European Journal of Human Genetics (2003) 11, 64–68
© 2003 Nature Publishing Group All rights reserved 1018–4813/03 $25.00
www.nature.com/ejhg

ARTICLE

PTPN11 mutations are not responsible for the Cardiofaciocutaneous (CFC) syndrome

MI Kavamura*[,1,2,3], MG Pomponi[1], M Zollino[1], R Lecce[1], M Murdolo[1], D Brunoni[2], MMA Alchorne[3], JM Opitz[1,4] and G Neri[1]

[1]*Istituto di Genetica Medica, Università Cattolica del Sacro Cuore, Rome, Italy;* [2]*Centro de Genética Médica, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil;* [3]*Departamento de Dermatologia, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil;* [4]*Pediatrics (Medical Genetics), Human Genetics, Obstetrics and Gynecology, University of Utah, Salt Lake City, USA*

Cardiofaciocutaneous (CFC) syndrome is a multiple congenital anomalies/mental retardation syndrome characterized by congenital heart defects, characteristic facial appearance, short stature, ectodermal abnormalities and mental retardation. It was described in 1986, and to date is of unknown genetic etiology. All reported cases are sporadic, born to non-consanguineous parents and have apparently normal chromosomes. Noonan and Costello syndromes remain its main differential diagnosis. The recent finding of *PTPN11* missense mutations in 45–50% of the Noonan patients studied with penetrance of almost 100% and the fact that in animals mutations of this gene cause defects of semilunar valvulogenesis, made *PTPN11* mutation screening in CFC patients a matter of interest. We sequenced the entire coding region of the *PTPN11* gene in ten well-characterised CFC patients and found no base changes. We also studied *PTPN11* cDNA in our patients and demonstrated that there are no interstitial deletions either. The genetic cause of CFC syndrome remains unknown, and *PTPN11* can be reasonably excluded as a candidate gene for the CFC syndrome, which we regard as molecular evidence that CFC and Noonan syndromes are distinct genetic entities.
European Journal of Human Genetics (2003) **11,** 64–68. doi:10.1038/sj.ejhg.5200911

Keywords: Cardiofaciocutaneous syndrome; *PTPN11* gene; chromosome 12

Introduction

Cardiofaciocutaneous (CFC) syndrome (MIM 115150) is a multiple congenital anomalies/mental retardation syndrome characterised by congenital heart defects, characteristic facial appearance, short stature, ectodermal abnormalities, and mental retardation.[1] A recent review has identified 54 published classical cases of CFC syndrome. All cases were sporadic, with apparently normal chromosomes, born to non-consanguineous parents and presenting the same unique combination of findings and natural history.[2]

The CFC phenotype comprises a characteristic facial appearance, congenital heart defects (pulmonic stenosis, 37%; atrial septal defect, 35%; hypertrophic cardiomyopathy, 11%), mental retardation/developmental delay (81%), speech delay (46%), short stature (78%) and hypotonia (28%). The typical craniofacial manifestations include relative macrocephaly, high forehead, bitemporal constriction, supraorbital hypoplasia, short nose with depressed nasal bridge and anteverted nostrils, apparently low-set and posteriorly angulated ears, hypertelorism, ptosis, epicanthal folds, and downslanting of palpebral fissures. All patients had an ectodermal abnormality consisting of sparse, slowly growing and curly hair, sparse or absent eyelashes and eyebrows, follicular keratosis or *ulerythema ophryogenes*, ichthyosis, hyperkeratosis, generalized hyperpigmentation, haemangiomas, hyperelastic skin, cutis marmorata, *café-au-lait* patches, and/or slowly growing nails.

*Correspondence: Maria Inês Kavamura, Rua Dom Armando Lombardi, 717 CEP 05616-011, São Paulo-SP, Brazil; E-mail: kavamura@uol.com.br
Received 19 June 2002; revised 24 September 2002; accepted 25 September 2002

In spite of the controversy about the distinction between CFC and Noonan syndrome (NS) most of the time a refined clinical evaluation is sufficient for the differential diagnosis. Although phenotypic similarity between CFC and NS exists, especially regarding cardiac defects, craniofacial findings, short stature and congenital lymphedema, there are clear differences in clinical manifestations and natural history that separate the two conditions.[3–5]

Autosomal dominant inheritance is evident in many NS families, and genetic heterogeneity has also been demonstrated.[6] Linkage studies have defined a region of approximately 7.5 Mb on the long arm of chromosome 12 (12q24) as a candidate region for a gene(s) causing NS[6–9] (Figure 1). The recent demonstration of mutations in *PTPN11*, the gene encoding the non-receptor-type protein tyrosine phosphatase SHP-2 (src homology region 2-domain phosphatase-2)[10] (Figure 2), in 45–50% of the studied patients of NS,[9,11] has enabled an initial genotype–phenotype analysis. This has also shown that pulmonic stenosis is more prevalent in the group of NS patients who had a *PTPN11* mutation than in the group without a mutation (70.6 *vs* 46.2%; $P<0.01$). The identification of a *PTPN11* mutation in a family segregating the Noonan-like/multiple giant-cell lesion syndrome,[11] and in LEOPARD syndrome,[12] suggests a considerable phenotypic range due to mutations in this gene making it necessary to study the possible role of this gene in CFC syndrome.

Patients

We studied ten CFC patients (nine males and one female) (Figure 3), randomly selected from a larger group of CFC patients followed by a group of clinical geneticists in Brazil, Italy and North America, experienced with CFC syndrome and its nosology with the Noonan and Costello syndromes. In all cases the clinical diagnosis was considered incontrovertible. The CFC Index[2] was applied to all patients. The patients selected for this study are originally from Brazil and Italy and in each case a consent form was signed by the parents.

Materials and methods

DNA extraction

Genomic DNA was isolated from the patients' peripheral-blood leukocytes or from Epstein–Barr virus-transformed lymphocytes using standard procedures.

Gene sequencing

The 15 exons of the *PTPN11* gene, described at http://genome.ucsc.edu//cgi-bin/hgTracks?position=NM 002834&Submit=Submit and at http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?dispmax=1&showndispmax=1&val=NT 009575.8&view=graph& from=758671& to=857626& sfrom=0& strand=2, and their intron–exon boundaries were sequenced bi-directionally (Table 1, Figure 2). These

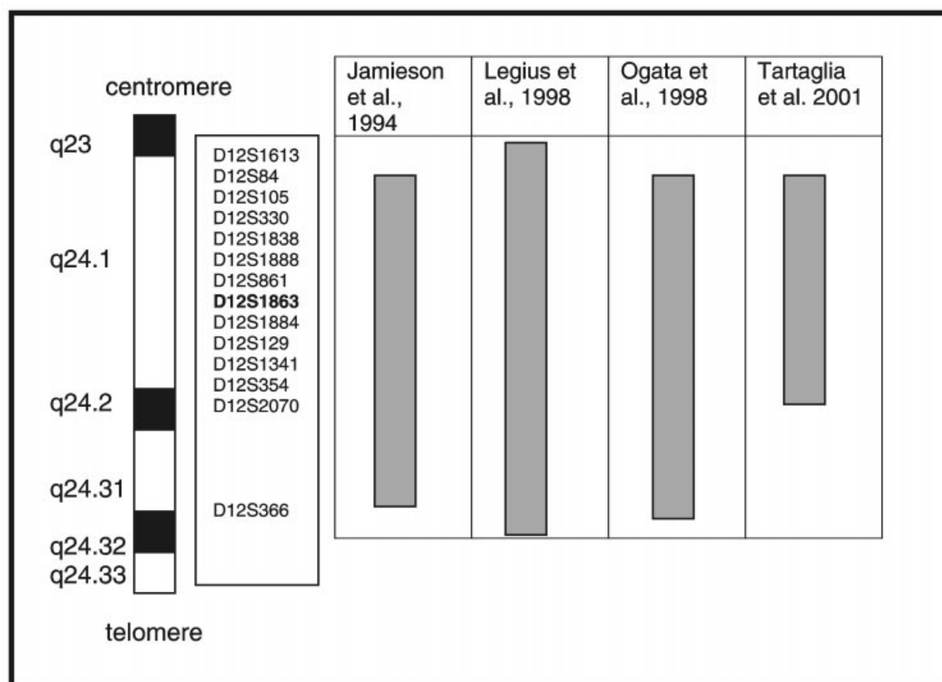

Figure 1 Representation of the 12q24 chromosome region, microsatellite markers that span the Noonan syndrome critical region and results of linkage studies in NS families. Microsatellite in bold corresponds to the *PTPN11* localization.

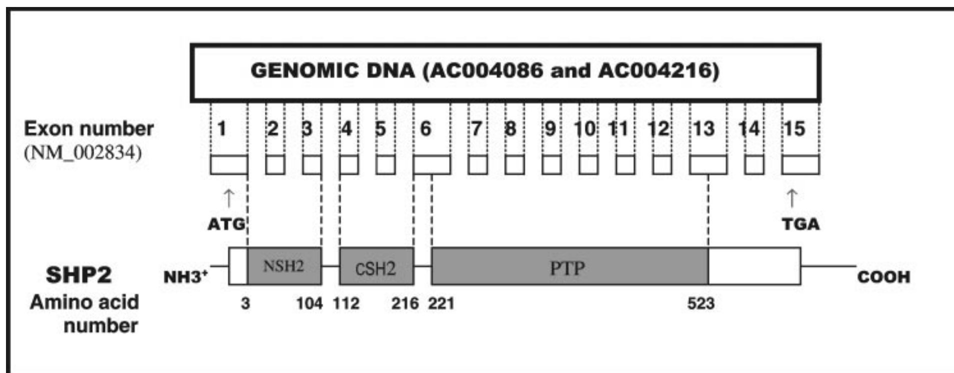

Figure 2 PTPN11 organization and SHP-2 structure.

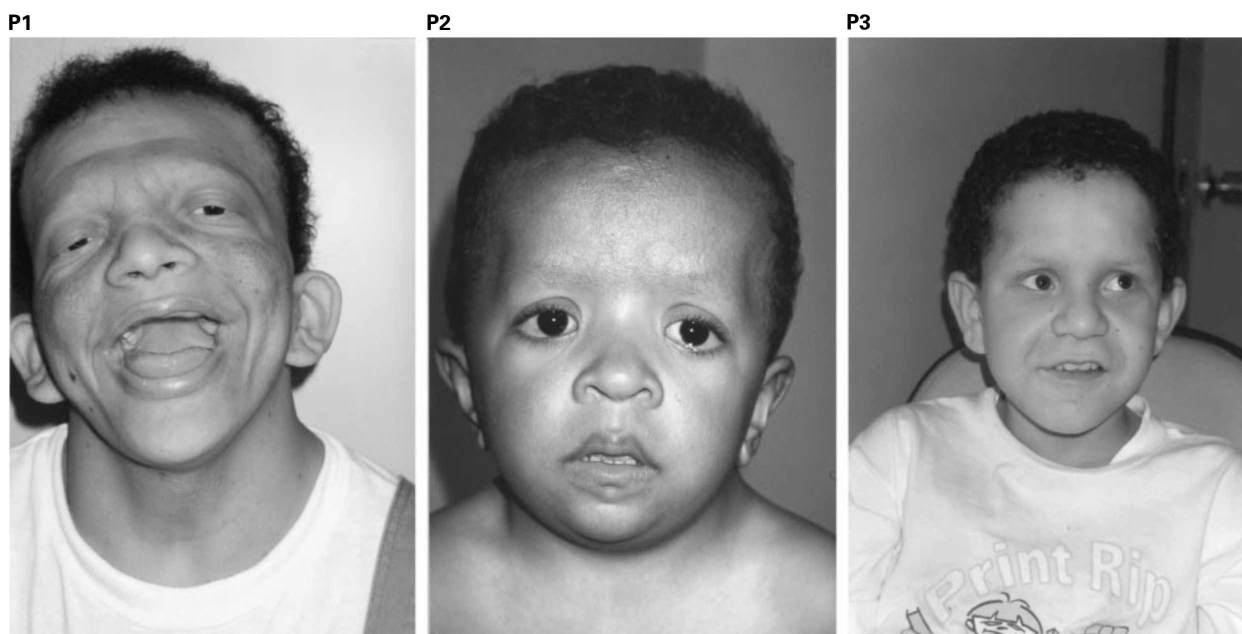

Figure 3 Three of the CFC patients studied. Patient 1, male, 28 years old; patient 2, male, 3 years old; patient 3, male, 8 years old.

regions were amplified from genomic DNA using the primers shown in Table 2, which were selected by the Macintosh program Primer Express.

PRC reactions were carried out under standard conditions, containing: 50 ng of genomic DNA, 1 mM of each forward and reverse primers and 10 μl of Promega TAX Master Mix, as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C (1 min), annealing at 53–60°C (1 min) and extension at 72°C (1 min), followed by final extension at 72°C for 5 min, using the Gene Amp PCR System 9600 - Perkin Elmer.

PRC products were purified with a PCR product pre-sequencing kit, composed of exonuclease 1 and shrimp alkaline phosphatase, by USB Corporation, according to their instructions, and later using the Centri-Sep Spin Columns by Applied Biosystems. Sequencing reactions were carried out using the ABIPRISM dye terminator cycle sequencing kit, and the products were analysed on an ABIPRISM 310 Genetic Analyzer DNA sequencer. Sequences were analysed manually.

Study of the cDNA

In order to detect the presence of deletions within the coding region, which could be overlooked by analysing the sequences, we constructed five pairs of primers to amplify overlapping cDNA fragments of about 550 bp (Table 3). These fragments were amplified by PCR, carried out under standard conditions, containing: 50 ng of cDNA, 1 mM of each forward and reverse primers and 10 μl of

Table 1 Intron–exon boundaries for the *PTPN11* gene, exons length and their position in the mRNA (NM_002834). Intronic sequences are represented in lower case, and exonic, in upper case

Exon Number	*intron/EXON... EXON/intron*	PTPN11 *mRNA (bp)*	*Exon length (bp)*
1	ggatcccccag/GCCTGGAGGG...CATCGCGGAG/gtgaggagcc	234-394	161
2	tctttttaag/ATGGTTTCAC...TTTCCGTTAG/gtaagttgga	397-517	123
3	actattttag/AAGAAATGGA...CCTCTGAAAG/gtcagtaaca	518-712	195
4	aaaactttag/GTGGTTTCAT...TCGCTGTCAG/caggtgagca	713-905	193
5	atcttgaaag/GAACTGAAAT...ACAACTCAAG/ctttctcctt	906-1022	117
6	actcgatcag/CCCCTTAACA...AGAATTTGAG/gtaagttatt	1023-1136	114
7	ttctttccag/ACACTACAAC...ATCCTGCCCT/gtaagtatca	1137-1233	97
8	tttcttctag/TTGATCATAC...TATCATCATG/gtaagctttg	1234-1313	80
9	aaatttctag/CCTGAATTTG...GAGAGGAAAG/gtaaatcaca	1314-1472	159
10	tctcttccag/AGTAAATGTG...GGTTGGACAA/gtaagtatat	1473-1604	132
11	tctactccag/GGGAATACGG ...TGCACTGCAG/gtgacagctc	1605-1759	155
12	ctgcccgcag/TGCTGGAATT...AGAGAGAAAG/gtgggtcatc	1760-1827	68
13	tccttcgtag/GTGTTGACTG...AGAAGAGCAG/gtaccagcct	1828-1979	152
14	caaatttcag/AAAAGCAAGA...CCTGTGCAGA/gtaagtagtg	1980-2092	113
15	ttctctccag/AATGAGAGAA...ACAGAAATAG/gtatttaaat	2093-2194	102

Table 2 Primers and PCR conditions used to amplify the coding region of the *PTPN11* gene

Exon	*Primer forward (5′→3′)*	*Primer reverse (5′→3′)*	*Exon length (bp)*	*T annealing (°C)*	*Size of the PCR product*
1	GCTGACGGGAAGCAGGAAGTGG	CTGGCACCCGTGGTTCCCTC	161	60	589
2	ACTGAATCCCAGGTCTCTACCAAG	CAGCAAGCTATCCAAGCATGGT	123	60	405
3	TTA GAA GAA ATG GAG CTG TCA	GAG ACT CAG GGC ACA AGG	195	55	254
4	AAC AAC ATG AAC CCA TAG TAG AGC	CCA TTT TTC AAC TGG AGA TTT AC	193	57	271
5	ATG TGT TTA TCT TGA AAG GAA CT	AAA ATT CTC TAT TAG GAT TTG TTT T	117	53	201
6	GCA TTA ACA CCG TTT TCT GTA AT	GCA ACT TCT CCT CCA CTA AAA AT	114	58	241
7	ATG CTG ATC CAG GCT TTT TT	GAG TGA CTA TTA CTG AGC GGA A	97	55	205
8/9	GCA GTC CAG GAC TTA TGT GA	AAA CAT GGC CAA TCT GAC A	80/159	55	477
10	TTT CAG AGT TCA CAG AAT TAA CTT T	ATT CCT ACA CAC CAT CAA CAG T	132	58	235
11	CGG GTG ATT CCT CAA CCT CTT G	GCG CTA GGA GAC AGG GAC AGG	155	60	272
12	TTG AGT CTG AAA CCC CCA TG	TGT TTT CGT GAG CAC TTT CC	68	55	201
13	TCT TCA TGA TGT TTC CTT CGT A	GCC TAG CAA GAG AAT GAG AAT C	152	60	222
14	ATC CCC TTA AAA TAA CCA TTG T	GAC CAG GTA AAA AGA ATT TCC T	113	55	201
15	TTT TGT AAA TGT CTT TCT TTT TCT T	AAT TAT GGT ACA CTT TGC TAA AAC A	102	55	240

Table 3 Primes and PCR conditions to amplify overlapping fragments of *PTPN11* cDNA

cDNA position from the ATG	*Primer forward (5′→3′)*	*Primer reverse (5′→3′)*	*T annealing (°C)*	*Size of PCR product*
−69 to 544	gatgtgaccgagcccagg	caacgtcgtatttcagttcctgac	64	613
246 to 811	ggaacatcacgggcaattaaa	gcctttgaccctcttttcgg	58	566
696 to 1269	actaagcaaattagctgagaccacaga	ccaggtccgaaagtggtattg	64	574
1135 to 1696	gaatatggcgtcatgcgtgt	ttggagtacaaggcgggaga	55	562
1484 to 1874	agatggtgcggtctcagagg	tcacataaactttcttgcgtctgtt	55	391

Promega TAX Master Mix, as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C (1 min), annealing at 55–64°C (1 min) and extension at 72°C (1.5 min), followed by extension period of 10 min, using the Gene Amp PCR System 9600 - Perkin Elmer. Every PCR reaction included 10 CFC patients and one control subject. PCR products were electrophoresed on a 2% agarose gel to verify the actual size of the fragments obtained from each patient.

Results

The CFC Index of all ten patents included in this study ranged from −1 SD to +1 SD. Three facial pictures are shown in Figure 3.

The coding-region of *PTPN11* and its intron–exon boundary regions were sequenced in 10 CFC patients and no base changes were identified.

The sizes of the amplified cDNA fragments were as expected and single bands were detected from all patients in all five fragments.

Discussion

The actual relationship between CFC and Noonan syndromes has been the object of a heated controversy, and many opinions have been expressed as to whether they are different genetic entities, allelic conditions, a contiguous gene syndrome, or even different phenotypes of the same condition.[2–5,13–15] The finding of one of the causes of Noonan syndrome is a very important step towards the clarification of this matter.

The protein encoded by *PTPN11* is a member of the protein tyrosine phosphatase (PTP) family. PTPs regulate a variety of cellular processes, including cell growth, differentiation, mitotic cycle and oncogenic transformation and, in mouse, is involved in cardiac semilunar valvogenesis.[16] The finding of 45–50% *PTPN11* mutation prevalence in a sample of Noonan syndrome patients with almost 100% penetrance[9,11] demonstrates that it is one of the genes causing Noonan syndrome. Mutations found in NS patients were all missense changes affecting amino acid residues that are conserved among the vertebrate SHP-2 orthologs.

PTPN11 mutations have also been found in conditions such as the Multiple-Lentigines/LEOPARD syndrome[12] and Noonan-like/multiple giant-cell lesion syndrome.[11] These data are of great importance in extending the phenotype of Noonan syndrome. It is also important to better classify some patients whose diagnosis is controversial. The finding of the same *PTPN11* mutation in the two patients from a large family with NS who were previously diagnosed as having CFC syndrome[17,18] demonstrates that those patients also have Noonan syndrome as already evident by their published phenotype. Therefore, to date, there are no familial cases of CFC syndrome. We have demonstrated that CFC syndrome patients do not have missense changes on the coding region of the *PTPN11*, nor small or large intragenic deletions, which we regard as molecular evidence that CFC and Noonan syndromes are distinct genetic entities.

We encourage *PTPN11* mutation screening in other CFC patients to confirm our results.

Acknowledgements

We thank the CFC Family Network and its President, Brenda Conger and CAPES-FADA (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), São Paulo - SP, Brazil, for financial support and for their contribution to this work. We also thank Drs: Giuseppina Corona, Livia Garavelli, Fernanda Teresa de Lima, Debora Pallos, Maria Piccione, Jane Sanchez and Gioacchino Scarano, for referral of patients.

We thank Drs Charles Schwartz and Ilaria Zito for their valuable suggestions to this work.

References

1 Reynolds JF, Neri G, Herrmann JP *et al*: New multiple congenital anomalies/mental retardation syndrome with cardio-facio-cutaneous involvement – the CFC syndrome. *Am J Med Genet* 1986; **25**: 413–427.
2 Kavamura MI, Peres CA, Alchorne MMA, Brunoni D: CFC Index for the diagnosis of cardiofaciocutaneous syndrome. *Am J Med Genet* 2002; **112**: 12–16.
3 Neri G, Zollino M, Reynolds JF: The Noonan–CFC controversy. *Am J Med Genet* 1991; **39**: 367–370, [editorial comment].
4 Neri G, Zollino M: More on the Noonan-CFC controversy. *Am J Med Genet* 1996; **65**: 100, [editorial comment].
5 Grebe TA, Clericuzio C: Neurologic and gastrointestinal dysfunction in cardio-facio-cutaneous syndrome: identification of a severe phenotype. *Am J Med Genet* 2000; **95**: 135–143.
6 Jamieson CR, Van Der Brugt I, Brady AF *et al*: Mapping a gene for Noonan syndrome to the long arm of chromosome 12. *Nat Genet* 1994; **8**: 357–360.
7 Legius E, Schollen E, Matthijs G, Fryns JP: Fine mapping of Noonan/cardio-facio-cutaneous syndrome in a large family. *Eur J Hum Genet* 1998; **6**: 32–37.
8 Ogata T, Muroya K, Tsukahara M: Noonan syndrome: genotype analysis of the Noonan syndrome critical region at chromosome 12q in a three-generation family. *Am J Med Genet* 1998; **79**: 153–154.
9 Tartaglia M, Mehler EL, Goldberg R *et al*: Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. *Nat Genet* 2001; **29**: 465–468.
10 Hof P, Pluskey S, Dhe-Paganon S, Ect MJ, Shoelson SE: Crystal structure of the tyrosine phosphatase SHP-2. *Cell* 1998; **92**: 441–450.
11 Tartaglia M, Kalidas K, Shaw A *et al*: *PTPN11* mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *Am J Hum Genet* 2002; **70**: 1555–1563.
12 Digilio MC, Conti E, Sarkozy A *et al*: Grouping of Multiple-Lentigines/LEOPARD and Noonan syndromes on the *PTPN11* gene. *Am J Hum Genet* 2002; **71**: 389–394.
13 Fryer AE, Holt PJ, Hughes HE: The Cardio-facio-cutaneous (CFC) syndrome and Noonan syndrome: Are they the same? *Am J Med Genet* 1991; **38**: 548–551.
14 Leichtman LG: Are cardio-facio-cutaneous syndrome and Noonan syndrome distinct? A case of CFC offspring of a mother with Noonan syndrome. *Clin Dysmorph* 1996; **5**: 61–64.
15 Lorenzetti ME, Fryns JP: Retinitis pigmentosa in a young man with Noonan syndrome: Further evidence that Noonan syndrome (NS) and the cardio-facio-cutaneous syndrome (CFC) are variable manifestations of the same entity? *Am J Med Genet* 1996; **65**: 97–99.
16 Chen B, Bronson RT, Klaman LD *et al*: Mice mutants for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. *Nat Genet* 2000; **24**: 296–299.
17 Fryns JP, Volcke PH, Van Den Berghe H: The Cardio-facio-cutaneous (CFC) syndrome: autosomal dominant inheritance in a large family. *Genet Counsel* 1991; **3**: 19–24.
18 Schollen E, Matthijs G, Legius E, Fryns J: Mutation in the gene for protein tyrosine phosphatase SHP-2 (*PTPN11*) in a large family with Noonan/cardio-facio-cutaneous syndrome. *Eur J Hum Genet* 2002; **10** (Suppl 1): 238, [abstract].