ROLE OF ALTERNATIVE SPLICING DURING SEX DETERMINATION IN MICE

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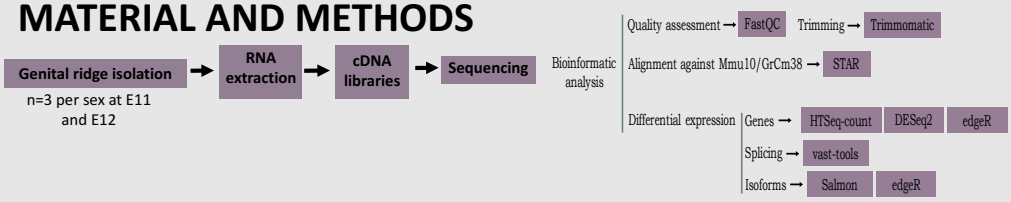
INTRODUCTION

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. In many insects, such as *Drosophila*, sex is determined by mRNA splicing. Also, differential intron retention is implicated in sex determination in some reptile and fish species. The genes that determine gonadal sex determination (GSD) in mice are known, but knowledge of the molecular pathways specifying GSD is still incomplete. mRNA isoforms are molecules of different exon composition and length, which may code for different forms of the corresponding protein. They may be produced from different transcriptional starting sites, terminated at different polyadenylation sites, or as a consequence of alternative 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

The aim of this study was to identify differentially expressed isoforms (DEI), differentially expressed genes (DEG) and alternative splicing (AS) changes during GSD in mice.

MATERIAL AND METHODS



RESULTS

Peak of *Sry* expression was first identified at E11.5 (Fig. 1). Gene analysis identified 729 and 1691 DEGs between males and females at E11 and E12 (Fig. 2), respectively. Hundreds of these genes are related with GSD and early sex differentiation and could be good candidate genes for sex reversal; also, many of them appeared to be grouped in clusters on several chromosomes (Fig. 8). Interestingly, increased expression at E11 in males was significantly enriched in RNA splicing and mRNA processing gene ontology (GO) terms. At E11 there is differential expression of Sox4 between male and female, and similar expression of Sox9 (Fig. 4). At E12 there is a higher expression of Sox2 in females and higher expression of Sox6, 7, 8, 9, 10 and 13 in males (Fig. 4). When we compare the differences between E11 and E12 we can observe that there is increased expression of Sox8, Sox9 and Sox10 in males, and a decreased expression in females. There is also an increase of expression of Sox6, Sox7 and Sox13 between E11 and E12 only in males, and an increase of Sox30 only in females (Fig. 4). The remaining genes follow the same pattern in both sexes. Isoforms analysis identified 705 and 1348 DEIs between males and females at E11 and E12, respectively (Fig. 3). We found 15 genes at E11 and 18 genes at E12 with different protein coding isoforms expressed in males and females (Fig. 5). Many DEI did not shown differences in the DEG analysis. In addition to the isoforms, 1167 differentially AS events were observed between females and males at E11. At E11 there was an enrichment in intron retention (IR) in females, and at E12 there was enrichment in IR and exon skipping in females (Fig. 6). Eighty-five genes exhibited expression of different AS events in both males and females at E11, and 184 at E12. Some of these AS genes are transcription factors that could play an important role in GSD, like *Jarid2*a Jumonji family member essential for AS sex determination in reptiles

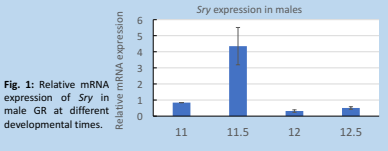


Fig. 1: Relative mRNA expression of *Sry* in male GR at different developmental times.

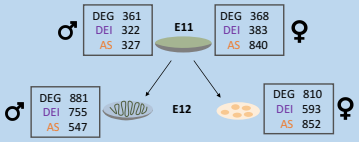


Fig. 2: Number of DEG, DEI, and AS events differentially expressed between male and female at E11 and E12.

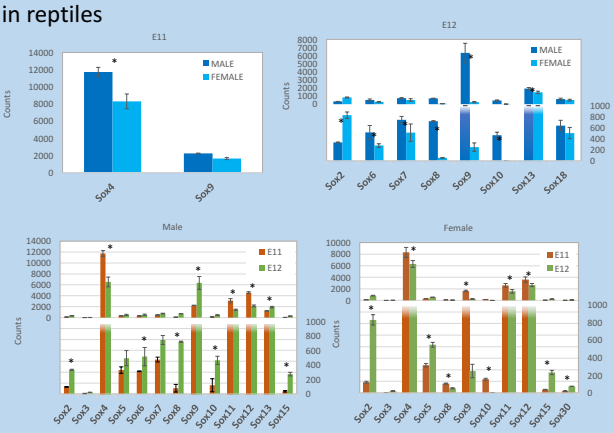


Fig. 4: *Sox*-gene family expression (Counts). Upper panel represents differential expression between male and female at E11 (left) and E12 (right) GR. Lower panel represents differential expression between E11 and E12 of male (left) and female (right) GR.

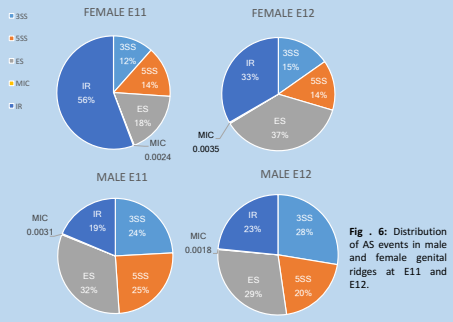


Fig . 6: Distribution of AS events in male and female genital ridges at E11 and E12.

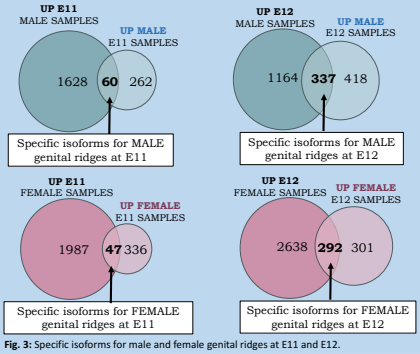


Fig. 3: Specific isoforms for male and female genital ridges at E11 and E12.

Some are related to important functions in sex determination:

Son: control of mRNA splicing

Xpo1: nuclear cytoplasmic transport

Mtm1: male sexual development.

Taf1 (Tra2): testis specific splicing

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

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)	202 (25)
Xpo1 *	-1.46	204 (5)	202 (2)
Mtm1	NS	203 (50)	213 (852)
Taf1 * (Tra2)	-1.42	203 (38)	206 (14)

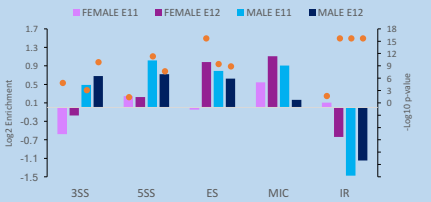


Fig. 7: Distribution of AS events normalized according to the overall distribution of AS in mice.

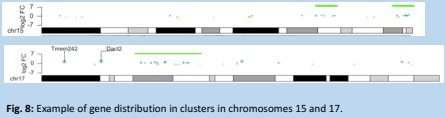


Fig. 8: Example of gene distribution in clusters in chromosomes 15 and 17.

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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