RESEARCH ARTICLE

Mutations of *ZFPM2/FOG2* Gene in Sporadic Cases of Tetralogy of Fallot

Antonio Pizzuti,[1,2]* Anna Sarkozy,[1,2] Anthea L. Newton,[3] Emanuela Conti,[2] Elisabetta Flex,[1,2] Maria Cristina Digilio,[4] Francesca Amati,[5] Debora Gianni,[1,2] Caterina Tandoi,[1,2] Bruno Marino,[6] Merlin Crossley,[3] and Bruno Dallapiccola[1,2]

[1]*Dipartimento di Medicina Sperimentale e Patologia, Università di Roma "La Sapienza," Rome, Italy;* [2]*Ospedale Casa Sollievo della Sofferenza San Giovanni Rotondo, Istituto Mendel Rome, Italy;* [3]*School of Molecular and Microbial Biosciences, University of Sydney, Australia;* [4]*Ospedale Pediatrico Bambino Gesù, Rome, Italy;* [5]*Dipartimento di Biopatologia e Diagnostica per Immagini, Università di Roma "Tor Vergata," Rome, Italy;* [6]*Dipartimento di Pediatria, Università di Roma "La Sapienza," Rome, Italy*

Communicated by Arnold Munnich

Two out of 47 patients with sporadic tetralogy of Fallot (TOF), the most common cyanotic conotruncal heart defect (CTD), showed heterozygous missense mutations of the *ZFPM2/FOG2* gene. Knockout mice carrying mutations in the *ZFPM2/FOG2* gene have similarly been found to exhibit TOF. While both mutant ZFPM2/FOG2 proteins, E30G (c.88A>G) and S657G (c.1968A>G), retain the ability to bind the partner protein GATA4 and repress GATA4 mediated gene activation, the S657G, but not the E30G, mutation is subtly impaired in this function. *ZFPM2/FOG2* gene mutations may contribute to some sporadic cases of TOF. Hum Mutat 22:372–377, 2003. © 2003 Wiley-Liss, Inc.

KEY WORDS: ZFPM2; FOG2; tetralogy of Fallot; TOF; conotruncal heart defect; CTD; GATA4

DATABASES:
ZFPM2 – OMIM: 603693, 187500 (TOF), 217095 (CTD); GenBank: AF119334 (cDNA), NT_008046 (genomic)

INTRODUCTION

Tetralogy of Fallot (TOF, MIM# 187500) is the most common cyanotic conotruncal heart defect (CTD, MIM# 217095), accounting for 2.5–3.5 per 10,000 live births [Ferencz et al., 1985]. CTDs are assumed to be multifactorial hereditary disorders. Analysis of recurrence risks, based on computer-modeling methods [Farral and Holder, 1992], has suggested that the best-fitting genetic model for these disorders would involve three loci acting by multiplication [Burn et al., 1998]. Familial cases of isolated TOF, either with autosomal recessive or dominant inheritance, have been described [Eldadah et al., 2001]. A total of 7% of trisomy 21 patients have TOF, which also occurs in other chromosomal disorders [Schinzel et al., 2001]. TOF is present in several less common syndromes [Marino et al., 1996], including DiGeorge/Velo-cardio-facial syndrome associated with deletion of chromosome 22q11.2, where it is found in 17% of the patients [Ryan et al., 1997]. Exclusion of 22q11.2 deletions does not modify the recurrence risk in isolated nonsyndromic TOFs, supporting the view that other gene(s), outside the 22q11.2 region, may be the cause of these defects [Digilio et al., 1997]. Benson et al. [1999] reported a single TOF individual with a missense mutation in the *NKX2.5* gene (MIM# 600584) that is a consistent cause of atrial, septal, and atrioventricular conduction defects. Low penetrant mutations in the same gene were detected in 4% of isolated TOF by Goldmuntz et al. [2001]. A missense mutation in the *JAG1* gene (MIM# 601920) was found in an extended kindred with autosomal dominant TOF and reduced penetrance [Eldadah et al., 2001].

ZFPM2/FOG2 (MIM# 603693) is an 1151–amino acid protein with eight zinc finger motifs, which is expressed during early heart development, and acts as a coregulator of the transcription factor GATA4 (MIM# 600576) [Svensson et al., 1999; Tevosian et al., 2000]. The orthologous human gene has been mapped to chromosome 8q22. Targeting experiments have recently shown that *ZFPM2/FOG2* gene mutations in mice can cause congenital heart defects (CHD), including tricuspid atresia and TOF [Tevosian et al., 2000; Svensson et al., 2000a]. We therefore screened the

Received 19 March 2003; accepted revised manuscript 29 May 2003.

*Correspondence to: Antonio Pizzuti, Istituto C.S.S.-Mendel, Viale Regina Margherita 261, 00198, Rome, Italy.
E-mail: a.pizzuti@css-mendel.it

Grant sponsors: Italian Ministry of Health; Italian Ministry of the University; Australian National Health and Medical Research Council; Grant sponsor: ItalianTelethon Foundation; Grant number: E723.

DOI 10.1002/humu.10261
Published online in Wiley InterScience (www.interscience.wiley.com).

human *ZFPM2/FOG2* gene for mutations in a series of sporadic nonsyndromic TOF patients.

MATERIALS AND METHODS

Mutation Detection

Forty-seven Italian patients, ranging in age from birth to 16.5 years (mean age ± SD = 3.2 ± 1.7 years), were selected based on strict phenotypic and cardiac criteria. Diagnoses of TOF were obtained by echocardiography and cardiac cateterization in all children. Patients showed neither major nor minor extracardiac features of DGS/VCFS, nor deletion of 22q11 and 10p13 DiGeorge syndrome regions, that were excluded using standard molecular protocols. Parental informed consent was obtained. The *ZFPM2/FOG2* gene coding regions, including exon–intron boundaries, were PCR amplified from genomic DNA. Primers were designed based on the cDNA sequence available in GenBank (AF119334) and the corresponding genomic regions (NT_008046). PCR products were screened by single strand conformation polymorphism analysis (SSCP; Genephor Unit; Amersham Pharmacia Biotech, Uppsala, Sweden). All fragments showing anomalous mobility shifts were sequenced (ABI PRISM 310 Genetic Analyser automated sequencer; Applied Biosystems, Foster City, CA). Samples showing DNA base changes were PCR amplified and sequenced twice.

Protein–Protein Interactions

The expression of glutathione-*S*-transferase (GST) fusion protein was performed in *Escherichia coli* BL21 as previously described [Holmes et al., 1999]. The GST fusion protein contained the N-terminal zinc finger of GATA4 (residues 200–254) cloned downstream and in frame with the GST protein contained in the vector pGEX2T. ^{35}S-labelled ZFPM2/FOG2 or ZFPM2/FOG2, containing the E30G or S657G mutations, were prepared by in vitro translation from pcDNA3-FLAG/ZFPM2/FOG2, pcDNA3-FLAG/ZFPM2/FOG2 E30G, or pcDNA3-FLAG/ZFPM2/FOG2 S657G using the TNT system (Promega, Madison, WI). In vitro binding assays were performed in 0.3 mL buffer (150 mM NaCl, 20 mM Tris HCl pH 7.5, 0.5% NP-40, 10 μM $ZnSO_4$, 0.25% bovine serum albumin, 1 mM β-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride). One microgram fusion protein attached to glutathione agarose beads was incubated with 2 μL radiolabelled ZFPM2/FOG2. After 1 hr incubation at 4°C, the agarose beads were washed four times with 1 mL binding buffer. The beads were recovered and bound material analysed by SDS-PAGE followed by imaging (Phosphorimager, Molecular Dynamics, Sunnyvale, CA).

Repression Assays

Cell culture manipulations were carried out using standard techniques. Briefly, NIH3T3 cells were cotransfected with 2 μg reporter construct, 1 μg GATA4 in pMT2, and 0, 0.5, 1, or 2 μg of wild-type, E30G or S657G ZFPM2/FOG2 in pcDNA3, using the calcium phosphate method as previously described [Fox et al., 1998, 1999]. The reporter consisted of the Brain Natriuretic Peptide (BNP) promoter (from –120 to +20) upstream of the firefly luciferase reporter gene in pGL3. Assays involving the minimal N-terminal repression domain of ZFPM2/FOG2 were also performed. NIH3T3 cells were cotransfected with 2 μg reporter construct and either 0, 5, 10, or 20 ng wild-type or E30G ZFPM2/FOG2 (residues 1–45) in pcDNA3-Gal4 DBD. The reporter consisted of five Gal4 sites upstream of the firefly luciferase reporter gene. All data shown are the results from duplicate experiments and have been normalised to Renilla luciferase levels derived from cotransfection with the control vector pRL-CMV (Promega).

RESULTS

We studied 47 sporadic TOF patients, eight of them with additional pulmonary atresia. All subjects underwent complete physical examination, excluding additional extra cardiac malformations. Cardiac examination was performed, as well as chest roentgenography and echocardiography. The diagnosis was confirmed at cardiac catheterisation and/or surgery. All patients showed viscero-atrial situs solitus, d-ventricular loop, and normally related great arteries. Chromosome 22q11 deletion was excluded in all the DNA samples by FISH analysis. Informed consent was obtained from all the studied subjects. *ZFPM2/FOG2* gene screening was performed by PCR exon amplification, SSCP, and automatic sequencing. In addition to common polymorphic changes, unique heterozygous missense mutations were found in two unrelated TOF patients. Both were A to G transitions: the first at codon 30 in exon 2 (c.88A>G, cDNA sequence GenBank Accession Number: AF119334) resulting in a Glu to Gly change (E30G); and the second at codon 657 in exon 8 (c.1968A>G), causing a Ser to Gly mutation (S657G) (Fig. 1). The mutations alter amino acid residues that are conserved between human and mouse ZFPM2/FOG2 sequences (Fig. 2). Given that Glycine has significantly different properties from the negatively charged residue (Glutamate) and the polar residue (Serine), we considered that these changes might alter the properties of ZFPM2/FOG2 and decided to investigate further.

The patient heterozygous for the E30G mutation showed TOF with pulmonary atresia, hypoplastic confluent pulmonary arteries, major aorto-pulmonary collateral arteries, and left aortic arch. This cardiac phenotype is prevalent in children with TOF and chromosome 22q11 deletion [Digilio et al., 1996] and is the same in three patients with *NKX2.5* gene mutations observed by Goldmuntz et al. [2001], confirming the role of genetic factors in this particular heart defect [Digilio et al., 1996]. The patient carrying the S657G mutation had TOF with a classic anatomy and left aortic arch. She presented episodes of ventricular tachicardia with atrio-ventricular dissociation. To address the possibility that these changes represented polymorphisms, rather than disease-causing mutations, 120 additional control DNAs obtained from unaffected individuals were screened by SSCP. No anomalous migration pattern, similar to those observed in the patients, was found. The whole *ZFPM2/FOG2* coding region was sequenced in both patients and no other mutation was identified. In order to trace the origin of the nucleotide changes, we sequenced the unaffected parents. The exon 2 mutation was inherited from the heterozygous father (Fig. 1A) and the exon 8 mutation from the mother. The mutation carrier parents underwent physical examination and echocardiography that resulted normal. The grandparents were not available for analysis.

We then tested whether the mutations altered the function of the ZFPM2/FOG2 protein. ZFPM2/FOG2 is

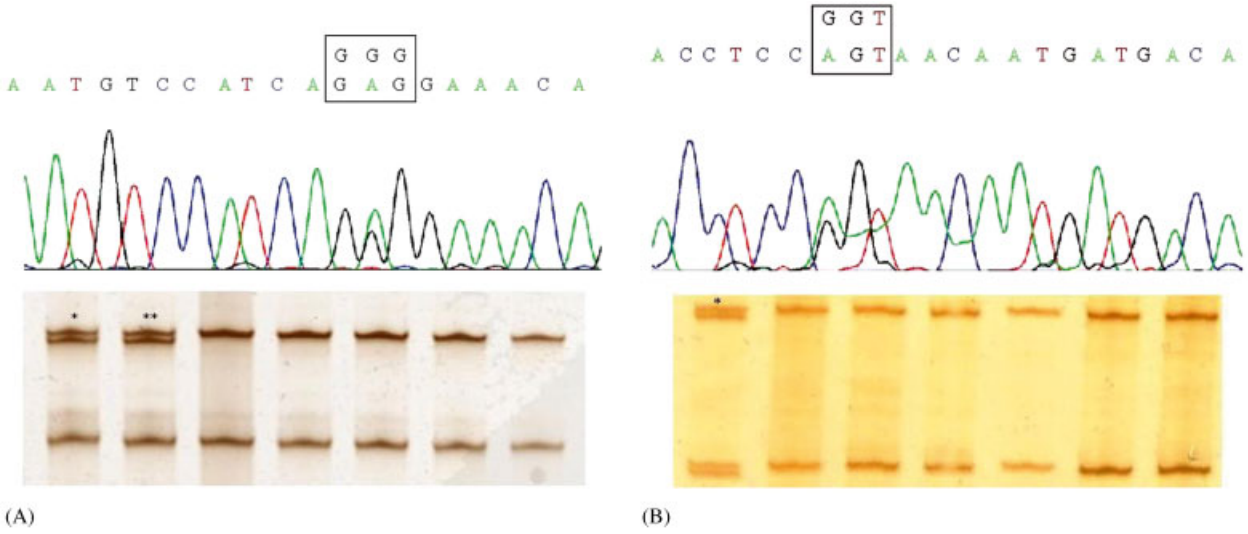

FIGURE 1. **SSCP and sequence analysis of the patients carrying the E30G (A) and the S657G (B) mutations. Anomalous patterns of DNA SSCP in TOF patients are indicated by asterisks (*). Two asterisks (**) label the anomalous SSCP pattern in the heterozygous unaffected father of the E30G patient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

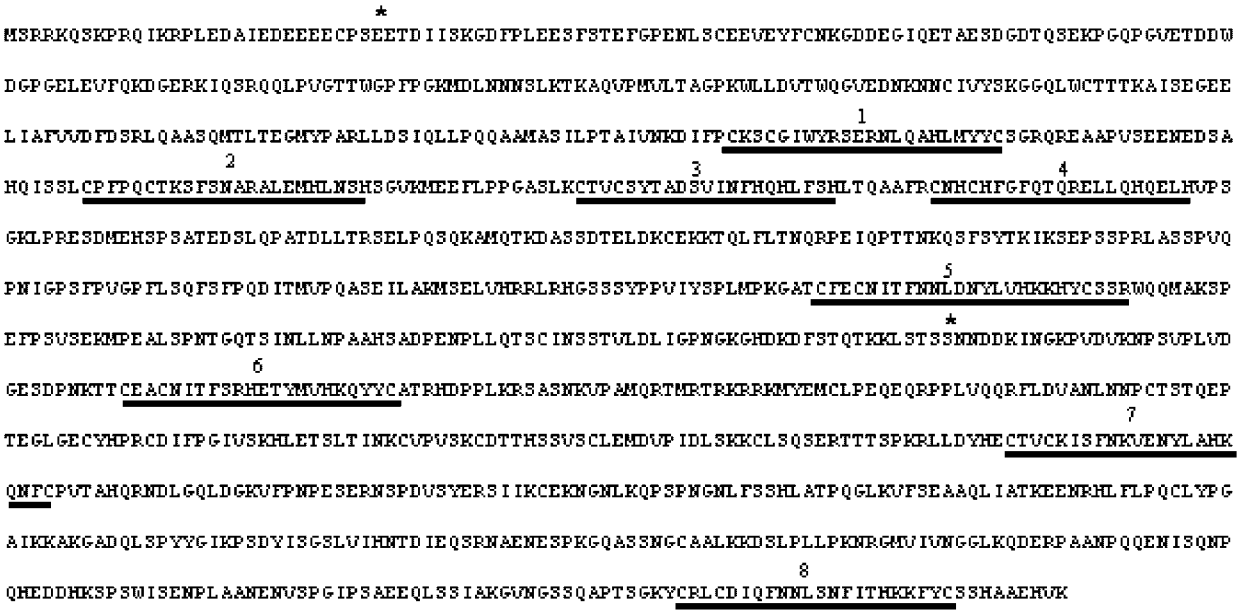

FIGURE 2. **Amino acid sequence of the human ZFPM2/FOG2 protein. The eight zinc finger motifs are underlined. The position of the E30G and S657G mutations are labelled with asterisks (*).**

known to function by binding to its partner, GATA4, and either activating or repressing GATA4 mediated gene expression, depending on promoter context [Holmes et al., 1999, Lu et al., 1999, Svennson et al., 2000a, 2000b, Tevonsian et al., 2000]. We first assessed whether the mutations interfered with the physical interaction between ZFPM2/FOG2 and GATA4, using the previously reported GST-pulldown assay [Holmes et al., 1999]. Radiolabelled normal ZFPM2/FOG2, ZFPM2/FOG2 carrying the E30G mutation, and ZFPM2/FOG2 carrying the S657G mutation were prepared by in vitro transcription and translation. The N-terminal zinc finger of GATA4 (the domain that is known to contact ZFPM2/FOG2) was prepared as a GST-GATA4-N-finger fusion protein [Holmes et al., 1999]. This material was immobilized on glutathione sepharose beads and its ability to retain the radiolabelled ZFPM2/FOG2 and mutant derivatives assessed (Fig. 3). As can be seen from the figure, the GATA4 N-finger was able to retain the normal and mutant forms of ZFPM2/FOG2, indicating that the mutations did not impair physical contact with GATA4.

Having established that the ZFPM2/FOG2 variants could still bind GATA4, we next investigated whether

they retained the ability to influence the transcriptional activity of GATA4. We tested their effect on expression of the Brain Natiuretic Peptide (BNP) promoter, a promoter which can be potently activated by GATA4 [Thuerauf et al., 1994]. We observed approximately 10-fold activation of this promoter by GATA4 (data not shown). On this promoter, ZFPM2/FOG2 serves to reduce GATA4-mediated activation (Fig. 4A). We cotransfected a reporter plasmid containing the BNP promoter driving the luciferase reporter gene together with a GATA4 expression vector and increasing amounts of a vector encoding normal ZFPM2/FOG2, or the mutant versions of ZFPM2/FOG2. We observed that normal ZFPM2/FOG2 repressed GATA4-mediated activation as expected. We noted that the E30G mutant and the S657G mutant versions of ZFPM2/FOG2 also reduced expression. It was notable, however, that while the repression mediated by the E30G mutant was comparable to that achieved by wild-type ZFPM2/FOG2, that mediated by the S657G variant was less than that observed with wild-type ZFPM2/FOG2 at all concentrations tested. This indicates that the S657G mutation causes a reproducible, but modest, reduction in the functional activity.

We wondered whether the failure to observe any major effect of the E30G mutation was due to the presence of additional repression domains in ZFPM2/FOG2 that may obscure subtle effects caused by a single mutation. It is known that ZFPM2/FOG2 contains more than one repression domain [Svennson et al., 2000b]. One domain lies at the N-terminus and encompasses the E30 site. A second domain lies further downstream and contains a PXDLS interaction motif that binds the corepressor C-terminal Binding Protein [Holmes et al., 1999]. We sought to test the activity of the N-terminal repression domain in isolation. We used the approach previously utilized by Svennson et al. [2000b]. The N-terminal repression domain was fused to the Gal4 DNA-binding domain and tested for its ability to repress a Gal4 target promoter driving luciferase activity. As shown in Figure 4B, and consistent with previous results [Svennson et al., 2000b], this domain represses gene expression. We then tested an equivalent construct carrying the E30G mutation. We observed equivalent repression. In conclusion, these assays do not show any significant effect of the E30G mutation on ZFPM2/FOG2 function.

DISCUSSION

Family studies have suggested that low penetrance gene mutations may cause nonsyndromic TOF. The most notable example is the 275 descendants of a man with TOF, 11 of which had CHDs, including TOF in three [Pitt, 1962]. Low penetrance of mutations was also observed in TOF patients with *NKX2.5* gene mutations [Goldmuntz et al., 2001]. Evidence suggesting that one of the TOF responsible genes is *ZFPM2/FOG2* derive from both targeting experiments in mice [Tevosian et al., 2000; Svensson et al., 2000a], and clinical observations showing that about 38% of patients with recombinant 8 syndrome have TOF [Gelb et al., 1991]. In these individuals, one of the chromosomal breakpoints is located at 8q22, where the *ZFPM2/FOG2* gene has been mapped, suggesting a possible relationship with *ZFPM2/FOG2* haploinsufficiency.

The precise mechanisms through which ZFPM2/FOG2 functions are not yet understood and the means by which the mutations alter its activity are accordingly difficult to define. The E30G mutation does not lie directly within one of the zinc finger domains through which ZFPM2/FOG2 contacts its partner GATA4 (i.e., zinc fingers 1, 5, 6, and 8) [Fox et al., 1999]. Accordingly, we did not observe any impairment of the ability of ZFPM2/FOG2 carrying this mutation to interact with GATA4. The E30G mutation lies within the N-terminal transcriptional repression domain of ZFPM2/FOG2 [Svennson et al., 2000b]. The mutation inserts a small neutral Glycine within a stretch of negatively charged amino acids and we expected this might alter the activity of ZFPM2/FOG2. Nevertheless, we did not observe any reduction in the ability of full length ZFPM2/FOG2 carrying the E30G mutation to repress GATA4-mediated transcription at the BNP promoter. When we tested the N-terminal repression domain in isolation, using a Gal4 fusion protein and a Gal4 dependent promoter, we did not observe any reduction in activity. The results are, however, limited by the fact that we have examined only two target genes and carried out assays only in a single test cell line (NIH3T3 cells). It is known that the in vivo activity of ZFPM2/FOG2 depends on both cell and promoter context and when other putative target genes are defined it will be interesting to test the effect of this mutation on the activity of other promoters. Previous work on the ZFPM2/FOG2-related protein, the erythroid/megakaryocytic coregulator FOG1, has shown that large segments of the protein can be deleted without affecting FOG1 (MIM# 601950) activity in cultured cells [Cantor et al., 2002]. Given these remarkable findings, a single missense mutation in the N-terminal repression domain might well be expected to have a relatively minor impact and be consistent with a low penetrance phenotype in vivo.

The S657G mutation does not lie within any of the zinc finger domains known to contact GATA4 and, consistent with this, we did not observe any reduction in the physical interaction between ZFPM2/FOG2 and GATA4. We did, however, observe a consistent reduction in repression of GATA4 mediate transactivation of the BNP promoter. The mechanism by which it impairs ZFPM2/FOG2 function is not known. A Serine to Glycine change could potentially disrupt the structure and folding of the protein (given that Glycine is less easily accommodated in α-helices than Serine, it is not polar and cannot make hydrogen bonds). Moreover, since Serine residues can be targets of phosphorylation, it is possible that the change to Glycine could prevent such a modification. Finally, it is possible that the change causes a structural change that alters protein–protein contacts made by ZFPM2/FOG2 or reduces its stability in vivo. In our assays, the effect on repression was

relatively modest, suggesting that the mutation slightly impairs, but does not ablate, the activity of the protein.

The present study shows that a few isolated TOF patients may harbor *ZFPM2/FOG2* gene mutations, similar to what has been observed for *NKX2.5* mutations [Goldmuntz et al., 2001], and supports the view that single gene defects can make a significant contribution to congenital heart disease, as mice model studies have suggested. The small number of mutation-positive patients does not allow any detailed phenotype/genotype correlation, even if pulmonary atresia may be more frequent as commented by Goldmuntz et al. [2001] for the *NKX2.5* mutations. In particular, the subtype with pulmonary atresia and major aorto-pulmonary collateral arteries seems to be in strong relation with genetic factors, since it is prevalent in association with deletion of chromosome 22q11 [DiGilio et al., 1996] and with *NKX2.5* mutations [Goldmuntz et al., 2001]. As for the TOF cases with *NKX2.5* involvement, the variability in expression, and the low penetrance associated with these monogenic mutations, suggests an important influence of environmental and other genetic factors in determining the cardiovascular phenotype, such as mutation/polymorphisms of the interacting transcription factor(s) and their enhancer sequences. A similar synergic activity between Tbx5 and Nkx2.5 promotes cardiomyocyte differentiation [Hintoi et al., 2001]. We analyzed the coding sequences of *GATA4* and *NKX2.5* genes in the *ZFPM2/FOG2*-mutated patients without finding any change.

Identification of the specific genes that may contribute to the development of congenital heart disease will help clinicians and researchers to better understand how this disease develops and provide improved counseling to families.

REFERENCES

Benson D-W, Silberbach G-M, Kavanaugh-McHugh A, Cottrill C, Zhang Y, Riggs S, Smalls O, Johnson M-C, Watson M-S, Seidman J-G, Seidman C-E, Plowden J, Kugler J-D. 1999. Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J Clin Invest 104:1567–1573.

Burn J, Brennan P, Little J, Holloway S, Coffey R, Somerville J, Dennis N-R, Allan L, Arnold R, Deanfield J-E, Godman M, Houston A, Keeton B, Oakley C, Scott O, Silove E, Wilkinson J, Pembrey M, Hunter A-S. 1998. Recurrence risks in offspring of adults with major heart defects: results from first cohort of British collaborative study. Lancet 351:311–316.

Cantor A-B, Katz S-G, Orkin S-H. 2002. Distinct domains of the GATA-1 cofactor FOG-1 differentially influence erythroid versus megakaryocytic maturation. Mol Cell Biol 22: 4268–4279.

Digilio M-C, Marino B, Grazioli S, Agostino D, Giannotti A, Dallapiccola B. 1996. Comparison of occurrence of genetic syndromes in ventricular septal defects with pulmonic stenosis (classic tetralogy of Fallot) versus ventricular septal defects with pulmonary atresia. Am J Cardiol 77:1375–1376.

Digilio M-C, Marino B, Giannotti A, Toscano A, Dallapiccola B. 1997. Recurrence risk figures for isolated tetralogy of Fallot after screening for 22q11 microdeletion. J Med Genet 34: 188–190.

Eldadah Z-A, Hamosh A, Biery N-J, Montgomery R-A, Duke M, Elkins R, Dietz H-C. 2001. Familial tetralogy of Fallot caused by mutation in the jagged1 gene. Hum Mol Genet 10:163–169.

Farrall M, Holder S. 1992. Familial recurrence-pattern analysis of cleft lip with or without cleft palate. Am J Hum Genet 50: 270–277.

Ferencz C, Rubin J-D, McCarter R-J, Brenner J-I, Neill C-A, Perry L-W, Hepner S-I, Downing J-W. 1985. Congenital heart disease: prevalence at livebirth. The Baltimore-Washington infant study. Am J Epidemiol 121:31–36.

Fox A-H, Kowalski K, King G-F, Mackay J-P, Crossley M. 1998. Key residues characteristic of GATA N-fingers are recognized by FOG. J Biol Chem 273:33595–33603.

Fox A-H, Liew C, Holmes M, Kowalski K, Mackay J, Crossley M. 1999. Transcriptional cofactors of the FOG family interact with GATA proteins by means of multiple zinc fingers. EMBO J 18:2812–2822.

Gelb B-D, Towbin J-A, McCabe E-R, Sujansky E. 1991. San Luis Valley recombinant chromosome 8 and tetralogy of Fallot: a review of chromosome 8 anomalies and congenital heart disease. Am J Med Genet 40:471–476.

Goldmuntz E, Geiger E, Benson W. 2001. NKX2.5 mutations in patients with tetralogy of Fallot. Circulation 104:2565–2568.

Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, Nagai R, Komuro I. 2001. Tbx5 associates with Nkx2-5 and synergistically promotes cardiomyocyte differentiation. Nat Genet 28:276–280.

Holmes M, Turner J, Fox A, Chisholm O, Crossley M, Chong B. 1999. hZFPM2/FOG2, a novel zinc finger protein, binds the co-repressor mCtBP2 and modulates GATA-mediated activation. J Biol Chem 274:23491–23498.

Lu J-R, McKinsey T-A, Xu H, Wang D-Z, Richardson J-A, Olson E-N. 1999. ZFPM2/FOG2, a heart- and brain-enriched cofactor for GATA transcription factors. Mol Cell Biol 19:4495–4502.

Marino B, Digilio M-C, Grazioli S, Formigari R, Mingarelli R, Giannotti A, Dallapiccola B. 1996. Associated cardiac anomalies in isolated and syndromic patients with tetralogy of Fallot. Am J Cardiol 77:505–508.

Pitt D-B. 1962. A family study of Fallot's tetrad. Ann Med 11: 179–183. (Australian)

Ryan A-K, Goodship J-A, Wilson D-I, Philip N, Levy A, Seidel H, Schuffenhauer S, Oechsler H, Belohradsky B, Prieur M, Aurias A, Raymond F-L, Clayton-Smith J, Hatchwell E, McKeown C, Beemer F-A, Dallapiccola B, Novelli G, Hurst J-A, Ignatius J, Green A-J, Winter R-M, Brueton L, Brondum-Nielsen K, Scambler P-J. 1997. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet 34:798–804.

Schinzel A. 2000. Catalog of unbalanced chromosome aberrations in man. Berlin: Walter de Gruyter 264p.

Svensson E-C, Tufts R-L, Polk C-E, Leiden J-M. 1999. Molecular cloning of ZFPM2/FOG2: a modulator of transcription factor GATA4 in cardiomyocytes. Proc Natl Acad Sci USA 96: 956–961.

Svensson E-C, Huggins G-S, Lin H, Clendenin C, Jiang F, Tufts R, Dardik F-B, Leiden J-M. 2000a. A syndrome of tricuspid atresia in mice with a targeted mutation of the gene encoding ZFPM2/FOG2. Nat Genet 25:353–356.

Svensson E-C, Huggins G-S, Dardik F-B, Polk C-E, Leiden J-M. 2000b. A functionally conserved N-terminal domain

of the friend of GATA-2 (ZFPM2/FOG2) protein represses GATA4-dependent transcription. J Biol Chem 275: 20762–20769.

Tevosian S-G, Deconinck A-E, Tanaka M, Schinke M, Litovsky S-H, Izumo S, Fujiwara Y, Orkin S-H. 2000. ZFPM2/FOG2, a cofactor for GATA transcription factors, is essential for heart morphogenesis and development of coronary vessels from epicardium. Cell 101:729–739.

Thuerauf D-J, Hanford D-S, Glembotski C-C. 1994. Regulation of rat brain natriuretic peptide transcription. A potential role for GATA-related transcription factors in myocardial cell gene expression. J Biol Chem 269:17772–17775.