Genetics of Bleeding Disorders
ID77

EXPLORING THE CAUSES OF SKEWED X-CHROMOSOME INACTIVATION: *XIST* GENETIC ANALYSIS OF CASES AND CONTROLS

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Introduction & Objective

In mammals, random X-chromosome inactivation (XCI) of one of the two X-chromosomes in females achieves dosage equivalency for X-linked genes with males XY. Skewed XCI is a strong deviation random XCI (>80%) and among others causes may be associated with deletions in *XIST* (X-inactivation specific transcript), non coding gene responsible for initiation, spreading and maintenance of XCI. The presence of genetic variants as SNP (Single Nucleotide Polymorphism) may modify *XIST* expression and/or function impacting its allelic ability to inactivate.

Objective: This work is aimed to investigate the association between *XIST* SNP variants and skewed XCI.

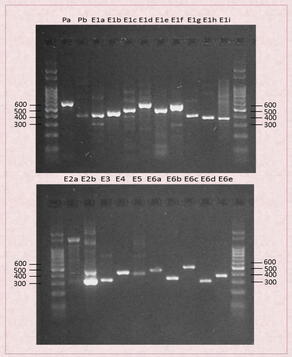
Materials & Methods

The studied population included 22 women, 11 Cases with extremely skewed XCI (>90%) and 11 Controls with random XCI (50-55%). Cases: carriers (symptomatic and asymptomatic) and non-carriers of severe Haemophilia A and Duchenne Muscular Dystrophy mutations. A comprehensive genetic variant screening of *XIST* was performed by CSGE (conformation sensitive gel electrophoresis) and Sanger sequencing. Most relevant sequences of *XIST* were studied. The Promoter (2 amplicons) and exons 2 to 5 with their splicing site consensus were entirely PCR-amplified and screened (4 amplicons with 64, 137, 209 and 164bp), and exon 1 (11,372bp) and exon 6 (7,325bp) including only regions with SNPs associated with known polymorphic allelic frequencies (9 and 5 amplicons, respectively).

Results

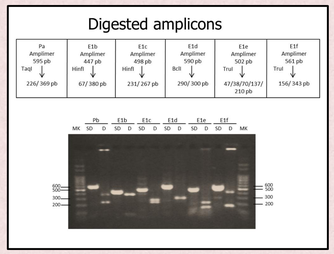
The Table below shows each of the 20 analyzed amplimers, their product length and the number of expected and observed SNPs. The identity and size of each amplicon was analyzed by agarose gel electrophoresis and result in positive and a specific amplification signal in each case.

Exon Region	Amplimers	Product Length [bp]	Expected SNP	Observed SNP
Promoter	Pa	595*	10	
	Pb	392	6	
1	E1a	413	4	
	E1b	529*	2	
	E1c	498*	7	
	E1d	415*	4	
	E1e	502*	1	1
	E1f	561*	1	
	E1g	378	5	
	E1h	422	3	1
	E1i	371	-	
2	E2a	1494	-	
	E2b	329	-	
3	E3	329	2	
4	E4	422	3	
5	E5	396	3	
6	E6a	444	3	
	E6b	370	6	1
	E6c	304	6	2
	E6d	493	10	1
	E6e	337	4	1

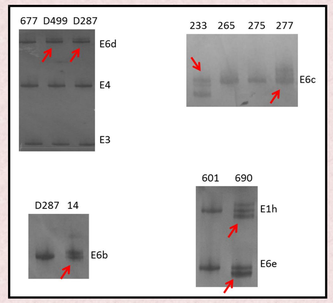


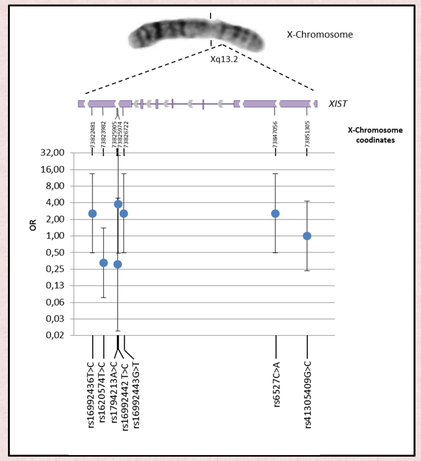
The Figure on the right panel shows examples of CSGE patterns on red arrows indicate signals with differential CSGE mobility.

Seven Cases and seven Controls resulted homozygous for SNPs within the analyzed region of first exon of *XIST*. Two Cases and three Controls resulted homozygous for all studied SNPs within all 20 studied amplicons.



The Figure above shows the products with more than 500bp that were digested to be within the detection range of a CSGE screening technique.





The Figure on the left panel shows the OR (odds ratio) and IC90 (confidence interval 90%) associated with informative SNPs. The analysis of 440 amplicons showed no new mutations but revealed 7 allelic variants of the 80 studied SNPs within *XIST*. Statistical analysis: OR associated with 2 SNPs on exon 6 (rs1794213 and rs1620574) suggested a protective effect (0.30≤OR≤0.33); a SNP in the Promoter (rs41305409) resulted clearly neutral (OR=1) and 4 SNPs, 3 in exon 6 (rs16992443, rs16992436 and rs16992442) and 1 in exon 1 (rs6527), suggested an association with skewed XCI (2.6≤OR≤3.8). Perhaps because of population sizes none of the observed differences reached statistical significance (Fisher exact test, P<0.05).

Conclusions

To of our knowledge, this is the first Case/Control study of *XIST* variants *vs* XCI skewing performed so far.

The SNP rs187705242, which was reported associated with skewed XCI twice (Plenge et al, 1997; Tomkins et al, 2002), was not found in our population, in agree with a study with more than 50 women with skewed XCI (Pereira & Zanz, 1999).

The observed trends associated with some *XIST* variants, either preventing or stimulating XCI skewing, although no statistically significant, encourage us to deepen these studies increasing the number of Cases and Controls (n>44) and extending the targeted regions to the entire exons 1 and 6 and deep intronic sequences.

In addition, genomic dosage analysis should be performed on *XIST* particularly in those homozygous cases for all tested SNPs to investigate deletions as the cause for XCI skewing.

Acknowledgements

We would like to thank the hematologist Miguel de Tezanos Pinto, Miguel Candela and Mariana Bonduel, and the scientist Leonela Luce for their help in different phases of the work. This study was supported by grants from the Florencio Fiorini Foundation, the National Research Council (CONICET), the National Agency for Science and Technology Promotion (ANPCyT) and a fellowship from the Word Federation of Hemophilia. The assistance to this Congress was supported by Pfizer. No other conflict of interests should be declared.

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