

Family	Origin	# af. ind.	IUGR	Lax. Wristy Skin	Hip Discomfort	Hernias	Osteopenia	Abnormal Corpus Cal.	Mental Retardation	Alcoholism	Cataplexy	Initial Diagnosis	Site	cDNA	Consequence	Exon	Literature
JL	Iraq	1	+	++	+	-	+	-	-	-	-	WSS	hom	c.138+1G>A	?	2	
TP	Pakistani	1	+	+	n.d.	n.d.	n.d.	+	+	+	-	WSS	hom	c.616G>T	p.Gly208Trp	5	Hamamy et al.
SP	Pakistani	3	+	++	+	-	+	+/	+	-	-	GOMWS	hom	c.617_633del	?	5	Al-Gazali et al.
IK	Kuwait	2	+	++	++	++	++	-	+	-	-	GOMWS	hom	c.707G>A	p.Arg208Gln + Splicing	6	Nanda et al.
FS	Kuwait	1	+	+	++	++	++	-	+	-	-	GOMWS	hom	c.707G>A	p.Arg208Gln + Splicing	6	Nanda et al.
SJ	Jordan	5	+	++	-	+	n.d.	+	+	-	-	WSS	hom	c.707+2_707+5del	p.Lys215_Asp130del	8	Nanda et al.
DI	Italy	1	+	++	-	++	++	n.d.	+	-	-	GO	het	c.116del	p.Gly465Glu	1	
SO	Oman	1	-	++	+	-	+	-	+	-	-	GO	het	c.355C>G	p.Arg119Gly	4	
												GO	het	c.356G>A	p.Arg119His	4	
GO	Oman	3	n.d.	++	-	-	+	n.d.	+	-	-	GO	hom	c.666C>T	p.Arg119His	5	
HB	Bahrain	2	+	++	++	+	-	n.d.	+	-	-	GO	hom	c.356G>A	p.Arg119His	4	Rejeb et al.
MA	Austria	1	+	+	++	+	++	n.d.	+	-	-	GO	hom	c.535G>A	p.Arg170Thr	4	
MQ	Qatar	1	n.d.	++	+	+	++	n.d.	+	-	-	GO	hom	c.540+1G>A	?	4	
HU	USA	1	+	+	-	-	-	+	+	-	-	GO	hom	c.616G>A	p.Gly208Arg	5	
NA	Australia	1	n.d.	++	n.d.	n.d.	-	+	+	-	-	GO	hom	c.833+1G>C	?	5	
												GO	het	c.633+1G>C	?	5	
AS	Syria	1	+	+	-	+	+	+	n.d.	-	-	GO	het	c.707+1G>T	?	6	
DT	Turkey	1	+	++	+	-	n.d.	n.d.	+	-	-	GO	hom	c.707+2_707+5del	p.Lys215_Asp130del	6	Al-Gazali et al.
MF	Italy	1	+	++	n.d.	++	n.d.	n.d.	+	-	-	de Barsy	het	c.355C>G	p.Arg119Gly	4	
												de Barsy	het	c.356G>A	p.Arg119His	4	Guerra et al.
ST	Turkey	2	+	+	-	-	n.d.	+	+	+	+	de Barsy	hom	c.540+1G>A	?	6	
EU	USA	1	+	++	+	++	n.d.	+	+	-	-	de Barsy	hom	c.752G>A	p.Arg251His	6	
JA	Australia	3	+	++	-	-	n.d.	n.d.	++	+	+	de Barsy	hom	c.707G>A	p.Arg257Thr	6	
KT	Turkey	1	-	+	+	-	n.d.	+	+	-	-	de Barsy	hom	c.707G>A	p.Arg257Thr	6	Kunze et al.
IT	Turkey	1	+	+	+	-	n.d.	-	+	-	-	de Barsy	hom	c.707+2_707+5del	p.Lys215_Asp130del	6	

af. ind., number of affected individuals in family. IUGR, intrauterine growth retardation. Corpus Cal., Corpus callosum. n.d., not determined. +/-, mild; +, average; ++, strong. For Hernias: +, unilateral; ++, bilateral.

Table 1

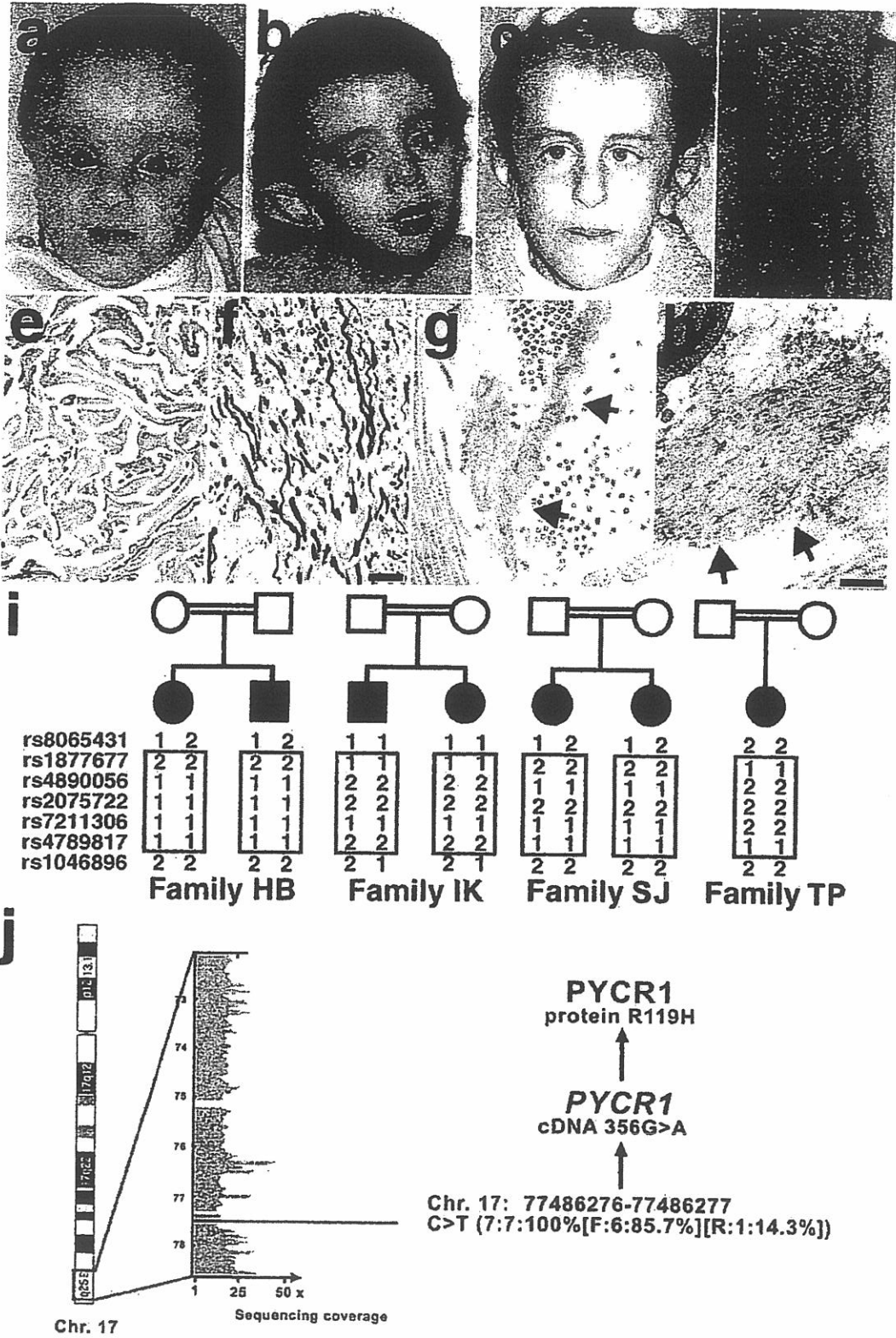
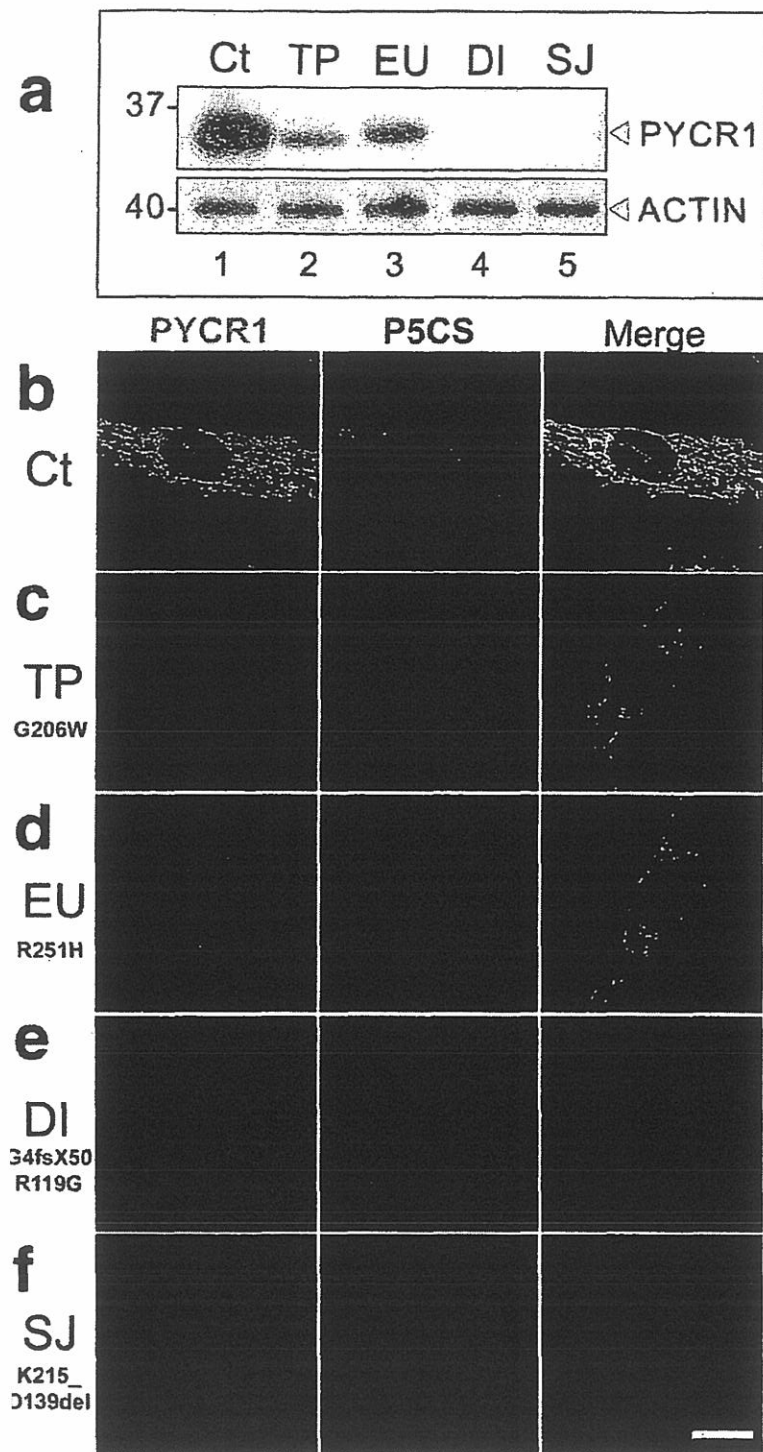
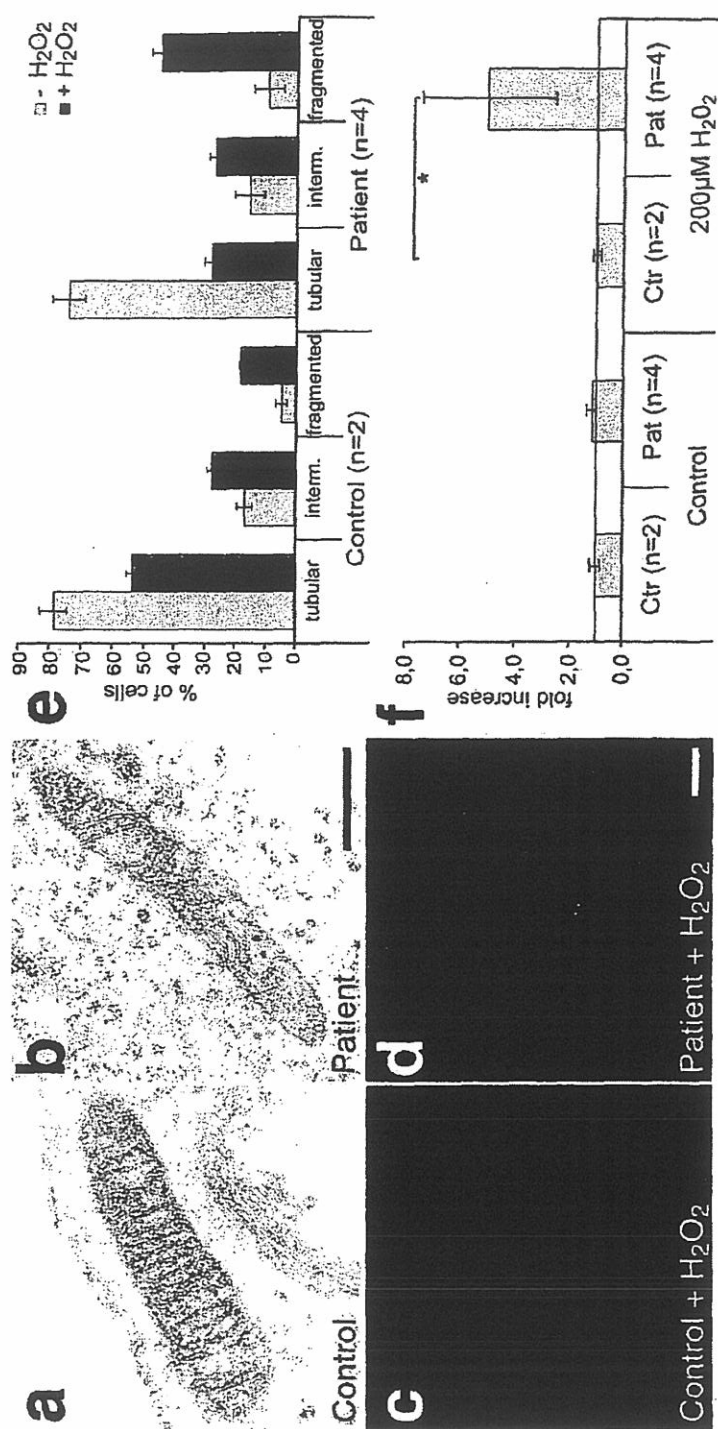


Figure 1





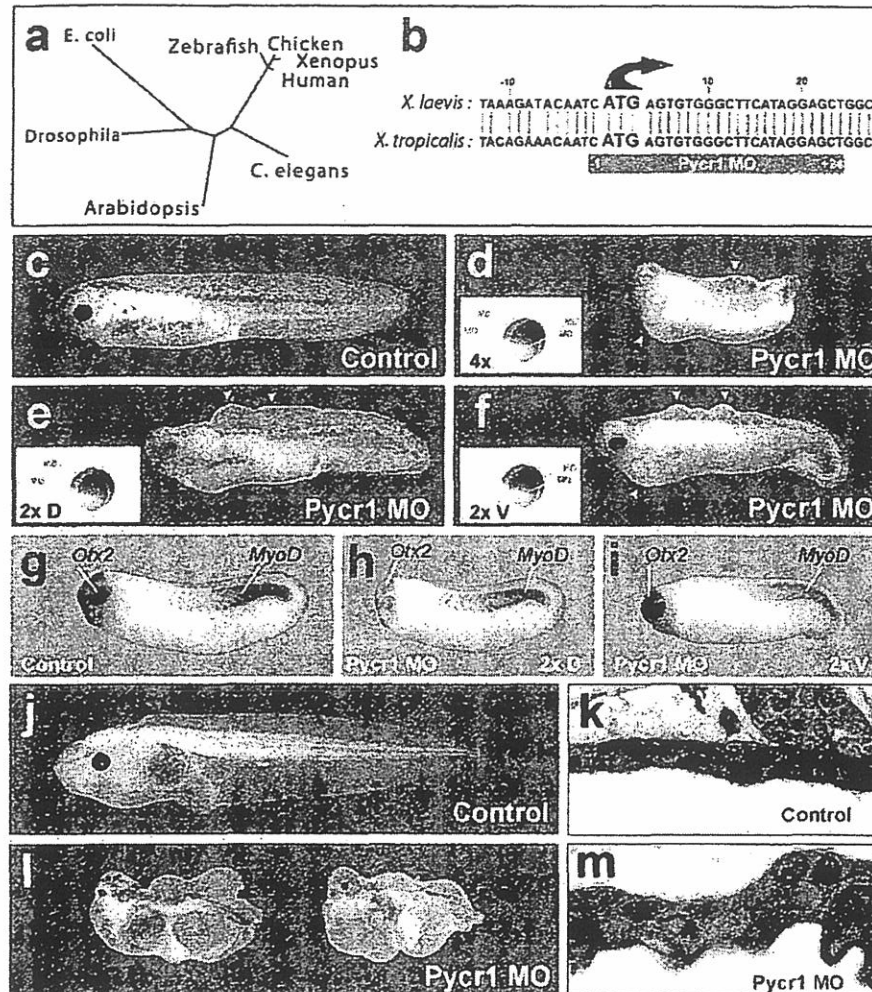


Figure4

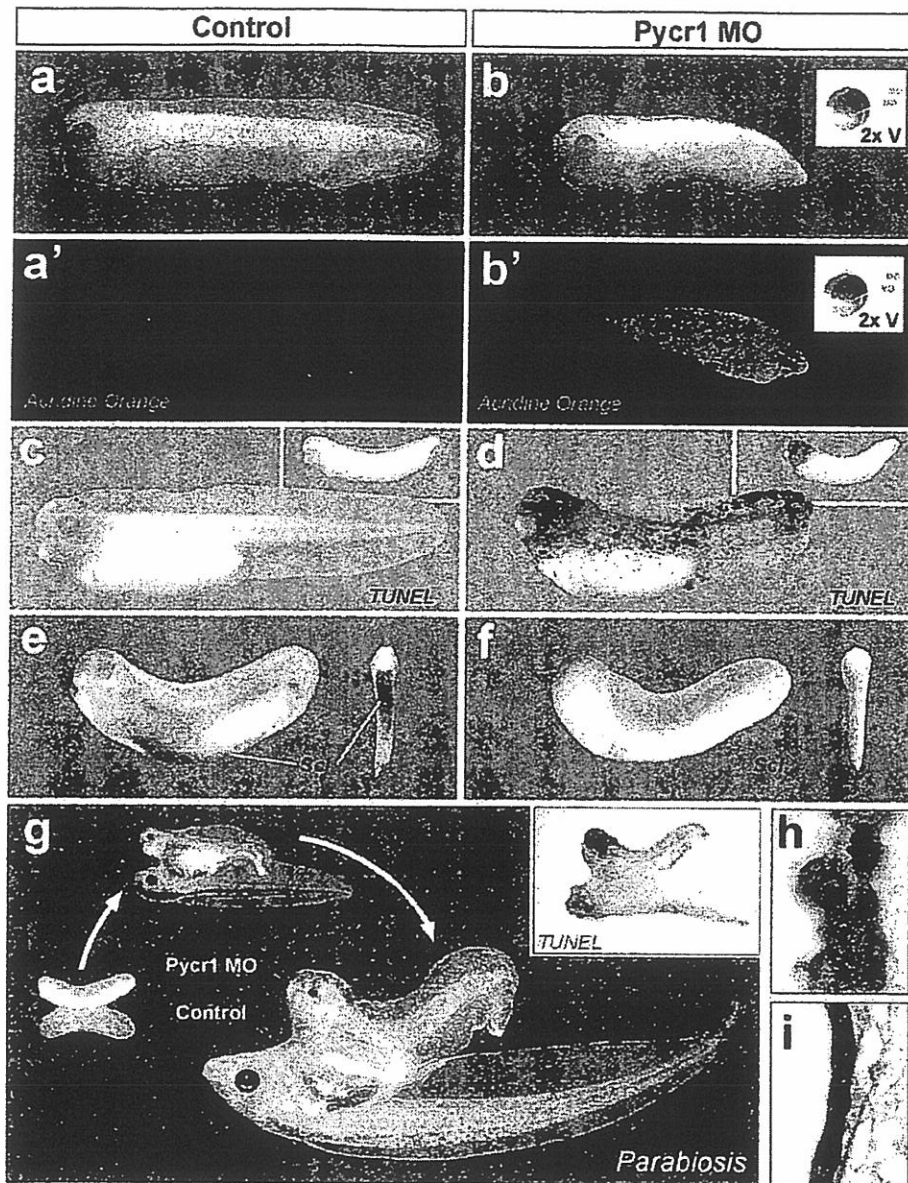
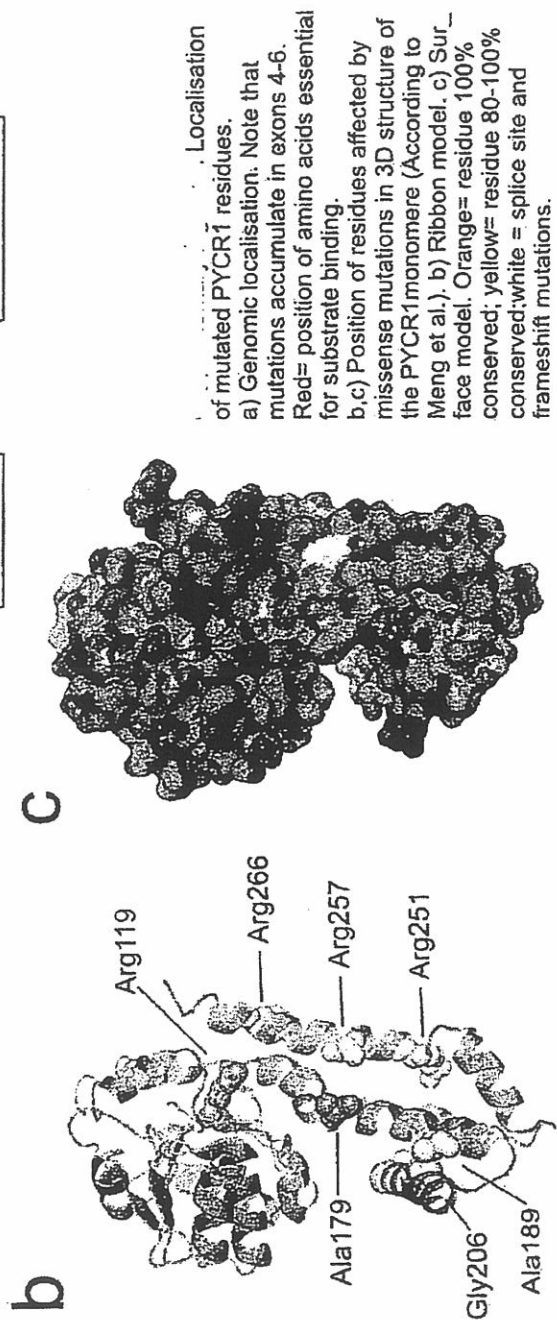
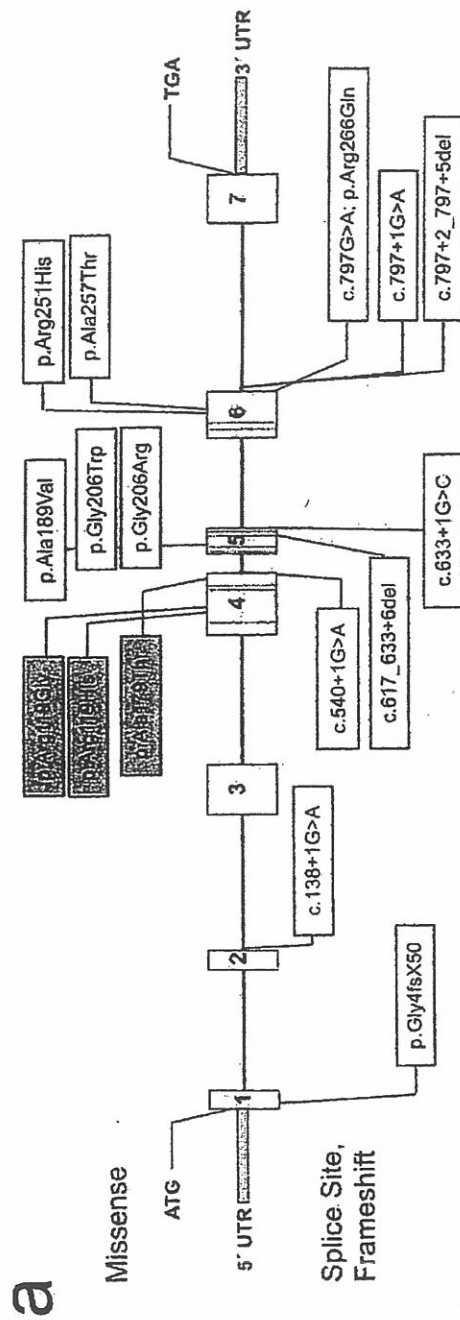


Figure5

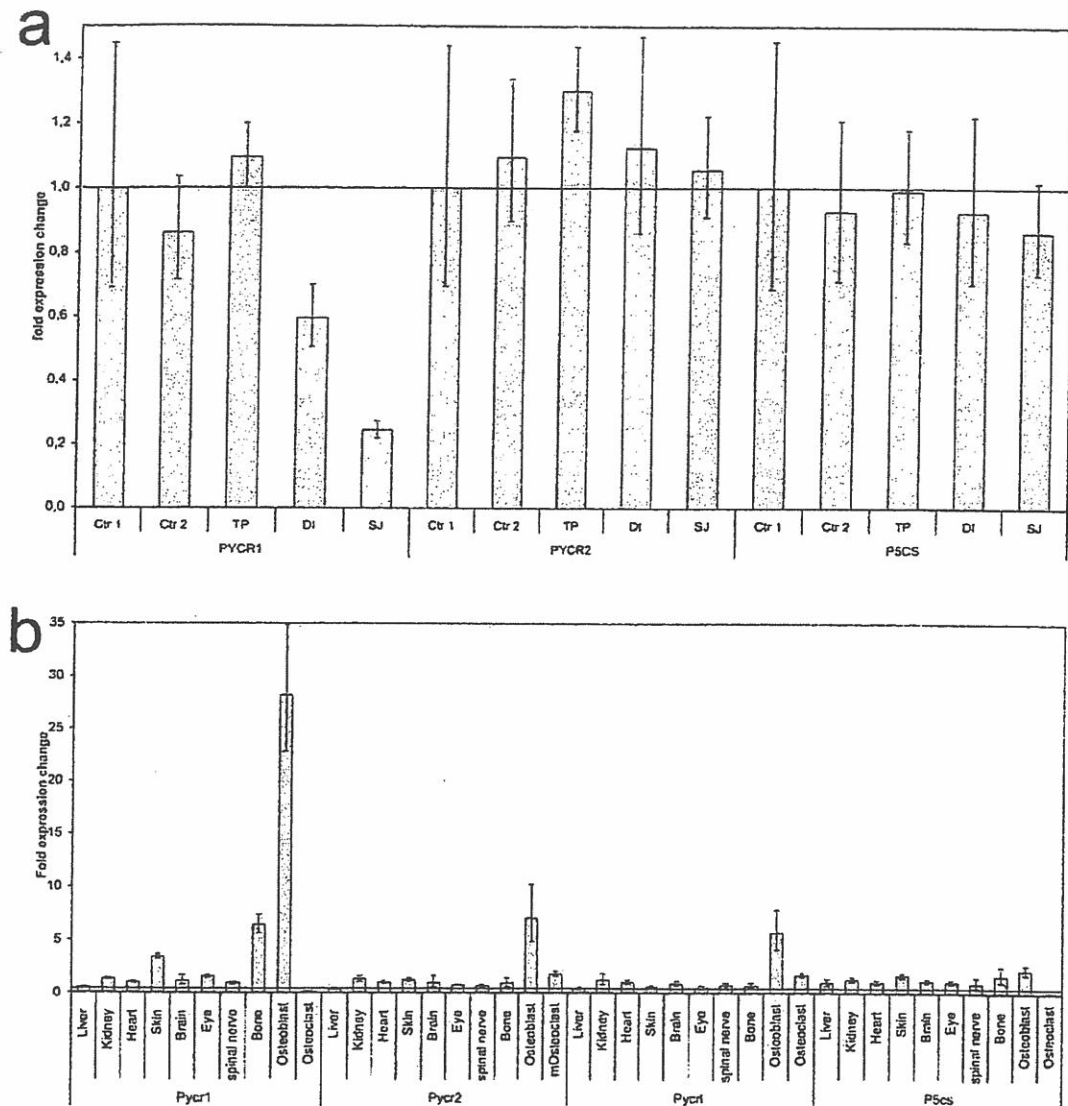


Localisation of mutated PYCR1 residues.

a) Genomic localisation. Note that mutations accumulate in exons 4-6. Red= position of amino acids essential for substrate binding.

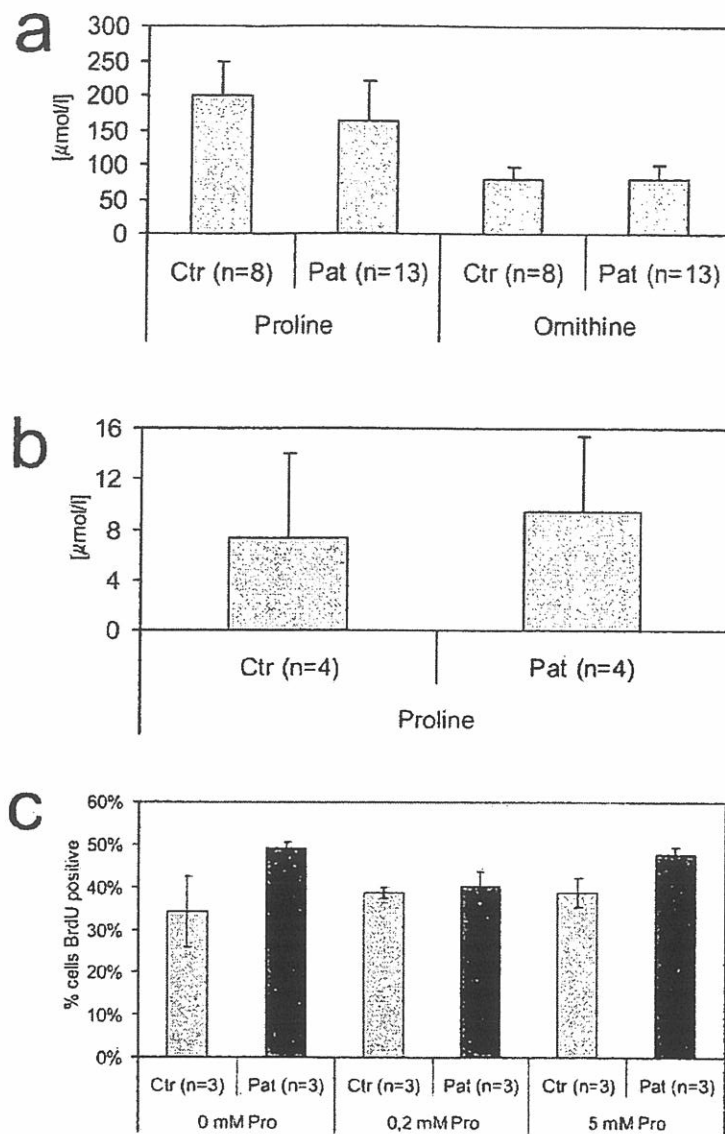
b,c) Position of residues affected by missense mutations in 3D structure of the PYCR1 monomer (According to Meng et al.). b) Ribbon model. c) Surface model. Orange= residue 100% conserved; yellow= residue 80-100% conserved; white = splice site and frameshift mutations.

Figure 6



a) Gene expression changes in fibroblasts from individuals with PYCR1 mutations and controls determined by quantitative PCR. While the Gly206Trp mutation in patient TP does not affect PYCR1 expression levels the mutations in DI and SJ clearly reduce mRNA levels due to nonsense-mediated decay. Expression of PYCR2 and P5CS is not affected. b) Expression of genes involved in proline metabolism in tissues from 4 day old mice normalized to expression levels in the heart determined by quantitative PCR. Note extremely high Pycr1 expression levels in osteoblasts and skin. Pycr2 and Pycr1 are also most highly expressed in osteoblasts, but otherwise show uniform expression, as does P5cs.

Figure 7



a) Proline and ornithine levels in individuals with PYCR1 mutations (Pat) compared to controls (Ctr). Note non-significant ($p=0,051$) reduction of proline concentrations in affected individuals. b) Proline levels in fibroblast lysates from controls and from individuals with PYCR1 mutation. No significant differences were detectable. c) Proliferation rates of fibroblasts from individuals with PYCR1 mutations (Pat) and controls (Ctr) grown in medium with different proline concentrations. No significant differences were observed, but mutant cells showed slightly higher proliferation. Representative result of two independent experiments. Ctr = control; Pat = patient.

Figure 8

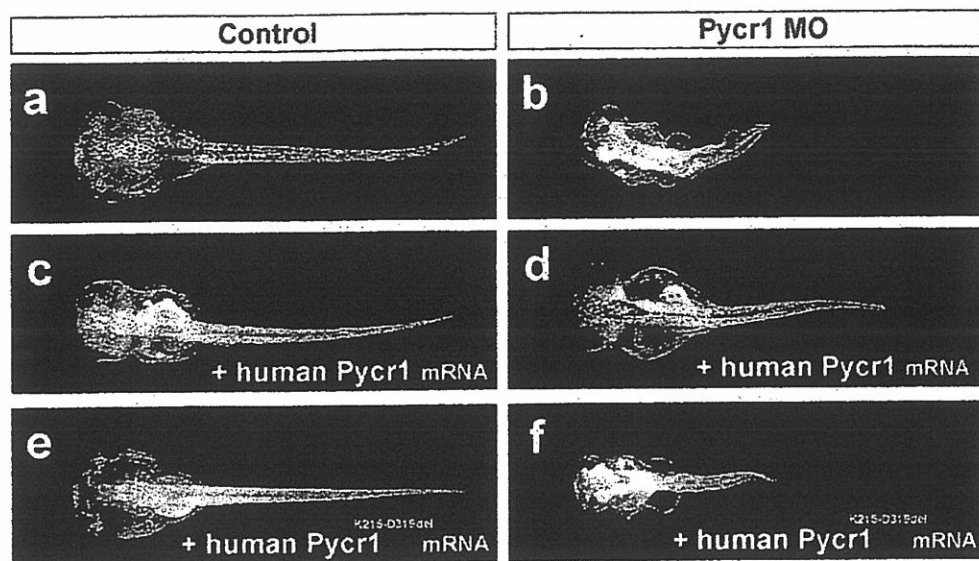


Figure 9

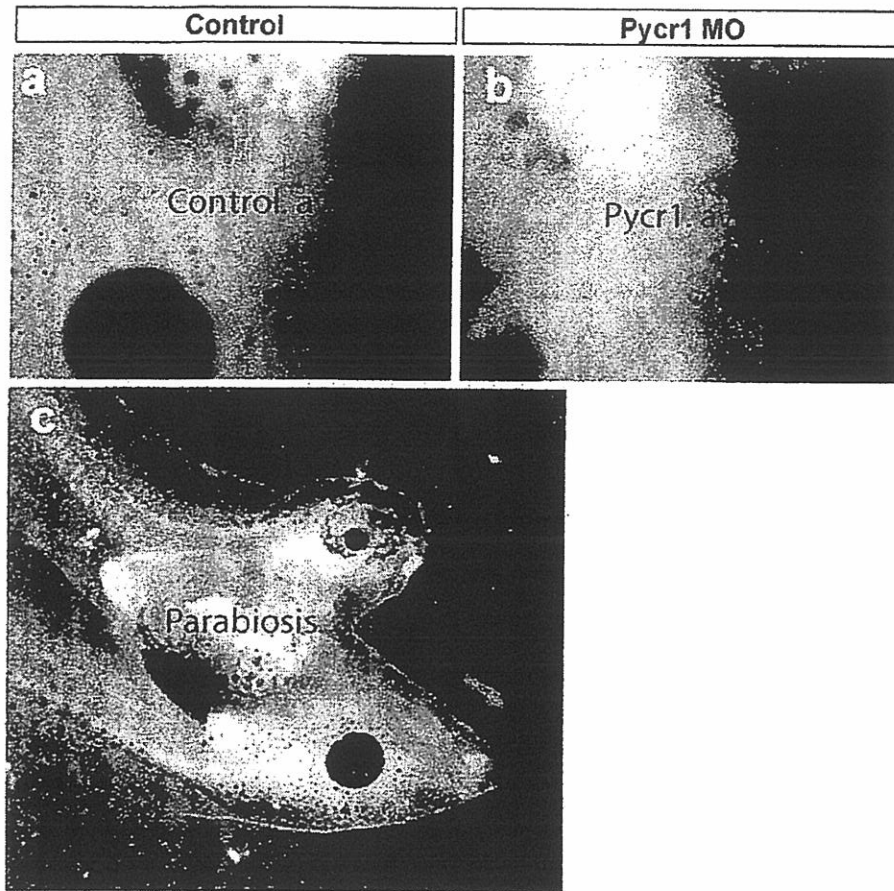
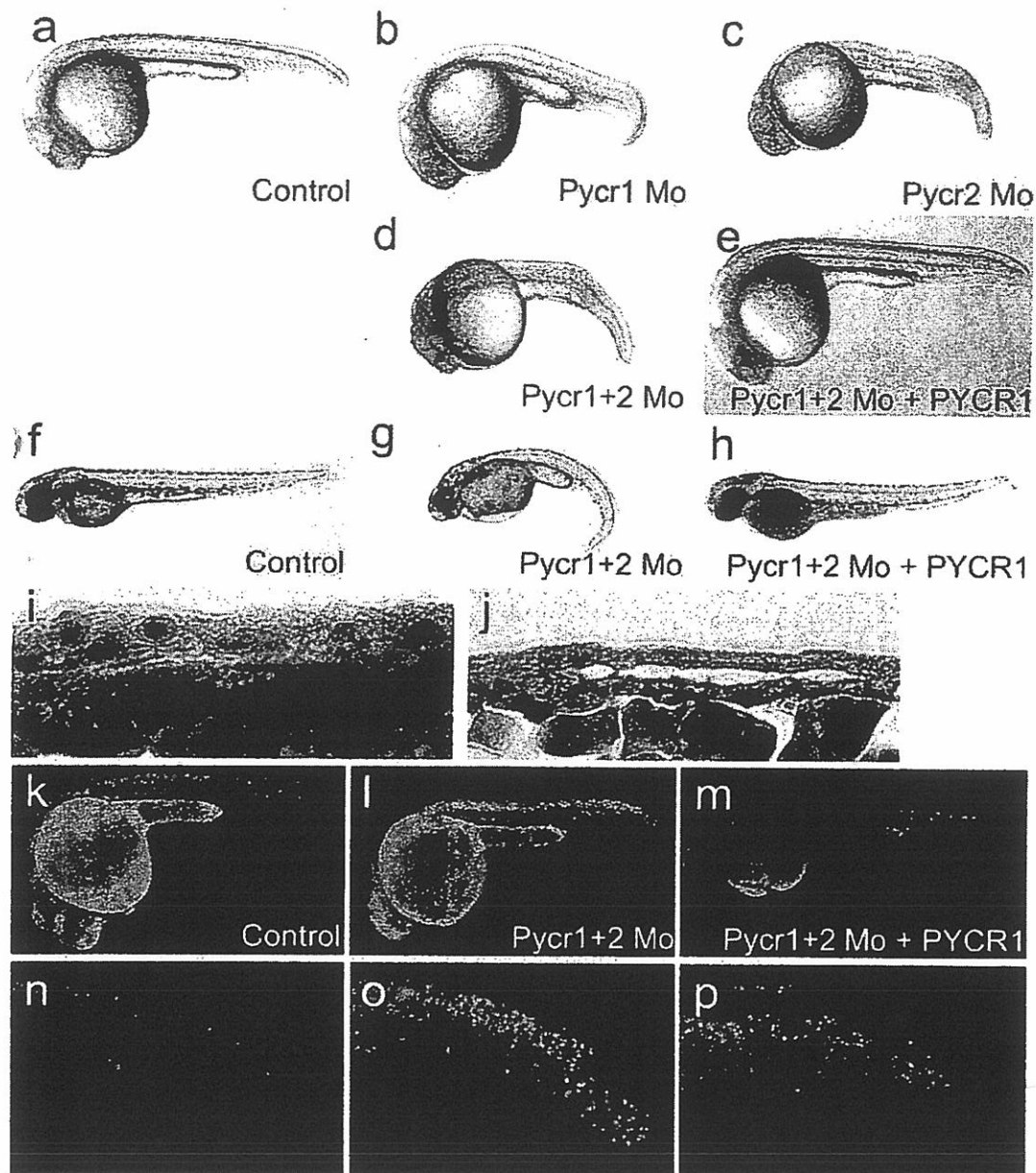


Figure 10



Knockdown of Pycr1+2 in zebrafish leads to malformation, epidermal atrophy and increased apoptosis. (a-h) Control, Pycr1 morphant, Pycr2 morphant, Pycr1+2 morphant and Pycr1+2 morphant zebrafish injected with 20pg human PYCR1 mRNA at stage 24h. (i-h) Control, Pycr 1+2 morphant and Pycr1+2 morphant zebrafish injected with 20pg human PYCR1 mRNA at stage 48h. Note reduced length of morphant fishes, abnormally bowed tail and loosening of the epidermis around the yolk sac. The severity of the phenotype increases when Pycr1+2 are both knocked down. Only subtle changes are seen after rescue of the morphant phenotype with 20pg of human PYCR1 mRNA. (i,j) Dermal atrophy in Pycr1+2 morphants. In 48h old control fish (i) a double-layered epidermis surrounds the yolk sac. In Pycr1+2 morphants this epidermis is atrophic. Goldner trichrome staining. (k-m) Acridine orange staining of apoptotic cells at stage 24h. Note strongly increased number of apoptotic cells in morphants and almost complete reversion of the phenotype by co-injection of the human PYCR1 mRNA. (n-p) Acridine staining of apoptotic cells in the tail, higher magnification.

Figure 11

10 20 30 40 50 60
 MSVGFIGAGQ LAFALAKGFT AAGVLAHAKI MASSPDMDLA TVSALRKMGV KLTPHNKETV

70 80 90 100 110 120
 QHSDVLFLAV KPHIIPFILD EIGADIEDRH IVVSCAAGVT ISSIEKKLSA FRPAPRVIRC

130 140 150 160 170 180
 MTNTPVVVRE GATVYATGTH AQVEDGRLME QLLSSVGFC T EVEEDLIDAV TGLSGSGPAY

190 200 210 220 230 240
 AFTALDALAD GGVKMGLPRR LAVRLGAQAL LGAAKMLLHS EQHPGQLKDN VSSPGGATIH

250 260 270 280 290 300
 ALHVLESGGF RSLINAVEA SCIRTRELQS MADQEQVSPA AIKKTILDKV KLDSPAGTAL

310
 SPSGHTKLLP RSLAPAGKD

Figure 12