

# Male fertility genes located in Y-chromosomal regions display differential mRNA profiles in response to GnRH treatment of cryptorchidism-dependent infertility

Faruk Hadziselimovic<sup>1</sup> Katharina Gegenschatz-Schmid<sup>1</sup> Gilvydas Verkauskas<sup>2</sup> Michael B. Stadler<sup>3,4</sup> <sup>1</sup>Cryptorchidism Research Institute, Kindermedizinisches Zentrum Liestal, 4410 Liestal, Switzerland <sup>2</sup>Children's Surgery Centre, Faculty of Medicine, Vilnius of University, 01513 Vilnius, Lithuania <sup>3</sup>Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland <sup>4</sup>Swiss Institute of Bioinformatics, Basel, Switzerland

**Objectives and aim;** Undescended testes in patients with defective mini-puberty contain germ cells that fail to differentiate normally into Ad spermatogonia and ultimately leads to infertility. Six months treatment with the gonadotropin-releasing hormone GnRH increases luteinizing hormone and testosterone secretion and rescues fertility in the majority of pathological cryptorchid testes. Several Y chromosomal genes in the male-specific Y region (MSY) are essential for spermatogenesis, testis development and function, and were associated with azoospermia, infertility and cryptorchidism. In this study, we analyzed the expression of MSY genes in testes with Ad spermatogonia (low infertility risk patients) as compared to testes lacking Ad spermatogonia (high infertility risk) before and after curative GnRH treatment..

**Patients and Methods;** We selected 15 patients with isolated cryptorchidism, based on histological results, and divided them into 2 groups. Seven belonged to the Ad- (lacking Ad spermatogonia) and 8 to the Ad+ (presenting Ad spermatogonia) group. The patients had a median age of 18.5 months (range 8–59 months) and were age matched. Data from Ad- bilateral cryptorchid boys treated with GnRH (10 µg intranasally on alternate day) following the first orchidopexy (surgery) (4 patients) were retrieved from a randomized study. Initial biopsies revealed no Ad spermatogonia, indicating defective mini-puberty (Ad- group). The second testis was managed by orchidopexy and biopsied 6 months after the initial surgery. Thus, results from 21 biopsies were compared. RNA sequencing data were used to analyze manually selected marker genes. Only genes with at least one read per million, in at least two samples, were included. P values and fold-changes were calculated for the treatment factor and differentially expressed genes were defined as those displaying a false discovery rate (FDR) of less than 0.05 and an absolute change in expression of at least two-fold.

**Results;** We found 21 genes that are significantly differentially expressed between Ad- and Ad+ samples (FDR<0.05). Furthermore, we identified 23 differentially expressed genes when we compared GnRH treated and untreated Ad- patient samples, all of which showed significant differences (FDR<0.05).

Symbol	GeneName	MSY location	FC <sup>Ad-/Ad+</sup>		FC <sup>untreated/treated</sup>		FDR <sup>untreated/treated</sup>		Median <sup>Ad-</sup>		Median <sup>Ad+</sup>		Median <sup>untreated</sup>		Median <sup>treated</sup>	
			FC <sup>Ad-/Ad+</sup>	logFC <sup>Ad-/Ad+</sup>	FDR <sup>Ad-/Ad+</sup>	FC <sup>untreated/treated</sup>	logFC <sup>untreated/treated</sup>	FDR <sup>untreated/treated</sup>	Median <sup>Ad-</sup>	MAD <sup>Ad-</sup>	Median <sup>Ad+</sup>	MAD <sup>Ad+</sup>	Median <sup>untreated</sup>	MAD <sup>untreated</sup>	Median <sup>treated</sup>	MAD <sup>treated</sup>
RPS4Y1	ribosomal protein S4, Y-linked 1	X-degenerate			n.s.	1.57732	-0.65748	0.00836			161.73	24.64	188.66	21.44		
ZFY	zinc finger protein, Y-linked				n.s.	1.87302	-0.90536	0.00042			5.66	1.29	7.89	0.49		
TGIF2LY	TGFB-induced factor homeobox 2-like, Y-linked	X-transposed	8.75685	-3.13041	0.00038		n.s.		0.13	0.08	1.46	0.63				
TSPY2	testis specific protein, Y-linked 2		6.42406	-2.68349	0.00020		n.s.		0.29	0.12	2.21	0.93				
TSPY4	testis specific protein, Y-linked 2		3.98676	-1.99522	0.00039	2.12311	1.08618	0.03249	0.29	0.08	1.26	0.41	1.65	0.91	0.31	0.47
TSPY8	testis specific protein, Y-linked 8		7.17067	-2.84211	0.00020		n.s.		0.20	0.08	1.66	0.61				
TSPY3	testis specific protein, Y-linked 3		4.87896	-2.28658	0.00053		n.s.		0.41	0.16	1.65	0.60				
FAM19Y5	family with sequence similarity 197, Y-linked, member 5, pseudogene	Amplificonic			n.d.	7.92319	2.98608	0.00103					1.65	0.64	0.13	0.07
TSPY1	testis specific protein, Y-linked 1		5.63307	-2.49392	0.00032		n.s.		0.34	0.09	1.84	0.80				
FAM19Y2	family with sequence similarity 197, Y-linked, member 2, pseudogene				n.d.	5.29255	2.40396	0.00029			1.95	0.91	0.23	0.04		
TTY2	testis-specific transcript, Y-linked 2 (non-protein coding)				n.d.	6.71383	2.74714	0.00391			0.37	0.18	0.03	0.01		
TTY15	testis-specific transcript, Y-linked 15 (non-protein coding)				n.s.	1.56402	-0.64526	0.00560			14.35	0.56	16.01	1.67		
USP9Y	ubiquitin specific peptidase 9, Y-linked		1.15903	0.21292	0.04713	1.88670	-0.91587	0.00062	33.99	1.95	30.83	1.76	24.43	1.98	34.14	1.84
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box helicase 3, Y-linked				n.s.	2.01671	-1.01200	0.00017			25.38	2.28	36.68	2.69		
UTY	ubiquitously transcribed tetratricopeptide repeat containing, Y-linked	X-degenerate	1.20133	0.26464	0.01275	1.72317	-0.78507	0.00176	24.85	1.08	22.28	1.24	18.87	1.83	22.87	1.25
TMSB4Y	thymosin beta 4, Y-linked				n.s.	1.49553	-0.58065	0.04459			3.41	0.47	3.81	0.31		
VCY	variable charge, Y-linked	Amplificonic	8.04195	-3.00755	0.00022		n.s.		1.10	0.45	6.71	3.26				
NLGN4Y	neuroligin 4, Y-linked	X-degenerate			n.s.	1.71092	-0.77477	0.00119			3.48	0.12	4.15	0.29		
XXRY	XX, Kell blood group complex subunit-related, Y-linked				n.d.	3.65300	1.86908	0.01275			0.47	0.15	0.09	0.06		
CDY2A	chromodomain protein, Y-linked, 2A				n.d.	5.01151	2.32524	0.01151			0.25	0.23	0.04	0.03		
HSFY2	heat shock transcription factor, Y-linked 2	Amplificonic			n.s.	3.43257	1.77929	0.00038			0.84	0.38	0.18	0.03		
BCORP1	BC16 corepressor pseudogene 1				n.s.	3.27597	1.71192	0.00273			0.59	0.46	0.13	0.06		
TXLNGY	taxilin gamma pseudogene, Y-linked		1.17276	0.22991	0.02109	2.12919	-1.09031	0.00007	8.98	0.65	8.14	0.38	5.11	0.92	8.48	0.75
KDM5D	lysine (K)-specific demethylase 5D	X-degenerate			n.s.	1.48838	-0.57374	0.01619			25.51	0.96	27.57	0.89		
TTY10	testis-specific transcript, Y-linked 10 (non-protein coding)		1.44367	0.52974	0.01332		n.s.		9.32	1.02	6.15	1.05				
EIF1AY	eukaryotic translation initiation factor 1A, Y-linked				n.s.	2.08432	-1.05957	0.00007			11.01	2.76	19.15	2.46		
RBM1B	RNA binding motif protein, Y-linked, family 1, member B		3.81730	-1.93255	0.00040	2.24993	1.16988	0.00234	0.38	0.10	1.30	0.56	2.03	0.97	0.40	0.50
RBM1E	RNA binding motif protein, Y-linked, family 1, member E		3.74046	-1.90321	0.00194	2.48825	1.31513	0.00099	0.31	0.12	1.37	0.65	2.41	1.37	0.47	0.51
RBM1F	RNA binding motif protein, Y-linked, family 1, member F		4.07882	-2.02815	0.00080		n.s.		0.30	0.09	1.12	0.49				
RBM12P	RNA binding motif protein, Y-linked, family 2, member F pseudogene		3.17672	-1.66754	0.00120		n.s.		0.18	0.05	0.50	0.18				
RBM1J	RNA binding motif protein, Y-linked, family 1, member J		3.86974	-1.95224	0.00064	1.78302	0.83432	0.01583	0.25	0.11	1.11	0.46	1.74	0.85	0.47	0.40
TTY4	testis-specific transcript, Y-linked 4 (non-protein coding)	Amplificonic			n.d.	7.37719	2.88307	0.00179			0.33	0.36	0.03	0.02		
BPY2	basic charge, Y-linked, 2				n.d.	17.71327	4.14676	0.00105			0.36	0.27	0.01	0.01		
DAZ1	deleted in azoospermia 1		4.25639	-2.08963	0.00377		n.s.		0.16	0.05	0.63	0.34				
DAZ2	deleted in azoospermia 2		4.81189	-2.26660	0.00125		n.s.		0.12	0.05	0.48	0.30				
DAZ3	deleted in azoospermia 3		5.28714	-2.40249	0.00094		n.s.		0.11	0.05	0.54	0.37				
DAZ4	deleted in azoospermia 4		4.93808	-2.30395	0.00102		n.s.		0.12	0.04	0.56	0.34				
CDY1	chromodomain protein, Y-linked, 1				n.d.	7.43764	2.89484	0.00050			0.37	0.24	0.04	0.01		

**Conclusions;** Our findings implicate Y-chromosome genes known to be important for spermatogenesis in the curative hormonal treatment of cryptorchidism-induced infertility. *RBM1Y* is critical for male fertility in a mouse and constitutes a major candidate for molecular functions that may help explain the curative effect of GnRH treatment.

Nothing to declare