromosomes, prepared as described elsewhere,[4] defined the extension of the 4p trisomic region as a 4p15.1→pter segment (fig 2, right). No apparent deletion of 2q bands was observed. The patient appeared trisomic for the distal part of 4p, without any apparent deletion of the 2q region apart from the probable loss of the 2q telomere. The patient's karyotype was 46,XY,−2,+der(2)t(2;4)(q37.3;p15.1). Paternal and maternal karyotypes were normal.

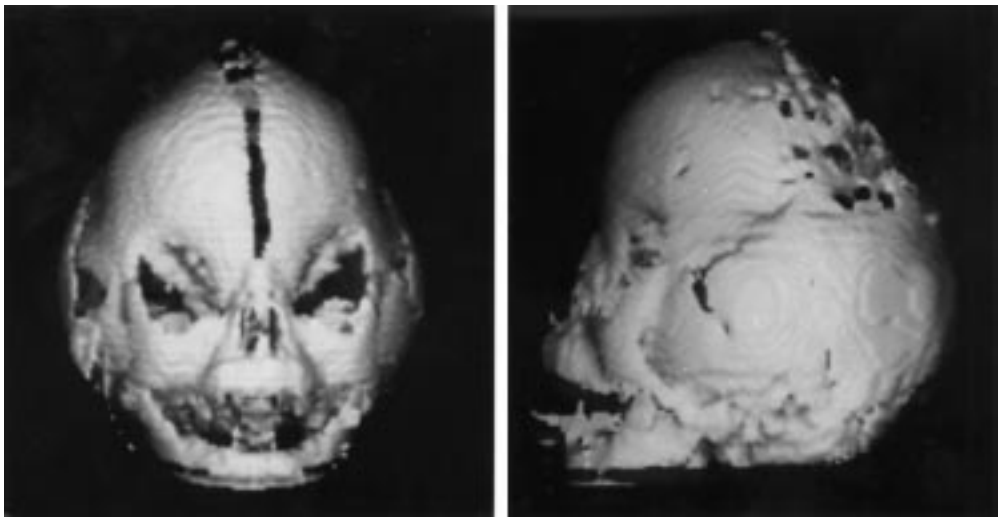

Figure 1 Cranial 3D CT scan of the patient, front and lateral view.

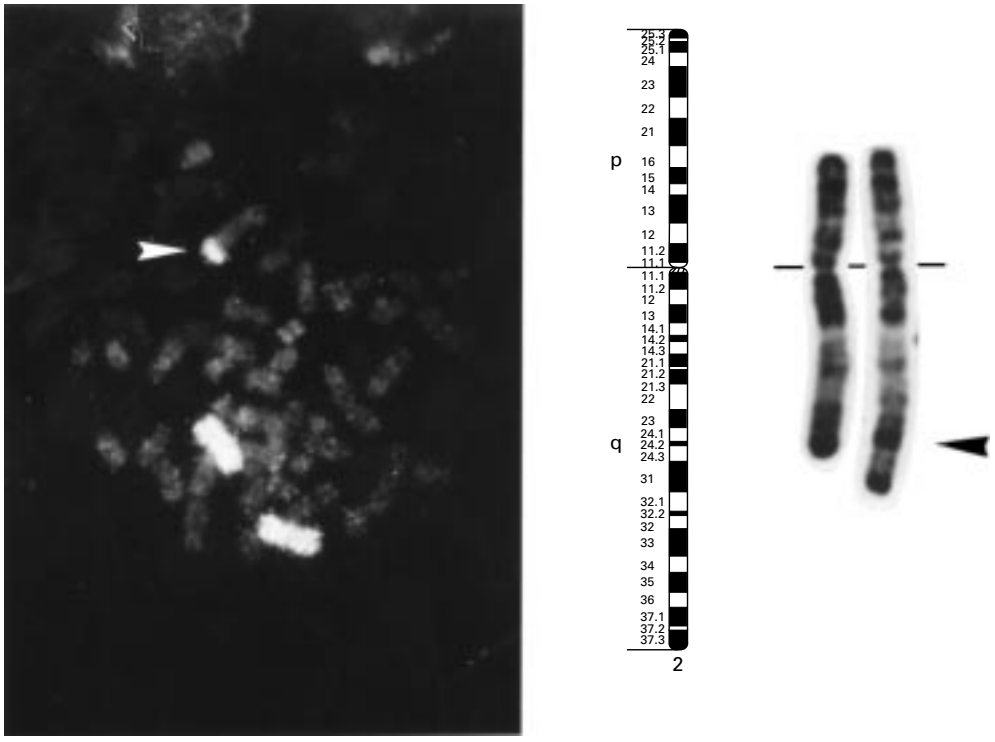

Figure 2 FISH performed in the patient using chromosome 4 painting library: arrowhead indicates part of chromosome 4 translocated onto chromosome 2 (left). RHG banded partial karyotype of the proband: chromosome translocation 2;4 (right).

Molecular analysis (data not shown) performed using PCR on DNA from a lymphoblastoid cell line of the proband and from the parents' peripheral lymphocytes showed that the patient was monosomic for a distal 2q telomeric marker, D2S125 (GDB ID:187994),[5] and heterozygous for a $(AC)_n$ repeat of the ALPP gene (PLAP, GDB ID:180439),[5] which maps to 2q37.1. On the other hand the analysis of the 4p markers disclosed the presence in the patient of three alleles for both a HOX7 $(CA)_n$ repeat (GDB ID:176982)[5] and D4S126 (GDB ID:197926),[5] respectively mapping to 4p16.1 and 4p16.3, the extra allele deriving from an error in paternal meiosis I.

Both environmental and genetic causes have been proposed for the pathogenesis of CS[1] but only three cases of CS have been found associated with cytogenetic aberrations so far. The first two cases reported CS associated with partial trisomy of 15q and 13q,[1] whereas, more recently, Van Allen *et al*[2] reported a child with multiple congenital anomalies, including CS, and an unbalanced 2q;15q translocation. This resulted in trisomy for the 15q26→qter region, and the authors hypothesised a cluster of genes crucial for the closure of cranial sutures located on chromosome 15q. Our patient and that reported by Van Allen *et al*[2] seem to share the same deletion of band 2q37. Even if microcephaly has been observed in some patients with 2q terminal deletion, this cytogenetic aberration has never been correlated with cloverleaf skull anomaly.[6 7] Furthermore, these patients exhibited a breakpoint more proximal than that found in our patient,[6] whereas deletions mapped distally to 2q37.1 were associated with normal cranial circumference or even macrocephaly.[7]

On physical examination, our patient exhibited many clinical features found in the classical trisomy 4p syndrome,[8] including iris coloboma, depressed nasal bridge, pointed chin, vertebral and rib abnormalities, camptodactyly, club feet, cryptorchidism, generalised hypertonia, cardiac defect, and respiratory problems, which can be explained by the presence of trisomy of a large part of the 4p region. However, his overall phenotype appears more severe than that described in the classical trisomy 4p syndrome. Specifically, CS is an extreme form of craniosynostosis compared to the variable pattern of sutural fusion observed in the classical trisomy 4p syndrome, which ranges from protruding glabella (30%) or prominent continuous supraorbital ridge fused across the glabella (23%), to microcephaly, prominent forehead, or other less specific cranial abnormalities, which have been described with a variable degree of severity even within the same family members.[8]

Several of the classical findings of the trisomy 4p syndrome can be caused by the duplication of about 2.1 Mb extending from the telomere and involving the 4p16.1→16.3 region.[9] In order to understand how cranial involvement is a main feature of the trisomy 4p syndrome, particularly in our patient, some of the genes located in the critical region should be taken into account. The gene encoding the fibroblast growth factor receptor 3 (FGFR3) maps to 4p16.3[10] and mutations within its different domains have recently been associated with syndromic and non-syndromic craniosynostosis.[10] A constitutive FGFR3 activation seems to be the mechanism underlying the craniosynostosis.[10] In this respect, three copies of the wild type FGFR3 gene might result in a greater production of protein, thus mimicking the effect of FGFR3 mutations as observed in some craniosynostoses.

The same activating mechanism could be envisaged for MSX1, which maps to 4p16.1. Even if MSX1 has never been associated with craniosynostosis, MSX2, one of its homologues,[11] has been found associated with the Boston type of craniosynostosis through a gain of function mutational mechanism.[12] Furthermore, MSX1 deficiency has been recently associated with failure of development of alveolar bone and teeth in man[13]; overexpression of MSX1 could result in hypertrophy of the alveolar process, as observed in our patient.

Further studies on both FGFR3 and MSX1 will probably clarify the effective involvement of these genes in the determination of craniosynostosis in the trisomy 4p syndrome.

D DE BRASI
L PERONE
P DI MICCO
G ANDRIA
G SEBASTIO
Dipartimento di Pediatria, Università "Federico II", Via S Pansini 5, I-80131 Naples, Italy

E IACCARINO
L PINTO
Divisione di Patologia Neonatale, Azienda Ospedaliera "Santobono-Pausilipon", Naples, Italy

F ALIBERTI
Divisione di Neurochirurgia, Azienda Ospedaliera "Santobono-Pausilipon", Naples, Italy

1 Gorlin JR, Cohen MM, Jr, Levin LS. Syndromes with craniosynostosis: general aspects and well-known syndromes. In: *Syndromes of the head and neck*. 3rd ed. New York: Oxford University Press, 1990:536-9.
2 Van Allen MI, Siegel-Bartelt J, Feigenbaum A, Teshima IE. Craniosynostosis associated with partial duplication of 15q and deletion of 2q. *Am J Med Genet* 1992;**43**:688-92.
3 Pinkel D, Straume T, Gray J. Cytogenetic analysis using quantitative, high sensitive, fluorescence in situ hybridization. *Proc Natl Acad Sci USA* 1986; **83**:2934-8.
4 Gosden CM, Davidson C, Robertson M. Lymphocyte culture. In: Rooney DE, Czepulkowski BH. *Human cytogenetics, a practical approach*. 2nd ed. Vol I. Oxford: Oxford University Press, 1992:31-54.
5 The Genome Database (version 6.4), http://www.gdb.org/
6 Wang TH, Johnston K, Hsieh CL, Dennery PA. Terminal deletion of the long arm of chrmosome 2 in a premature infant with karyotype: 46,XY,del(2)(q37). *Am J Med Genet* 1994;**49**:399-401.
7 Conrad B, Dewald G, Christensen E, Lopez M, Higgins J, Pierpont ME. Clinical phenotype associated with terminal 2q37 deletion. *Clin Genet* 1995;**48**:134-9.
8 Patel SV, Dagnew H, Parckh AJ, *et al*. Clinical manifestations of trisomy 4p syndrome. *Eur J Pediatr* 1995;**154**:425-31.
9 Wyandt HE, Milunsky J, Lerner T, *et al*. Characterization of a duplication in the terminal band of 4p by molecular cytogenetics. *Am J Med Genet* 1993; **46**:72-6.
10 Webster MK, Donoghue DJ. FGFR activation in skeletal disorders: too much of a good thing. *Trends Genet* 1997;**13**:178-82.
11 Davidson D. The function and evolution of Msx genes: pointers and paradoxes. *Trends Genet* 1995;**11**:405-11.
12 Ma L, Golden S, Wu L, Maxson R. The molecular basis of Boston-type craniosynostosis: the Pro148→His mutation in the N-terminal arm of the MSX2 homeodomain stabilizes DNA binding without altering nucleotide sequence preferences. *Hum Mol Genet* 1996;**5**:1915-20.
13 Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet* 1996;**13**:417-21.