

## Forkhead genes and human disease

Robert P. ERICKSON

Angel Charity for Children – Wings for Genetic Research, Steele Memorial Children's Research Center, Department of Pediatrics, Section of Medical and Molecular Genetics, University of Arizona College of Medicine, Tucson, Arizona, USA

**Abstract.** Forkhead, or Fox-box genes, code for winged helix transcription factors that make up a multi-gene family. Two human genetic diseases have recently been associated with loss of function of one allele of different Fox-box genes: Axenfeld-Rieger anomaly of the anterior eye chamber associated with haploinsufficiency of FOXC1 and lymphedema-distichiasis associated with haploinsufficiency of FOXC2. Earlier, both genes had been studied intensively for their transcription patterns and for the phenotypes of knockouts. These studies are reviewed and related to the phenotypes found in the two human disorders.

**Key words:** forkhead genes, winged helix domains, mesodermal development, Axenfeld-Rieger anomaly, lymphedema-distichiasis.

The forkhead homology is a 100-amino acid motif that was first identified as a region of similarity between the product of the homeotic *Drosophila* gene forkhead and hepatocyte nuclear factor (HNF3) (WEIGEL, JACKLE 1990). The unifying feature of this family of transcription factors is the shared 100-amino acid DNA-binding domain known as a "FOX", for forkhead box, domain (KAESTNER et al. 2000). The X-ray crystallography of the complex of HNF3 with its DNA-binding target has led to an understanding of the structure of this DNA-binding motif (CLARK et al. 1993); a variant of the helix-turn-helix motif made up of three  $\alpha$ -helices and two characteristic large loops, or "wings" (Figure 1). Thus, the Fox-box domain has also been termed a "winged helix" DNA-binding domain.

Received: December 5, 2000. Accepted: December 8, 2000.

Correspondence: R.P. ERICKSON, Department of Pediatrics/4341A, 1501 N. Campbell Avenue, P.O. Box 245073, Tucson, Arizona 85724-5073, USA, e-mail: erickson@peds.arizona.edu

Outside the DNA-binding domain, Fox-box transcription factors can be quite different. Variation in the DNA-binding domain allows the clustering of chordate Fox-box sequences into 15 sub-classes based on the phylogenetic tree program, Clustal W (KAESTNER et al. 2000). Thus, studies comparing various forkhead genes in development or disease must take into account, not only the non-Fox-box sequence but also the subclass for the Fox-box domain.

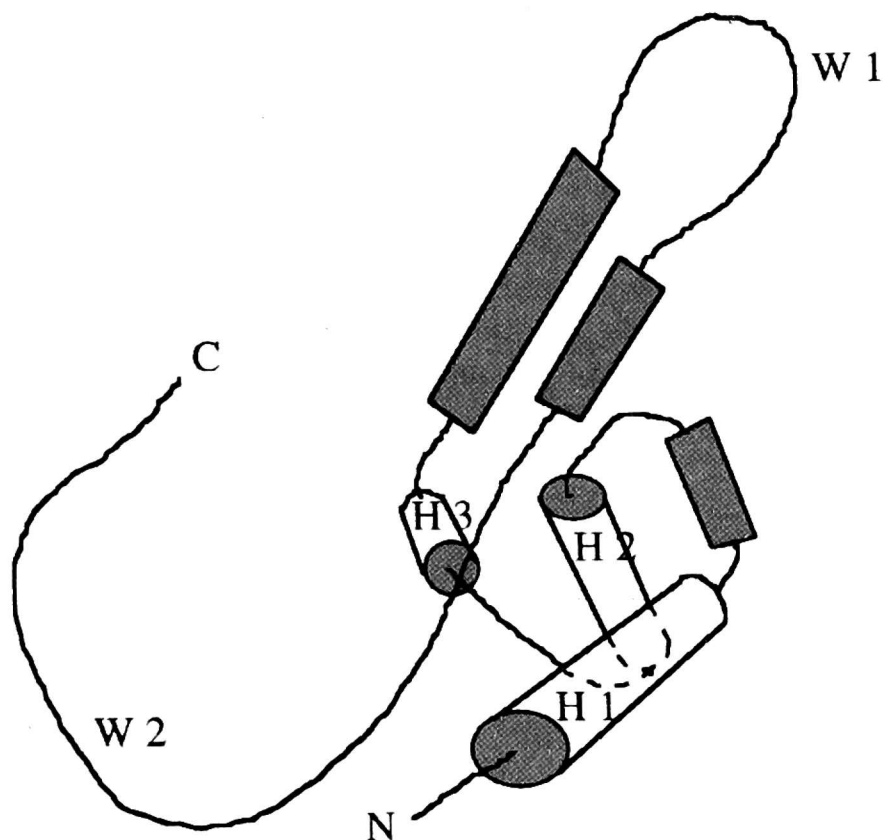


Figure 1. Structure of winged-helix (structure determined in association with DNA) after CLARK et al. 1993. "Tubes": 3 alpha-helices (H1-H3); "tapes":  $\beta$ -structure; single lines (dotted under H1) open structure of amino acid chain forming wings 1 and 2.

This paper reviews studies of two genes: 1) forkhead-like 7 (FK4L7) in man and MF1 in mice, now known as *FOXC1* and *Foxc1*, respectively and 2) *MFH1* in man and mice, known as *FOXC2* and *Foxc2*, respectively.

Studies of forkhead genes in yeast have amplified the importance of these conserved DNA-binding domains in transcription and identified a role in gene silencing. This work was first reported in *Genetics* (HOLLENHORST et al. 2000) and subsequently confirmed in *Nature* (ZHU et al. 2000). The roles of forkhead homologue 1 (*FKH1*) and forkhead homologue 2 (*FKH2*) in transcriptional silencing and pseudohyphal growth were explored. Although these two genes had the same DNA-binding domain (proven by a Fox-box switch between the two proteins) (HOLLENHORST et al. 2000), they differ in that *FKH2* has an additional 300 C-terminal amino acids. The two proteins were found to be redundant in controlling pseudohyphal growth, while having opposite effects on gene silencing. *FKH1*

has a positive role in silencing while *FKH2* has a negative role (HOLLENHORST et al. 2000). The different effects on transcriptional silencing were reflected in differences in the expression of *CLB2*, a G2/M-phase cyclin. Deletion of *FKH1* caused an increase in rate of progression through the G2/M-phases of the cell cycle while the *FKH2* deletion strain progressed more slowly through the cell cycle. These differences were reflected in elevated levels of *CLB2* mRNA in the former case and decreased levels in the latter (HOLLENHORST et al. 2000). A microarray analysis confirmed these and many other gene changes, most of them in a *CLB2* cluster (a group of genes including *CLB2* whose transcription peaks early in mitosis) in double *FKH1/FKH2* deletions (ZHU et al. 2000). The role of *FKH2* was specifically demonstrated using chromatin immunoprecipitation/polymerase chain reaction assays to identify a positive regulator, Ndd1, in association with *FKH2* (KORANDA et al. 2000). In addition, a negative effect on cell-cycle regulation of AFX-like forkhead transcription factors has been demonstrated in mammalian cells (MEDEMA et al. 2000). This Fox-box transcription factor inhibited the cyclin cdk2 complex and the endogenous PI(3)K/PKB signaling pathway – through increased expression of p27<sup>kip1</sup> (MEDEMA et al. 2000).

The role of forkhead genes in cell cycle regulation in mammals is supported by the involvement of forkhead fusion genes in cancer. Chimeric proteins involving forkhead genes have been implicated in leukemia and rhabdomyosarcoma. The *MLL* gene involved in myelogenous leukemia has been fused with AFX (BORKHARDT et al. 1997), and AF6q21 (HILLION et al. 1997), two forkhead genes, in translocations found in acute leukemic patients. The *FKHR* forkhead gene has been fused with *PAX3* (GALILI et al. 1993) or *PAX7* (DAVIS et al. 1994) in alveolar rhabdomyosarcoma. The *PAX3-FKHR* fusion protein has been shown to activate a myogenic program when the fusion gene was transfected into NIH 3T3 cells and the resultant pattern of gene transcription studied by microarray (KHAN et al. 1999). The fusion breakpoints are in the FOX box at very similar locations in all these fusion proteins.

Because of the interest in the role of forkhead transcription factors in development, there have been multiple studies of the expression of *Foxc1* and *Foxc2* in early embryos. KAESTNER et al. (1996) studied the expression of *Foxc2* during mouse embryogenesis. Using the mouse TATA-box-binding protein chain as an internal control in RNA nuclease protection assays, they found an over 2000-fold molar excess of *Foxc2* mRNA in total day 11.5 embryos with molar ratios ranging in the hundreds for later embryos and in adult tissues, including brain, muscle, heart, fat, kidney, lung, liver, uterus, and ovaries (TATA-box-binding protein is not a good control in the latter two tissues). Their *in situ* hybridizations, however, showed even earlier expression (not attempted in total embryos by RNA nuclease protection) in the embryonic mesoderm of day 7.5 embryos, both anterior and posterior to the node, but not including the extra-embryonic mesoderm. As development continued, *Foxc2* transcripts were detected in somites, mesoderm, head, and endocardium at day 8.25. By day 10.5, expression was very strong in the somites

and in the dorsal aorta (KAESTNER et al. 1996). *Foxc2* expression in the head region was particularly strong in the cartilaginous condensation around the optic vesicle and the skull underlying the midbrain and hindbrain (LIDA et al. 1997). This mesenchyme gives rise to palatal processes and the outer layer of the optic vesicle. The mandibular component of the first branchial arch also showed strong expression of *Foxc2*. At day 11.5, *Foxc2* expression was prominent surrounding the optic cup, including the prospective sclerotic coat of the eyeball (LIDA et al. 1997).

Studies on *Foxc1* expression were facilitated by the use of a knockout which replaced the coding region of the gene with a  $\beta$ -galactosidase recorder (KUME et al. 1998). Expression at day 9.5 was in the pharyngeal arch and the cardiac endothelium. At day 10.5, *Foxc1* expression includes the mesenchyme of the aortic arches but not the epithelia (WINNIER et al. 1999, KUME et al. 1998). From day 11.5 onward, *Foxc1* was expressed in the mesenchyme surrounding the aorta and pulmonary arteries and in the mesenchyme of the semilunar valves. At birth, *Foxc1* is expressed in the smooth muscle of the pulmonary trunk and the aortic arch and continues to be expressed here and also in the endocardium, smooth muscle and endothelium of coronary vessels (WINNIER et al. 1999).

The role of these forkhead genes in development was further explored with knockouts using embryonic stem cell technology. Some *Foxc2* homozygous embryos died *in utero* and other sat birth with heart defects (LIDA et al. 1997). Almost all of the *Foxc2* embryos had interruptions, coarctation or tubular hypoplasia of the aortic arch. All newborns had complete cleft secondary palate. There were abnormalities of the skull, including abnormal formation of the optic canal, absence of the posterior wall of the foramen ovale, and fusion of the malleus and incus, primarily defects of neural crest origin (LIDA et al. 1997). Axial skeletal anomalies with short vertebral bodies, spina bifida, and spina bifida occulta were found.

The *Foxc1* knockout replicated the spontaneous mutation in *Foxc1*, congenital hydrocephalus, a long-studied mouse mutation (GRUNEBERG 1943, GREEN 1970). The homozygous knockouts died shortly after birth with hydrocephalus, cardiac anomalies and skeletal defects (KUME et al. 1998). The frequency of aortic arch interruption, coarctation, or narrowing was much lower than in *Foxc2* mutants and the death at birth is thought to result from an inability to breathe secondary to absence of the sternum (GRUNEBERG 1943). The *Foxc1* homozygous knockout is also characterized by severe abnormalities of the eye with absent anterior chamber (KIDSON et al. 1999). Eye defects in heterozygous *Foxc1* deficiency were also seen on some genetic backgrounds (HONG et al. 1999).

The interactions of *Foxc1* and *Foxc2* in cardiac development were shown by creating double heterozygotes for the two knockouts (WINNIER et al. 1999). Because single heterozygotes were thought to be completely recessive (without detectable abnormalities) finding such severe cardiac abnormalities in double heterozygotes strongly implicated the interaction of *Foxc1* and *Foxc2*. Thus,



*Foxc1* and *Foxc2* proteins, which have nearly identical DNA binding sequences, must have similar functions in the cells in which they are co-expressed and can only partially compensate for each other. In the double mutant, the threshold for genes downstream from them must be below an essential level to prevent the cardiac defects (WINNIER et al. 1999). Alternatively, expression in differing cell types with signaling between them, might not reach critical thresholds.

The role of Sonic hedgehog in signalling and *Foxc2* transcription was explored by FURUMOTO et al. (1999). They found that patterns of *Pax1* and *Foxc2* expression in ventral lateral sclerotomes were very similar at days 9.5, 10.5 and 11.5 of the mouse gestation. Using Danforth's short-tail mouse mutant which has an absent notochord, they showed the importance of the notochord in controlling this expression by finding markedly decreased *Foxc2* expression in this mutant (FURUMOTO et al. 1999). Using a chicken model of notochord induction, they showed that this failure could be corrected with Sonic hedgehog expressing cells. FURUMOTO et al. (1999) made double heterozygotes of the *Pax1* knockout and the *Foxc2* knockout and found that they were normal, whereas the double homozygous knockouts had missing dorsal medial structures of the vertebrae, causing spina bifida, with subcutaneous myelomeningocele; vertebral bodies and intervertebral discs were absent. However, there were no effects of the double knockout of *Pax1* and *Foxc2* on the neurocranium (FURUMOTO et al. 1999). They further explored the mechanism of this failure by showing decreased mitosis in the ventral sclerotome of the double knockout mice.

*Foxc1* and *Foxc2* have also been shown to play important roles in kidney development (KUME et al. 2000). At day 10.5, *Foxc1* is transcribed in the mesonephric mesenchyme, and although *Foxc2* transcripts are lower initially, higher levels are found at day 10.5 around the ureter bud (KUME et al. 2000). Homozygous *Foxc1* knockout mice had hydronephrosis and hydroureter on some genetic backgrounds, other genetic backgrounds showed very low penetrance with only 2% of homozygotes showing kidney abnormalities. Taking advantage of these differences in genetic backgrounds, KUME et al. (2000) made the double compound heterozygote *Foxc1*<sup>+/-</sup>, *Foxc2*<sup>+/-</sup> on the genetic background with a low frequency of kidney defects in *Foxc1*<sup>-/-</sup> mice. This demonstrated the interaction of the two genes with one-third showing hypoplastic kidneys and two-thirds having a single hydroureter. Thus, *Foxc1* and *Foxc2* have overlapping roles in renal development as well as in cardiac development.

Human disorders involving FOX-box genes include Axenfeld-Rieger anomaly (ARA). This term refers to abnormal development of the anterior eye chamber which may result from posterior embryotoxon with or without iris stromal atrophy. Mild forms of this anomaly occur in "normal" individuals and in association with various syndromes. Its importance rests in the increased likelihood of glaucoma. Axenfeld-Rieger syndrome (MIM 180500) is an autosomal dominant syndrome in which Axenfeld-Rieger anomaly is associated with a particular facial appearance and hypodontia.

A gene responsible for Axenfeld-Rieger syndrome (ARS) has been mapped to 4q25 and cloned as *RIEG* (now called *PITX2*) (SEMINA et al. 1996). The locus responsible for ARA without the extraocular manifestations of ARS appears not to map to 4q25. In a family of 13 in which 7 members were affected with ARA, linkage analysis showed significant linkage to 6p25 (GOULD et al. 1997). Recently, a candidate gene mapping to 6p25 – the forkhead transcription factor gene *FOXC1* – has been found to be responsible for Axenfeld-Rieger anomaly and iris hypoplasia in patients with chromosomal anomalies involving 6p25 (NISHIMURA et al. 1998) and for ARS in other patients (MIRZAYANS et al. 2000). One of the two patients identified in the study had an unbalanced translocation, resulting in loss of the 6p25→ pter region. It was confirmed in the study by NISHIMURA et al. (1998) that this patient only possessed one copy of the *FOXC1* gene and, as no mutations were detected, it was inferred that haploinsufficiency alone could account for the ARA phenotype. Surprisingly, duplication of *FOXC1* has also been associated with iris hypoptasia and glaucoma in one family (LEHMANN et al. 2000). However the breakpoints were not mapped and could disrupt some other gene(s). For instance, haploinsufficiency of the nearby gene, *AP-2a*, which is involved in eye development (WEST-MAYS et al. 1999), may also be important (DAVIES et al. 1999) and could be the second 6p25 gene implicated in ARA (MEARS et al. 1998).

Lymphedema-distichiasis (LD) (MIM 153400) is an autosomal dominant disorder that may present with lymphedema of the lower limbs with variable age of onset and abnormal eyelashes (distichiasis, second eyelashes growing from the meibomian glands) that may produce corneal abrasions (FALLS, KERTESZ 1964, NEEL, SCHULL 1954). Other complications may include cardiac defects, cleft palate and extradural cysts (FALLS, KERTESZ 1964, ROBINOW et al. 1970, GOLDSTEIN et al. 1964, SCHWARTZ et al. 1980, CORBETT et al. 1982), suggesting a gene defect with pleiotropic effects acting during development. We previously reported neonatal lymphedema similar to that found in Turner syndrome associated with t(Y;16)(q12;q24.3) (ERICKSON et al. 1995). Our search for a causative gene at the translocation breakpoints began with the Y chromosome, where we did not find a candidate gene (DRURY et al 1998). Our focus shifted to the chromosome 16 breakpoint when MANGION et al. (1999) reported mapping a gene for LD to a 16cM region at 16q24.3. By FISH, we determined that our breakpoint was within this critical region and further narrowed the breakpoint to a 20 kb interval. Because the translocation did not interrupt a reading frame, we considered candidate genes in the immediate region that might be inactivated by position effect. We identified inactivating mutations (a nonsense and a frameshift mutation) in the *FOXC2* (*MFH-1*) gene in two unrelated families with lymphedema-distichiasis (FANG et al. 2000).

Both mutations found in the two families described would be predicted to truncate and inactivate one allele of *FOXC2* in affected patients. The results are consistent with an autosomal dominant mode of inheritance due to

haploinsufficiency. This mechanism of mutation and mode of inheritance is known to result in variable phenotypes in a number of human genetic disorders, especially when found in a developmental gene with pleiotropic effects. This likely accounts for the variable phenotype found in lymphedema-distichiasis syndrome. We hypothesized that the translocation in our index case inactivates the *FOXC2* gene and, perhaps other genes in the region by position effect such as those observed with *PAX6* and *SOX9* inactivation in humans (KLEINJAN, van HEYNINGEN 1998). *SOX9* inactivation from breakpoints as far as 850 kb has been observed. Our breakpoint is approximately 120 kb 3' to the *FOXC2* gene. Because the *FKH6* gene maps between the two, it could also be inactivated and have phenotypic effects in this patient.

The finding of inactivating mutations in one allele of *FOXC2* in two unrelated families with LD and a likely position effect inactivation in a third individual provide strong evidence that these are responsible for the disorder. Hereditary lymphedema is a heterogeneous disorder and mutations in the *FLT4*, *VEGF* receptor-3 gene at 5q35 have been shown to be one cause of hereditary lymphedema type I, or Milroy's disease (KARKKAINEN et al. 2000). Genetic heterogeneity is clearly present with some evidence for multiple interacting genes in this apparently dominantly inherited disorder (HOLBERG et al. 2000).

It is interesting to note that Axenfeld-Rieger anomaly has not yet been noted in lymphedema-distichiasis patients but was noted in *Foxc2* knockout heterozygotes (SMITH et al. 2000). This recent finding is in contrast to the original report that *Foxc2* heterozygous embryos were completely normal (LIDA et al. 1997). Perhaps the difference could result from variations in genetic background or in the degree to which the abnormalities were sought. It was also noted that the double heterozygote *Foxc1*<sup>+/-</sup>/*Foxc2*<sup>+/-</sup> had a similar range of ocular abnormalities but with a more severe deficiency of mesenchyme-derived iris stroma (SMITH et al. 2000). The different eye findings reported by these two groups (LIDA et al. 1997 versus SMITH et al. 2000) provides a caveat to the absence of lymphedema also described for the heterozygous knockout. Given the short distances in mice that the extravascular tissue fluid needs to travel before being returned to the central circulation, it is not surprising that mice do not usually manifest lymphedema (which, of course, is most frequently in the lower extremities in our two legged species). However, it is possible that more careful examination of a larger number of heterozygous *Foxc2* knockouts might detect minor lymphedema in some tissues. It would also be important to exam fetuses; many human fetuses show nuchal hygroma that resolves later. Similarly, *in utero* lymphedema could be only transient in *Foxc2* heterozygous knockouts.

Forkhead genes have just been implicated in two more developmental disorders: FOXP3 in the immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX; BENNETT et al. Nature genetics 27:20, 2001) and FOXL2 in Hephazophimosis/ptosis/epiconthus inresus syndrome (CRISPENI et al. Nature Genetics 27:159, 2001).



**Acknowledgments.** I thank M. Michael COHEN, Jr. for comments on the manuscript, Ms. Carole MEYER for secretarial help, and the Muscular Dystrophy Association for research support.

**Note added in proof:** Forkhead genes have just been implicated in two more developmental disorders: *FOXP3* in the immune dysregulation, polyendocrinopathy, enteropathy X-linked syndrome (IPEX; BENNETT et al., *Nature Genetics*, 27: 20, 2001) and *FOXL2* in blepharophimosis/ptosis/epicanthus inversus syndrome (CRISPENI et al., *Nature Genetics*, 27: 159, 2001).

## REFERENCES

- BORKHARDT A., REPP R., HAAS O.A., LEIS T., HARBOTT J., KREUDER J., HAMMERMANN J., HENN T., LAMPERT F. (1997). Cloning and characterization of AFX, the gene that fuses to MLL in acute leukemias with a t(X;11)(q13;q23). *Oncogene* 14: 195-202.
- CLARK K.L., HALAY E.D., LAI E., BURLEY S.K. (1993). Co-crystal structure of the HNF-3/forkhead DNA-recognition motif resembles histone H5. *Nature* 364: 412-420.
- CORBETT C.R.R., DALE R.F., COLTART D.J., KINMONTH J.B. (1982). Congenital heart disease in patients with primary lymphoedemas. *Lymphology* 15: 85-90.
- DAVIES A.F., MIRZA G., SEKHON G., TURNPENNY P., LEROY F., SPELEMAN F., LAW C., van REGEMORTER N., VAMOSE E., FLINTER F., RAGOISSIS J. (1999). Delineation of two distinct 6p deletion syndromes. *Hum. Genet.* 104: 64-72.
- DAVIS R.J., D'CRUZ C.M., LOVELL M.A., BIEGEL J.A., BARR F.G. (1994). Fusion of PAX7 to FKHR by the variant t(1;13)(p36;q14) translocation in alveolar rhabdomyosarcoma. *Cancer Res.* 54: 2869-2872.
- DRURY S.S., ERICKSON R.P., GLOVER T.W. (1998). Y;16 translocation breakpoint associated with a partial Turner phenotype identifies a foamy virus insertion. *Cytogenet. Cell Genet.* 80: 199-203.
- ERICKSON R.P., HUDGINS L., STONE J.F., SCHMIDT S., WILKE C., GLOVER T.W. (1995). A balanced Y;16 translocation associated with Turner-like neonatal lymphedema suggests the location of a potential anti-Turner gene on the Y chromosome. *Cytogenet. Cell Genet.* 71: 163-167.
- FALLS H.F., KERTESZ E.D. (1964). A new syndrome combining pterygium colli with developmental anomalies of the eyelids and lymphatics of the lower extremities. *Trans. Am. Ophthalmol. Soc.* 62: 248-275.
- FANG J.M., DAGENAIS S.L., ERICKSON R.P., ARLT M.F., GLYNN M.W., GORSKI J.L., SEAVER L.H., GLOVER T.W. (2000). Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *Am. J. Hum. Genet.* 67: 1382-1388.
- FURUMOTO T., MIURA N., AKASAKA T., MIZUTANI-KOSEKI Y., SUDO H., FUKUDA K., MAEKAWA M., YUASA S., FU Y., MORIYA H., TANIGUCHI M., IMAI K., DAHL E., BALLING R., PAVLOVA M., GOSSLER A., KOSEKI H. (1999). Notochord-dependent expression of MFH1 and PAX1 cooperates to maintain the proliferation of sclerotome cells during the vertebral column development. *Dev. Biol.* 210: 15-29.



- GALILI N., DAVIS R.J., FREDERICKS W.J., MUKHOPADHYAY S., RAUSCHER R.J. 3D, EMANUEL B.S., ROVERA G., BARR F.G. (1993). Fusion of a fork head domain gene to PAX3 in the solid tumour alveolar rhabdomyosarcoma. *Nat. Genet.* 5: 230-235.
- GOLDSTEIN S., QAZI Q.H., FITZGERALD J., GOLDSTEIN L., FRIEDMAN A.P., SAWYER P. (1964). Distichiasis, congenital heart defects and mixed peripheral vascular anomalies. *Am. J. Med. Genet.* 20: 283-394.
- GOULD D.B., MEARS A.J., PEARCE W.G., WALTER M.A. (1997). Autosomal dominant Axenfeld-Rieger anomaly maps to 6p25. *Am. J. Hum. Genet.* 61: 765-768.
- GRUNEBERG H. (1943). Congenital hydrocephalus in the mouse: a case of spurious pleiotropism. *J. Genet.* 45: 1-21.
- GREEN M. (1970). The developmental effects of congenital hydrocephalus (ch) in the mouse. *Dev. Biol.* 23: 585-608.
- HILLION J., LE CONIAT M., JONVEAUX P., BERGER R., BERNARD O.A. (1997). AF6q21, a novel partner of the MLL gene in t(6;11) (q21;q23), defines a forkhead transcriptional factor subfamily. *Blood* 90: 3714-3719.
- HOLBERG C.J., ERICKSON R.P., BERNAS M.J., WITTE M.H., FULTZ K.E., ANDRADE M., WITTE C.L. (2000). Segregation analyses and a genome-wide linkage search confirm genetic heterogeneity and suggest oligogenic inheritance in some Milroy congenital primary lymphedema families. Accepted: *Am. J. Med. Genet.*
- HOLLENHORST P.C., BOSE M.E., MIELKE M.R., MULLER U., FOX C.A. (2000). Forkhead genes in transcriptional silencing, cell morphology and the cell cycle: overlapping and distinct functions for *FKH1* and *FKH2* in *Saccharomyces cerevisiae*. *Genetics* 154: 1533-1548.
- HONG H.-K., LASS J.H., CHAKRAVARTI A. (1999). Pleiotropic skeletal and ocular phenotypes of the mouse mutation congenital hydrocephalus (ch/Mf1) arise from a winged helix-forkhead transcription factor gene. *Hum. Mol. Genet.* 8: 625-637.
- KAESTNER K.H., BLECKMANN S.C., MONAGHAN A.P., SCHLONDORFF J., MINCHEVA A., LICHTER P., SCHUTZ G. (1996). Clustered arrangement of winged helix genes *fhx-6* and *MFH-1*: possible implications for mesoderm development. *Development* 122: 1751-1758.
- KAESTNER K.H., KNOCHEL W., MARTINEZ D.E. (2000). Unified nomenclature for the winged helix/forkhead transcription factors. *Gene Dev.* 14: 142-146.
- KARKKAINEN M.J., KFERRELL R.E., LAWRENCE E.C., KIMAK M.A., LEVINSON K.L., MC TIGUE M.A., ALITALO K. (2000). Missense mutations interfere with VEGFR-3 signaling in primary lymphedema. *Nat. Genet.* 25: 153-159.
- KHAN J., BITTNER M.L., SAAL, L.H., TEICHMANN U., AZORSA D.O., GOODEN G.C., PAVAN W.J., TRENT J.M., MELTZER P.S. (1999). cDNA microarrays detect activation of a myogenic transcription program by the PAX3-FKHR fusion oncogene. *Proc. Natl. Acad. Sci. USA* 96: 13264-13269.
- KIDSON S.H., KUME T., DENG K., WINFREY V., HOGAN B.L.M. (1999). The forkhead/winged helix gene, *Mf1*, is necessary for the normal development of the cornea and formation of the anterior chamber in the mouse eye. *Dev. Biol.* 211: 306-322.
- KLEINJAN D.-J., van HEYNINGEN V. (1998). Position effect in human genetic disease. *Hum. Mol. Genet.* 7: 1611-1618.

- KORANDA M., SCHLEIFFER A., ENDLER L., AMMERER G. (2000). Forkhead-like transcription factors recruit Ndd1 to the chromatin of C2/M-specific promoters. *Nature* 406: 94-98.
- KUME T., DENG K.Y., WINFREY V., GOULD D.B., WALTER M.A., HOGAN B.L. (1998). The forkhead/winged helix gene *Mfl* is disrupted in the pleiotropic mouse mutation congenital hydrocephalus. *Cell* 93: 985-996.
- KUME T., DENG K., HOGAN B.L.M. (2000). Murine forkhead/winged helix genes *Foxc1* (*Mfl*) and *Foxc2* (*Mfh1*) are required for the early organogenesis of the kidney and urinary tract. *Development* 127: 1387-1395.
- LEHMANN O.J., EBENEZER N.D., JORDAN T., FOX M., OCAKA L., PAYNE A., LEROY B.P., CLARK B.J., HITCHINGS R.A., POVEY S., KHAW P.T., BHATTACHARYA S.S. (2000). Chromosomal duplication involving the forkhead transcription factor gene FOXC1 causes iris hypoplasia and glaucoma. *Am. J. Hum. Genet.* 67: 1129-1135.
- LIDA K., KOSEKI H., KAKINUMA H., KATO N., MIZUTANI-KOSEKI Y., OHUCHI H., YOSHIOKA H., NOJI S., KAWAMURA K., KATAOKA Y., UENO F., TANIGUCHI M., YOSHIDA N., SUGIYAMA T., MIRUA N. (1997). Essential roles of the winged helix transcription factor MFH-1 in aortic arch patterning and skeletogenesis. *Development* 124: 4627-4638.
- MANGION J., RAHMAN N., MANSOUR S., BRICE G., ROSBOTHAM J., CHILD A.H., MURDAY V.A. (1999). A gene for lymphedema-distichiasis maps to 16q24.3. *Am. J. Hum. Genet.* 65: 427-432.
- MEARS A.J., JORDON T., MIRZAYANS F., DUBOIS S., KUME T., PARLEE M., RITCH R., KOOP B., KUO W.-L., COLLINS C., MARSHALL J., GOULD D.B., PEARCE W., CARLSSON P., ENERBACK S., MORISSETTE J., BHATTACHARYA S., HOGAN B., RAYMOND V., WALTER M.A. (1998). Mutations of the forkhead/winged-helix gene, *FKHL7*, in patients with Axenfeld-Rieger anomaly. *Am. J. Hum. Genet.* 63: 1316-1328.
- MEDEMA R.H., KOPS G.J.P., BOS J.L., BURGERING B.M.T. (2000). AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27<sup>kip1</sup>. *Nature* 404: 782-787.
- MIRZAYANS F., GOULD D.B., HEON E., BILLINGSLEY G.D., CHEUNG J.C., MEARS A.J., WALTER M.A. (2000). Axenfeld-Rieger syndrome resulting from mutation of the *FKHL7* gene on chromosome 6p25. *Eur. J. Hum. Genet.* 8: 72-74.
- NEEL J.V., SCHULL W.J. (1954). *Human Heredity*. University of Chicago Press, Chicago: 50-51.
- NISHIMURA D.Y., SWIDERSKI R.E., ALWARD W.L.M., SEARBY C.C., PATIL S.R., BENNET S.R., KANIS A.B., GASTIER J.M., STONE E.M., SHEFFIELD V.C. (1998). The forkhead transcription factor gene *FKHL7* is responsible for glaucoma phenotypes which map to 6p25. *Nat. Genet.* 19: 140-147.
- ROBINOW M., JOHNSON G.F., VERHAGEN A.D. (1970). Distichiasis-lymphoedema. A hereditary syndrome of multiple congenital defects. *Am. J. Dis. Child.* 119: 343-347.
- SCHWARTZ J.F., O'BRIEN M.S., HOFFMAN JR., J.S. (1980). Hereditary spinal arachnoid cysts, distichiasis, and lymphedema. *Ann. Neurol.* 7: 340-343.
- SEMINA E.V., REITER R., LEYSENS N.J., ALWARD W.L., SMALL K.W., DATSON N.A., SIEGEL-BARTLET J., BIERKE-NELSON D., BITOUN P., ZABEL B.U., CAREY J.C.,

- MURRAY J.C. (1996). Cloning and characterization of a novel biocide-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat. Genet.* 14: 392-399.
- SMITH R.S., ZABALETA A., KUME T., SAVINOVA O.V., KIDSON S.H., MARTIN J.E., NISHIMURA D.Y., ALWARD W.L., HOGAN B.L.M., JOHN S.W.M. (2000). Haploinsufficiency of the transcription factors FOXC1 and FOXC2 results in aberrant ocular development. *Hum. Mol. Genet.* 9: 1021-1032.
- WEIGEL D., JACKLE H. (1990). The fork head domain: A novel DNA binding motif of eukaryotic transcription factors. *Cell* 63: 455-456.
- WEST-MAYS J.A., ZHANG J., NOTTOLI T., HAGOPIAN-DONALDSON S., LIBBY D., STRISSEL K.J., WILLIAMS T. (1998). *AP-2 $\alpha$*  transcription factor is required for early morphogenesis of the lens vesicle. *Dev. Biol.* 206: 46-62.
- WINNIER G.E., KUME T., DENG K., ROGERS R., BUNDY J., RAINES C., WALTER M.A., HOGAN B.L.M., CONWAY S.J. (1999). Roles for the winged helix transcription factors MF-1 and MFH1 in cardiovascular development revealed by nonallelic non-complementation of null alleles. *Dev. Biol.* 213: 418-431.
- ZHU G., SPELLMAN P.T., VOLPE T., BROWN P.O., BOTSTEIN D., DAVIS T.N., FUTCHER B. (2000). Two yeast forkhead genes regulate the cell cycle and pseudohyphal growth. *Nature* 406: 90-94.