

Electronic letter

J Med Genet 2000;37 (<http://jmedgenet.com/cgi/content/full/37/12/e47>)

CHARGE association in a child with de novo chromosomal aberration 46,X,der(X)t(X;2)(p22.1;q33) detected by spectral karyotyping

EDITOR—The CHARGE association is an increasingly recognised pattern of congenital anomalies comprising colobomata, heart defects, choanal atresia, retarded growth and development, hypoplastic genitalia, and ear abnormalities/deafness.¹ We report a case of CHARGE association with a de novo chromosomal aberration, 46,X,+der(X)t(X;2), which was detected by spectral karyotyping.

The proband, an 11 year old girl, was the term product of a normal pregnancy and delivery to healthy and unrelated parents. She has a healthy younger brother. The family history is unremarkable for mental retardation and congenital malformations. Birth weight was 2870 g and Apgar scores were 7 and 7 at one and five minutes, respectively. Bilateral choanal atresia was diagnosed at birth and she was admitted to hospital with an airway for two months. At the age of 8 months, she underwent a surgical repair but left choanal stenosis persisted. At the age of 5 years, after recurrences of purulent otitis media, she underwent an adenoidectomy. At the age of 8 years, a heart murmur was heard and an echocardiogram and cardiac catheterisation showed minimal valvar pulmonary stenosis and partial anomalous venous return. Renal ultrasound showed mild enlargement of the left kidney with calculi. Because of additional growth and developmental delay, a chromosome analysis was performed on blood lymphocytes at the age of 6 years and showed 46,X,add(X)(p?) (fig 1). FISH studies using X chromosome paint probe showed that the derivative chromosome came from an autosomal chromosome. The parents' karyotypes were normal.

On present examination at 11 years, the patient's OFC is 44 cm (−6 SD) and her height is well below the 5th centile. She functions in the mild mental retardation range. Her eye examination showed exotropia and esophoria and she has posterior embryotoxon. She exhibits a number of minor anomalies including a high nasal bridge, short columella, bulbous nasal tip, hypertelorism, simple auricles with small ear lobes, mild prognathism (figs 2 and 3), clinodactyly, and a hypertrophic clitoris with normal labia.

A CT scan performed when she was 8 years old showed mild cerebral atrophy. Auditory tests and tympanometry indicated partial conductive and sensorineural hearing loss.

In order to determine the origin of the derivative chromosome, spectral karyotyping analysis (SKY) was performed, as previously described.² Briefly, chromosome specific libraries generated by PCR from flow sorted human chromosomes were directly labelled with nucleotides conjugated to four different dyes (FITC, Rodamine, Texas Red, Cy5). All 24 chromosome libraries were hybridised simultaneously to the metaphases. Slides were washed and stained with 4'6-diamidino-2 phenylindole (DAPI) in antifade medium. Discrimination between the different spectra was done using the SD200 spectral bioimaging system (Applied Spectral Imaging Ltd, Migdal Ha'emek, Israel) and showed the correct karyotype: 46,X,+der(X)t(X;2) (fig 4). Only after re-examination of the G banded chromosomes were the breakpoints in chromosomes 2 and X assigned, and it appeared that the breakpoints were at Xp22.1 and 2q33 and the karyotype was 46,X,add(Xp?)ish. der(X)t(X;2)(p22.1;q33)(wcpX+,wcp2+).

The combination of anomalies (bilateral choanal atresia, congenital heart defect, short stature, external ear anomalies with conductive hearing loss, mental retardation, and minor anomalies) found in our patient is consistent with the diagnosis of CHARGE association. Colobomata were not found in our patient. In a recent evaluation of 47 patients with CHARGE syndrome for the frequency of major anomalies, coloboma was found only in 79% of them.³ The cause of the CHARGE association remains

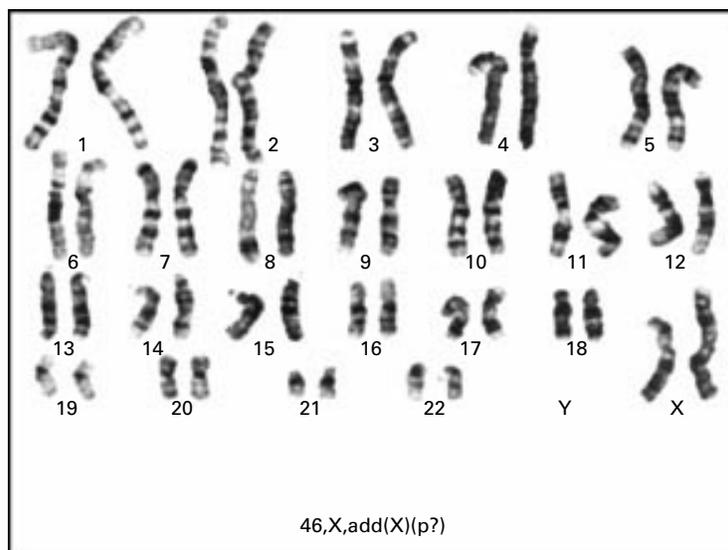


Figure 1 G banded karyotype of the patient 46,X,add(X)(p?).



Figure 2 Front view of the patient at the age of 11 years.



Figure 3 Side view of the patient at 11 years.

unknown. Most cases reported are sporadic. However, many aspects favour the view that a genetic abnormality is involved, particularly the concordance of phenotype in monozygotic twins and discordance in dizygotic twins, absence of environmental factors, a significantly higher paternal age at conception,⁴ the existence of chromosomal anomalies including $t(2;18)$ and $t(3;22)$,⁵ de novo $t(6;8)$,⁵ a microdeletion in the 22q11.2 region in two patients,^{6,7} and inverted duplication (14)(q22-q24.3).⁸ However, in a recent review of 47 patients with the CHARGE association, no chromosomal aberrations were found.³

There are rare descriptions of familial cases suggestive of autosomal recessive or dominant transmission.⁹⁻¹³ This suggests the possible role of a de novo dominant mutation or a hitherto undetected chromosomal abnormality in CHARGE patients. The combination of malformations observed in CHARGE syndrome strongly evokes a

polytopic developmental field defect involving the neural crest cells,⁵⁻¹⁸ and supports the view that the CHARGE complex is a syndrome.¹⁹

Trisomy for the terminal part of chromosome 2 (q33→qter) was found in our patient. The most serious manifestations seen in patients with partial trisomy 2q is severe psychomotor retardation, often associated with pronounced muscular hypotonia.^{20,21} Only 13 cases with “pure” duplications of the distal part of the long arm of chromosome 2 have been reported and reviewed in a recent article. Most of them had typical facial features, limb and genital anomalies, and mental and developmental delay, and none of them had the CHARGE association.²² However, our patient has the CHARGE association with mild mental retardation and a few minor additional findings. A reduction in the symptoms in our patient would be expected owing to the spreading effect of X inactivation

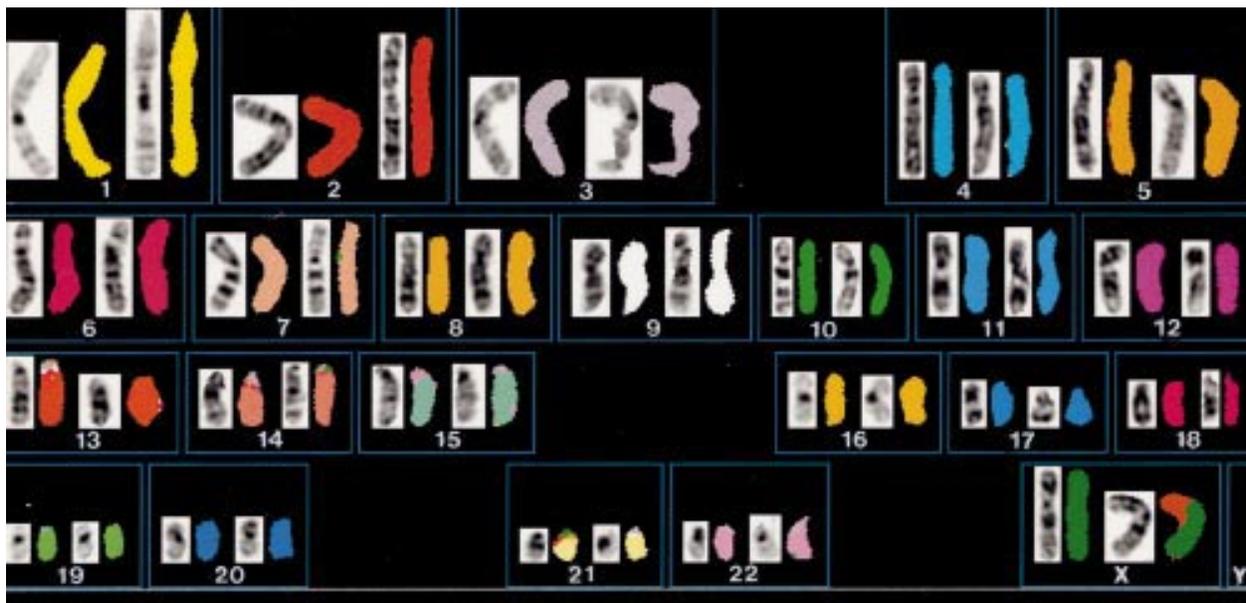


Figure 4 Spectral karyotyping analysis: 46,X,der(X)t(X;2).

onto the translocated 2q segment. Although the X inactivation pattern was not determined in our patient, we would expect non-random X inactivation of the translocated X.²³

The previously described chromosome 2 rearrangement in a case of CHARGE association involved chromosomes 2 and 18 and it was a result of unbalanced familial translocation. The breakpoints in that case were in 2q37, while in our patient the breakpoints were in 2q33.¹⁴

Here we report for the first time the use of SKY for the identification of the correct karyotype in a patient with CHARGE association. SKY is a recently described molecular cytogenetic technology. A 24 colour FISH using spectral karyotyping (SKY) permits simultaneous visualisation of all human chromosomes in 24 different colours and has been shown to be a powerful new technique in characterising chromosome rearrangements in clinical and cancer cytogenetics.^{24 25} This technique was especially useful in our case where the derivative could have originated from any one of 22 different chromosomes. Spectral karyotyping application proved to be the preferred technique for delineation of the correct karyotype, but the breakpoints in chromosome 2 were assigned following re-examination of the G banded chromosomes. We recommend the application of the SKY technique to help clarify the origin of additional chromosomal material in patients with complicated karyotypes.

DORIT LEV*†
ORLY NAKAR†
IRIT BAR-AM‡
ALEXANDER ZUDIK*
NATHAN WATEMBERG†
SHARON FINKELSTIEN‡
NIRIT KATZIN‡
TALLY LERMAN-SAGIE†

*Institute of Medical Genetics, Wolfson Medical Centre, Holon, Israel 58100

†Paediatric Metabolic Neurogenetic Clinic, Wolfson Medical Centre, Holon, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

‡Applied Spectral-Imaging, Migdal Ha'Emek, Israel

Correspondence to: Dr Lev, dorlev@post.tau.ac.il

- Pagon RA, Graham JM, Zonana J, Yong SL. Coloboma, congenital heart disease and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr* 1981;**99**:223-7.
- Schrock E, Veldman T, Padilla-Nash H, Ning Y, Spurbeck J, Jalal S, Shaffer LG, Papenhausen P, Kozma C, Phelan MC, Kjeldsen E, Scharberg SA, O'Brian P, Biesecker L, du Manoir S, Ried T. Spectral karyotyping cytogenetic diagnosis of constitutional chromosomal abnormalities. *Hum Genet* 1997;**101**:255-62.

- Tellier AL, Cormier-Daire V, Abadie V, Amiel J. CHARGE syndrome: report of 47 cases and review. *Am J Med Genet* 1998;**76**:402-9.
- Tellie AL, Lyonnet S, Cormier-Daire V. Increased paternal age in CHARGE association. *Clin Genet* 1996;**50**:548-50.
- Hurst JA, Meinecke P, Baraitser M. Balanced t(6;8)(6p8p;6q8q) and the CHARGE association. *J Med Genet* 1991;**28**:54-5.
- Emanuel BS, Budarf ML, Sellinger B, Goldmuntz E, Driscoll DA. Detection of microdeletions of 22q11.2 with fluorescence in situ hybridization (FISH): diagnosis of DiGeorge (DGS), velo-cardio-facial (VCF) syndrome, CHARGE association and conotruncal cardiac malformations. *Am J Hum Genet* 1992;**51**:A3.
- Devriendt K, Swillen A, Fryns JP. Deletion in chromosome region 22q11 in a child with CHARGE association. *Clin Genet* 1998;**53**:408-10.
- North KN, Wu BL, Cao BN, Whitemen DAH, Korf BR. CHARGE association in a child with de novo inverted duplication (14)(q22-q24.3). *Am J Med Genet* 1995;**47**:610-14.
- Levin DL, Muster AJ, Newfeld EA, Paul MH. Concordant aortic arch anomalies in monozygotic twins. *J Pediatr* 1973;**83**:459-61.
- Mitcell JA, Giangiacomo J, Hefner MA, Thelin JW, Pickens JM. Dominant CHARGE association. *Ophthalmic Paediatr Genet* 1985;**6**:271-6.
- Metlay LA, Smithe PS, Miller ME. Familial CHARGE syndrome: clinical report with autopsy findings. *Am J Med Genet* 1987;**26**:577-81.
- Hall BD, Blumberg BD. CHARGE association in affected half siblings through a normal mother. *Am J Hum Genet* 1988;**43**:A54.
- Blake KD, Ratcliffe JM, Wyse RK. CHARGE association in two monozygous triplets. *Int J Cardiol* 1989;**25**:339-41.
- Clementi M, Tenconi R, Turolla L, Silvan C, Bortotto L, Artifoni L. Apparent CHARGE association and chromosome anomaly: chance or contiguous gene syndrome? *Am J Med Genet* 1991;**41**:246-50.
- Siebert JR, Graham JM Jr, Macdonald C. Pathologic features of the CHARGE association: support for involvement of the neural crest. *Teratology* 1985;**31**:331-6.
- Lin AE, Chin AJ, Devine W, Park SC, Zackai E. The pattern of cardiovascular malformation in the CHARGE association. *Am J Dis Child* 1987;**141**:1010-13.
- Couly G, Le Douarin NM. Head morphogenesis in embryonic avian chimeras: evidence for a segmental pattern in the neuroectoderm corresponding to the neuromeres. *Development* 1990;**108**:543-58.
- Kirby ML, Waldo KL. Role of neural crest in congenital heart disease. *Circulation* 1990;**82**:332-40.
- Lubinski MS. Properties of associations: identity, nature and clinical criteria, with a commentary on why CHARGE and Goldenhar are not associations. *Am J Med Genet* 1994;**49**:21-5.
- Zankl M, Schwanitz G, Schmid P, Zankl H, Dockter G, Rodewald A, Zankl KD, Grosse KP. Distal 2q duplication: report of two familial cases and an attempt to define a syndrome. *Am J Med Genet* 1979;**4**:5-16.
- Plessis G, Couturier J, Turleau C, Despoisses S, Delavenne J. "Pure" partial trisomy 2q in a male owing to malsegregation of a maternal translocation t(X;2)(p22.3;q32.1). *J Med Genet* 1985;**22**:70-3.
- Frietz B, Muller-Navia J, Hillig U, Kohler M, Aslan M, Rehder H. Trisomy 2q35-37 due to insertion of 2q material into 17q25: clinical, cytogenetic, and molecular cytogenetic characterization. *Am J Med Genet* 1999;**87**:297-301.
- Couturier J, Dutrillaux B. Replication studies and demonstration of position effect in rearrangements involving human X chromosome. Basic mechanisms of X-chromosome behaviour. In: Sandberg AA, ed. *Progress and topics in cytogenetics. The cytogenetics of mammalian X-chromosome*. Vol 3. Part A. New York: Alan R Liss, 1983:375-404.
- Schrock E, du Manoir S, Veldman T, Schoell B, Weinberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. Multicolor spectral karyotyping of human chromosomes. *Science* 1996;**273**:494-7.
- Veldman T, Vignon C, Schrock E, Rowley J, Ried T. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nat Genet* 1997;**15**:406-10.