P-531 hCFTR p.G970D mutation causes Sertoli Cell-only Syndrome (SCOS) and Congenital bilateral absence of the vas deferens (CBAVD)

J. Hou[1], X. Li[1], L. Wang[2], W. Xu[1]

[1]*Sichuan University- West China Second University Hospital, West China School of Medicine, Chengdu, China ;*
[2]*Gansu Provincial Masternity and Child-care Hospital, the Center of Reproductive Medicine, Gansu, China*

Study question: Whether CFTR is a pathogenic gene for azoospermia?
Summary answer: CFTR p.G970D affects spermatogenesis and leads to male infertility by affecting the proliferation and survival of Germ Cell.
What is known already: Male infertility is a multifactorial and heterogeneous pathological condition affecting 7% of the general male population. However, up to now, only a relatively low number of genic factors have a clear relationship with spermatogenesis. Although, increased frequency of CFTR mutations or impaired CFTR expression in men with non-obstructive azoospermia or oligospermia as compared to the fertile men has been reported, but there is no direct evidence CFTR mutations cause azoospermia. Compared to F508Del mutations in Caucasians, p.G970D mutation is the most frequent CFTR mutation identified in Chinese CF patients. However, p.G970D has not been reported involved with male infertility.
Study design, size, duration: In this study, began in an infertile man suffering CBAVD and SCOS with no CF-like phenotype related symptoms up to now. By identifying the patient with CFTR p.G970D mutation, we further verified the function of the mutation in spermatogenesis in spermatogonia cell lines. Control testicular tissue sample was obtained from fertility man donors.
Participants/materials, setting, methods: WES was performed for probands and relatives and the mutation was confirmed by Sanger sequencing. Hematoxylin-eosin (HE) staining and immune fluorescence (IF) was performed on seminiferous tubules from the patient and control to characterize the structural anomalies present in the patient. GC2 mCFTRG965D cells was knocked in by the CRISPR/Cas9 gene editing system. The effects of mutations on the growth and proliferation of GC2 cells were detected by CCK8, IF, WB, BCECF staining and RT-PCR.
Main results and the role of chance: First, we identified the CBAVD and SCOS patient with homozygous missense mutations p.G970D in the CFTR gene, and his mutation inherited from both parents. The patient has normal general parameters and fertility parameters except for smaller testes, lower semen volume and pH. His testicular histopathology and co-location of CFTR and DDX4 which is the marker of spermatogonia likewise showed SCOS. Second. given that the amino acid sequence is conserved and the same expression and localization patterns of CFTR between human and mouse, we generated mouse derived cell lines model (mCFTRG965D) that carried a homozygous mutation equivalent to the *CFTR* variant in patients, using CRISPR/Cas9-mediated genome editing. mCFTRG965D affects the proliferation of Germ Cell, but has less effect on Sertoli cells, which is similar to the SCOS patient's phenotype. Third, lower mature CFTR were observed in the GC2 mCFTRG965D groups cells compared to those in wild type groups, and CFTR protein is not evident in the GC2 mCFTRG965D groups' cell membrane, which demonstrated the mutation affecting the anchoring of CFTR to the cell membrane. What's more, the missense mutation will affect the function of CFTR in regulating pH, thus affecting cell homeostasis.
Limitations, reasons for caution: The low number of biological samples, we need more patients to confirm this mutation and azoospermia. We only validated at the cellular level, not in an animal model. It is noteworthy that, the CFTRF508del mice are fertility.
Wider implications of the findings: Our study reveals that CFTR has a broader indication than just the absence of the vas deferens. We recommend to take further understanding of CFTR playing important role in spermatogenesis by affecting germ cell survival not just regulating cell volume during spermiogenesis.
Trial registration number: not applicable