Mutations in *CBX2* (*CHROMOBOX* 2)associated with gonadal anomalies in 46,XY and 46,XX individuals

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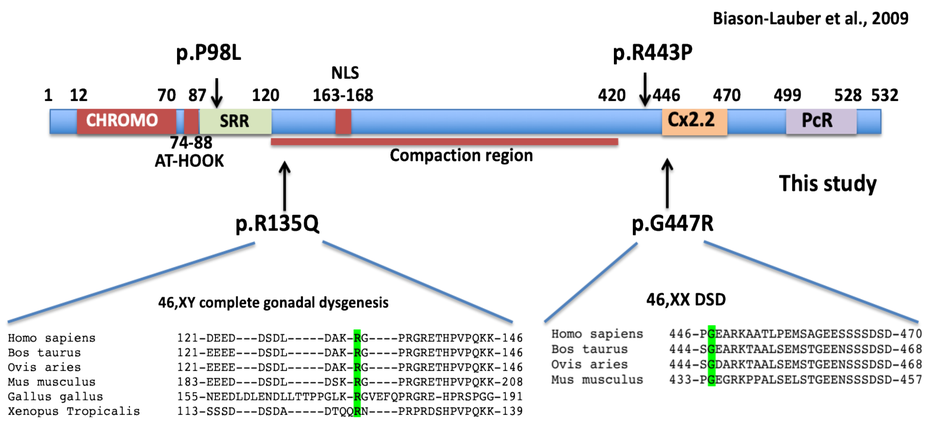
ABSTRACT

The Polycomb Repressive Complex 1 (PRC1) represses gene expression through CBX2, which binds to H3K27me3 and promotes chromatin expression. Recently, CBX2 has been shown to function in testis-formation by directly repressing Wnt4's downstream target, *Lef1*, in Sertoli cells rather than positively controlling *Sry* expression, as previously thought.

Here, we describe two Iindividuals carrying missense mutations in CBX2. The first is a female with 46,XY complete gonadal dysgenesis and the second is a 46,XX individual with intellectual deficiency, facial dysmorphia, a small uterus with no ovaries. In an exome sequencing approach, the first patient carried a *CBX2*, c.G404A mutation that is predicted to result in an p.R135Q amino acid change, whilst the latter carried a *CBX2*, c.G1339A mutation that is predicted to result in an p.G447R amino acid change. Both mutations are predicted to be damaging to the protein by multiple prediction tools. The p.R135Q mutation is absent from all public SNP databases and the p.G447R change has been reported in gnomAD at a minor allelic frequency of $6.6x10^{-5}$ in South Asian populations. Analysis of our exome data and public SNP databases also indicate that the smaller CBX2 isoform 2.2 is unlikely to be functional as healthy controls carry multiple loss-of-function mutations in this isoform. Mice lacking *Cbx2* have been reported to have small ovaries associated with a spectrum of meiotic anomalies in germ cells.

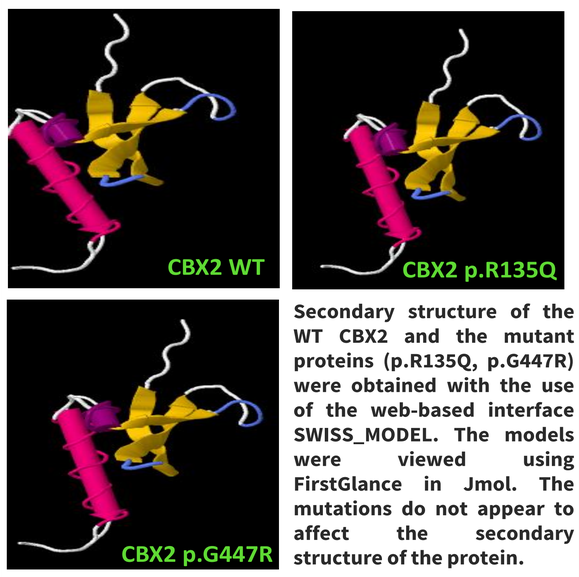
Our data suggest that mutations in the human *CBX2* gene could be a novel cause of ovarian insufficiency along with 46,XY gonadal dysgenesis.

Protein structure of CBX2 with conserved functional domains, position of variants reported in association with DSD and the conservation of amino acids affected in the two patients.



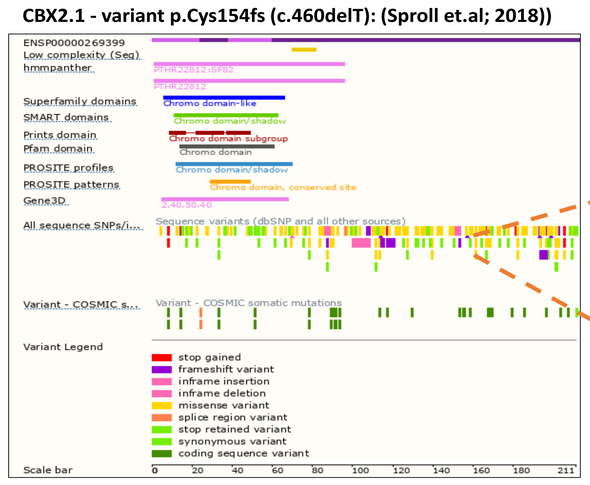
CHROMO, chromodomain; AT-HOOK DNA-binding motif; SRR, Serine rich region; NLS, nuclear localization sequence; Cx2.2 conserved CBX2 motif rich in serine, aspartic acid and glutamic acid residues. PcR, conserved Polycomb repressor box.

Secondary structure of CBX2 WT and CBX2 p.R135Q and CBX2 p.G447R

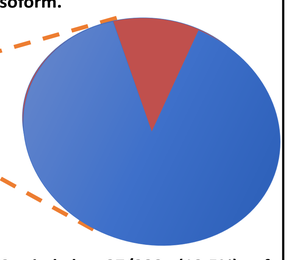


Secondary structure of the WT CBX2 and the mutant proteins (p.R135Q, p.G447R) were obtained with the use of the web-based interface SWISS_MODEL. The models were viewed using FirstGlance in Jmol. The mutations do not appear to affect the secondary structure of the protein.

The p.R135Q is absent from all public SNP databases and the p.G447R shows population specific frequencies



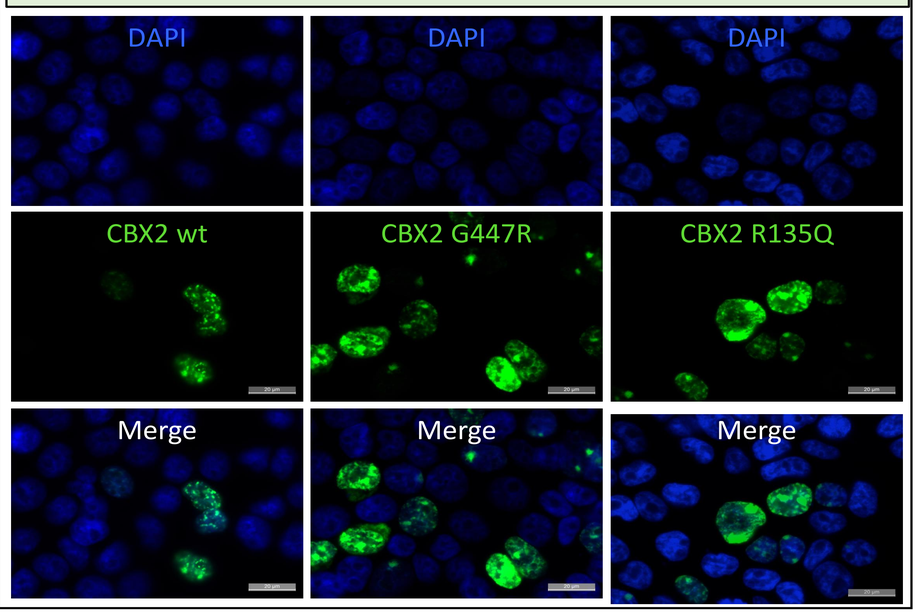
Analysis of our exome data and public SNP databases also indicate that the smaller CBX2 isoform 2.2 is unlikely to be functional as healthy controls carry multiple loss-of-function mutations in this isoform.



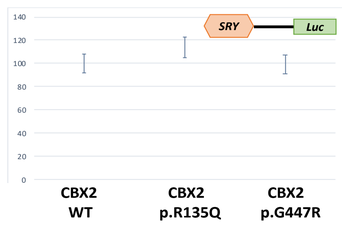
Carried by 37/339 (12.5%) of North African normospermic /fertile men

The p.R135Q mutation is absent from all public SNP databases and the p.G447R change has been reported in gnomAD at a minor allelic frequency of $6.6x10^{-5}$ in South Asian populations.

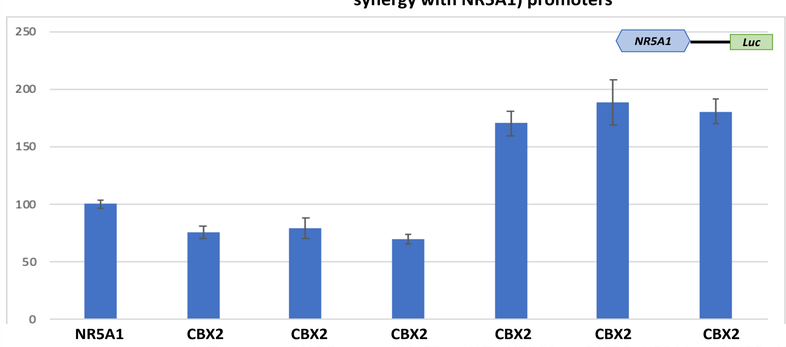
Both the wild-type and mutant CBX2 can localize to the nucleus



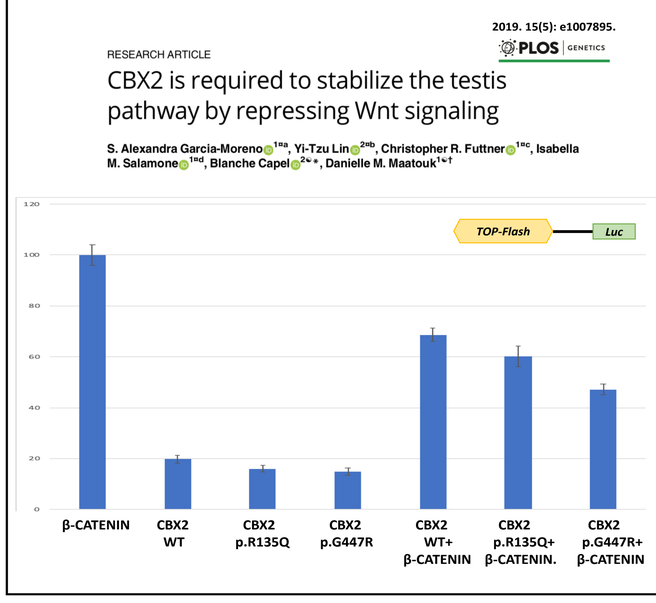
Both the wild-type and mutant CBX2 can activate SRY and NR5A1 promoters *in-vitro*



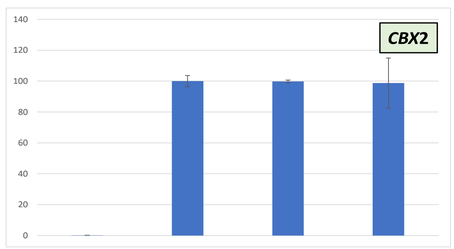
The transcriptional activities of CBX2 WT, p.R135Q and p.G447R were studied using the human *SRY and NR5A1* promoters as reporters, following transfection in HEK 293-T cells. All data were standardized for Renilla activity. The results are expressed as relative percentage of activation by CBX2-WT (for *SRY-luc (*100%; top panel)) or NR5A1 (for *NR5A1-luc (*100%; bottom panel). Both the WT and mutant CBX2 do not show a difference in their ability of activate *SRY* and *NR5A1* (with or without synergy with NR5A1) promoters

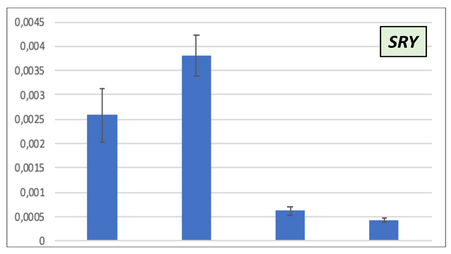


Both the wild-type and mutant CBX2 can repress WNT/ β-CATENIN signaling *in-vitro*

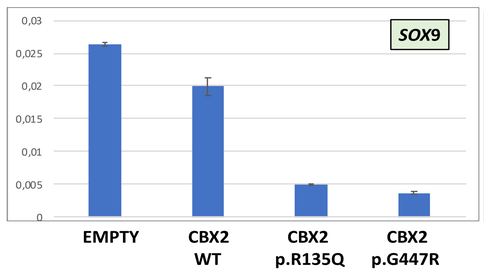


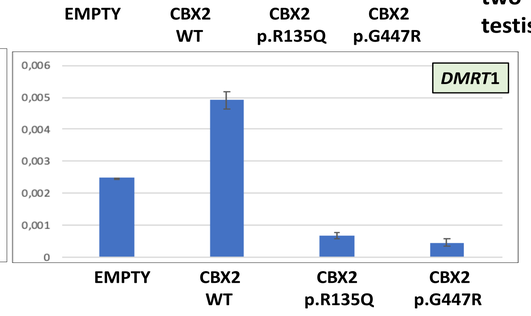
CBX2p.R135Q and p.G447R appear to suppress the endogenous pro-testis transcripts in NTERA-2 cells

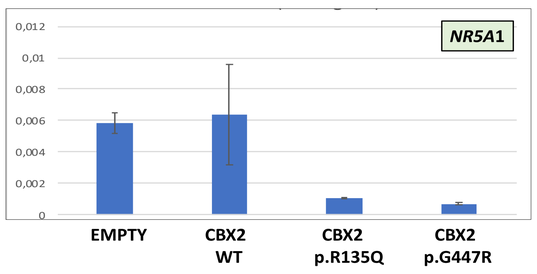




Pluripotent human embryonal carcinoma, NTERA-2 cl.D1 [NT2/D1] cells were transfected with plasmids encoding CBX2-wild type, CBX2p.R135Q or CBX2p.G447R expression vectors. After transfections, the levels of and endogenous CBX2 and Sertoli (*SRY*, *SOX9, NR5A1*, *DMRT1*) transcripts were measured by qRT-PCR. Data are presented as mean 2-ΔΔCt values, normalized to the housekeeping 18S rRNA normalizer gene (*RPL*19). The two mutants appear to suppress the endogenous pro-testis transcripts.







SUMMARY

This study expands the number of pathogenic variants of CBX2 associated with DSD. The data suggest apart from causing 46,XY complete gonadal dysgenesis as previously reported (Biason-Lauber et.al 2009), variants in the gene may be a cause of impaired ovarian development in 46,XX individuals, which is similar to the mouse knockout model (Katoh-Fukui et.al 1998).

It is unclear if the somatic anomalies present in the 46,XX DSD patient are caused by the variant CBX2 or due to other, as yet unidentified, genetic changes. A further genomic analysis of DSD patients with related phenotypes is required. The current functional analysis suggests that the mutant CBX2 proteins may suppress the transcription of key testis transcript.

Our genetic data also indicate that the CBX2.2 isoform is non-functional and should not be included in routine genetic analysis.

1- Garcia-Moreno SA et.al. (2019) PLoS Genet. 15(5):e1007895.
2- Sproll P et.al. (2018) Mol Genet Genomic Med. 6(5):785-795.
3- Biason-Lauber A et.al. (2009) Am J Hum Genet. 84(5):658-63.
4- Katoh-Fukui Y et.al. (1998) Nature. 393(6686):688-92.

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Sex differentiation, gonads and gynaecology or sex endocrinology

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