Differential isoform expression and alternative splicing in sex determination in mice

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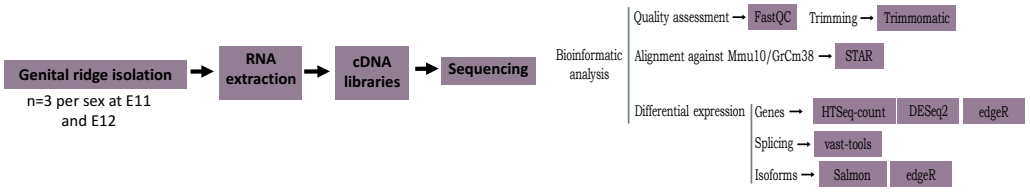
INTRODUCTION

Sex determination is a highly plastic process through which a bipotential gonad develops into a testis or ovary. The mechanisms of sex determination are remarkably variable among organisms. The majority of the principal genes related with sex determination in mammals are known; however, not much is known about RNA isoforms and AS variants during germline development. This mechanisms are responsible for sex determination in some insects including flies, honeybees, and wasps, and in some reptiles. It has been reported that some transcription factors important for sex determination in mammals, such as Wt1, Sry and Sox6, control mRNA splicing. During sex differentiation in mice, Sox9 not only regulates transcription of its target genes directly, but also influences their RNA splicing. As isoform changes may be masked by gene-level measurements, estimation of isoform expression provides a better resolution than gene expression to evaluate dynamic developmental processes.

OBJECTIVE

To explore the genome-wide transcriptomic landscape to identify gene-, isoform-, and AS-level expression features related with sex determination and early differentiation in mice.

MATERIAL AND METHODS



RESULTS

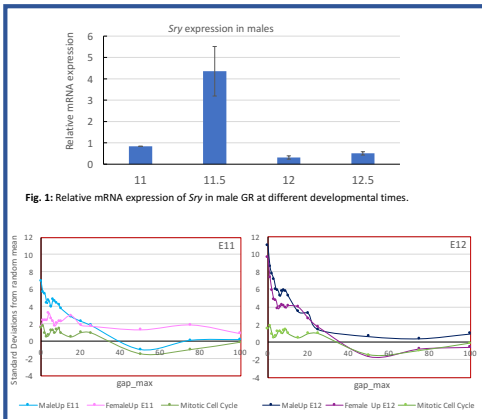


Fig. 1: Relative mRNA expression of *Sry* in male GR at different developmental times.

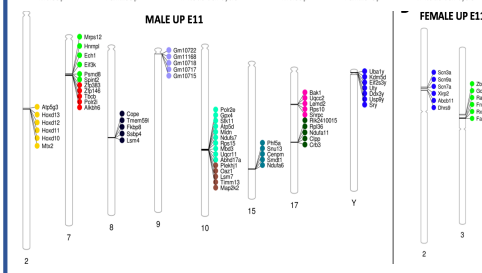


Fig.2: Clustering of DEGs.

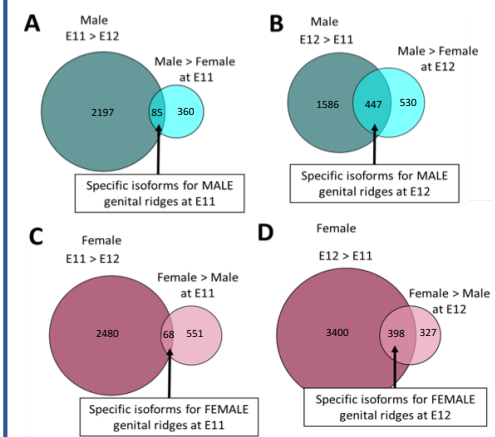


Fig. 3: Specific isoforms for male and female genital ridges at E11 and E12.

GENDER ANALYSIS

	E11			E12		
	Male Up	Female Up	Total	Male Up	Female Up	Total
DEG	697	531	1228	957	892	1849
DEI	445	619	1064	977	725	1702
Common events	104	96	200	676	495	1171
% DEI commons	23.37	15.67	18.80	69.19	68.28	68.80

TIME –COURSE ANALYSIS

	Female			Male		
	E11 Up	E12 Up	Total	E11 Up	E12Up	Total
DEG	2882	4184	7066	1897	1685	3582
DEI	2548	3798	6346	2282	2033	4315
Common events	1988	2948	4944	1590	1333	2923
% DEI commons	78.02	77.80	77.62	69.68	65.57	67.74

Fig.4: Comparison between DEIs and DEGs detected.

DIFFERENTIALLY EXPRESSED ISOFORMS (DEI)

Genes with different isoforms expressed in males and females

Some are related to important functions in sex determination:

Xpo1: nuclear cytoplasmic transport

Son: control of mRNA splicing

Mga: repressor of germ cell-related gene expression

Taf1 (Tra2): testis specific splicing

Genes with different protein coding isoforms expressed in both, male and female E11 gonads

Gene	DEG EdgeR FC	Male isoform (FC)	Female isoform (FC)
Son *	NS	205 (11.9)	202 (-29)
Xpo1 *	-0.6	204 (3.4)	202 (-2.1)
Mga	-0.8	202(3797)	215(-8690)
Taf1 * (Tra2)	-0.56	203 (3306)	206 (-10.6)

Fig.5: Genes with different protein coding isoforms expressed in males and females.

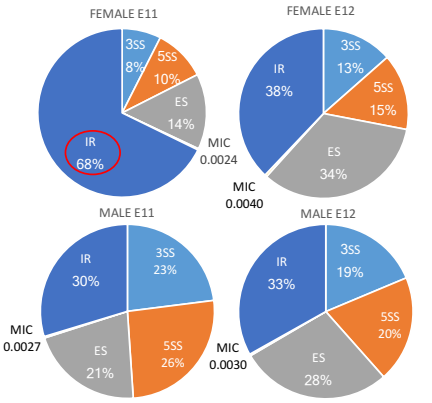


Fig . 6: Distribution of AS events in male and female genital ridges at E11 and E12.

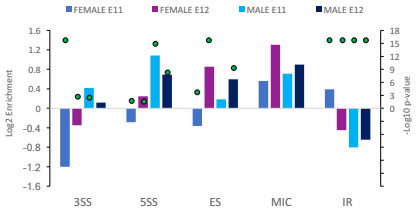


Fig. 7: Distribution of AS events normalized according to the overall distribution of AS in mice.

Peak of *Sry* expression was first identified at E11.5 (Fig. 1). Differential expressed gene (DEG) analysis identified hundreds of genes related with GSD and early sex differentiation that could be good candidates for sex reversal; also, many of them grouped in clusters on specific chromosomes (Fig 2). Increased expression at E11 in males was significantly enriched in RNA splicing and mRNA processing gene ontology (GO) terms. Differentially expressed isoform (DEI) analysis identified hundreds of specific isoforms related with GSD, many of which did not show differences in the DEG analysis (Fig. 3 and 4). Interestingly, 30 genes at E11 exhibited expression of a different isoform in males and females related to important functions (Fig. 5). Hundreds of AS events were identified as modified at E11 and E12. Female E11 gonads exhibit sex-biased up-regulation of intron retention (Fig. 6) (genes related with regulation of transcription, protein phosphorylation, protein transport and mRNA splicing) and exon skipping (genes related with chromatin repression) suggesting AS as a post-transcription mechanism that controls sex determination of the bipotential fetal gonad.

CONCLUSION

The results suggest that important steps in the mammalian sex determination process are likely to operate at the post-transcriptional level. RNA isoforms expression and splicing regulatory mechanisms constitute a common feature among sex determination in distant phyla, including mammals.

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