

## Supplementary data:

# Mutational landscape of the human Y chromosome-linked genes and loci in patients with hypogonadism

Deepali Pathak, Sandeep Kumar Yadav, Leena Rawal and Sher Ali

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**Table 1.** Details showing age, sex, karyotype, clinical features and diagnosis results of the patients with H.

Patient study ID	Karyotype	Clinical features	Fertility status	Diagnosis	Hormone profile		
					T (ng/dL)	LH (I U/L)	FSH (I U/L)
*H1	46 XY	Cryptorchidism	NR	PH	0.01	0.1	2.4
*H2	46 XY	Micropenis/small bilaterally descended testes	NR	PH	0.05	0.6	1.2
*H3	46 XY	Micropenis	NR	PH	0.04	0.9	1.6
H4	46 XY	Aspermia	–	HH	1.8	2.5	2.8
H5	46 XY	Micropenis	Azoospermia	HH	0.02	0.9	0.6
H6	46 XY	NR	Oligospermia	HH	0.9	2.8	3.6
*H7	46 XY	Cryptorchidism	NR	PH	0.05	1.5	1.3
*H8	46 XY	Cryptorchidism	NR	PH	0.01	0.1	1.6
H9	46 XY	NR	NR	HH	0.2	0.6	0.8
H10	46 XY	Micropenis	Azoospermic	HH/KS	1.2	0.1	0.6
*H11	46 XY	Cryptorchidism/anosmia	NR	PH/KS	0.03	0.2	1.17
*H12	46 XY	Cryptorchidism/anosmia	NR	PH/KS	0.02	0.8	0.4
*H13	46 XY	Cryptorchidism/anosmia	NR	PH/KS	0.2	0.5	0.8
H14	46 XY	anosmia	NR	HH/KS	0.5	3.2	4.5
H15	46 XY	NR	NR	HH	1.0	3.7	1.2
H16	46 XY	Aspermia	–	HH	0.5	4.3	3.0
H17	46 XY	Cryptorchidism	Azoospermia	HH	1.2	0.1	0.6
H18	46 XY	NR	Azoospermia	HH	0.2	3.9	4.9
H19	46 XY	Bilateral gynecomastia	NR	HH	2.0	0.3	0.3

Serum T (testosterone) level range in normal males adult from 3 to 11 ng/dL; FSH, follicle stimulating hormone; LH, luteinizing hormone levels 5–20 I U/L. \*Prepubertal cases of hypogonadism (PH); HH, hypogonadotropic hypogonadism; KS, Kallmann syndrome; M, male; NR, fertility status not reported.

**Table 2.** Details of primers/STSs used for PCR and sequencing analysis.

Gene	Forward primer	Reverse primer	$T_m$ (°C)
Y-linked genes			
<i>SRY1</i> *	GAATCTGGTAGAAGTGAGTTTTGGA	GCCTTTATTAGCCAGAGAAAAGAAA	55
<i>SRY2</i>	CTTCTGCTATGTTAAGCGTATTCAA	CAGCTTTGTCCAGTGGGCTTGTA	60
<i>ZFY</i> *	GATCCAGAGATGTATGAGAGCATTT	CCATTCTCCAAATTCTTATTAACCA	55
<i>AMELY</i> *	TGTTTGCATTAGCAGTCCCCTGG	GCGGTGCAAACACTGTCCCTC	60
<i>USP9Y1</i>	TGCAAGATGTTTTGTCCTTGAA	AATCATTCAGGACATGTTTCACAA	60
<i>USP9Y2</i>	GAGCCCATCTTTGTCAAGTTTAC	CTGCCAATTTTCCACATCAACCC	60
<i>USP9Y3</i>	GCCATTGTAGCTTTTTCTACCAATTA	CTTGAAATCACTTATGACCCAGTCT	55
<i>DBY1</i>	TATTGGCAATCGTGAAAGAC	TGCCGGTTGCCTCTACTGGT	60
<i>DBY2</i>	ATCGACAAAGTAGTGGTTCC	AGATTCAGTTGCCCCACCAG	60
<i>DUXY</i> *	CCGACACCTTCGGACAGCAC	GTGGTCTGGGATCCGGTGAC	61
<i>KALY</i> (sY182)*	TCAGAAGTGAAACCCTGTATG	GCATGTGACTCAAAGTATAAGC	60
Housekeeping genes			
$\beta$ -actin	AGATGACCCAGATCATGTTTGAGA	CTAAGTCATAGTCCGCCCTAGAAGC	60
<i>GAPDH</i>	GCCACATCGCTCAGACACCAT	ACCAGGCGCCAATACG	60
Region	STS screened		No. of STS
<i>AZFa</i>	sY83, sY84, sY86, sY87, sY88, sY746, sY1179, sY1180, sY1181, sY1064, sY1065, sY1182, sY1183, sY1184, sY1185, sY1186, sY1066, sY1316 ( <i>USP9Y</i> exon 26), sY1317 ( <i>USP9Y</i> exon 3), sY1234 ( <i>DBY</i> exon 9), sY1231 ( <i>UTY</i> exon 8)		21
<i>AZFb</i>	sY113, sY117, sY124, sY125, sY127, sY129, sY131, sY627 ( <i>RBMV</i> exon 12), sY1233 ( <i>EIF1AY</i> exon 1)		9
<i>AZFc</i>	sY152, sY153, sY155, sY148, sY146, sY147, sY159, sY254, sY255, sY258, sY277, sY279, sY283, sY254, sY1161, sY1191, sY1291, sY1206, sY1201, sY1197, sY1258, sY160, sY121, sY142, sY1054, sY1125, sY1190, sY1246, sY1263, sY1322		30
Other MSY-STS markers	sY14 ( <i>SRY</i> exon 1), sY238 ( <i>ZFY</i> intron 2), sY1161 ( <i>PRY</i> intron 2), sY274 ( <i>RPS4Y1</i> intron 4), sY1254 ( <i>TGIF2LY</i> exon 1), sY1240 ( <i>PCDH11Y</i> intron 3), sY1256 ( <i>TSPY</i> intron 5), sY276 ( <i>AMELY</i> exon 4/intron 4), sY1238 ( <i>TBL1Y</i> exon 11), sY637 ( <i>PRKY</i> 5' upstream of gene), sY1319 ( <i>PRKY</i> 3'UTR), sY1250 (proximal boundary of <i>TSPY</i> array), sY1251 (boundary between centromere and Yq), *sY182 ( <i>KALY</i> ), sY1230 ( <i>TMSB4Y</i> exon 1/intron 1), sY1220 ( <i>VCY</i> exon 2), sY1239 ( <i>NLGN4Y</i> exon 1), sY210 ( <i>STSP</i> intron 5), sY280 ( <i>JARID1D</i> ( <i>SMCY</i> ) exon 9/intron 9), sY1682 ( <i>RPS4Y2</i> exon 1), sY1237 ( <i>HSFY</i> exon 2), sY1260 ( <i>CDY2</i> exon 1), sY1235 ( <i>XKRY</i> exon 1), sY1258 (boundary between unique sequence u1 and blue amplicon b1 in <i>AZFc</i> ), sY1035 ( <i>BPY2</i> intron 5), sY1318 ( <i>DAZ</i> exon 11), sY1246 (proximal portion of distal Yq heterochromatin), sY160 (satellite-3(DYZ1) sequences in distal Yq heterochromatin)		27
Total			87

\*Primers used for cloning and sequencing.

**Table 3.** Details of *DAZ*-SNV typing and their status in H patients.

(a) <i>DAZ</i> -SNV	I	II	III (sY586)	V (sY587)	VII (sY581)	<i>DAZ</i> (sY152)	<i>SRY</i> (sY14)	No. and frequency of <i>DAZ</i> SNVs affected
Location within gene	5' UTR exon 1	Exon 3–4	Intron 7A/B	Intron 9	Intron 3	Intron 6a	Exon	
Restriction enzyme	<i>FspI</i>	<i>MboI</i>	<i>TaqI</i>	<i>DraI</i>	<i>Sau3AI</i>	Controls		
Haplotype/ fragments size (bp)	A - 709 B- 398 + 311	A-182 B-122 + 60	A-301 B-184 +117	A-195 + 49 B-122 +73 + 49	A-189 + 63 B-130 + 63 + 59	125	470	
Amplicon copy	A- <i>DAZ1</i> , <i>DAZ2,DAZ3</i> B- <i>DAZ4</i>	A- <i>DAZ1</i> B- <i>DAZ2,DAZ3</i> , <i>DAZ4</i>	A- <i>DAZ2</i> B- <i>DAZ1, DAZ3</i> , <i>DAZ4</i>	A- <i>DAZ3</i> , <i>DAZ4</i> B- <i>DAZ1</i> , <i>DAZ2</i>	A- <i>DAZ1</i> , <i>DAZ4</i> B- <i>DAZ2</i> , <i>DAZ3</i>	<i>DAZ</i> (1+4)		
(b) Patient ID	Amplicon copy							
*H1	A+B	A+B	A+B	A+B	A+B	+	+	–
*H2	A+B	A+B	A+B	A+B	A+B	+	+	–
*H3	A+B	A+B	A+B	A+B	A+B	+	+	–
H4	B	–	B	–	A+B	+	+	4/5 (0.8%)
H5	A+B	A+B	A+B	–	A+B	+	+	1/5 (0.2%)
H6	B	A+B	A+B	–	A+B	+	+	2/5 (0.4%)
*H7	A+B	A+B	A+B	A+B	A+B	+	+	–
*H8	A+B	A+B	A+B	–	A+B	+	+	1/5 (0.2%)
H9	A+B	A	B	–	A+B	+	+	3/5 (0.6%)
H10	A+B	A+B	A+B	A+B	A+B	+	+	–
*H11	A+B	A+B	A+B	A+B	A+B	+	+	–
*H12	A+B	A+B	A+B	A+B	A+B	+	+	–
*H13	A+B	A+B	A+B	A+B	A+B	+	+	–
H14	A+B	A	A+B	A+B	A+B	+	+	1/5 (0.2%)
H15	A+B	A+B	A+B	–	A+B	+	+	1/5 (0.2%)
H16	B	A+B	A+B	A+B	A+B	+	+	1/5 (0.2%)
H17	A+B	A+B	A+B	A+B	A+B	+	+	–
H18	A+B	A+B	A+B	–	A+B	+	+	1/5 (0.2%)
H19	A+B	A	B	–	A+B	+	+	3/5 (0.6%)

–, uncut template.

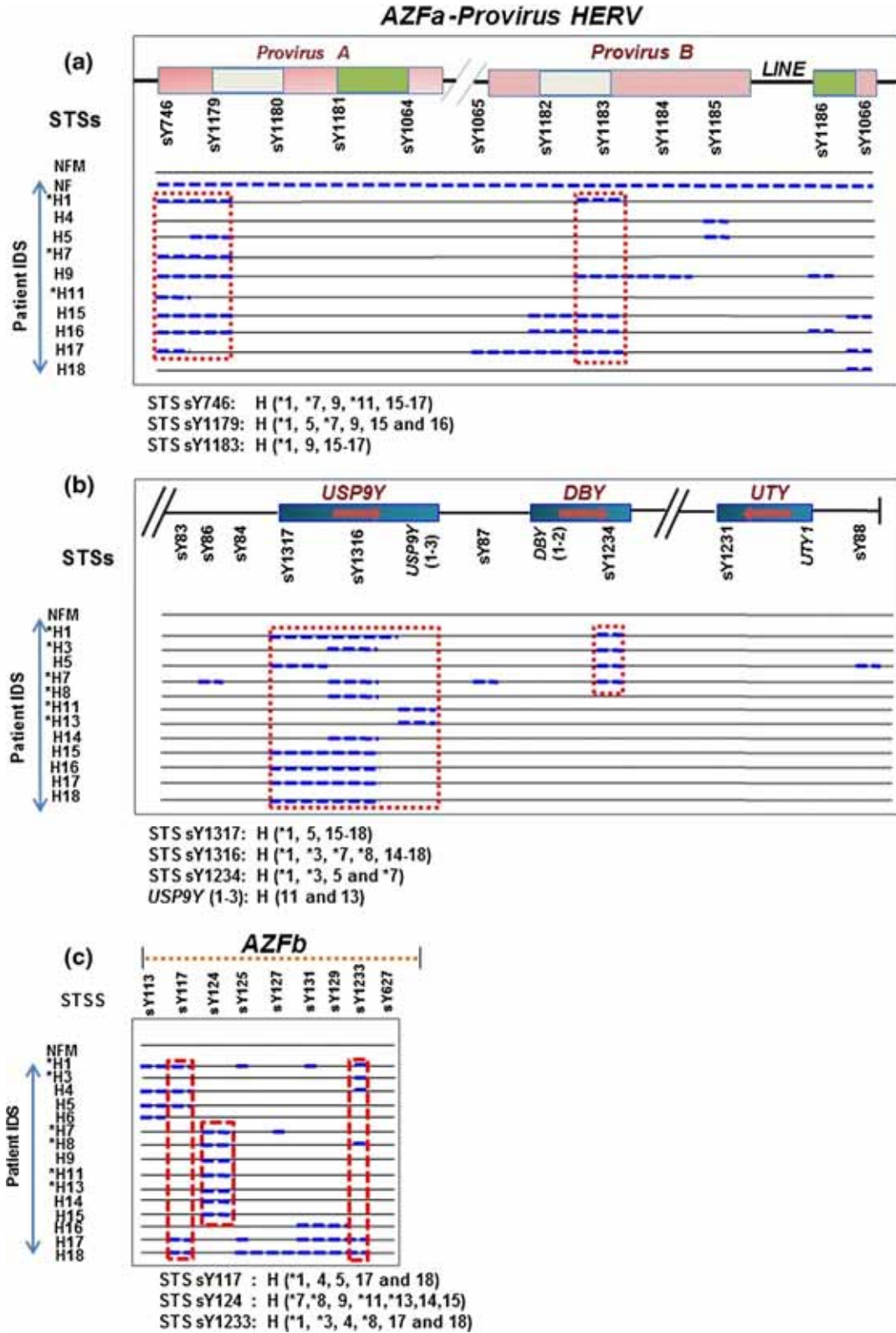
**Table 4.** Primers and assays used for copy number and expression analysis in current study.

Primer name	Primer sequence	Reporter/ quencher dye	Cytoband location
Copy number primers/assays			
<i>SRY</i>	Hs00243216_s1	FAM/TAMRA	Yq11.3
<i>SARBPY2F</i>	TGGAGTCTGCCAAAACAAGGG		Yq11.223
<i>SARBPY2R</i>	CAGAGCAGGAGAGTCTCATCAC		
<i>SARBPY2P</i>	CACATATTGCGGAGTCCAGCACCCAGG	FAM/TAMRA	
<i>SARHSFYF</i>	TCAATGAGGCTCCTTATCCTAACCT		
<i>SARHSFYR</i>	GCAGCCGATGTATCAAATGTCATAG		Yq.11.222
<i>SARHSFYF</i>	CCAGCAGGCAACCAG	FAM/TAMRA	
<i>SARDAZF</i>	TGACTGGACACCTAGTTTCATGAAC		Yq11.3 (sY587)
<i>SARDAZR</i>	GTCAAGAGGCATCAAGTGAAAGTTG		
<i>SARDAZP</i>	CACCCTGTCTCCAACCC	FAM/TAMRA	
<i>SARPRYF</i>	CAGGATGAAGGGATGCAGTGA		Yq11.223
<i>SARPRYR</i>	CTTAGAGGTGGGTGTCAGTGAAG		
<i>SARPRYP</i>	CAAGAGCCCAACCTTC	FAM/TAMRA	
<i>SARAMELYF</i>	CCCTGGGCTCTGTAAAGAATAGTG		Yp11.2
<i>SARAMELYF</i>	ATCAGAGCTTAAACTGGGAAGCTG		
<i>SARAMELYP</i>	CATCCCAAATAAAGTG	FAM/TAMRA	
<i>SARXKRYF</i>	TCCCTCTTATCAGTTGTGTATGGG		Yq11.221
<i>SARXKRYR</i>	TGAGATAGTCACCAAGGAATGCC		
<i>SARXKRYP</i>	TCACCGCAACATACTAGCAATCCACACC	FAM/TAMRA	
<i>DYZ1F</i>	GTGGGCCGCTCTAGACACCA	SYBR	Yq12
<i>DYZ1R</i>	TGCCAAATCATTGCATTCTTTCC		
Expression primers/assays			
<i>SRY</i>	Hs00243216_s1	FAM/TAMRA	Location in the gene Single exon
<i>UTY</i>	Hs00273053_m1	FAM/TAMRA	Probe spans exons
<i>VCY1</i>	Hs00855141_sH	FAM/TAMRA	Single exon
<i>DAZ1</i>	Hs00414014_m1	FAM/MGB	Probe spans exons
<i>HPRT1</i>	Hs99999909_m1	FAM/TAMRA	Probe spans exons
<i>GAPDH</i>	GCCACATCGCTCAGACACCAT ACCAGGCGCCAATACG	SYBR	Probe spans exons

**Table 5.** Relative expression values for *SRY*, *UTY* and *VCY* genes in blood lymphocytes of patients with H.

Patients ID/genes	Expression analysis $2^{-\Delta\Delta Ct}$		
	<i>SRY</i>	<i>UTY</i>	<i>VCY</i>
NFM	9.452907	0.468628367	0.309197623
		H/KS	
H4	503.7129366	147.7221468	<i>cb</i>
H5	404.2793849	144.3217796	32.78135025
*H7	291.5573325	64.32200401	7.31594304
*H8	328.5065626	67.76747041	8.36019396
H9	265.4699956	86.76363609	11.48938816
H10	155.6717859	66.41435025	7.53831936
*H11	235.9987251	37.83357081	77.17563933
*H12	0.003666302	63.11031364	19.41929867
*H13	113.15747	106.504464	23.90818816
H14	1.131138602	67.1735964	10.09269361
H15	178.6633223	<i>cb</i>	9.74251369
H16	0.676424003	70.16240169	9.132786203
H17	236.845944	36.40070889	16.378209
H18	<i>cb</i>	150.1127292	8.830703722
H19	165.8338695	145.4496301	29.15568016

*cb*, calibrator; NFM, average  $\Delta\Delta Ct$  values for all the normal fertile males. H, hypogonadism.



**Figure 1.** Microdeletions in *AZFa/AZFb/AZFc* regions of H patients. Diagrammatic representation shows (a) *AZFa*-provirus HERV and interstitial region between STSs, sY1064 and sY1065 covering *USP9Y*, *DBY* and *UTY* genes (b). Panel (c) signifies *AZFb* region representing STSs, sY113-sY627. Line in blue represents deleted STSs. STSs absent in majority of the patients are marked in red box. IDs are mentioned on left side and STSs on top of respective a, b and c panels. NFM, normal fertile male controls; \*marked IDs, PH cases suspected of HH.

NFM GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 59  
H18 GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TA-GTTTCGAACTCTGGCACCTTTCA 58  
\*H3 GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCCTTTCA 59  
\*H7 GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 59  
\*H8 GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 58  
\*H2 GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 60  
H6 GAATCTGGTAGAAGTGAGTTTTGGATA-TAAA--TAAGTTTCGAACTCTGGCACCTTTCA 57  
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NFM ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119  
H18 ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 118  
\*H3 ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119  
\*H7 ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119  
\*H8 AATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 118  
\*H2 ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 120  
H6 ATTTTGTGCGACTCTCCTTGTTTTTGACAATGCAATCAATGCTTCTGCTATGTTAAGCG 117  
\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

NFM TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 179  
H18 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAATAATTCCTGCTCTCCGGAGAA 178  
\*H3 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 179  
\*H7 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 179  
\*H8 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 178  
\*H2 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 180  
H6 TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCTGCTCTCCGGAGAA 177  
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NFM GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 239  
H18 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 238  
\*H3 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 239  
\*H7 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 239  
\*H8 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 238  
\*H2 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 240  
H6 GCTCTTCCTTCCCTTGGCACTGAAAGCTGTAACCTAAGTATCAGTGTGAAACGGGAGAAA 237  
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NFM ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299  
H18 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 298  
\*H3 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299  
\*H7 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299  
\*H8 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 298  
\*H2 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 300  
H6 ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 297  
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NFM CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 359  
H18 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 358  
\*H3 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 359  
\*H7 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 359  
\*H8 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 358  
\*H2 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 360  
H6 CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCAGAATGCGAAACTCAGAGATCA 357  
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NFM GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419  
H18 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 418  
\*H3 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419  
\*H7 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419  
\*H8 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 418  
\*H2 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 420  
H6 GCAAGCAGCTGGGATACCAAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 417  
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NFM AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 479  
H18 AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 478  
\*H3 AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 479  
\*H7 AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 479  
\*H8 AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 478  
\*H2 AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 480

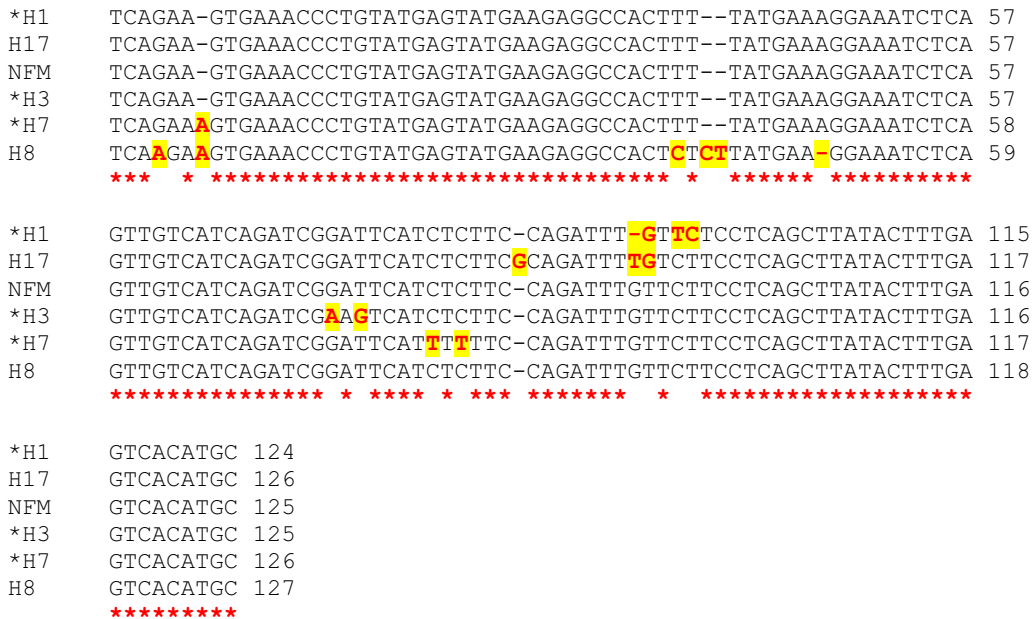
Figure 2. (continues)

H6	AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC	477
	*****	
NFM	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	539
H18	CTGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	538
*H3	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	539
*H7	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	539
*H8	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	538
*H2	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	540
H6	CTCGTCGGAAGGCGAAGATGCTGCCGAAGAATTGCAGTTTGCTTCCCGCAGATCCCGCTT	537
	** *****	
NFM	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	599
H18	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	598
*H3	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	599
*H7	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	599
*H8	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	598
*H2	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	600
H6	CGGTACTCTGCAGCGAAGTGCAACTGGACAACAGGTTGTACAGGGATGACTGTACGAAAG	597
	***** *	
NFM	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	658
H18	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	657
*H3	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	658
*H7	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	659
*H8	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	657
*H2	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	659
H6	CCACACACTCAAGAATGGAGCACCAGCTAGGCCACTTACCGCCC-ATCAACGCAGCCAGC	656
	*****	
NFM	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	718
H18	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	717
*H3	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	718
*H7	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	719
*H8	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	717
*H2	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	719
H6	TCACCGCAGCAACCGGGACCGCTACAGCCACTGGACAAAGCTGTAGGACAATCGGGTAACA	716
	*****	
NFM	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	777
H18	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	775
*H3	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	777
*H7	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	778
*H8	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	776
*H2	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	778
H6	TTGGCTACAAAGACCTACCTAGATGCTCCTTTTT-ACGATAACTTACAGCCCTCACTTTC	774
	*****	
NFM	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	824
H18	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	822
*H3	-TTATG-TT-AGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	823
*H7	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	824
*H8	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	823
*H2	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	825
H6	-TTATG-TTTAGTTTC-AATATTGTTTTCTTTTCTCTGGCTAATAAAGGC	821
	*****	

**Figure 2.** ClustalW alignment of *SRY* gene sequences in H males. Nucleotide changes are highlighted in yellow and marked in red. *SRY*-CDS region is highlighted in grey. NFM, normal fertile males. Accession number for respective patients is mentioned in table 3.

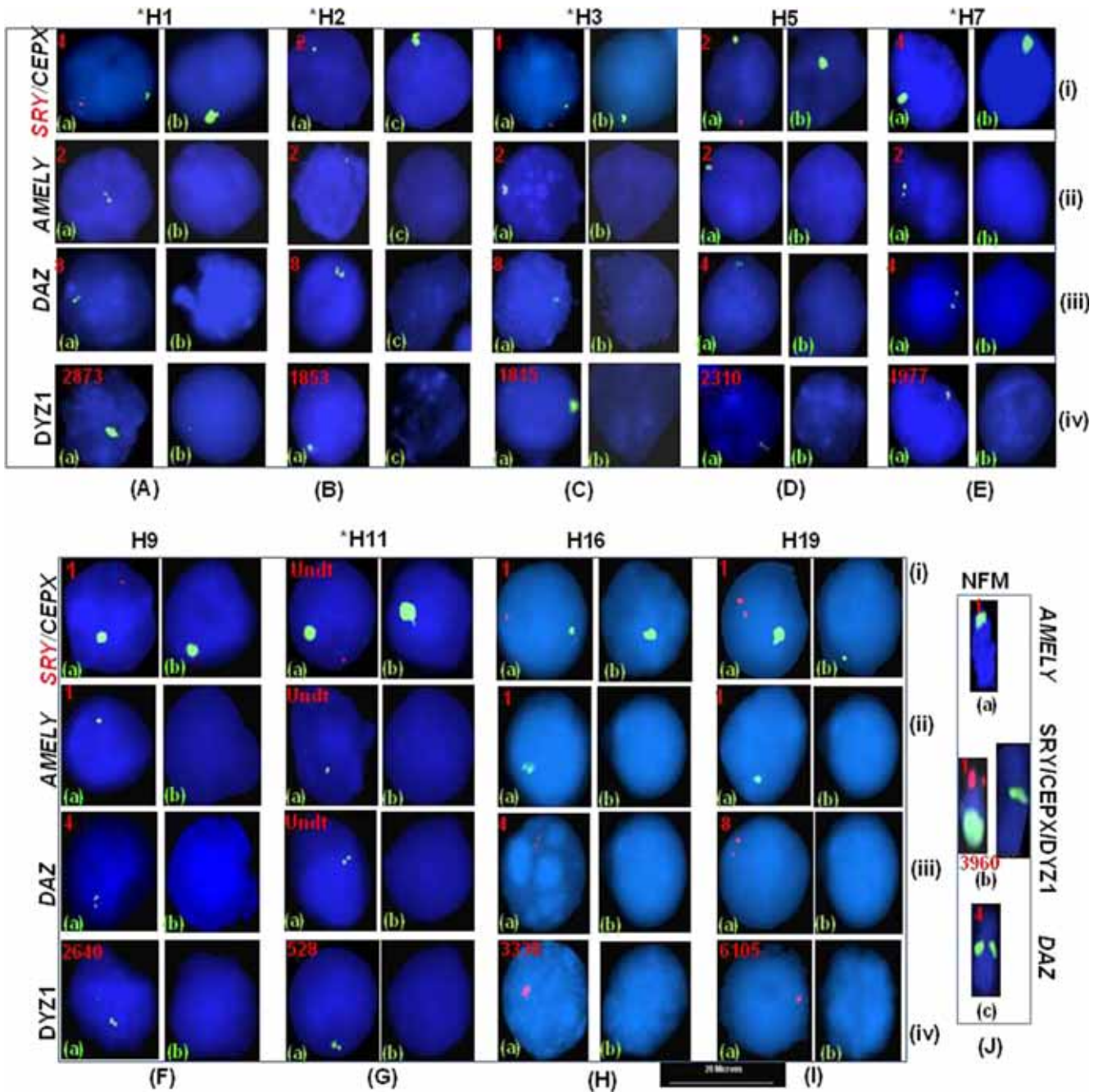


**Figure 3.** ClustalW alignment of *SRY* gene. Amino acid (aa) changes in *SRY* CDS in representative H patients. The box represents HMG region and aa changes are marked in red and highlighted with yellow. H, hypogonadism; NFM, normal fertile control males. Accession number for respective patients is mentioned in table 3.

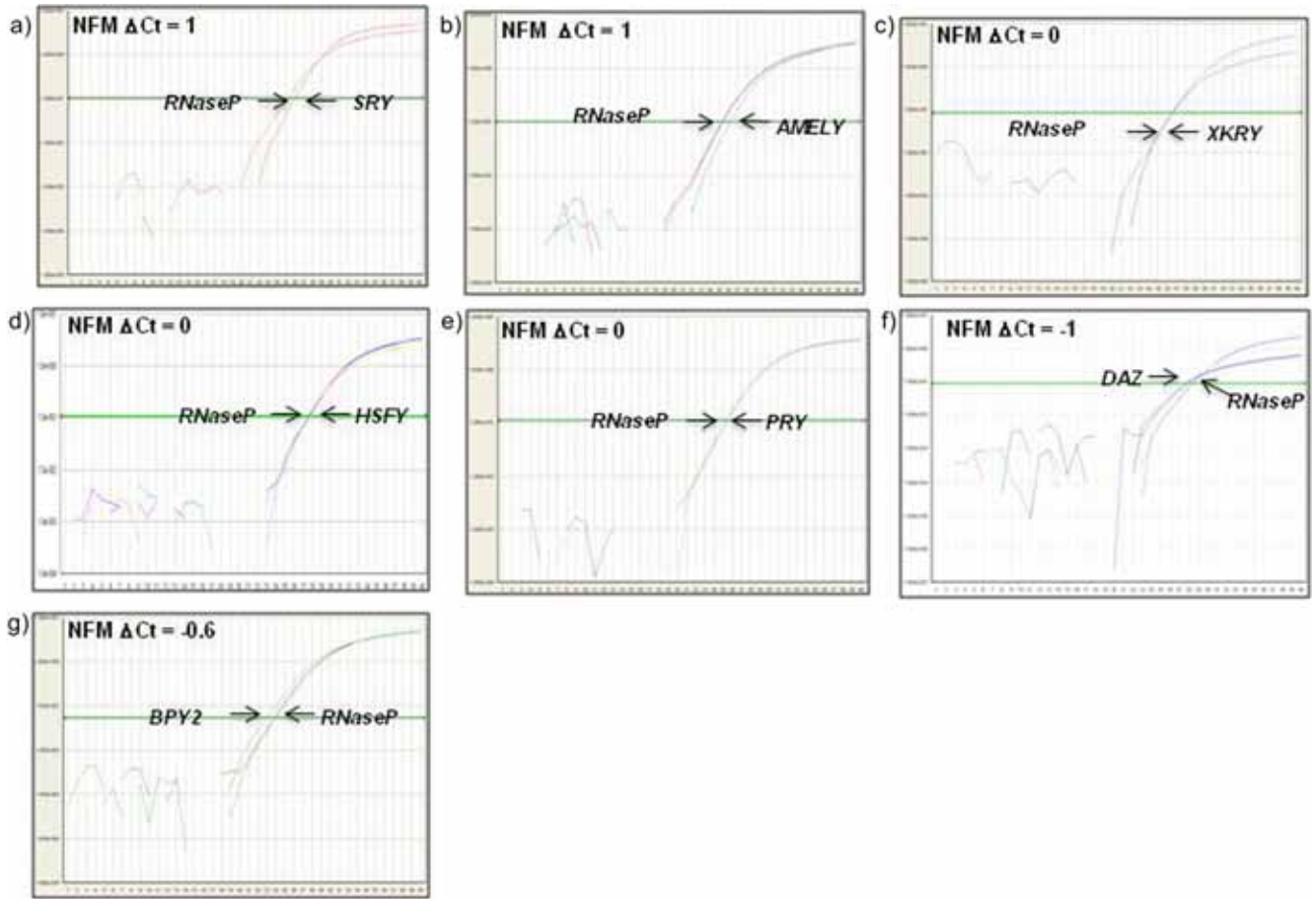


**Figure 4.** ClustalW alignment of STS sY182, sequence in patients with H. Changes are marked in red and highlighted in yellow. NFM, normal fertile males.

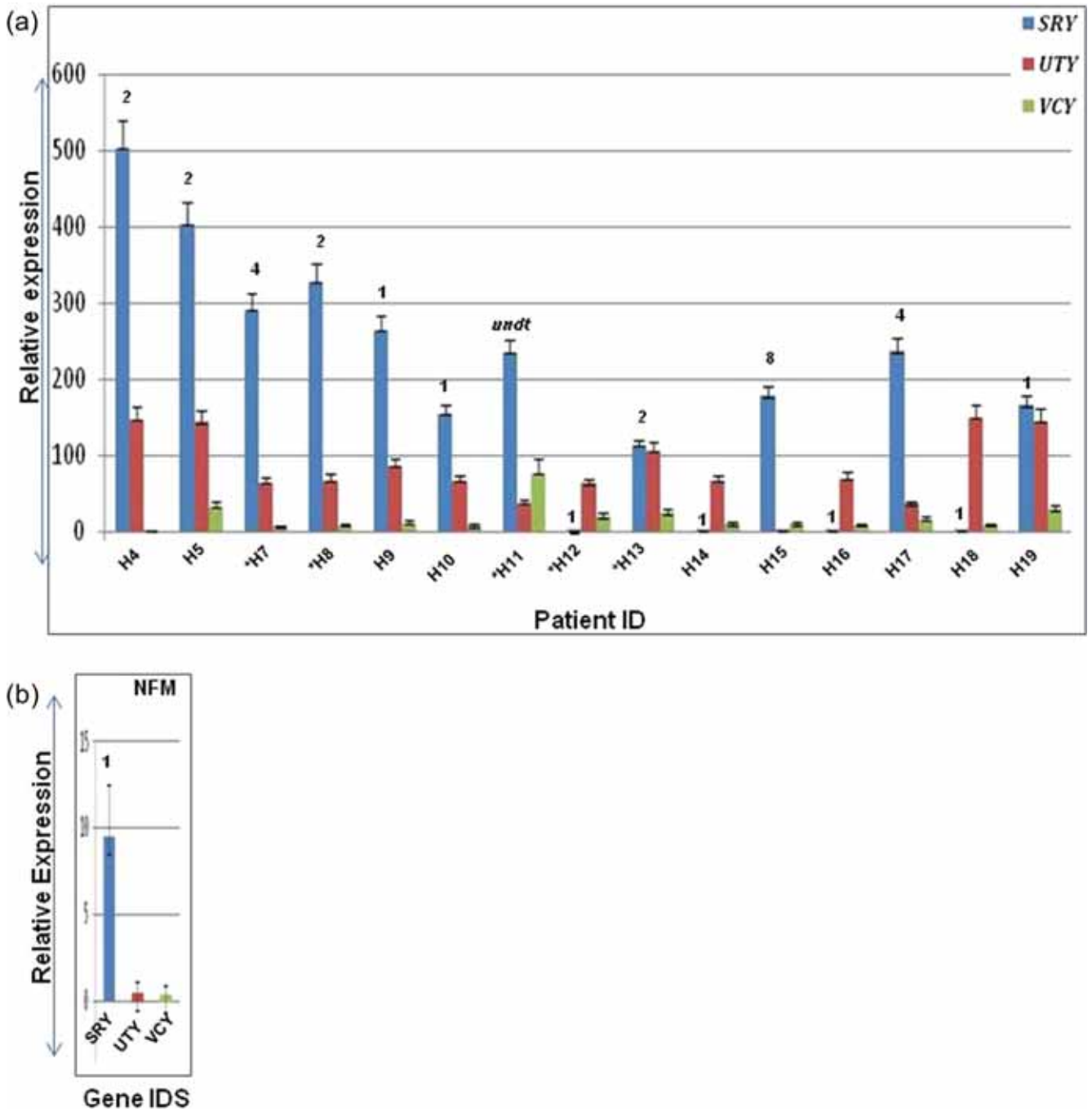




**Figure 5.** FISH of *AMELY*, *SRY/CEPX*, *DAZ* genes and *DYZ1* arrays. Representative panels shows location of *AMELY*, *SRY/CEPX*, *DAZ* and *DYZ1* arrays on the interphase nuclei of H patients (A–I (i–iv)). Note the mosaicism of signal for the four probes (a and b). Panel J (a–c) represents position of the four probes on Y chromosome of NFM. Patient IDs are mentioned on top of the panels and copy number on left. Undt, undetermined copy number. Scale is provided at the bottom of the panel. \*PH cases.



**Figure 6.** Real-time amplification plot. Copy number analysis of (a) *SRY*, (b) *AMELY*, (c) *XKRY*, (d) *HSFY*, (e) *PRY*, (f) *DAZ*, (g) *BPY2* in NFM. Interpretation of  $\Delta C_t$  values are given in the text. *RNaseP* present in two copies was used as an internal calibrator.



**Figure 7.** Transcriptional analysis of *SRY*, *UTY* and *VCY* genes. Bar diagram showing varying expressional status of *SRY*, *UTY* and *VCY* genes in the blood of representative H patients, panel (a) and normal fertile males (NFM), panel (b) ( $P < 0.001$ ). Data is represented as mean  $\pm$  SEM. Copy number of *SRY* gene in corresponding patients is given on top of each bar. The sign ‘undt’ denotes undetermined copies. A colour code for each bar is given on top right corner of the panel (a).