Supplementary data:

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

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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	Micopenis	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		
*SRY1**	GAATCTGGTAGAAGTGAGTTTTGGA	GCCTTTATTAGCCAGAGAAAAGAAA	55
SRY2	CTTCTGCTATGTTAAGCGTATTCAA	CAGCTTTGTCCAGTGGGCTTGTA	60
*ZFY**	GATCCAGAGATGTATGAGAGCATTT	CCATTCTCCAAATTCTTATTAACCA	55
*AMELY**	TGTTTGCATTAGCAGTCCCCTGG	GCGGTGCAAACACTGTCCCTC	60
USP9Y1	TGCAAGATGTTTTGTCCTTGAA	AATCATTCAGGACATGTTTCACAA	60
USP9Y2	GAGCCCATCTTTGTCAGTTTAC	CTGCCAATTTTCCACATCAACCC	60
USP9Y3	GCCATTGTAGCTTTTTCTACCAATTA	CTTGAAATCACTTATGACCCAGTCT	55
DBY1	TATTGGCAATCGTGAAAGAC	TGCCGGTTGCCTCTACTGGT	60
DBY2	ATCGACAAAGTAGTGGTTCC	AGATTCAGTTGCCCCACCAG	60
*DUXY**	CCGACACCTTCGGACAGCAC	GTGGTCTGGGATCCGGTGAC	61
KALY (sY182)*	TCAGAAGTGAAACCCTGTATG	GCATGTGACTCAAAGTATAAGC	60
	Housekeeping genes		
β-actin	AGATGACCCAGATCATGTTTGAGA	CTAAGTCATAGTCCGCCTAGAAGC	60
GAPDH	GCCACATCGCTCAGACACCAT	ACCAGGCGCCCAATACG	60

Region	STS screened	No. of STS
AZFa	sY83, sY84, sY86, sY87, sY88, sY746, sY1179, sY1180, sY1181, sY1064, sY1065, sY1182, sY1183 sY1184, sY1185, sY1186, sY1066, sY1316 (*USP9Y* exon 26), sY1317(*USP9Y* exon 3), sY1234 (*DBY* exon 9), sY1231 (*UTY* exon 8)	21
AZFb	sY113, sY117, sY124, sY125, sY127, sY129, sY131, sY627(*RBMY* exon 12), sY1233 (*EIF1AY* exon 1)	9
AZFc	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 (*SRY* exon 1), sY238 (*ZFY* intron 2), sY1161 (*PRY* intron 2), sY274 (*RPS4Y1* intron 4), sY1254 (*TGIF2LY* exon 1), sY1240 (*PCDH11Y* intron 3), sY1256 (*TSPY* intron 5), sY276 (*AMELY* exon 4/intron 4), sY1238 (*TBL1Y* exon 11), sY637 (*PRKY* 5' upstream of gene), sY1319 (*PRKY* 3'UTR), sY1250 (proximal boundary of *TSPY* array), sY1251 (boundary between centromere and Yq), *sY182 (*KALY*), sY1230 (*TMSB4Y* exon 1/intron 1), sY1220 (*VCY* exon 2), sY1239 (*NLGN4Y* exon 1), sY210 (*STSP* intron 5), sY280 (*JARID1D* (*SMCY*) exon 9/intron 9), sY1682 (*RPS4Y2* exon 1), sY1237 (*HSFY* exon 2), sY1260 (*CDY2* exon 1), sY1235 (*XKRY* exon 1), sY1258 (boundary between unique sequence u1 and blue amplicon b1 in *AZFc*), sY1035 (*BPY2* intron 5), sY1318 (*DAZ* 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)

DAZ-SNV	I	II	III (sY586)	V (sY587)	VII (sY581)	*DAZ* (sY152)	*SRY* (sY14)	No. and frequency of *DAZ* SNVs affected
Location within gene	5′ UTR exon 1	Exon 3–4	Intron 7A/B	Intron 9	Intron 3	Intron 6a	Exon	
Restriction enzyme	*Fsp*I	*Mbo*I	*Taq*I	*Dra*I	*Sau3A*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- *DAZ*1, *DAZ*2,*DAZ*3 B- *DAZ*4	A- *DAZ*1 B- *DAZ*2,*DAZ*3, *DAZ*4	A- *DAZ*2 B-*DAZ*1, *DAZ*3, *DAZ*4	A- *DAZ*3, *DAZ*4 B- *DAZ*1, *DAZ*2	A- *DAZ*1, *DAZ*4 B- *DAZ*2, *DAZ*3	*DAZ* (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			
SRY	Hs00243216_s1	FAM/TAMRA	Yq11.3
SAR*BPY2*F	TGGAGTCTGCCAAAACAAGGG		Yq11.223
SAR*BPY2*R	CAGAGCAGGAGAGTCTCATCAC		
SAR*BPY2*P	CACATATTGCGGAGTCCAGCACCCAGG	FAM/TAMRA	
SAR*HSFY*F	TCAATGAGGCTCCTTATCCTAACCT		
SAR*HSFY*R	GCAGCCGATGTATCAAATGTCATAG		Yq.11.222
SAR*HSFY*P	CCAGCAGGCAACCAG	FAM/TAMRA	
SAR*DAZ*F	TGACTGGACACCTAGTTTCATGAAC		Yq11.3 (sY587)
SARDAZR	GTCAAGAGGCATCAAGTGAAAGTTG		
SAR*DAZ*P	CACCCTGTCTCCAACCC	FAM/TAMRA	
SAR*PRY*F	CAGGATGAAGGGATGCAGTGA		Yq11.223
SAR*PRY*R	CTTAGAGGTGGGTGTCAGTGAAA		
SAR*PRY*P	CAAGAGCCCAACCTTC	FAM/TAMRA	
SAR*AMELY*F	CCCTGGGCTCTGTAAAGAATAGTG		Yp11.2
SAR*AMELY*F	ATCAGAGCTTAAACTGGGAAGCTG		
SAR*AMELY*P	CATCCCAAATAAAGTG	FAM/TAMRA	
SAR*XKRY*F	TCCCTCTTATCAGTTGTGTATGGG		Yq11.221
SAR*XKRY*R	TGAGATAGTCACCAAGGAATGCC		
SAR*XKRY*P	TCACCGCAACATACTAGCAATCCACACC	FAM/TAMRA	
*DYZ1*F	GTGGGCCGCTCTAGACACCA	SYBR	Yq12
*DYZ1*R	TGCCAAATCATTGCATTCCTTTCC		
Expression primers/assays			Location in the gene
SRY	Hs00243216_s1	FAM/TAMRA	Single exon
UTY	Hs00273053_m1	FAM/TAMRA	Probe spans exons
VCY1	Hs00855141_sH	FAM/TAMRA	Single exon
DAZ1	Hs00414014_m1	FAM/MGB	Probe spans exons
HPRT1	Hs99999909_m1	FAM/TAMRA	Probe spans exons
GAPDH	GCCACATCGCTCAGACACCAT ACCAGGCGCCCAATACG	*SYBR*	Probe spans exons

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

	Expression analysis $2^{-\Delta\Delta Ct}$		
Patients ID/genes	*SRY*	*UTY*	*VCY*
NFM	9.452907	0.468628367	0.309197623
	H/KS		
H4	503.7129366	147.7221468	*cb*
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	*cb*	9.74251369
H16	0.676424003	70.16240169	9.132786203
H17	236.845944	36.40070889	16.378209
H18	*cb*	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.

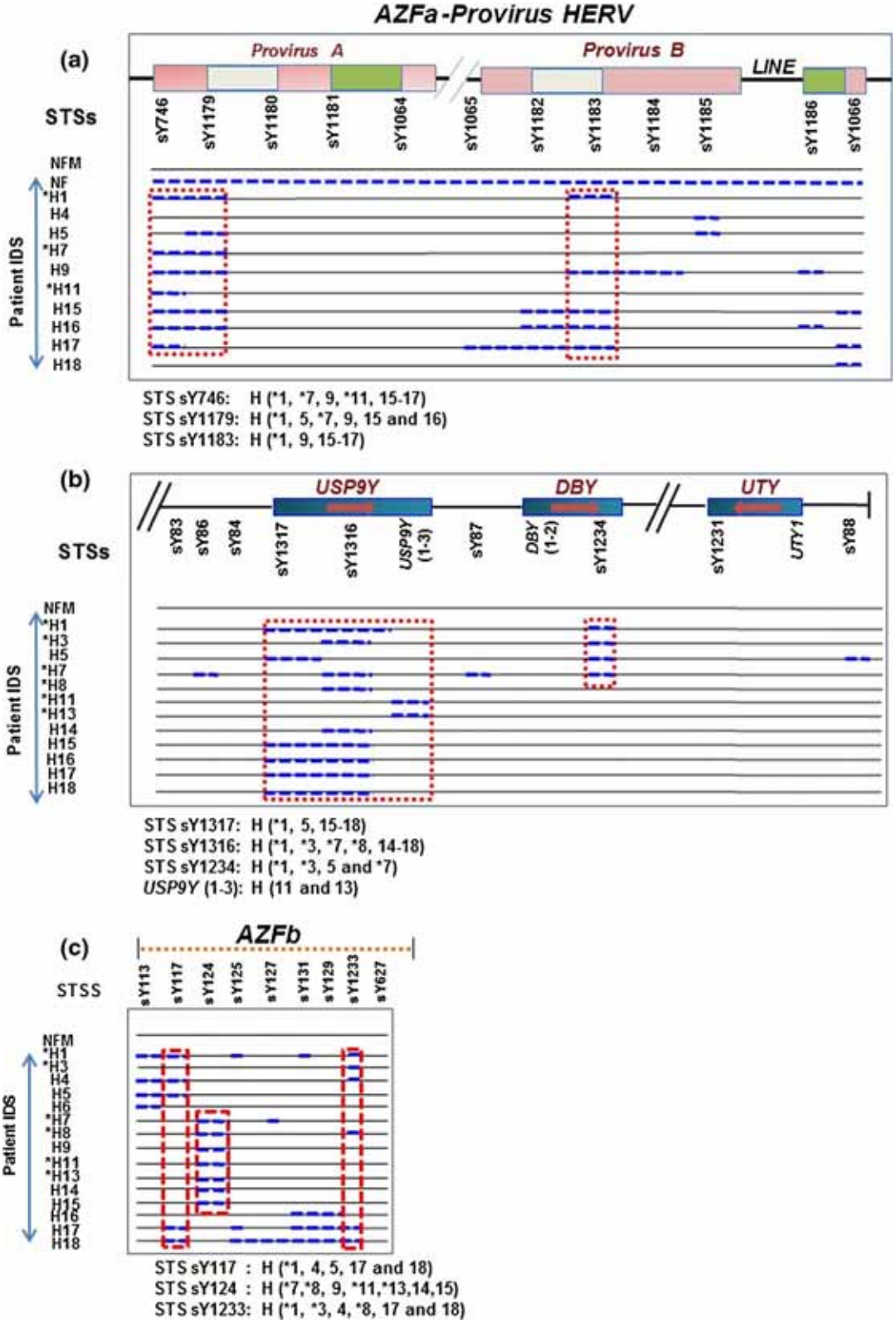

Figure 1. Microdeletions in *AZFa/AZFb/AZFc* regions of H patients. Diagrammatic representation shows (a) *AZFa*-provirus HERV and interstitial region between STSs, sY1064 and sY1065 covering *USP9Y*, *DBY* and *UTY* genes (b). Panel (c) signifies *AZFb* region representing STSs, sY113-sY627. Line in 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.

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NFM          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 59
H18          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TA-GTTTCGAACTCTGGCACCTTTCA 58
*H3          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCGCCTTTCA 59
*H7          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCACCTTTCA 59
*H8          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAA-TAAGTTTCGAACTCTGGCAC-TTTCA 58
*H2          GAATCTGGTAGAAGTGAGTTTTGGATAGTAAAAATAAGTTTCGAACTCTGGCACCTTTCA 60
H6           GAATCTGGTAGAAGTGAGTTTTGGATA-TAAA--TAAGTTTCGAACTCTGGCACCTTTCA 57
             *************************** ****  ** *************** * *****

NFM          ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119
H18          ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 118
*H3          ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119
*H7          ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 119
*H8          AATTTTGTCGCACTCTCCTTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 118
*H2          ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCATATGCTTCTGCTATGTTAAGCG 120
H6           ATTTTGTCGCACTCTCCTTGTTTTTGACAATGCAATCAAATGCTTCTGCTATGTTAAGCG 117
             * ******************  ****************** **********************

NFM          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 179
H18          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAAAATATTCCCGCTCTCCGGAGAA 178
*H3          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 179
*H7          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 179
*H8          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 178
*H2          TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 180
H6           TATTCAACAGCGATGATTACAGTCCAGCTGTGCAAGAGAATATTCCCGCTCTCCGGAGAA 177
             ************************************ **********************

NFM          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 239
H18          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 238
*H3          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 239
*H7          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 239
*H8          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 238
*H2          GCTCTTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 240
H6           GCTCCTCCTTCCTTTGCACTGAAAGCTGTAACTCTAAGTATCAGTGTGAAACGGGAGAAA 237
             **** ************************************************************

NFM          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299
H18          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 298
*H3          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299
*H7          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 299
*H8          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 298
*H2          ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 300
H6           ACAGTAAAGGCAACGTCCAGGATAGAGTGAAGCGACCCATGAACGCATTCATCGTGTGGT 297
             ************************************************************

NFM          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 359
H18          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 358
*H3          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCGGAGATCA 359
*H7          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 359
*H8          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 358
*H2          CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 360
H6           CTCGCGATCAGAGGCGCAAGATGGCTCTAGAGAATCCCAGAATGCGAAACTCAGAGATCA 357
             ************************************************** *******

NFM          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419
H18          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 418
*H3          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419
*H7          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 419
*H8          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 418
*H2          GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 420
H6           GCAAGCAGCTGGGATACCAGTGGAAAATGCTTACTGAAGCCGAAAAATGGCCATTCTTCC 417
             ************************************************************

NFM          AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 479
H18          AGGAGGCACAGAAATTTCAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 478
*H3          AGGAGGCACAGAAATTACAGGCCATGCGCAGAGAGAAATACCCGAATTATAAGTATCGAC 479
*H7          AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 479
*H8          AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 478
*H2          AGGAGGCACAGAAATTACAGGCCATGCACAGAGAGAAATACCCGAATTATAAGTATCGAC 480
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Figure 2. (*continues*)

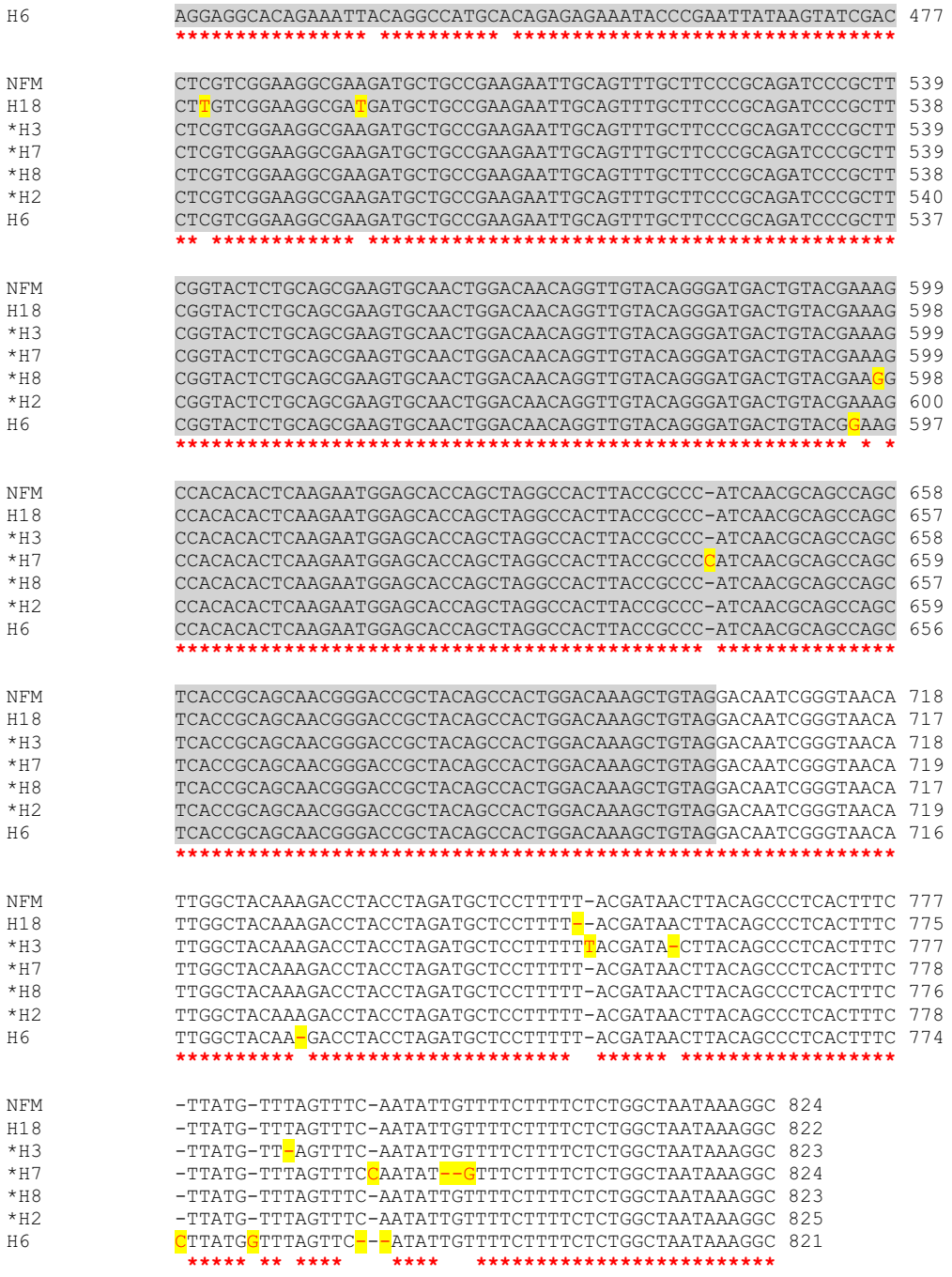

Figure 2. ClustalW alignment of *SRY* gene sequences in H males. Nucleotide changes are highlighted in yellow and marked in red. *SRY*-CDS region is highlighted in grey. NFM, normal fertile males. Accession number for respective patients is mentioned in table 3.

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.

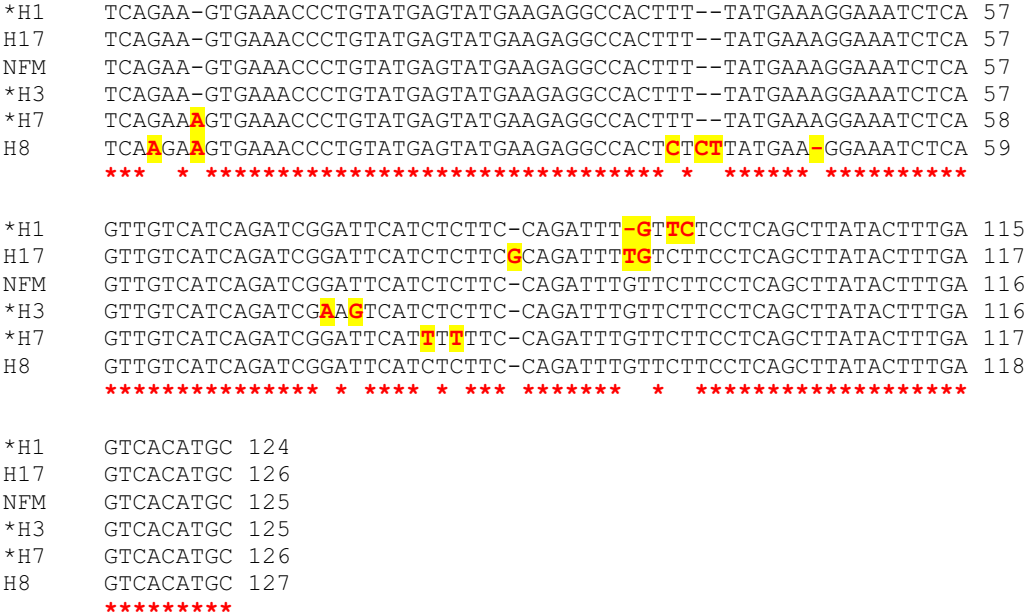

Figure 4. ClustalW alignment of STS sY182, sequence in patients with H. Changes are marked in red and highlighted in yellow. NFM, normal fertile males.

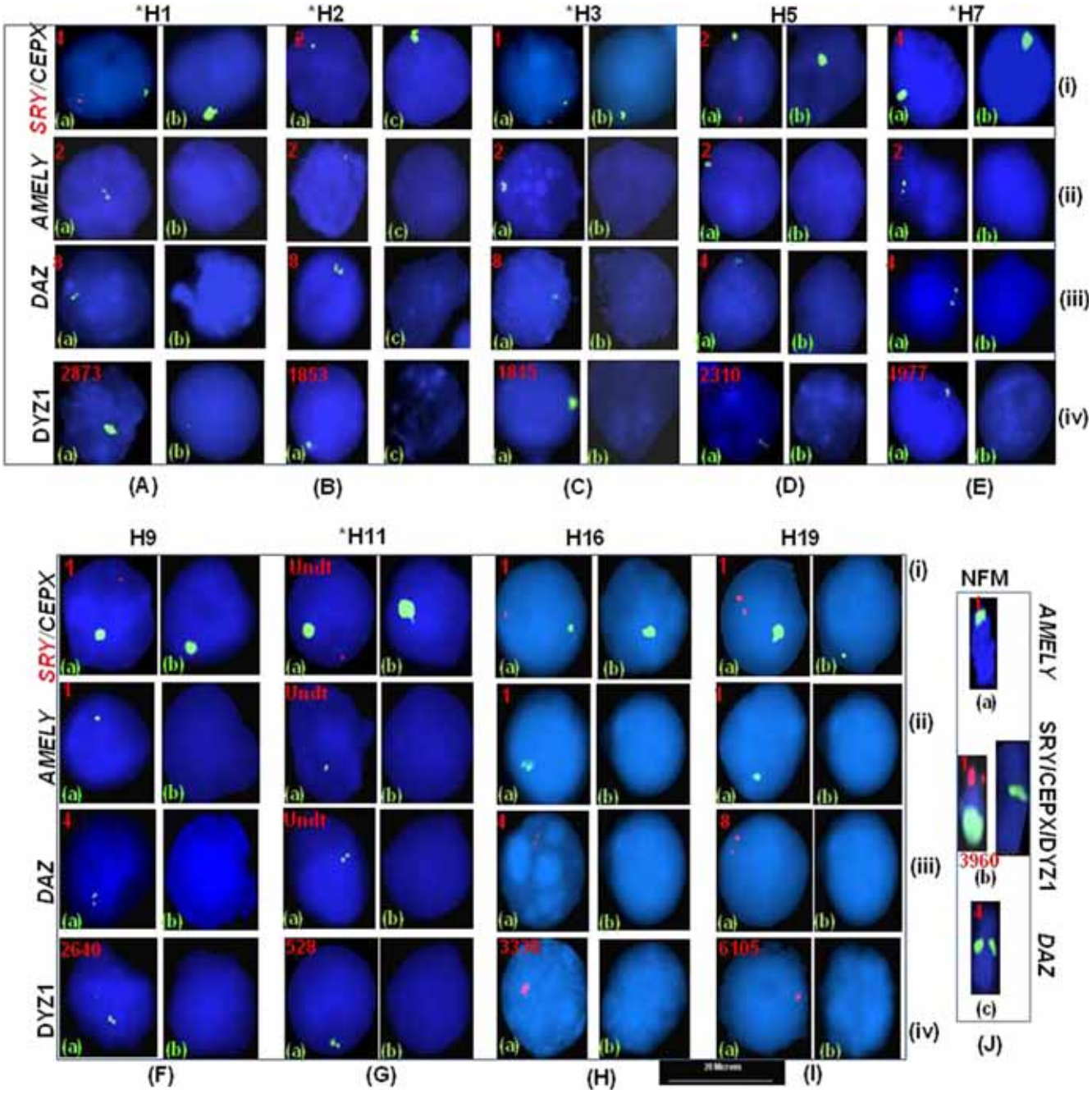

Figure 5. FISH of *AMELY, SRY/CEPX, DAZ* genes and DYZ1 arrays. Representative panels shows location of *AMELY, SRY/CEPX, DAZ* and DYZ1 arrays on the interphase nuclei of H patients (A–I (i–iv)). Note the mosaicism of signal for the four probes (a and b). Panel J (a–c) represents position of the four 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.

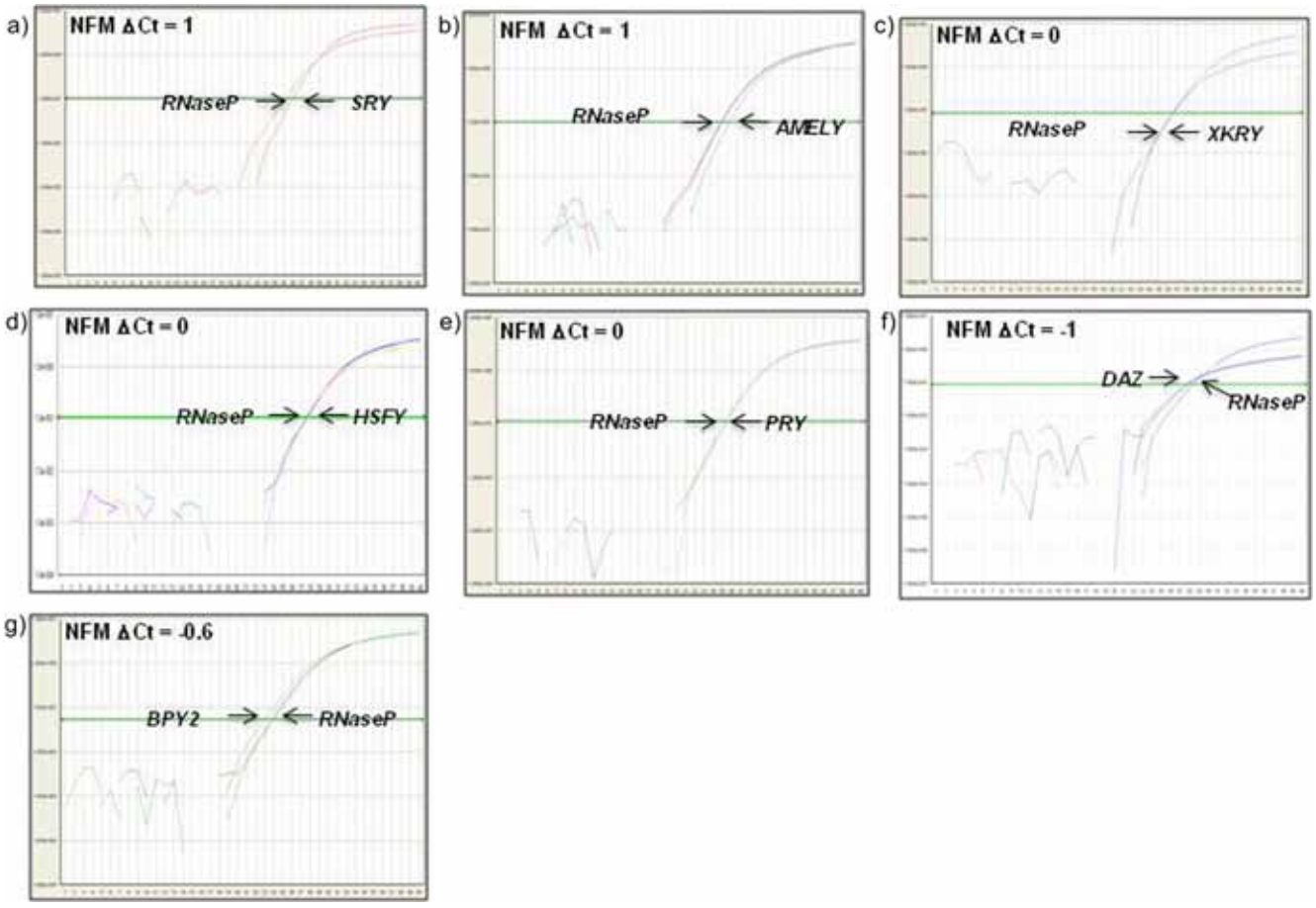

Figure 6. Real-time amplification plot. Copy number analysis of (a) *SRY*, (b) *AMELY*, (c) *XKRY*, (d) *HSFY*, (e) *PRY*, (f) *DAZ*, (g) *BPY2* in NFM. Interpretation of ΔCt values are given in the text. *RNase*P present in two copies was used as an internal calibrator.

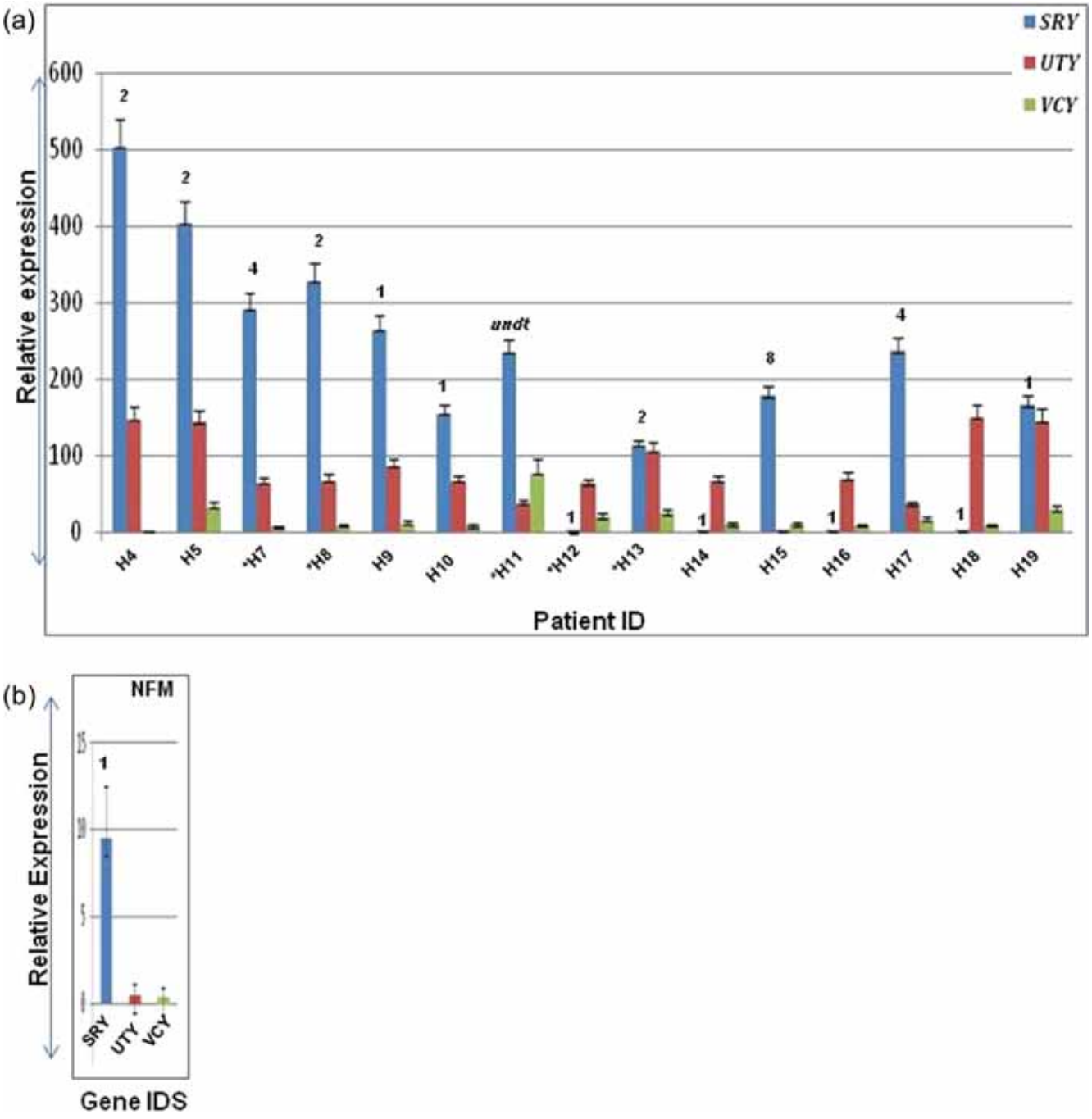

Figure 7. Transcriptional analysis of *SRY, UTY* and *VCY* genes. Bar diagram showing varying expressional status of *SRY, UTY* and *VCY* genes in the blood of representative H patients, panel (a) and normal fertile males (NFM), panel (b) ($P < 0.001$). Data is represented as mean ± SEM. Copy number 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).