CMLS, Cell. Mol. Life Sci. 58 (2001) 1296–1300
1420-682X/01/091296-05 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Human Genome and Diseases: Review

Transcription factor GATA3 and the human HDR syndrome

H. Van Esch and K. Devriendt*

Centre for Human Genetics, University of Leuven, Herestraat 49, 3000 Leuven (Belgium), Fax: +32 16 34 6051, e-mail: Koen.Devriendt@med.kuleuven.ac.be

Received 12 February 2001; received after revision 28 March 2001; accepted 28 March 2001

Abstract. Recently, a member of the GATA-binding family of transcription factors was shown to be involved in the human hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome. Deletion-mapping studies and subsequent mutation analysis revealed that haploinsufficiency for GATA3 is the underlying mechanism of the HDR syndrome. Here we discuss the clinical characteristics of the HDR syndrome and present an overview of the role of GATA3 and related GATA-binding transcription factors during vertebrate embryonic development and their involvement in human disease.

Key words. GATA3; transcription factor; HDR syndrome; DiGeorge; embryonic development.

The human HDR syndrome

The combination of hypoparathyroidism, sensorineural deafness and renal anomalies was described for the first time in 1992 by Bilous et al. [1] in a family with autosomal dominant hypoparathyroidism, sensorineural deafness and renal dysplasia (fig. 1), and termed the HDR syndrome. This autosomal dominant malformation syndrome represented a new clinical entity and was given a separate entry in the Mendelian Inheritance in Man catalogue (MIM 146255, [2]). Since then, only a few other patients with HDR have been reported; they include a patient with HDR reported by Beetz et al. [3] and a family with partial HDR reported by Watanabe et al. [4]. This latter family features autosomal dominant hypoparathyroidism and sensorineural deafness without renal dysplasia. Besides these patients, two further families were reported by Barakat et al. [5] and Shaw et al. [6], but in these families, the pattern of inheritance is not clear and the renal anomalies are not well defined.

Clinically, the hypoparathyroidism is characterised by low serum calcium as a result of deficient parathyroid hormone (PTH) secretion. PTH levels measured in serum of HDR patients range from low-normal to undetectable. This leads to symptomatic or subclinical manifestations such as hypocalcaemic seizures, calcium deposits and bone demineralisation. The deafness found in HDR patients is of the sensorineural type, bilateral and present at birth. The hearing loss is more pronounced at the higher frequencies and the severity ranges from moderate to severe, necessitating the use of hearing aids. The renal anomalies observed in HDR patients belong to a spectrum of malformations including renal hypo- and dysplasia, vesico-ureteral reflux and agenesis of the kidney. Penetrance of these renal anomalies is variable, since some HDR patients lack renal abnormalities. However, renal malformations may be subtle or even resolve spontaneously with age, and remain therefore undetected.

Unravelling the molecular basis of the HDR syndrome

The gene responsible for the HDR syndrome was identified during a detailed study of individuals with the DiGeorge syndrome (MIM 188400). These patients present abnormalities in organs derived from the third and fourth

* Corresponding author.

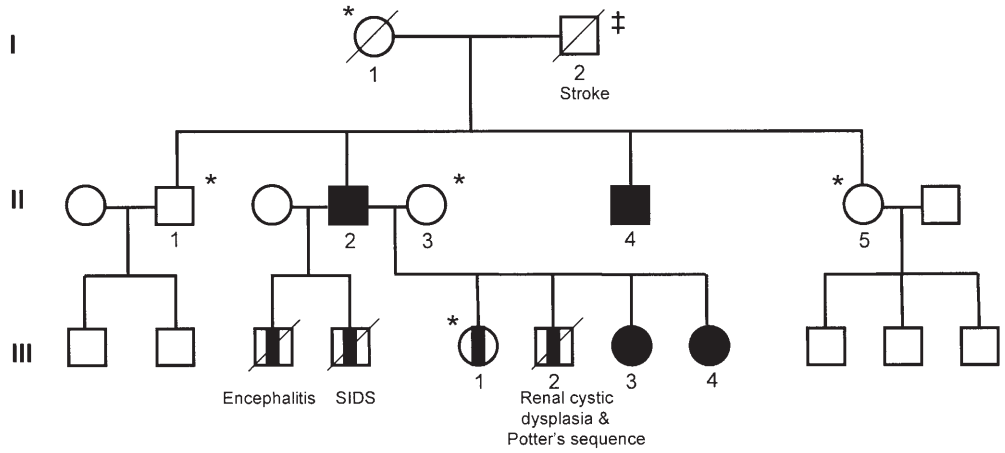

Figure 1. Pedigree of the family reported by Bilous et al. [1] with autosomal dominant hypoparathyroidism, sensorineural deafness and renal dysplasia. Solid symbols represent family members affected with HDR. Striped symbols represent family members who were possibly affected. The cause of death in the deceased family members is listed below the symbol. SIDS, sudden infant death syndrome; * normocalcaemia and normal audiometric results; ‡ normal kidneys and parathyroids on post-mortem examination.

branchial arches, including the parathyroid glands, thymus and outflow tract of the heart. A microdeletion in chromosome 22q11 is present in the majority of DiGeorge patients. However, in a small group of patients, there is evidence of deletion or aberration in chromosome 10p. Since the first description by Elliot et al. [7], more than 40 patients with a "DiGeorge-like" phenotype and a partial deletion of chromosome 10p have been reported in the literature [8]. Molecular deletion analyses of these patients have delineated two non-overlapping regions on chromosome 10p that contribute to this phenotype. Terminal 10p deletions (10p14–10pter) are associated with hypoparathyroidism, renal anomalies and sensorineural deafness, whereas interstitial deletions (10p13–14) are associated with cardiac malformations and immune deficiency (fig. 2) [9–13].

As has often been the case in disease gene discovery, a patient carrying a rare cytogenetic reciprocal insertional translocation [13] enabled cloning of the gene responsible for the HDR syndrome. Using deletion-mapping studies in this patient and in the original reported HDR family [1], a critical HDR region on chromosome 10p14–15 was delineated, which contains the *GATA3* gene (fig. 2). Mutation analysis in HDR patients without cytogenetic abnormalities confirmed the involvement of GATA3 in the human HDR syndrome [14]. All mutations found thus far result in absence of DNA binding by the mutant GATA3 protein, leading to haploinsufficiency.

GATA3 belongs to an important family of transcription factors

GATA3 belongs to a family of transcription factors that bind to a GATA consensus motif (A/TGATAA/G) through a highly conserved C4 zinc finger domain [15]. Six members (GATA1–GATA6) of this family have been identified, all showing distinctive tissue-specific expression [16], and playing an essential role during vertebrate development [17–20]. Among different vertebrate species, the GATA proteins are also highly conserved with, for example in the case of GATA3, a 96% amino acid (aa) identity between human and mouse, 92% aa identity between human and chicken and 80% aa identity between human and Xenopus species. Even in invertebrates, GATA family members have been identified, such as the *Caenorhabditis elegans* protein *elt-1* [21] and several single-finger GATA-binding fungal factors [22–24].

The expression pattern of *GATA3* during embryonic development, at least at the tissue level, is highly conserved among different vertebrates [25–28]. In human embryos, *GATA3* expression can be detected from the beginning of the 4th week of gestation [29]. From then on, *GATA3* transcripts are observed in various developing embryonic and foetal tissues, including the developing kidney [30], the parathyroids and the inner ear [29], consistent with the human HDR phenotype.

A homozygous *Gata3* knock-out mouse has been reported by Pandolfi et al. [18] and displays multiple organ abnormalities, especially of the central nervous system together with a total block of T-cell differentiation and massive internal bleeding, resulting in lethality at midgestation. This embryonic lethality is partially due to a noradrenaline deficiency of the sympathetic nervous system, as shown by pharmacological rescue of mutant mice using catechol intermediates [31]. In these mice, later-occurring embryonic defects mimicking the human pathology could be detected, encompassing the kidney and cephalic neural crest [31]. In contrast to human GATA3 haploinsufficiency, heterozygous *Gata3* knock-out mice

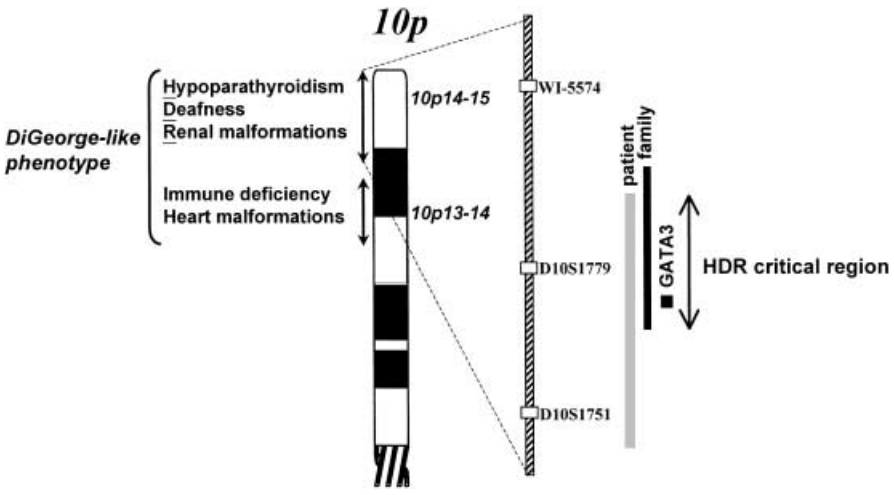

Figure 2. Schematic representation of the two non-overlapping regions on chromosome 10p, the HDR region at 10p14–15 and the region for heart malformations and immune deficiency at 10p13–14. The microdeletions (grey and black solid bars) found in the translocation patient and in the reported HDR family overlap, thereby delineating a critical HDR region (between the arrowheads). The GATA3 gene (black square) maps within the HDR critical region. Nearby polymorphic microsatellite markers are shown (open boxes). Not drawing to scale.

were reported to be normal. However, careful re-examination of these animals showed the presence of sensorineural deafness in the heterozygous mutants (F. Grosveld, personal communication). This was already suspected from detailed *GATA3* expression studies during ear morphogenesis in normal and mutant mice [32].

The precise pathogenesis of the developmental anomalies caused by GATA3 haploinsufficiency is unknown and difficult to assess at this time. To date, no potential target genes of GATA3 are known in the developing kidney, inner ear or parathyroids. It will be interesting to determine if related GATA3-regulated developmental pathways are present in these functionally and morphogenetically different organs. However, the expression of *GATA3* in human and mouse embryos is not restricted to these organs alone. *GATA3* expression can also be detected in the foregut, the liver, the developing eye, the branchial arches and in different regions of the developing brain and peripheral nervous system [25, 29]. This indicates that these developing tissues and organs might be less susceptible to GATA3 haploinsufficiency. Alternatively, redundancy by other GATA family members in these organs cannot be excluded. So far, redundancy for GATA transcription factors has been observed in haematopoiesis, where GATA2 [19], and GATA3 can partially rescue GATA1 loss of function [33]. A close interaction between GATA2 and GATA3 has also been detected in the developing hindbrain. Temporally, *GATA2* expression precedes that of *GATA3* and is required to initiate *GATA3* expression in rhombomere 4 [34].

Thus far, in two HDR patients, no mutations in the coding part of the *GATA3* gene have been found [14]. The presence of mutations in the intron sequences or in the regulatory sequences flanking the gene, however, cannot be excluded. These transcriptional regulatory elements are located at substantial distances 5′ and 3′ to the gene, as shown in experiments with transgenic mice [35, 36]. On the other hand, the absence of GATA3 mutations in these patients may indicate non-allelic genetic heterogeneity for the HDR syndrome. The existence of specific GATA3-interacting proteins may present a possible alternative. Two GATA cofactors have been identified. Friend of GATA1 (FOG1), a multitype zinc finger protein, interacts with GATA1 and is predominantly expressed in haematopoietic lineages, as is GATA1 [37]. Mice lacking FOG1 display a similar block in erythroid cell maturation as is seen in GATA1 null mutants [38], indicating close co-operation during erythroid and megakaryocytic cell differentiation. In humans, the importance of this GATA1-FOG1 interaction was recently illustrated in a family with dyserythropoietic anaemia and thrombocytopenia due to a *GATA1* mutation affecting this multi-protein complex [39]. For GATA4, a similar interaction with another cofactor, FOG2, is present. *GATA4* is expressed in the developing heart, gut and gonads [40] and *Gata4* knock-out mice display defects in early cardiac and gut morphogenesis [17, 41]. In humans, also, GATA4 haploinsufficiency is suspected to cause the cardiac malformations observed in patients with partial chromosome 8p deletions which include the *GATA4* gene [42]. It is of interest that targeted mutation of the *FOG2* gene in mice also leads to a cardiac malformation, namely tricuspid valve atresia [43].

Besides these two cofactors, a whole array of other proteins has been described that interact with GATA proteins and function as transcriptional activators or repressors

[44]. The tissue and organ specificity of these widely expressed GATA transcription factors is likely orchestrated by cell type-specific interactions with other transcription factors, which are expressed in a restricted pattern.

Perhaps one of the most unexpected findings is the absence of immune deficiency in patients with GATA3 haploinsufficiency. None of the patients that were tested suffered from recurrent or clinically significant infections, and their lymphocyte counts, B and T subsets and functional tests were normal [14]. This is surprising, since GATA3 is known to play an essential role in T-cell development [18, 45]. GATA3, GATA1 and GATA2 are necessary factors for haematopoiesis: gene disruption of any of them results in major haematopoietic defects in the mouse [19, 20, 45]. However, *GATA1, GATA2* and *GATA3* display different lineage-restricted patterns of expression. GATA3 is expressed mainly in very immature haematopoietic progenitors and subsequently only in the T-cell and natural killer-cell lineages. In *Gata3–/–* mice, T-cell differentiation is blocked at the earliest stages of thymocyte development [45]. Based on these findings, and in analogy with GATA1 mutations, one might expect T-cell abnormalities in patients lacking one functional GATA3 allele. However, thus far, no abnormalities have been found. Redundancy by other GATA factors can be excluded, since *GATA3* is the only GATA factor expressed in the T-cell lineage. This suggests that these haematopoietic developmental processes are probably less or not susceptible to haploinsufficiency of GATA3, compared to the morphogenetic events. The immune abnormalities observed in patients with large 10p deletions must be caused by other genes on 10p, most likely located within the region 10p13–14 (fig. 2).

In conclusion, besides unravelling the molecular cause of the human HDR syndrome, the finding that GATA3 haploinsufficiency results in a distinct pattern of malformations will stimulate further research into the developmental role of this transcription factor and related proteins.

1 Bilous R. W., Murty G., Parkinson D. B., Thakker R. V., Coulthard M. G., Burn J. et al. (1992) Autosomal dominant familial hypoparathyroidism, sensorineural deafness, and renal dysplasia. N. Engl. J. Med. **327:** 1069–1074

2 McKusick V. A. (1998) Medelian Inheritance in Man. Catalogs of Human Genes and Genetic Disorders, Johns Hopkins University Press, Baltimore, Ma, USA

3 Beetz R., Zorowka P., Schonberger W., Kruse K., Wilichowski E., Zabel B. et al. (1997) Hypoparathyroidismus und Innenohrschwerhorigkeit. Monatsschr. Kinderheilkd. **145:** 347– 352

4 Watanabe T., Mochizuki H., Kohda N., Minamitani K., Minagawa M., Yasuda T. et al. (1998) Autosomal dominant familial hypoparathyroidism and sensorineural deafness without renal dysplasia. Eur. J. Endocrinol. **139:** 631–634

5 Barakat A. Y., D'Albora J. B., Martin M. M. and Jose P. A. (1977) Familial nephrosis, nerve deafness, and hypoparathyroidism. J. Pediatr. **91:** 61–64

6 Shaw N. J., Haigh D., Lealmann G. T., Karbani G., Brocklebank J. T. and Dillon M. J. (1991) Autosomal recessive hypoparathyroidism with renal insufficiency and developmental delay. Arch. Dis. Child. **66:** 1191–1194

7 Elliott D., Thomas G. H., Condron C. J., Khuri N. and Richardson F. (1970) C-group chromosome abnormality (10p–?): Occurrence in a child with multiple malformations. Am. J. Dis. Child. **119:** 72–73

8 Van Esch H., Groenen P., Fryns J. P., Van de Ven W. and Devriendt K. (1999) The phenotypic spectrum of the 10p deletion syndrome versus the classical DiGeorge syndrome. Genet. Couns. **10:** 59–65

9 Dasouki M., Jurecic V., Phillips J. A., Whitlock J. A. and Baldini, A. (1997) DiGeorge anomaly and chromosome 10p deletions: one or two loci? Am. J. Med. Genet. **73:** 72–75

10 Gottlieb S., Driscoll D. A., Punnett H. H., Sellinger B., Emanuel B. S. and Budarf M. L. (1998) Characterization of 10p deletions suggests two nonoverlapping regions contribute to the DiGeorge syndrome phenotype. Am. J. Hum. Genet. **62:** 495–498

11 Lichtner P., Konig R., Hasegawa T., Van Esch H., Meitinger T. and Schuffenhauer S. (2000) An HDR (hypoparathyroidism, deafness, renal dysplasia) syndrome locus maps distal to the DiGeorge syndrome region on 10p13/14. J. Med. Genet. **37:** 33–37

12 Fujimoto S., Yokochi K., Morikawa H., Nakano M., Shibata H., Togari H. et al. (1999) Recurrent cerebral infarctions and del(10)(p14p15.1) de novo in HDR (hypoparathyroidism, sensorineural deafness, renal dysplasia) syndrome. Am. J. Med. Genet. **86:** 427–429

13 Van Esch H., Groenen P., Daw S., Poffyn A., Holvoet M., Scambler P. et al. (1999) Partial DiGeorge syndrome in two patients with a 10p rearrangement. Clin. Genet. **55:** 269–276

14 Van Esch H., Groenen P., Nesbit M. A., Schuffenhauer S., Lichtner P., Vanderlinden G. et al. (2000) GATA3 haplo-insufficiency causes human HDR syndrome. Nature **406:** 419–422

15 Labastie M. C., Bories D., Chabret C., Gregoire J. M., Chretien S. and Romeo P. H. (1994) Structure and expression of the human GATA3 gene. Genomics **21:** 1–6

16 Simon M. C. (1995) Gotta have GATA. Nat. Genet. **11:** 9–11

17 Kuo C. T., Morrisey E. E., Anandappa R., Sigrist K., Lu M. M., Parmacek M. S. et al. (1997) GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev. **11:** 1048–1060

18 Pandolfi P. P., Roth M. E., Karis A., Leonard M. W., Dzierzak E., Grosveld F. G. et al. (1995) Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis. Nat. Genet. **11:** 40–44

19 Pevny L., Simon M. C., Robertson E., Klein W. H., Tsai S. F., D'Agati V. et al. (1991) Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature **349:** 257-60

20 Tsai F. Y., Keller G., Kuo F. C., Weiss M., Chen J., Rosenblatt M. et al. (1994) An early haematopoietic defect in mice lacking the transcription factor GATA-2. Nature **371:** 221–226

21 Spieth J., Shim Y. H., Lea K., Conrad R. and Blumenthal T. (1991) Elt-1, an embryonically expressed *Caenorhabditis elegans* gene homologous to the GATA transcription factor family. Mol. Cell. Biol. **11:** 4651–4659

22 Minehart P. L. and Magasanik B. (1991) Sequence and expression of GLN3, a positive nitrogen regulatory gene of *Saccharomyces cerevisiae* encoding a protein with a putative zinc finger DNA-binding domain. Mol. Cell. Biol. **11:** 6216–6228

23 Lowry J. A. and Atchley W. R. (2000) Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain. J. Mol. Evol. **50:** 103–115

24 Venter J. C., Adams M. D., Myers E. W., Li P. W., Mural R. J., Sutton G. G. et al. (2001) The sequence of the human genome. Science **291:** 1304–1351

25 George K. M., Leonard M. W., Roth M. E., Lieuw K. H., Kioussis D., Grosveld F. et al. (1994) Embryonic expression and

cloning of the murine GATA-3 gene. Development **120:** 2673–2686
26 Kornhauser J. M., Leonard M. W., Yamamoto M., LaVail J. H., Mayo K. E. and Engel J. D. (1994) Temporal and spatial changes in GATA transcription factor expression are coincident with development of the chicken optic tectum. Brain Res. Mol. Brain Res. **23:** 100–110
27 Neave B., Rodaway A., Wilson S. W., Patient R. and Holder N. (1995) Expression of zebrafish GATA 3 (gta3) during gastrulation and neurulation suggests a role in the specification of cell fate. Mech. Dev. **51:** 169–182
28 Zon L. I., Mather C., Burgess S., Bolce M. E., Harland R. M. and Orkin S. H. (1991) Expression of GATA-binding proteins during embryonic development in *Xenopus laevis*. Proc. Natl. Acad. Sci. USA **88:** 10642–10646
29 Debacker C., Catala M. and Labastie M. C. (1999) Embryonic expression of the human GATA-3 gene. Mech. Dev. **85:** 183–187
30 Labastie M. C., Catala M., Gregoire J. M. and Peault B. (1995) The GATA-3 gene is expressed during human kidney embryogenesis. Kidney Int. **47:** 1597–1603
31 Lim K. C., Lakshmanan G., Crawford S. E., Gu Y., Grosveld F. and Engel J. D. (2000) Gata3 loss leads to embryonic lethality due to noradrenaline deficiency of the sympathetic nervous system. Nat. Genet. **25:** 209–212
32 Karis A., Pata I., Doorninck J. H. van, Grosveld F., Zeeuw C. I. de, Caprona D. de et al. (2001) Transcription factor GATA-3 alters pathway selection of olivocochlear neurons and affects morphogenesis of the ear. J. Comp. Neurol. **429:** 615–630
33 Tsai F. Y., Browne C. P. and Orkin S. H. (1998) Knock-in mutation of transcription factor GATA-3 into the GATA-1 locus: partial rescue of GATA-1 loss of function in erythroid cells. Dev. Biol. **196:** 218–227
34 Pata I., Studer M., Doorninck J. H. van, Briscoe J., Kuuse S., Engel J. D. et al. (1999) The transcription factor GATA3 is a downstream effector of Hoxb1 specification in rhombomere 4. Development **126:** 5523–5531
35 Lakshmanan G., Lieuw K. H., Grosveld F. and Engel J. D. (1998) Partial rescue of GATA-3 by yeast artificial chromosome transgenes. Dev. Biol. **204:** 451–463
36 Lakshmanan G., Lieuw K. H., Lim K. C., Gu Y., Grosveld F., Engel J. D. et al. (1999) Localization of distant urogenital system-, central nervous system-, and endocardium-specific transcriptional regulatory elements in the GATA-3 locus. Mol. Cell. Biol. **19:** 1558–1568
37 Tsang A. P., Visvader J. E., Turner C. A., Fujiwara Y., Yu C., Weiss M. J. et al. (1997) FOG, a multitype zinc finger protein, act as a cofactor for transcription factor GATA-1 in erythroid and megakaryocytic differentiation. Cell **90:** 109–119
38 Tsang A. P., Fujiwara Y., Hom D. B. and Orkin S. H. (1998) Failure of megakaryopoiesis and arrested erythropoiesis in mice lacking the GATA-1 transcriptional cofactor FOG. Genes Dev. **12:** 1176–1188
39 Nichols K. E., Crispino J. D., Poncz M., White J. G., Orkin S. H., Maris J. M. et al. (2000) Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in GATA1. Nat. Genet. **24:** 266–270
40 Arceci R. J., King A. A., Simon M. C., Orkin S. H. and Wilson D. B. (1993) Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. Mol. Cell. Biol. **13:** 2235–2246
41 Molkentin J. D., Lin Q., Duncan S. A. and Olson E. N. (1997) Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. Genes Dev. **11:** 1061–1072
42 Devriendt K., Matthijs G., Van Dael R., Gewillig M., Eyskens B., Hjalgrim H. et al. (1999) Delineation of the critical deletion region for congenital heart defects, on chromosome 8p23.1. Am. J. Hum. Genet. **64:** 1119–1126
43 Svensson E. C., Huggins G. S., Lin H., Clendenin C., Jiang F., Tufts R. et al. (2000) A syndrome of tricuspid atresia in mice with a targeted mutation of the gene encoding Fog-2. Nat. Genet. **25:** 353–356
44 Molkentin J. D. (2000) The zinc finger-containing transcription factors GATA-4, -5, and -6. J. Biol. Chem. **275:** 38949–38952
45 Ting C. N., Olson M. C., Barton K. P. and Leiden J. M. (1996) Transcription factor GATA-3 is required for development of the T-cell lineage. Nature **384:** 474–478