Abstract citation ID: bvae163.1708

Reproductive Endocrinology

8320

Integration of long-read sequencing, DNA methylation and gene expression reveals heterogeneity in Y-chromosome segment lengths in phenotypic males with 46,XX testicular disorder of sex development

Emma Bruun Johannsen, MSc[1], Agnethe Berglund, MD, PhD[1], Anne Skakkebæk, MD, PhD[1], Simon Chang[1], Julia Rohayem, MD, PhD[2], Sandra Laurentino, PhD[2], Arne Hørlyck, MD[1], Simon Opstrup Drue, PhD[1], Ebbe Norskov Bak, PhD[1], Jens Fedder, MD, PhD[3], Frank Tuttelmann[4], Joerg Gromoll, PhD[2], Jesper Just, PhD[5], and Claus Hojbjerg Gravholt, MD, PhD[1]

[1]Aarhus University Hospital, Aarhus, Denmark; [2]Münster University Hospital, Münster, Germany; [3]Odense University Hospital, Odense, Denmark; [4]University of Münster, Munster, Germany; [5]Aarhus Univeristy Hospital, Aarhus N, Denmark

Disclosure: E.B. Johannsen: None. **A. Berglund:** None. **A. Skakkebæk:** None. **S. Chang:** None. **J. Rohayem:** None. **S. Laurentino:** None. **A. Hørlyck:** None. **S.O. Drue:** None. **E.N. Bak:** None. **J. Fedder:** None. **F. Tuttelmann:** None. **J. Gromoll:** None. **J. Just:** None. **C.H. Gravholt:** None.

Background: 46,XX testicular disorder of sex development is a rare congenital condition affecting approximately three per 100,000 newborn males. It is characterized by a combination of the typical female sex chromosome constitution, 46,XX, and a variable male phenotype. In the majority of XX males, a Y chromosome segment containing the sex-determining region gene (*SRY)* has been translocated to the paternal X chromosome. However, the precise genomic content of the translocated segment, breakpoints, genome-wide effects, and the effect on other biological pathways essential for the phenotype remain unknown. **Methods:** We performed long-read DNA sequencing (Oxford Nanopore), RNA sequencing (paired-end, 100 bp) and DNA methylation (Infinium EPIC) analysis on blood samples from males with 46,XX testicular disorder of sex development (XX males; n = 11), male controls (46,XY; n = 12 for long-read DNA sequencing, n = 26 for RNA sequencing and DNA methylation) and female controls (46,XX; n = 12 for long-read DNA sequencing, n = 32 for RNA sequencing and DNA methylation), in addition to RNA sequencing and DNA methylation analysis on blood samples from males with Klinefelter syndrome (47,XXY, n = 22). We also performed clinical measures on all XX males and a subset of male controls (n = 10). **Results:** We identified variation in the translocated Y-chromosome segment of our XX male cohort, allowing for subcategorization into 1) XX males without Y chromosome material (n = 1), 2) those with short Yp arms (breakpoint at 2.7-2.8 Mbp, n = 2), 3) those with medium Yp arms (breakpoint at 7.3 Mbp, n = 1) and 4) those with long Yp arms, including *AMELY, TBLY1 deletions*, and in some cases *PRKY* deletions (n = 7). We also identified variable expression of the X-Y homologues *PRKY* and *PRKX*, appeared to have balanced expression level resulting in overall equilibrium. The Y-chromosomal transcriptome and methylome were affected, reflecting the Y-chromosome segment length. Genomic changes induced by the translocated Yp segment were evident in the transcriptome and methylome of autosomal and X-chromosomal regions, indicating global effects. Furthermore, the transcriptional changes in XX males correlated with phenotypic traits of the XX males, including lower height, decreased lean mass and smaller testicular size. **Conclusion:** Integration of long-read DNA sequencing, DNA methylation, and RNA sequencing analysis allowed for the identification of a heterogenous translocation process in XX males that exerted widespread effects on methylation and transcription.

Presentation: 6/2/2024