Title: The Non-random Location of Autosomal Genes that Participate in X Inactivation

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ABSTRACT

By transcribing XIST RNA, the human inactive X chromosome has a prime role in X-dosage compensation. Yet, the autosomes also play an important role in the process. In fact, multiple genes on human chromosome 1 interact with XIST RNA to silence the inactive Xs, no matter how many there are. And it is likely that multiple genes on human chromosome 19 prevent the silencing of the *single* active X, which is a highly dosage sensitive process. Previous studies of the organization of chromosomes in the nucleus and their genomic interactions indicate that most contacts are intra-chromosomal. Co-ordinate transcription or dosage regulation could explain the clustered organization of these autosomal genes on these two chromosomes that are critical for X dosage compensation in human cells. Unlike those on chromosome 1, the genes within the critical eight MB region of chromosome 19, have remained together in all mammals assayed, except rodents, indicating that their proximity in non-rodent mammals is evolutionarily conserved.

When female mammals compensate for sex differences in the dosage of X linked genes by inactivating X chromosomes, the X chromosome(s) to be silenced has a major role in the process. In all mammals, a non-coding RNA, encoded by the X, is essential to its being inactivated by epigenetic factors [1]. Clearly, the bi-directional spread of Xist RNA from its locus in the middle of the X chromosome initiates the silencing process in eutherian mammals [2, 3]. In addition, the other long non coding RNAs, implicated in the process, that is, the potential *Xist* repressors, rodent-specific *Tsix*, [4], and the primate specific *XACT* [5], are also encoded by the X chromosome. Once coated with enough Xist RNA, the future inactive X moves toward the nuclear lamina, where its chromatin is transformed from euchromatin to heterochromatin [6, 7].

The silencing of the future inactive X, or Xs, is attributable to a Rube-Goldberg type of mechanism that not only brings it close to the nuclear periphery (where inactive chromatin tends to reside), but also attracts the epigenetic factors that silence it. Ultimately, the binding of Xist RNA results in expulsion of the SWI/SNF complex from the inactive X [8]. The few active (escape) genes on that X chromosome manage to find their way out of the heterochromatic mass of inactive chromatin towards the center of the nucleus, where transcription occurs [9]. Yet, Xist RNA cannot do this alone, as autosomal gene products are essential to complete the silencing process [6, 7, 10].

In pursuit of autosomal genes that cooperate with the X chromosome, Percec et al., 2003 [11] used ENU chemical mutagenesis to screen for autosomal mutations involved in the initiation of X inactivation in mice. They identified regions of mouse chromosomes 5, 10 and 15, which seemed to affect the choice of the mouse inactive X. More recent studies in mice have elucidated the essential autosomal products that interact with Xist RNA to silence the chromosome [6, 7, 12, 13] (Table 1). These include the lamin B receptor *Lbr*, the satellite attachment factor A (*Saf-A*) and *Sharp*, (*Smrt* and Hdac Associated Repressor Protein, also called *Spen*). *SPEN*, *LBR* and *SAFA* map to human chromosome 1; *Lbr* and *Safa* also map to mouse chromosome 1, whereas *Sharp* is on mouse chromosome 4, (orthologous to human chromosome 1). Other genes that have been implicated in the silencing process are *RBM 15* and *SETDB1*, on human chromosome 1, and mouse chromosome 3 – also orthologous to human chromosome 1. Therefore, the genes on human chromosome 1 that play a role in silencing the future inactive X also map to mouse chromosome 1 or its orthologs (*Table 1, Figure 1a*). Conceivably, genes that were on three different chromosomes in mice have evolved to be on a single human chromosome to facilitate their interaction in silencing the X.

In prokaryotes, interactions between genes with a common function are facilitated because such genes are contiguous in the genome, organized into operons, with a common promoter [14]. On the other hand, most eukaryotic genes that interact with each other, do not share promotors, and are less well clustered [15]. Yet, it has become apparent that the spatial arrangement of genes in the mammalian nucleus is non-random; chromosome folding and intermingling enable the proximity of genes that reside on the same chromosome, by looping, and even on different chromosomes, by chromosome clustering. The likely advantage of interactions between genes is coordination of their expression – perhaps in the same transcription factory, thought to occur in a discrete

nuclear region [16].

Based on HI-C studies of the human genome [17], Thevenin et al. [18] showed that a significant number of functional groups (pairs of interacting proteins, complexes and pathways) are either located within the same chromosome or dispersed over several chromosomes. Those on different chromosomes tend to co-localize in space. In order to coordinate their expression, genes that function together tend to reside on fewer chromosomes than expected by chance. On the same chromosome, they are closer to each other than randomly chosen genes; on different chromosomes, they tend to be closer to each other in 3D space [18]. Among the best documented inter-chromosomal interactions are those between the mouse X chromosomal gene, *Xist*, and the autosomal epigenetic factors mentioned above that help silence the X chromosome from which the up-regulated *Xist* locus is being transcribed [15].

When extending her observations in mice to other mammals, Lyon suggested there was only a single *active* X, no matter the number of X's in a cell [19]; however, the literature has persisted in labeling the mammalian process of X dosage compensation, *X inactivation*, which focuses us on the process of silencing the inactive X. Therefore, the salient question has been, "How does one *choose* the X chromosome that becomes *inactive*?" Because Xist RNA is able to silence any chromosome into which it is inserted [20, 21], it is surprising that few ask the pertinent question, "What protects the single active X from silencing by its own *Xist* locus?"[22, 23].

Further, it has not been easy to show how the mouse *inactive* X is chosen. Earlier studies suggested that an infrequent physical association (kissing) between the *Xist* loci of the two X chromosomes in mouse embryos determined the choice of inactive X [24, 25], but more recent studies indicate that neither the expression of *Xist* nor *Tsix*, its antisense RNA, is affected by the interaction [26, 27].

In addition, Inoue et al. [28] and Harris et al. [29] recently showed that in mice, the choice of *active* X is determined prenatally. Having been imprinted during oocyte differentiation [28, 29], (as predicted by Lyon and Rastan [30]), the active X is always *maternal* in trophectoderm – the first tissue to undergo dosage compensation in the mouse embryo. Because X inactivation in the placenta occurs relatively early in mice, it is likely that the paternal X hasn't had time to erase the inactivation imprint imposed during the early stages of spermatogenesis [31]. It remains to be seen if the rodent specific Tsix RNA, which is transcribed only from the maternal X in trophectoderm, protects the active X, regardless of its parental origin, from silencing by *Xist* in other mouse embryonic tissues.

Because human oocytes do not express *PCR2*, which imprints the mouse oocyte, [29] and the human maternal X is not imprinted [32], and because human *TSIX* is ineffective, having been truncated during human evolution [32], another means of repressing the *XIST* locus on the future *active* human X is needed to protect it from being silenced. Therefore, to repress its *XIST* locus, recent studies suggest that the future human *active* X needs to interact with human chromosome 19. [23]. These studies reveal a

previously unsuspected eight MB region on the short arm of human chromosome 19 (19p13.3-13.2) which contains at least one dosage sensitive gene that is likely to play a role in silencing the *XIST* locus on *one* X chromosome in each cell, to protect it from heterochromatization [22, 23], (Table 2). Candidate genes include satellite attachment factors *SAFB* and *SAFB2*, a cluster of zinc finger proteins that surround *DNMT1* and its co-factor *UHRF1*, among many others. Although most of the zinc finger proteins clustered in the relevant region of human chromosome 19 arose after the split between rodents and humans, the other genes in this region can be found on mouse chromosomes 8,9 and 17, which are orthologous to human chromosome 19 (*Table 2, Figure 1b*). Again, perhaps human 19 evolved to facilitate the interaction of genes that protect the future active X.

In the genomics era, geneticists tend not to think about the individual autosomes which encode genes of interest; therefore, I was surprised to see that many of the major players that interact with *XIST* to silence the X are encoded by human chromosome 1 [23] ,*Table 1 & Figure 1a)*], and in the mouse, by the three orthologs of chromosome 1 (chromosomes 1, 3 and 4) (Table 1). In mice, these genes are bound to *Xist* at the same developmental stage [6]. To my knowledge, no one has examined the *Xist*-autosomal interactions by RNA FISH to determine if there is clustering of the three murine chromosome 1 orthologs. The positions of these genes on human chromosome 1 is of interest as some of the genes are present on opposing ends of the chromosome, which would require a large fold in the chromosome to facilitate any interaction (*Figure 1a*). Such intermingling and folding are frequently observed in the 3D nuclear space [17].

Table 3 presents conservation data obtained from the UCSC Genome Browser; it shows that of four relevant genes on chromosome 1, only SAFA and LBR have been on the chromosome since we evolved from marsupials. SPEN and RBM15 although on the same chromosome as SAFA and LBR in primates, are on other chromosomes in marmosets and non-primate mammals. In contrast, except in rodents (rat, mouse and rabbit), the region on chromosome 19 is preserved in primates such as gorilla, orangutang, and marmoset, and other mammals such as cat, dog, pig, horse, cow, and opposum, (Table 4). The exceptional genes include the long noncoding RNA, TINCR, and the MD3L3-5, methyl CPG binding domain proteins, which are on chromosome 19 in primates and in marmoset but are not found in all mammals. The conserved cluster in pig, horse and cow is in the reverse orientation (Table 4). These differences interrupt what would otherwise be an exceptionally long synteny block, but the preservation of so many genes in this region, in spite of multiple evolutionary structural alterations, suggests that the local landscape may be important to function. That the chromosome 19 genes in rodents are not conserved as a group argues that their process of ensuring that one X will remain active differs from that of other mammals [33], perhaps because only rodents have *Tsix* to protect the active X from silencing by *Xist.*

Most likely, the relevant genes on the same chromosome are co-regulated. The advantage of genes clustered in interphase is that they can be programmed for simultaneous transcription. To silence *XIST* on the future active X, some genes in the chromosome 19 cluster might be transcribed together, perhaps if they are close enough in

3D space, as a single transcript. The telomeric location of genes on primate chromosome 1 that participate in *XIST* silencing (*Figure 1a)* suggest that the two ends of the chromosome might physically interact at the time of transcription.

Several important questions remain unanswered: First, how do multiple genes in the inactivation (activation) pathway on human chromosome 1 (or chromosome 19) coordinately interact with each other? And then, how do autosomal genes encoding protein products, interact with the X chromosome?

Recent studies suggest that the intra-chromosomal gene interactions occur within the same topologically-associating-domain (TAD)[34, 35] and that TADS align with co-coordinately regulated gene clusters, fostering long-range contacts and preventing deleterious interactions between genes in different TADs [35] One would like to examine the candidate genes on human chromosomes 1 and 19, at the appropriate time in development, to determine if they are located within the same TAD, or are otherwise coordinately regulated. It is unlikely that the occurrence of multiple silencers of the inactive X on human chromosome 1 and *XIST* repressors on human chromosome 19 is coincidental.

The question of how genes on an autosome interact with the genes on the X chromosome is especially challenging because in the human species either one or several X chromosomes can be silenced within a cell, the number dependent upon the number of X chromosomes in the genome. All but one X chromosome are silenced no matter how many are in the cell, nor the sex of the individual [36]. Therefore, only one X chromosome *resists* silencing no matter the number of X chromosomes in the cell.

Clearly, suppressing the *XIST* locus on the future active X is easier for males than females. We know this because of the specific loss of females who reduplicate the essential chromosome 19 gene(s), presumably because reduplication enables both X's to be active – a known lethal event in diploid cells. At least five percent more pre-implantation human females are miscarried than are males [23]. If males reduplicate the *XIST* repressor, it has little consequence, but females who by chance inactivate both *XIST* loci, die before they implant into the uterus. This suggests that not only when this region of chromosome 19 is duplicated, but even, when the chromosome is normal, the required interaction is a difficult one, as either too little or too much *XIST* repressor would lead to a lethal event (too many active X's or no active X). The former does not occur as often in males who have only one X chromosome: too much repressor is not lethal, although too little might be.

And there is the question of gene dosage. How in a diploid cell do two autosomes cooperate to make an inhibitor for a single X chromosome? And in the case of more than two X chromosomes, how is the right dosage of gene product from chromosome 1 achieved? On one hand, Lyon [37] and more recently Nguyen, Lee & Wu [38] suggest that the two autosomes might pair to synthesize a single product. There is also the possibility of competitive inhibition. Once, a molecule of gene product arrives on one X chromosome then the other(s) are unable to be hit. On the other hand, perhaps, not all

attempts to activate or inactivate the chromosome are successful, and so the process is stochastic. That many errors occur while repressing *XIST* on the future active X might explain a significant loss of pre-implantation females, even in absence of gene reduplication.

To answer these questions one needs to identify genome interactions during the pre-implantation development of the human embryo, at the time of X inactivation. One can use chromosome capture such as Hi-C, 3D RNA-FISH [39] (to see if nascent transcripts are transcribed together). And single-cell RNA-Seq as has been recently described in the mouse [26], examining the candidate genes. The best human model would be the beginning of cleavage to embryonic day 10. The inability to study available human embryos is a decided disadvantage for American investigators, but I hope that my colleagues in other countries will carry out such studies. For the human X: 19 interaction, embryonic day 4-7 would probably be appropriate, whereas human embryonic day 6-9 should capture the chromosome 1: X interaction.

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TABLE 1: Location of mouse and human genes that silence the inactive X

Human GENE	Human CHROM	*5' location of Human Gene (GRch38)*	Mouse GENE	*5' location of Mouse Gene (GRCm38)*	*Citation for Mouse Genes*
SPEN	1p36.21	***1:15,847,863***	Sharp* (Spen)	***4:141,467,890***	*McHugh[6]* *Moindrot[7]*
RBM15	1p13.3	***1:110,338,928***	Rbm15	***3:107,325,421***	*McHugh* *Moindrot* *Patil [10]*
LBR	1q42.12	***1:225,401,501***	Lbr	***1: 181,815,315***	*McHugh* *Chen[12]*
HNRNPC	14q11.2	14:21,209,135	Hnrnpc	14:52,073,380	*McHugh*
RALYL	8q21.2	8:84,182,764	Raly	***3:13,471,655ralyl*** 2:154,791,096raly	*McHugh*
HNRNPM	19p13.2	19:8,444,574	Hnrnpc	17:33646233	*McHugh*
HDAC3	5q13.3	5:141,620,875	Hdac3	18:37936971	*McHugh*
HNRNPU	1q44	***1:244,850,299***	Hnrnpu or Safa	***1:178321108***	*McHugh*
CELF1	11p11.2	11:47,465,932	Celf1	2: 90940387	*McHugh*
PTBP1	19p13.3	19:797,391	Ptbp1	10:79854432	*Moindrot*
	Not found		Myef2	2:125,084,628	*McHugh*
NCOR1	17p12-p11	17:16,030,093	NCoR-Hdac3 complex	11:62316426	*Moindrot*
HNRNPK	9q21.32	9:83,968,082		2: 32363005	*Moindrot*
CIZ1	9q34.11	9:128,166,064	Ciz1	***3:95323525***	*Moindrot* *Sunwoo[13]*
SETDB1	1q21.3	***1:150,926,245***	Setdb1	17: 12966799	*Moindrot*
WTAP	6q25.3	6:159,726,695	Wtap	***4:129,516,104***	*Moindrot*
HDAC1		***1:32,292,102***	Hdac1		*This paper*

*SPEN (SMART/HDAC1 associated repressor protein =SHARP

TABLE 2: Location of mouse and human genes that maintain the active X

Human GENE	Human CHROM	*5' location of Human Gene (GRch38)*	Mouse GENE	*5' location of Mouse Gene (GRCm38)*
SAFB	19p13.3	***19: 5,623,034***	Safb	***17:56, 584,830***
SAFB2	19p13.3	***19: 5,586,992***	Safb2	***17:56, 560,965***
DNMT1	19p13.2	***19:10,133,343***	Dnmt1	***9:20,907,209***
UHRF1	19p13.3	***19: 4,903,079***	Uhrf1	***17: 56, 303,367***
HNRNPM	19p13.2	***19: 8,444,574***	Hnrnpm	***17: 33, 646,233***
MBD3	19p13.3	***19: 1,576,670***	Mbd3	10: 80,392,539
MBD3L-5L	19p13.2	***19: 8,842,392***	Mbd3l	***9: 18,478, 359***
PRMT4 or CARM1	19p13.2	***19:10,871,576***	Carm1	***9: 21,546,894***
ZNF69	19p13.2	***19:11,887,772***	Not found	
ZNF823	19p13.2	***19:11,832,080***	"	
ZNF443	19.p13.2	***19:12,4***	Znf 709	8:71,882,068
ZNF699	19p13.2	***19: 9,291,139***	Not found	
ZNF627	19p13.2	***19:11,575,254***	Znf 867	11:59, 461,197
ZNF44	19p13.2	***19:12,224,685***	Not found	
ZNF358 pli.87	19p13.2	***19: 7,580,178***	Zfp358	8: 3,493,138

** ***Bold italics***: Human chromosome 19 or mouse orthologs of human chromosome 19

TABLE 3: Conservation of Some Candidate Genes, and Not Others in Various Mammals

MAMMAL	GENE	CHROM	5' LOCATION (nucleotides)	GENE	CHROM	5' LOCATION (nucleotides)
Human	DNMT1	**19***	10,133,346	SPEN	**1**	15,847,864
	UHRF1	**19**	4,910,367	LBR	**1**	225,401,503
	SAFB	**19**	5,623,099	SAFA	**1**	244,850,297
	SAFB2	**19**	5,586,999	RBM15	**1**	110,286,375
Gorilla	DNMT1	**19**	9,911,947	SPEN	**1**	15, 818,157
	UHRF1	**19**	4,549,324	LBR	**1**	205,129,423
	SAFB	**19**	5,391,167	SAFA	**1**	224,804,897
	SAFB2	**19**	5,343,115	RBM15	**1**	111,770,116
Orangutan	DNMT1	**19**	10,128,395	SPEN	**1**	212,361,620
	UHRF1	**19**	4,819,523	LBR	**1**	24,182,913
	SAFB	**19**	5,532,720	SAFA	**1**	4,279,561
	SAFB2	**19**	5,496,867	RBM15	**1**	116,356,665
Marmoset	DNMT1	**22**	9,536,311	SPEN	7	50,174,237
	UHRF1	**22**	4,640,990	LBR	**19**	18,374,272
	SAFB	**22**	5,347,272	SAFA	**19**	35,988,006
	SAFB2	**22**	5,310,815	RBM15	7	146,230,306
Pig	DNMT1	**2**	68,982,341	SPEN	6	75,015,891
	UHRF1	**2**	73,898,195	LBR	**10**	13,389,915
	SAFB	**2**	73,300,630	SAFA	**10**	17,485,493
	SAFB2	**2**	73,334,753	RBM15	4	109,778,998
Cow	DNMT1	**7**	15,914,205	SPEN	**16**	52,882, 374
	UHRF1	**7**	20,436,673	LBR	**16**	29,148,981
	SAFB	**7**	19,846,024	SAFA	**16**	33,162,888
	SAFB2	**7**	19,908,323	RBM15	3	33,196,547
Sheep	DNMT1	**5**	12,315,683	SPEN	**12**	49,635,296
	UHRF1	**5**	16,747,203	LBR	**12**	26,512,015
	SAFB	**5**	16,167,299	SAFA	**12**	30,479,650
	SAFB2	**5**	16,230,105	RBM15	1	86,670,575
Horse	DNMT1	**7**	49,751,153	SPEN	2	37,048,480
	UHRF1	**7**	3,014,835	LBR	**30**	8,017,554
	SAFB	**7**	3,409,307	SAFA	**30**	0,184,656
	SAFB2	**7**	3,388,372	RBM15	5	57,896,671
Dog	DNMT1	**20**	50,880,023	SPEN	2	81,683,829
	UHRF1	**20**	54,858,675	LBR	**7**	39,291,511
	SAFB	**20**	54,381,519	SAFA	**7**	35,833,232
	SAFB2	**20**	54,381,353	RBM15	6	41,645,939
Cat	DNMT1	**A2**	7,689,975	SPEN	1	11,528,828
	UHRF1	**A2**	3,678,067	LBR	**F1**	1,574,749
	SAFB	**A2**	4,176,193	SAFA	**F1**	5,103,486
	SAFB2	**A2**	4,143,427	RBM15	1	94,297,141
Opposum	DNMT1	**3**	431,238,772	SPEN	4	375,579,105
	UHRF1	**3**	441,797,772	LBR	**2**	137,055,167
	SAFB	**3**	443,046,263	SAFA	**2**	142,860,792
	SAFB2	**3**	443,045,746	RBM15	**2**	479,908,213

***chromosome numbers in bold indicate conservation**

TABLE 4. Site of 18 Clustered Human Chromosome 19 Genes in Other Mammals

MAMMAL	GENE	CHROMOSOME	SITE 5' (nucleotide)	Other Site(s)
HUMAN	SIRT6	19	4,174,109	
	PLIN3	19	4,852,208	
	UHRF1	19	4,910,367	
	KDM4B	19	4,969,121	
	TINCR	19	5,560,774	
	RFX2	19	5,993,164	
	VAV1	19	6,772,726	
	MBD3L4	19	7,037,748	
	INSR	19	7,112,226	
	ZNF358	19	7,516,118	
	MAP2K7	19	7,903,891	
	FBN3	19	8,130,286	
	HNRNPM	19	8,269,278	
	ZNF558	19	8,806,170	
	OLFM2	19	9,853,718	
	DNMT1	19	10,133,346	
	DNM2	19	10,828,755	
	CARM1	19	10,871,513	
ORANGUTAN	SIRT6	19	4,083,376	
	PLIN3	19	4,752,733	
	UHRF1	19	4,819,523	
	KDM4B	19	4,940,648	also 11
	TINCR	19	5,468,562	
	RFX2	19	5,907,338	
	VAV1	19	6,738,253	
	MBD3L4	19	7,005,357	
	INSR	19	7,065,165	
	ZNF358	19	7,328,128	
	MAP2K7	19	7,862,957	
	FBN3	19	8,037,199	also 5
	HNRNPM	19	8, 412,645	
	ZNF558	19	8,801,446	
	OLFM2	19	9,841,684	
	DNMT1	19	10,128,395	
	DNM2	19	10,719,521	
	CARM1	19	10,872.517	
MARMOSET	SIRT6	22	3,843,381	
	PLIN3	22	4,576,676	
	UHRF1	22	4,640,990	
	KDM4B	22	4,753,547	
	TINCR	22	5,280,800	also 22:19,138,083
	RFX2	22	5,714,269	also 22:13,197,841 and 1:104,992,215

	VAV1	22	6,482,055	
	MBD3L4	22	6,745,638	
	INSR	22	6,884,705	
	ZNF358	22	7,258,135	also 16
	MAP2K7	22	7,564,197	
	FBN3	22	7,702,224	also 10 & 2
	HNRNPM	22	8,116,508	
	ZNF558	22	8,418,995	also 2
	OLFM2	22	9,242,165	also 5
	DMNT1	22	9,536,311	
	DNM2	22	10,141,800	
	CARM1	22	10, 298,967	
PIG	SIRT6	2	74,568,548	
	PLIN3	2	73,970,200	
	UHRF1	2	73,898,195	
	KDM4B	2	73,747, 610	
	RFX2	2	72,949 979	
	TINCR	*not found*		
	VAV1	2	72,327,498	
	MBD3L4	2	72,012,690	
	INSR	2	71,797,542	
	ZNF358	2	71,615,476	
	MAP2K7	2	71,298,318	
	FBN3	2	71,104,118	
	HNRNPM	2	70,813,749	
	ZNF558	2	(70,582,106)	
	OLFM2	2	68,734,136	
	DMNT1	2	68,982,341	
	DNM2	2	69,474,069	
	CARM1	2	69,602,214	
HORSE	SIRT6	7	2,539,099	also 21
	PLIN3	7	2,972,664	
	UHRF1	7	3,014,835	
	KDM4B	7	3,087,218	also 23 & 2
	RFX2	7	3,649,694	also 23
	TINCR	*not found*		
	VAV1	7	4,329,609	
	MBD3L4	*7*	*52,446,746*	also X
	INSR	7	4,882,687	
	ZNF358	7	4,701,725	also 4,10, &13
	MAP2K7	7	5,229,948	
	FBN3	7	5,361,278	also 1 & 14
	HNRNPM	7	52,895,099	
	ZNF558	*7*	*52,539,233yes*	
	OLFM2	7	49,967,570yes	also 25
	DMNT1	7	49,751,153	
	DNM2	7	49,316,987	also 25
	CARM1	7	49,257,318	
COW	SIRT6	7	21,079,141	
	PLIN3	7	20,507,000	

	UHRF1	7	20,436,673	
	KDM4B	7	20,308,693	also 3,8 & 15
	RFX2	7	19,126,799	
	TINCR	*not found*		
	VAV1	7	18,866,379	also 3 & 11
	MD3L4	7	17,264,390	also 11
	INSR	7	17,276,143	also 21 & 3
	ZNF358	7	17,610,070	also 4,14,17 &18
	MAP2K7	7	17,891,887	
	FBN3	7	18,005,675	also 10 & 7:26,698,333
	HNRNPM	7	18,289,395	also 11
	ZNF558	7	17,220,537	also 19,11 & 25
	OLFM2	7	15, 550,353	
	DNMT1	7	15,914,205	
	DNM2	7	16,465,942	also 11
	CARM1	7	16,571,428	
DOG	SIRT6	20	55,416,563	
	PLIN3	20	54,924, 119	
	UHRF1	20	54,858,675	
	KDM4B	20	54, 715,308	also 11, 15, & 21
	RFX2	20	54,013, 618	also 1
	TINCR	*not found*		
	VAV1	20	53,482, 255	
	MBD3L4	20	53,213,540	
	INSR	20	52,017, 347	
	ZNF358	20	52,314,421	
	MAP2K7	20	52,594, 536	
	FBN3	20	52,723,997	also 11 & 30
	HNRNPM	20	52,997,963	
	ZNF558	20	51, 897, 297	
	OLFM2	20	51,148, 154	
	DMNT1	20	50,880,023	
	DNM2	20	50,399,784	
	CARM1	20	50, 331,081	
CAT	SIRT6	A2	3,162,759	
	PLIN3	A2	3,631,793	
	UHRF1	A2	3,678,067	
	KDM4B	A2	3,765,143	
	RFX2	A2	4,427,650	
	TINCR	*not found*		
	VAV1	A2	5,108,402	
	MBD3L4	A2	5,395,765	
	INSR	A2	6,443,171	
	ZNF358	A2	6,267,306	
	MAP2K7	A2	6,004,415	
	FBN3	A2	5,820,368	also A1
	HNRNPM	A2	5,569,560	
	ZNF558	A2	6,657,696	
	OLFM2	A2	7,484,626	
	DMNT1	A2	7,689,975	
	DNM2	A2	8,118,334	

	CARM1	A2	8,257,736	
OPOSSUM	SIRT6	3	440,652,009	
	PLIN3	3	441,702,797	
	UHRF1	3	441,797,772	
	KDM4B	3	441,910.670	
	RFX2	3	443,674,276	
	TINCR	*not found*		
	VAV1	3	444,980,624	
	MBD3L4	*not found*		
	INSR	3	463,520,164	
	ZNF388	*unassigned*		
	MAP2K7	3	462,757,443	
	FBN3	**3**	461,508,720	
	HNRNPM	3	460,359,655	
	ZNF558	***4***	*409,014310*	
	OLFM2	3	431,554,923	
	DMNT1	3	431,238,772	
	DNM2	3	430,280, 994	
	CARM1	3	430,212,862	
MOUSE	SIRT6	10	81,621,787	
	PLIN3	17	56,277,475	
	UHRF1	17	56,304.407	
	KDM4B	17	56,326,074	
	RFX2	17	56,775,897	
	TINCR	17	56,551,534	
	VAV1	17	57,279,100	
	MBD3L4	*not found*		
	INSR	8	3,150,922	
	ZNF358	8	3,493,154	
	MAP2K7	8	4,238,740	
	FBN3	18	58,012,265	
	HNRNPM	17	33,646,236	
	ZNF558	*not found*		
	OLFM2	9	20,672,332	
	DNMT1	9	20,,907,209	
	DNM2	9	21,425,244	
	CARM1	9	21,546,894	
RAT	SIRT6	7	10,937,622	
	PLIN3	9	10,774,869	
	UHFR1	9	10,738,211	
	KDM4B	9	10,656,035	
	RFX2	9	10,216,249	
	TINCR	9	10,499,290	
	VAV1	9	9,617,783	
	MBD3L4	*8*	*18,226,238*	*2014 assembly*
	INSR	12	1,678,623	
	ZNF358	12	2,046,542	
	MAP2K7	12	2,546,139	
	FBN3	18	53,070,463	
	HNRNPM	7	18,516,253	

	ZNF558	*not found*		
	OLFM2	8	21,684,494	
	DNMT1	8	21,922,515	
	DNM2	8	22,458,869	
	CARM1	8	22,527,213	
RABBIT	SIRT6	3	16,044,566	
	PLIN3	*not found*		
	UHRF1	1	47,672,908	
	KDM4B	1	47,085,460	Also 13 & 12 & 1:121,843,123
	RFX2	1	51,045,589	
	TINCR	*not found*		
	VAV1	13	56,144,807	
	MBD3L4	*unknown*		
	INSR	un0069	1,077,773	
	ZNF358	un0069	914,737	
	MAP2K7	un0069	665,019	
	FBN3	un0069	502,497	Also 3:11,898,428
	HNRNPM	un0069	252,960	Also X
	ZNF558	*not found*		
	OLFM2	un0135	375,668	
	DNMT1	un0135	156,550	
	DNM2	13	20,368,794	
	CARM1	1	51,421,465	

LEGENDS FOR FIGURES

Figure 1

A. Human Chromosome 1 with relevant genes, bent to show telomeres in proximity:

SPEN 1p36.2, HDAC1 1p35.2 RBM15 1p13.3, LBR 1q42.12, and SAFA, 1q44,
See Table 1.

B. Human Chromosome 19 insert with relevant genes, showing proximity of genes in 19p13.1-13.2:

UHRF1, Safb2, SAFB, ZNF 358, ZNF 699, SMARCA4, DNMT1, ZNF 823, ZNF 69
See Table 2.

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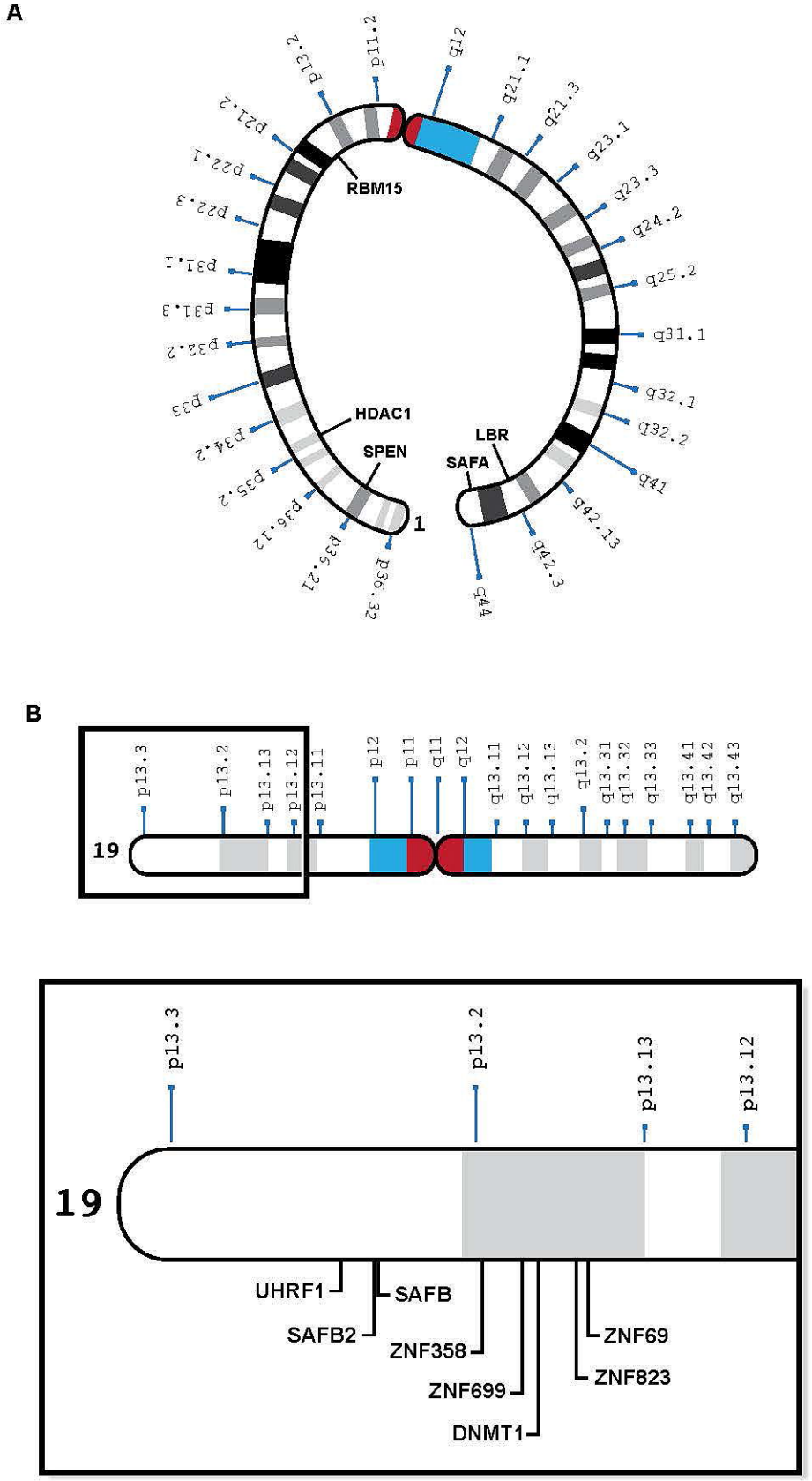
A
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p11.2
q12
q21.1
q21.3
q23.1
q23.3
q24.2
q25.2
q31.1
q32.1
q32.2
q41
q42.13
q42.3
q44
p36.32
p36.21
p36.12
p35.2
p34.2
p33
p32.2
p31.3
p31.1
p22.3
p22.1
p21.2
RBM15
HDAC1
SPEN
LBR
SAFA
1
B
19
p13.3
p13.2
p13.13
p13.12
p13.11
p12
p11
q11
q12
q13.11
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