

X chromosome-wide identification of SNVs in microRNA genes and non-obstructive azoospermia risk in han chinese population

SUPPLEMENTARY TABLES

Supplementary Table S1: NOA associated SNPs in human X-linked miRNAs identified in screening stage through next generation sequencing (NGS)

MiRNA	ACC	Location in X chr. (hg19)	SNP	Nucleotide change	Cases ^a	Controls ^a	OR	P value
hsa-mir-105-2	MI0000112	151562884-151562964	rs5970293	G > C	68/26	77/14	2.10(1.26-3.52)	0.004
hsa-mir-1277	MI0006419	117520357-117520434	rs5956046	T > C	47/46	32/57	0.55(0.36-0.84)	0.005
hsa-mir-1912	MI0008333	113886019-113886098	rs12836771	A > G	85/7	77/14	0.45(0.23-0.89)	0.020
hsa-mir-325	MI0000824	76225829-76225926	rs5981521	T > C	78/16	69/24	0.59(0.36-0.97)	0.038
hsa-mir-325	MI0000824	76225829-76225926	rs5938804	T > C	78/16	70/24	0.60(0.36-0.99)	0.044
hsa-mir-325	MI0000824	76225829-76225926	rs958410	A > G	74/17	60/25	0.55(0.34-0.91)	0.018
hsa-mir-3937	MI0016593	39520470-39520575	rs5917320	A > G	69/20	52/30	0.50(0.31-0.81)	0.004
hsa-mir-4329	MI0015901	112023946-112024016	rs11152741	G > A	61/32	79/15	2.76(1.69-4.53)	0.00004
hsa-mir-4329	MI0015901	112023946-112024016	rs2286063	G > T	64/30	79/15	2.47(1.50-4.06)	0.0003
hsa-mir-4330	MI0015902	150336694-150336798	rs547043	A > G	52/42	36/58	0.50(0.33-0.76)	0.0009
hsa-mir-506	MI0003193	146312238-146312361	rs5951785	A > G	77/14	87/10	1.86(1.02-3.36)	0.040
hsa-mir-507	MI0003194	146312502-146312595	rs5905010	C > G	75/18	84/10	2.02(1.12-3.63)	0.018
hsa-mir-509-3	MI0005717	146341170-146341244	rs2504174	C > T	45/49	35/59	1.55(1.03-2.34)	0.037
hsa-mir-510	MI0003197	146353853-146353926	rs61560773	G > C	85/9	76/17	0.47(0.26-0.87)	0.015
hsa-mir-510	MI0003197	146353853-146353926	rs1447394	G > T	84/10	73/19	0.46(0.25-0.82)	0.008
hsa-mir-510	MI0003197	146353853-146353926	rs1447393	C > G	83/5	73/13	0.34(0.16-0.73)	0.004
hsa-mir-510	MI0003197	146353853-146353926	rs112589319	T > A	86/8	75/18	0.39(0.21-0.73)	0.002
hsa-mir-652	MI0003667	109298557-109298654	rs5985440	A > G	89/5	77/17	0.25(0.12-0.53)	0.0001
hsa-mir-652	MI0003667	109298557-109298654	rs12841904	G > A	88/6	78/16	0.33(0.17-0.97)	0.001
hsa-mir-766	MI0003836	118780701-118780811	rs5909648	C > A	87/7	80/14	0.46(0.23-0.90)	0.022
hsa-mir-766	MI0003836	118780701-118780811	rs6646439	A > T	87/7	80/14	0.46(0.23-0.90)	0.022
hsa-mir-767	MI0003763	151561893-151562001	rs5970291	G > A	68/26	77/14	1.73(1.06-2.83)	0.027

Chr, chromosome; ACC, Accession number in miRbase; NOA, non-obstructive azoospermia; OR, odds ratio.

^a Major allele/minor allele.

^b OR and P values were calculated by allele model.

Supplementary Table S2: Validation of NOA associated SNPs near human X-linked miRNAs in large population (Stage I validation)

MiRNA	ACC	Location in X chr. (hg19)	SNP	Nucleotide change	Cases ^a	Controls ^a	OR ^b	P value ^b
hsa-mir-105-2	MI0000112	151562884-151562964	rs5970293	G > C	416/118	415/142	0.83(0.63-1.10)	0.188
hsa-mir-1277	MI0006419	117520357-117520434	rs5956046	T > C	343/186	350/207	0.92(0.72-1.17)	0.492
hsa-mir-1912	MI0008333	113886019-113886098	rs12836771	A > G	466/72	477/79	0.93(0.66-1.32)	0.692
hsa-mir-325	MI0000824	76225829-76225926	rs5981521	T > C	463/73	470/90	0.82(0.59-1.15)	0.255
hsa-mir-325	MI0000824	76225829-76225926	rs5938804	T > C	457/74	462/88	0.85(0.61-1.19)	0.342
hsa-mir-325	MI0000824	76225829-76225926	rs958410	A > G	452/81	448/107	0.75(0.55-1.03)	0.076
hsa-mir-3937	MI0016593	39520470-39520575	rs5917320	A > G	378/151	383/176	0.87(0.67-1.13)	0.291
hsa-mir-4329	MI0015901	112023946-112024016	rs11152741	G > A	415/117	425/133	0.90(0.68-1.20)	0.470
hsa-mir-4329	MI0015901	112023946-112024016	rs2286063	G > T	415/117	427/132	0.91(0.69-1.21)	0.524
hsa-mir-4330	MI0015902	150336694-150336798	rs547043	A > G	225/302	277/272	1.37(1.07-1.74)	0.011
hsa-mir-506/507	MI0003193	146312238-146312361	rs5951785	A > G	423/113	471/85	1.48(1.09-2.02)	0.013
hsa-mir-507	MI0003194	146312502-146312595	rs5905010	C > G	434/101	473/85	1.30(0.94-1.78)	0.109
hsa-mir-509-3	MI0005717	146341170-146341244	rs2504174	C > T	236/292	243/314	0.96(0.75-1.22)	0.723
hsa-mir-510	MI0003197	146353853-146353926	rs61560773	G > C	469/59	480/80	0.75(0.53-1.08)	0.125
hsa-mir-510	MI0003197	146353853-146353926	rs1447394	G > T	443/77	439/93	0.82(0.59-1.14)	0.239
hsa-mir-510	MI0003197	146353853-146353926	rs1447393	C > G	462/47	469/89	0.54(0.37-0.78)	0.001
hsa-mir-510	MI0003197	146353853-146353926	rs112589319	T > A	481/53	493/66	0.82(0.56-1.21)	0.319
hsa-mir-652	MI0003667	109298557-109298654	rs5985440	A > G	463/29	471/54	0.55(0.34-0.87)	0.012
hsa-mir-652	MI0003667	109298557-109298654	rs12841904	G > A	462/74	467/91	0.82(0.59-1.15)	0.248
hsa-mir-766	MI0003836	118780701-118780811	rs5909648	C > A	477/57	489/69	0.85(0.58-1.23)	0.382
hsa-mir-766	MI0003836	118780701-118780811	rs6646439	A > T	456/61	484/69	0.94(0.65-1.35)	0.734

Chr, chromosome; ACC, Accession number in miRbase; NOA, non-obstructive azoospermia; OR, odds ratio.

^a Major allele/minor allele.

^b OR and P values were calculated by additive model.

Supplementary Table S3: Validation of NOA associated SNPs near human X-linked miRNAs in large population (Stage II validation)

MiRNA	ACC	Location in X chr. (hg19)	SNP	Nucleotide change	Cases ^a	Controls ^a	OR ^b	P value ^b
hsa-mir-506/507	MI0003193	146312238-146312361	rs5951785	A > G	386/85	450/60	1.65(1.16-2.36)	0.006
hsa-mir-510	MI0003197	146353853-146353926	rs1447393	C > G	423/37	471/65	0.63(0.41-0.97)	0.035
hsa-mir-652	MI0003667	109298557-109298654	rs5985440	A > G	473/35	495/41	0.89(0.56-1.43)	0.637

Chr, chromosome; ACC, Accession number in miRbase; NOA, non-obstructive azoospermia; OR, odds ratio.

^a Major allele/minor allele.

^b OR and P values were calculated by additive model.

Supplementary Table S4: Predicted or reported targets of selected human X-miRNAs

Selected x-linked miRNA	Predicted targets	References	Algorithm			annotation
			Targetscan	miRanda	miRwalk	
MiRNA-506	<i>CDK4</i>	PMID:24811707				PMID:12754735 <i>CDK4</i> was expressed during spermatogenesis.
	<i>CDH2</i>	PMID:25230372				PMID:25631347 <i>CDH2</i> was associated with spermatogenesis failure.
	<i>GLI3</i>	PMID:24608427				PMID:9178901 <i>Gli3</i> is expressed during spermatogenesis.
	<i>PIK3C2A</i>		$P_{CT} > 0.99$ Context score = -0.6	mirSVR score = -1.84	—	PMID:21963845 <i>PIK3C2A</i> involved in cell proliferation.
	<i>STAT3</i>	PMID:24811707				PMID:24560784 <i>STAT3</i> promoted later spermatogonial differentiation steps.
MiRNA-507	<i>ADAM17</i>		Context score = -0.59	mirSVR score = -1.61	—	PMID: 25474107 <i>ADAM17</i> was associated with male germ cell apoptosis.
	<i>CAMK4</i>		Context score = -0.68	mirSVR score = -2.06	—	PMID:10932193 <i>CAMK4</i> was expressed during spermatogenesis.
	<i>NRF2</i>	PMID:24307696				PMID:23089668 <i>NRF2</i> associated with sperm motility.
MiRNA-510	<i>AGTPBP1</i>				√	
	<i>SFRP2</i>		Context score = -0.26	mirSVR score = -1.31	—	
	<i>PPP2R5E</i>		Context score = -0.69	mirSVR score = -2.32	—	
	<i>PRDX1</i>	PMID:23971998			—	PMID:22640168 <i>PRDX1</i> associated with sperm motility.

Supplementary Table S5: The binding site of human X-linked miRNAs in target genes

miR-506	Position of 131-137 of <i>CDK4</i> 3'UTR	5' ... CAGAGAUUACUUUGCUGCCUAA...
	hsa-miR-506	3' AGAUGAGUCUUCCCACGGAAU
	Position of 226-232 of <i>CDH2</i> 3'UTR	5' ... ACUGGGAUUUUAUGUGCCUUU...
	hsa-miR-506	3' AGAUGAGUCUUCCCACGGAAU
	Position of 1206-1211 of <i>GLI3</i> 3'UTR	5' ... AGUAGCUGUGUGGAUGCCUUU...
	hsa-miR-506	3' AGAUGAGUCUUCCCACGGAAU
	Position of 615-621 of <i>PIK3C2A</i> 3'UTR	5' ... AUGUUGUAAAUAUGGUGCCUUA...
	hsa-miR-506	3' AGAUGAGUCUUCCCACGGAAU
	Position of 1208-1214 of <i>STAT3</i> 3'UTR	5' ... GUUGUUUCUGUGGGUGCCUUA...
	hsa-miR-506	3' AGAUGAGUCUUCCCACGGAAU
MiR-507	Position of 16-22 of <i>ADAM17</i> 3'UTR	5' ...UUCUGACUUAAGUGUGCAAAA...
	hsa-miR-507	3' AAGUGAGGUUUUCCACGUUUU
	Position of 96-104 of <i>CAMK4</i> 3'UTR	5' ... UUCUGUUUUUGAGGUGCAAAA...
	hsa-miR-507	3' AAGUGAGGUUUUCCACGUUUU
	Position of 2501-2506 of <i>NRF2</i> 3'UTR	5' ... GUUUCUGUGUAAGUGUAAAU...
	hsa-miR-507	3' AAGUGAGGUUUUCCACGUUUU
MiR-510	Position of 255-262 of <i>AGTPBP1</i> 3'UTR	5' ...CCUUUAAAAAAAAAACCUGAGUA...
	hsa-miR-510	3' CACUAACGGUGAGAGGACUCAU
	Position of 161-167 of <i>SFRP2</i> 3'UTR	5' ...GCAUCCCCAGCAUUUCCUGAGUU...
	hsa-miR-510	3' CACUAACGGUGAGA-- GGACUCAU
	Position of 517-523 of <i>PPP2R5E</i> 3'UTR	5' ...UCUUUUGCACAUUCUCCUGAGUU...
	hsa-miR-510	3' CACUAACGGUGAGA--- GGACUCAU
	Position of 183-189 of <i>PRDX1</i> 3'UTR	5' ... CUUUGGUAGGAAUGGCCUGAGUU...
	hsa-miR-510	3' CACUAACGGUGAGA-GGACUCAU

Supplementary Table S6: The target regions of X-linked miRNAs on the customer-designed DNA capture array

(See Supplementary File 1)

Supplementary Table S7: The sequences of pre-miRNAs studied in this study.

Pre-miRNA name	Sequences of pre-miRNAs
pre-miR-506	GCCACCACCAUCAGCCAUACUAUGUGUAGUGCCUUAUUCAGGAAGGUGUUACUU AAUAGAUUAAUAAUUUGUAAGGCACCCUUCUGAGUAGAGUAAUGUGCAACAUGGA CAACAUUUGUGGUGGC
pre-miR-507	GUGCUGUGUGUAGUGCUUCACUUCAAGAAGUGCCAUGCAUGUGUCUAGAAAUAU GUUUUGCACCUUUUGGAGUGAAAUAUGCACAACAGAUAC
pre-miR-510	GUGGUGUCCUACUCAGGAGAGUGGCAAUCACAUGUAAUUAGGUGUGAUUGAAA CCUCUAAGAGUGGAGUAAACAC