

Online supplement 1. Evaluation of pathogenic potential of *FOXC1* and *PITX2* missense mutations

<i>FOXC1</i>				
Mutation	Grantham score^a	Polyphen	SIFT	Protein domain
p.Met109Val	21	Probably damaging	Affects protein function	FHD
p.Ser131Trp	177	Probably damaging	Affects protein function	FHD
p.Lys138Glu	56	Benign	Affects protein function	FHD
<i>PITX2</i>				
Mutation	Grantham score	Polyphen	SIFT	Protein domain
p.Phe58Leu	22	Probably damaging	Affects protein function	HD

^a A Grantham score of more than 60 is considered to be significant.

Online supplement 2. Sequence alignment of the forkhead domain (upper panel) and the homeodomain (lower panel)

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FOXC1_Homo_sapiens      FQF--QPKDMVKFPYSYIALITMAIQNAFDKKITLNGIYQFMDRFFPYRDNKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXC1_Mus_musculus     FQF--QPKDMVKFPYSYIALITMAIQNAFDKKITLNGIYQFMDRFFPYRDNKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXC1_Xenopus_laevis   FQF--QPKDMVKFPYSYIALITMAIQNAFDKKITLNGIYQFMERFFPYRDNKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXC1a_Danio_rerio    FQF--QPKDMVKFPYSYIALITMAIQNSPDKKITLNGIYQFMERFFPYRDNKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXC1b_Danio_rerio    FQF--QPKDMVKFPYSYIALITMAIQNSSDKKITLNGIYQFMERFFPYRDNKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXC2_Homo_sapiens     HHQPAAPKDLVKFPYSYIALITMAIQNAPEKKITLNGIYQFMDRFFPYRENKQGWQNSIRHNISLNECFYKVPRDDKKPGKGSYWTLDPDSYNYMFENGSLRARRRRFKKKD
FOXA3_Homo_sapiens     FKGYRRPLAHAKFPYSYISLITMAIQQAQPGKMLTSEIYQWIMDLFPYRENQQRWQNSIRHSISLNDCEYKVPARSPDKPGKGSYVALHFPSSGNMFENGSCYLRQKRFKLEE
FOXD1_Homo_sapiens     KNP-----LVKFPYSYIALITMAIQSPKKRLTSEICEHFSGRFPYREKFFAWQNSIRHNISLNDCEYKVPREFGNPGKGNWTLDPEADMFENGSLRARRRRFKKRD
FOXF1_Homo_sapiens     KKTNAGIRRPEKFPYSYIALIVMAIQSSPTKRLTSEIYQFQSRFPFFRGSYQGWKNSVRHNISLNECFYKVPKGLGRPGKGHYWTIDPASEFMEEEGSFRARRRQFRKAC
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PITX2a_Homo_sapiens    QRRQRTHFTSQQLQELEAFQNRNYPDMSTREEIAVWVNLTEARVRVWFQNRRAKWRKRE
PITX2a_Mus_musculus   QRRQRTHFTSQQLQELEAFQNRNYPDMSTREEIAVWVNLTEARVRVWFQNRRAKWRKRE
PITX2a_Gallus_gallus  QRRQRTHFTSQQLQELEAFQNRNYPDMSTREEIAVWVNLTEARVRVWFQNRRAKWRKRE
PITX2a_Danio_rerio    QRRQRTHFTSQQLQELEAFQNRNYPDMSTREEIAVWVNLTEARVRVWFQNRRAKWRKRE
PITX2a_Xenopus_laevis QRRQRTHFTSQQLQELEAFQNRNYPDMSTREEIAVWVNLTEARVRVWFQNRRAKWRKRE
ARX_Homo_sapiens      QRRYRITFTSYQLEELERAFQKT HYPDVFTREELAMRLDLTEARVQVWFQNRRAKWRKRE
PAX3_Homo_sapiens     QRRSRTTFTAELQLEELERAFERT HYPDIYTREELAQRAKLTEARVQVWFSNRRARWRKQA
MNX1a_Homo_sapiens    CRRPRTAFTSQQLLELEHAFKLNKYLSPKRFEVATSLMLTETQVKIWFQNRRMKWRKSK
LMX1B_Homo_sapiens    PKRPRTILTIQRRRAFKASFEVSKPCRKVRETAAETGLSVRVVQVWFQNRRAKWRKLA
PIT1_Homo_sapiens     LGYTQTNVGEALAAVHGSEFFSQT TICRFENLQLSFKNACKLKAILSKWLEEAQVVGALYN
TGIF1a_Homo_sapiens   ARRRLPDMLRKDGKDPNCFITISRRGAKISETSSVESVMGIKNFMPALEETPFHSCTAGP
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Online supplement 3. Comparison of phenotypes of patients carrying the same genotype.

c.286dupG - p.Asp96GlyfsX210			
Kawase et al., 2001¹		This study	
Identified in 15-year old female patient, diagnosed with congenital glaucoma at the age of ten. Other reported features were bilateral posterior embryotoxon, iris hypoplasia, and hypertelorism.		Identified 37-year old female with congenital glaucoma, bilateral posterior embryotoxon and anterior chamber angle abnormalities. Cataract and corneal opacities in the right eye.	
c.253-11A>G			
Semina et al., 1996²	Borges et al., 2002³	Riice et al., 2001⁴ and 2009⁵	This study
Reported in patient with ARM ocular features (not specified), hypodontia, and umbilical hernia.	Identified in three-generation ARM family with five affected family members, four of which were clinically examined and presented with glaucoma, corneal disease, iris atrophy, and systemic features. Two out of four were found to have anterior synechiae.	Identified in patient with correctopia in the right eye, bilateral microphthalmic eyes and bilateral hypoplasia of the iris stroma. In addition, in the left eye, iris tissue strands were shown to traverse the trabecular meshwork. The patient also displayed maxillary hypoplasia, hypodontia, and redundant periumbilical skin.	Identified in 11-year old male proband, his affected sister and mother. The proband displays unilateral posterior embryotoxon, bilateral correctopia, and pigment on the anterior lens capsule. To date, there is no evidence for glaucoma. He has maxillary hypoplasia, hypodontia, and failure of involution of the periumbilical skin. Patient was reported to have short stature.

References

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3. Borges AS, Susanna R, Jr., Carani JC, et al. Genetic analysis of PITX2 and FOXC1 in Rieger Syndrome patients from Brazil. *J Glaucoma* 2002;11:51-56.
4. Riise R, Storhaug K, Brondum-Nielsen K. Rieger syndrome is associated with PAX6 deletion. *Acta Ophthalmol Scand* 2001;79:201-203.
5. Riise R, D'Haene B, De Baere E, Gronskov K, Brondum-Nielsen K. Rieger syndrome is not associated with PAX6 deletion: a correction to *Acta Ophthalmol Scand* 2001: 79: 201-203. *Acta Ophthalmol* 2009.