Supplementary Material for the article:

Hertz et al., ***Human Mutation*** **18**:141–148, 2001.

Detection of mutations in the *COL4A5* gene by SSCP in X-linked Alport Syndrome

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PCR amplification

Genomic DNA was amplified in a reaction volume of 25 µl containing 200 ng DNA, 200 µM dNTP, 20 pmol of each primer and 1 U *Taq*-DNA polymerase (Boehringer Mannheim). The buffer used for PCR contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$. The PCR conditions were as follows: Initial denaturation at 94ºC for 4 min followed by 30 cycles of denaturation at 94ºC for 1 min, annealing for 1 min at a temperature as indicated for each primer pair in Table S1, and extension at 72ºC for 2 min. The final extension was 4 minutes at 72ºC. Hot start PCR was used for exons 25, 30, and 31, and hot start PCR and AmpliTaq Gold DNA Polymerase (Perkin Elmer) was used for exons 8, 10, 36, 37, 41, and 44.

Table S1: Primer sequences and conditions for PCR amplification and SSCP analysis used in the study

			PCR-amplification			SSCP analysis	
Exon	Forward primer (5' → 3')	Reverse primer (5' → 3')	Annealing temp. (°C)	Product size (bp)	Gel	Running conditions at 4 °C	at 15 °C
1	TCAATTGGTTAGAGCCA	AGCGTGAAAGAGCGGTGATG (28)	59	195	12.5%	150 Vh	110 Vh
2	GATGTGATTTCAGTTGAGCTGT(63)	CTAAGTACTGAGATAGAAGCT (77)	63	244	12.5%	60 Vh	50 Vh
3	TCTCAACCATGCCTGTGCTT (44)	TGATGTGACACCTACTCCCAC (54)	62	229	12.5%	250 Vh	175 Vh
4	AAAACCTAAAAATATTGCA (8)	GTATTTGATATAGAGAAAATAC (7)	45	101	12.5%	70 Vh	50 Vh
5	GTTTAAGGATTTTATTTCTT (7)	TCAAAGTAAAAAGTGAAATG (8)	45	100	12.5%	110 Vh	70 Vh
6	ATTATACATGTGTTATGTCG (10)	ACTACCCAAGATTAAATGGA (10)	45	123	12.5%	120 Vh	80 Vh
7	TCCATGCTCTTTATTTTTAA (10)	TCATCATAATCCCAAATTTT (9)	45	113	12.5%	120 Vh	80 Vh
8	CCTTTTCTTTTTAATAATAG (0)	CCCCAGAATGAGATG (5)	40*	67	12.5%	70 Vh	50 Vh
9	AGAACTTCCATTGATGGCTTCT (5)	AAAAAAGAAAATCACTGGATAC (4)	55	129	12.5%	150 Vh	90 Vh
10	GGCGACACAAGTGAGACTTT (78)	GAATGTTGAGAATGCATTATGTTTTC (81)	58*	268	12.5%	300 Vh	225 Vh
11/12	TATTTTCTCTTTTGTCTTCTCTTC(4)	GAATAACCAGCTCTCTTTCTTTAC (4)	55	224	12.5%	350 Vh	175 Vh
13	CCACTGTCTTATTTTATCTTGC (6)	CATTGACTTCCTCCCTACTTAC (4)	55	157	12.5%	150 Vh	90 Vh
14/15	GATTTCCTTTCCCCTACTACTG (5)	TGCAAAGAATATTAGCAGTTACATC (6)	55	264	12.5%	350 Vh	275 Vh
16	GCCCTATCATTTCTTTGTATCC (5)	AGGGGGAAAGAACCTTAGCTAC (4)	55	98	12.5%	70 Vh	50 Vh
17	CTGATGTCACCCTATCCTCTATG (8)	TAGACTAAACCAGTCACTTCAAAG (15)	55	124	12.5%	100 Vh	80 Vh
18	CTAACCTATTTTACAATTGC (9)	ATTTGATAAACGAAGACTAA (15)	55	106	H.D.	900 Vh	700 Vh
19	GCATTTCTTTATTTTTTTTTC (13)	CAAGGCCATAAATGCAATC (8)	55	195	H.D.	1000 Vh	800 Vh
20	AAGATGAAATCATTTTGATCAC (15)	GAACTTAATAGGAGAAAAATATAGC (4)	55	240	H.D.	900 Vh	700 Vh
21	GCTTGCTATCCTTTCTTTATCTTAC(4)	GAAGGAATGAATATGTTTGGAGATC (4)	55	142	12.5%	130 Vh	70 Vh
22	TGTTATTATGATTTCACTAG (0)	TTAGAAGGTTACCCTGAGGC (9)	55	142	12.5%	130 Vh	70 Vh

Table S1 (continued)

			PCR-amplification			SSCP analysis	
			Annealing	Product		Running conditions	
Exon	Forward primer (5' ® 3')	Reverse primer (5' ® 3')	temp. (°C)	size (bp)	Gel	at 4 °C	at 15 °C
23	AAGCTTACGTTATTGTGT (33)	TGTAAAATGCCTTCCTTC (36)	56	176	12.5%	160 Vh	300 Vh
24	CTTTTTTTCCTTACTCATTTC(2)	AACCAAAAATATCAAACCAAC (4)	57	240	20%	600 Vh	300 Vh
25	ATATGTTTCTGTATTAAAC(14)	TAAGCACCAAGTTTAAAAC (18)	44*	239	12.5%	250 Vh	225 Vh
26	ACTTCTCATTTACCATTGATTTAC(12)	GTTACTTTGAAATAAATTCCTCAC (4)	55	157	12.5%	130 Vh	90 Vh
27	GTTTTCCTTTCAATAACTGCTGTTTC(7)	ACTCTGCCTGCTACCCATTCC (7)	58	166	12.5%	150 Vh	80 Vh
28	TCCTTTGGTGGTTAAAAAATGAC(14)	GAGAAGGAATAAAGAAAAATGTCCC (4)	58	164	12.5%	150 Vh	80 Vh
29	ATGGGAGTTTTTGTTGTGTTTTGTC(15)	CAAGTTGAGATGCAGTGACAGCC (7)	60	221	12.5%	250 Vh	150 Vh
30	TTTAAACTGTATTTATTTCTTA (3)	TACAAAATGCACATTTACTCTA (7)	44*	168	20%	600 Vh	300 Vh
31	CTTATTAATATTGATATTGTATT (6)	AAATCAGAGAAAACTTTAAAC (7)	45*	225	12.5%	250 Vh	150 Vh
32	AATAGTTTTCTGGTTGACATC (68)	TATTCTGTACTGACATAAAGC (51)	55	251	12.5%	285 Vh	175 Vh
33	ATATTGTGTTTTCACACACTTTGA (6)	AAATATTCATAAATAAATTCATTCAC (4)	55	210	12.5%	200 Vh	130 Vh
34	CTTGCCTCTTCTACTCATTCTTG (4)	CAATTGCTACAAATGGCCTATCAC (6)	55	156	20%	500 Vh	300 Vh
35	TTAATTTTACCAATTTGACCTTCC (3)	CTAAATATTTGGAAGATTTTCATC (6)	53	147	20%	550 Vh	300 Vh
36	AATATTATATATCACATATTTTCAAC (2)	TGCCTAAAACTATATGCCAAAG (5)	54*	195	12.5%	150 Vh	85 Vh
37	ATTTACATCAAGTACTTACTGGAG (99)	AGTCTGCCAAATAAGAAGCTGC (65)	63*	337	12.5%	100 Vh	80 Vh
38	AAAGCAATGCAGTTTTTCTTTC (27)	AACAGCAAACTGTTATTTTTCATG (5)	55	159	12.5%	85 Vh	60 Vh
39	GGTGTAACCTGCTGTACTCAATT (6)	AATAGGAAAAATGAAAAACTACAG (3)	55	155	20%	550 Vh	300 Vh
40	TGTTTTGTTTTGTTTTGTACTCTG (4)	TTGATTTAGCATGTTTTATTAAGG (5)	53	108	12.5%	85 Vh	60 Vh
41	TTATCTTCTAATTATACTTTACTTTC (4)	AGACCATTCTCCTACCACTC (10)	55*	246	12.5%	250 Vh	225 Vh
42	AATGTCGTCATTTGCTGTGGATTA (5)	CATCAGATATCTACTTCCATTTCC (3)	55	191	12.5%	210 Vh	110 Vh
43	CAATCACCTTCCTCCCCTCG (40)	CAAATCAGAAAATGGCTATCTTG (50)	65	206	12.5%	250 Vh	150 Vh
44	AAAACTGTATGTACCTTCTGTG (3)	TATAACTATCTTCAGGAATAAGTC (4)	54*	125	20%	600 Vh	300 Vh
45	CCCTTCAAATTTGTGTGTTTTGTC (6)	GATAATAAAGATGATCTGCATTGG (3)	55	186	12.5%	210 Vh	110 Vh
46	TATTTGAATGCCTCATTCTTTTCC (5)	ACCAACAGCATGTTTTACTTGTC (4)	55	155	12.5%	175 Vh	125 Vh
47	TCTTGTATACTGATTATTTCGTGG (6)	CAGTAGGAAATTAGATATTGATTA (6)	55	273	H.D.	900 Vh	600 Vh
48	CTTTACTGTTTTCTCTCCAAATCT (6)	TAAAAGTCACAGCTAAATCAATGCC (4)	55	237	12.5%	375 Vh	275 Vh
49	ATTATGTTCCTTCTCCTTTTCCTT (5)	ATGACAAATGCAAGGAAGAGTGTA (6)	58	175	12.5%	250 Vh	150 Vh
50	TTGCGGCACATTTTTCCTTGTCTT (6)	GGACCTGAATTAAAGCTATAAGCAC (5)	55	233	12.5%	300 Vh	175 Vh
51	GATCTGATTGTCTTATTTCTTATT (6)	TAAAACACAAAAGGAATTCTTCAA	55	139	20%	350 Vh	300 Vh

Primers synthesized according to published sequence data (Zhou et al., 1994; Martin et al., 1998). Exonic sequences are underlined.
Number of intron bases between primer and exon are indicated in parentheses.
* Hot Start PCR; H.D. = High Density gel

REFERENCES

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