

# A novel *NR5A1* mutation with preserved fertility

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P1-D3-98

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We have nothing to disclose.

## Take home message

Phenotype of *NR5A1* mutations in 46,XY varies, ranging from complete female to normal fertile male.

## Backgrounds

Preserved fertility has been reported in only three 46,XY men who carry different heterozygous *NR5A1* mutations. Functional analyses of the mutations have not been performed.

## Objectives

To investigate the molecular function of a novel *NR5A1* mutation in which we identified in male siblings and their father, indicating preserved fertility.

## Patients

• Patient 1 was a 3-years-old boy, who presented with penoscrotal hypospadias. No other manifestations including adrenal deficiency were presented. He was the first child of healthy nonconsanguineous Japanese parents. Surgery for hypospadias was underwent at the age of 3 years. Physical and endocrinological evaluations are summarized in Table 1.

• Patient 2 was a 3-months-old boy, who is the younger brother of Patient 1. He presented with penoscrotal hypospadias and bifid scrotum. He had no other manifestations. Physical and endocrinological evaluations are summarized in Table 1.

Fig. 1 Pedigree

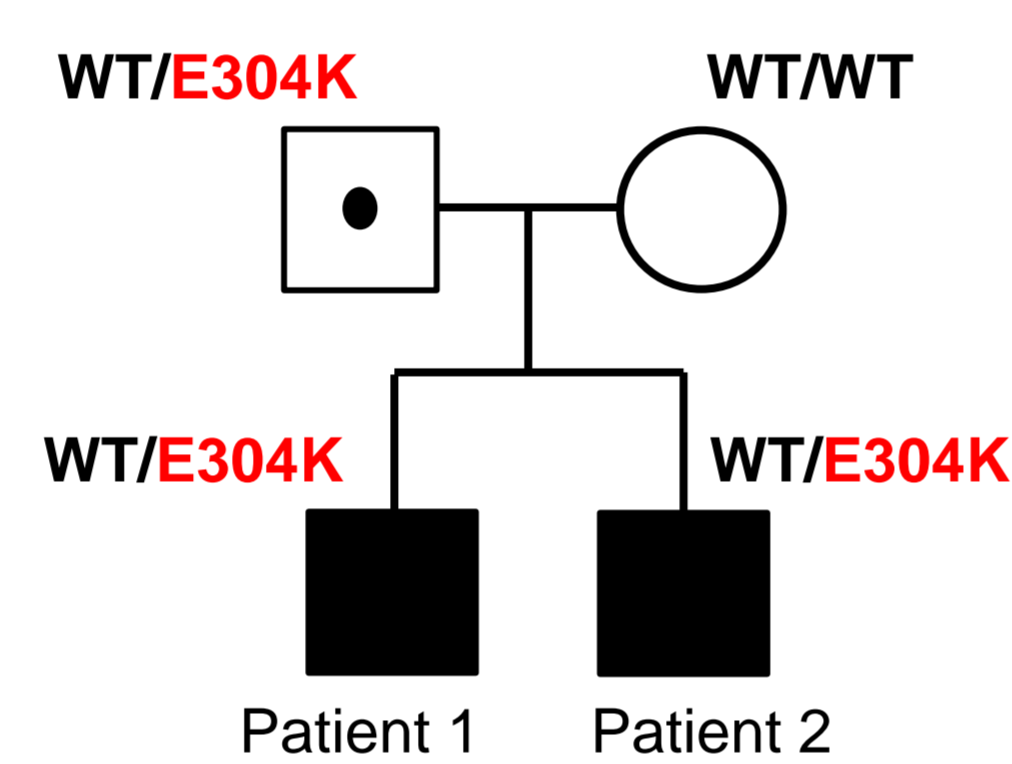


Table 1. Physical and Endocrinological evaluations

Patient		1 (older brother)	2 (younger brother)
Age at evaluation		3 years old	4 months old
Testes volume (ml)		1	1
Penile length (cm)		4.5	3.7
Karyotype		46,XY	
LHRH test	LH (mIU/ml)	0.50/3.82 (60 min)	1.45/9.04 (30 min)
Basal/Maximum	FSH (mIU/ml)	0.92/5.13 (90 min)	2.07/5.48 (90 min)
HCG test	T (ng/ml)	<0.1/2.8	1.0/7.8
Basal/Day 7	DHT (ng/ml)	ND/0.54	0.52/1.57
Age at evaluation		16 years old	13 years old
Testes volume (ml)		15	12
Pubic hair		Tanner 4	Tanner 3
Height (cm)		168.8	155.6
LH (mIU/ml)		7.12	2.59
FSH (mIU/ml)		5.04	4.30
T (ng/ml)		3.94	3.81
AMH (pmol/L)		11.30	5.88

## Methods

This study protocol was approved by the Institutional Ethical Review Board of the Tokyo Metropolitan Children's Medical Center and National Research Institute for Child Health and Development.

### 【Mutational analysis】

Genomic DNA was extracted from the peripheral leukocytes and saliva by using standard techniques. Genomic DNA were analyzed for mutations in 25 known causative/candidate/susceptible genes. A *NR5A1* mutation indicated by the screening analysis was confirmed by Sanger sequencing.

### 【Functional analysis】

We performed functional analyses of the novel mutation in transient expression system using COS1 cells. Western blotting, Subcellular localization analysis, Electrophoretic Mobility Shift Assay (EMSA) and Transcription analysis were performed using the Wild type(WT) and Mutant (E304K) *NR5A1* vectors.

## Results

Fig.2 *NR5A1* gene analysis

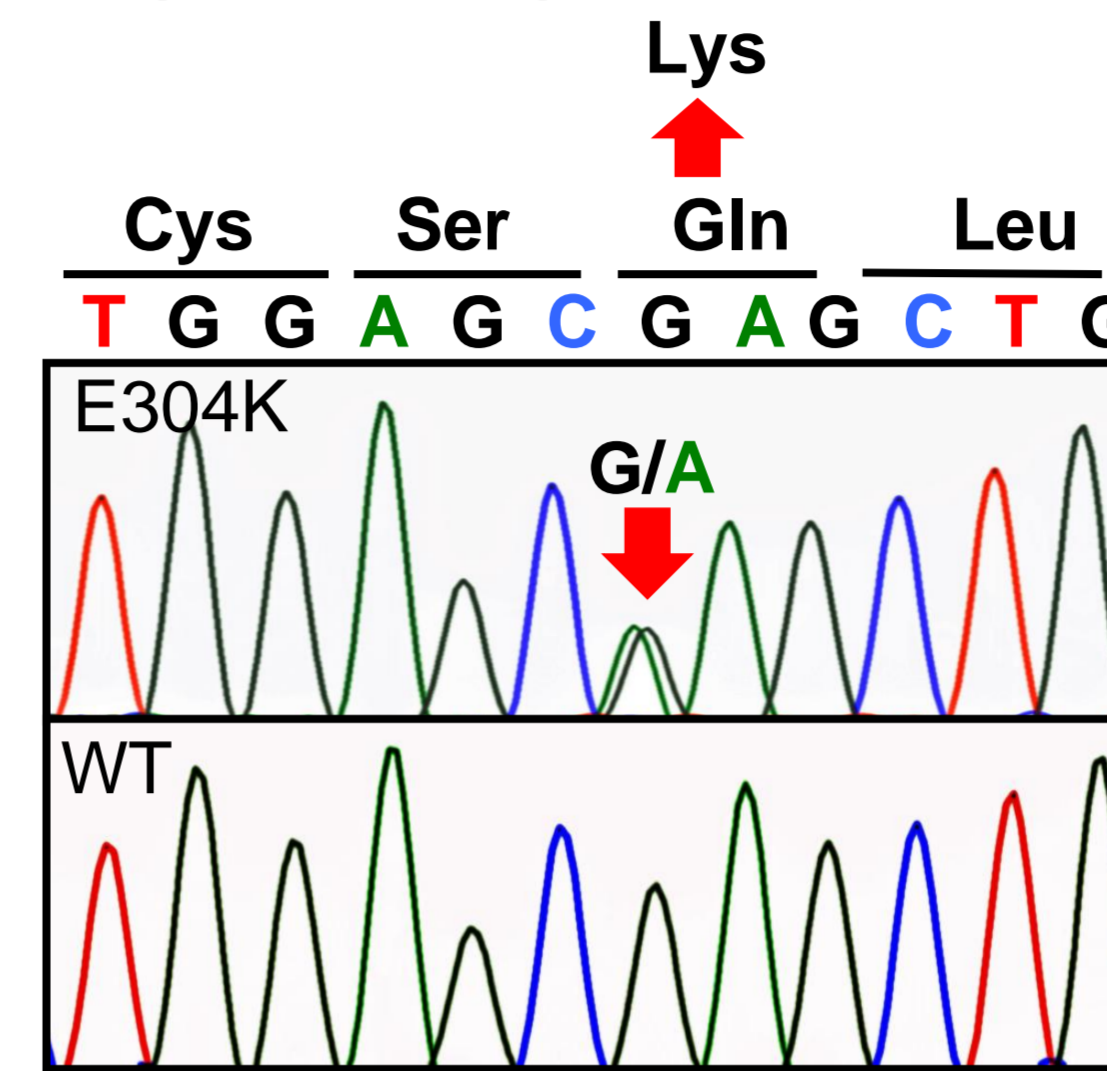


Fig.3 *NR5A1* functional domains and reported mutations that preserved fertility

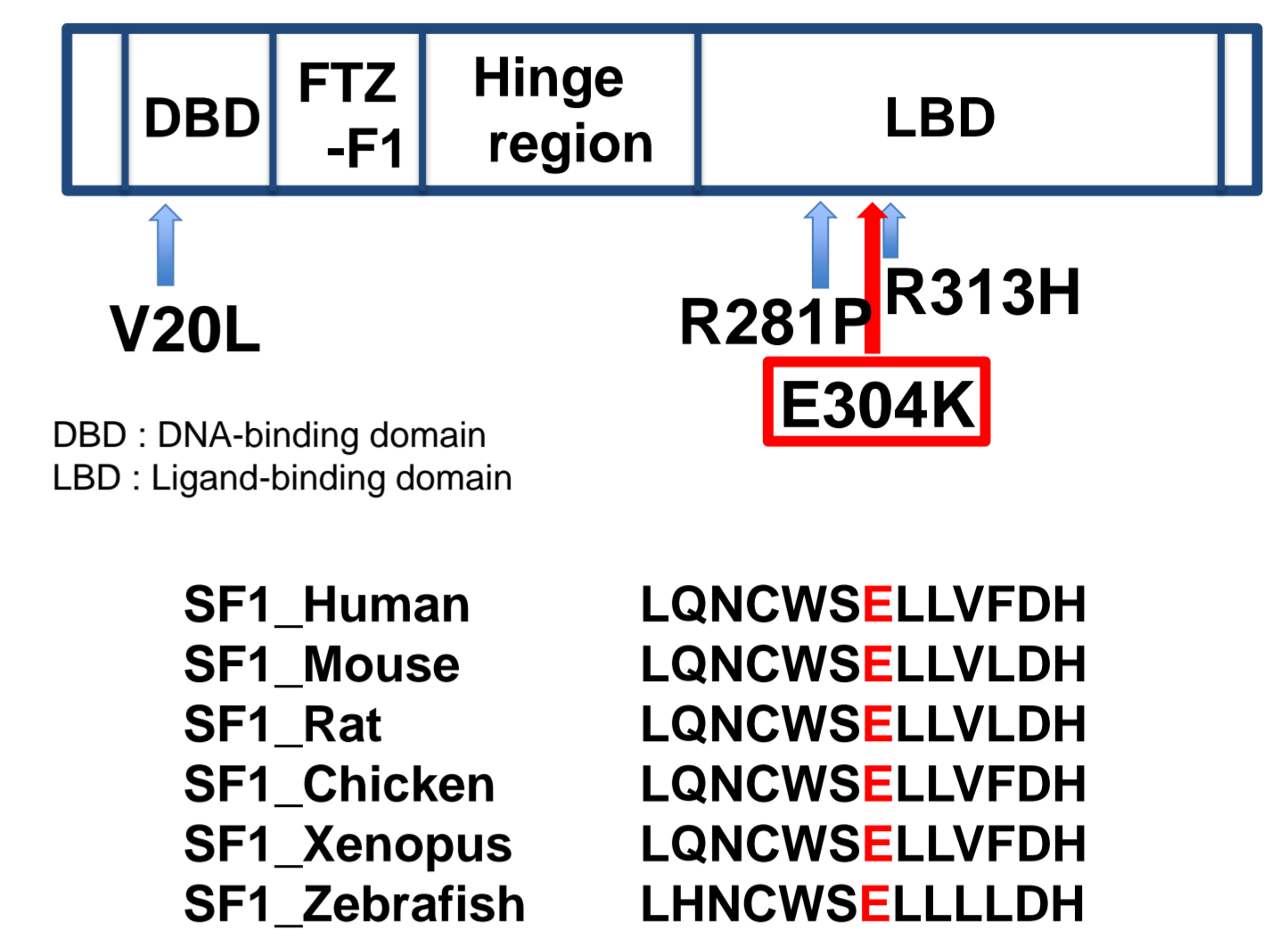


Fig.4 Crystal structure modeling of

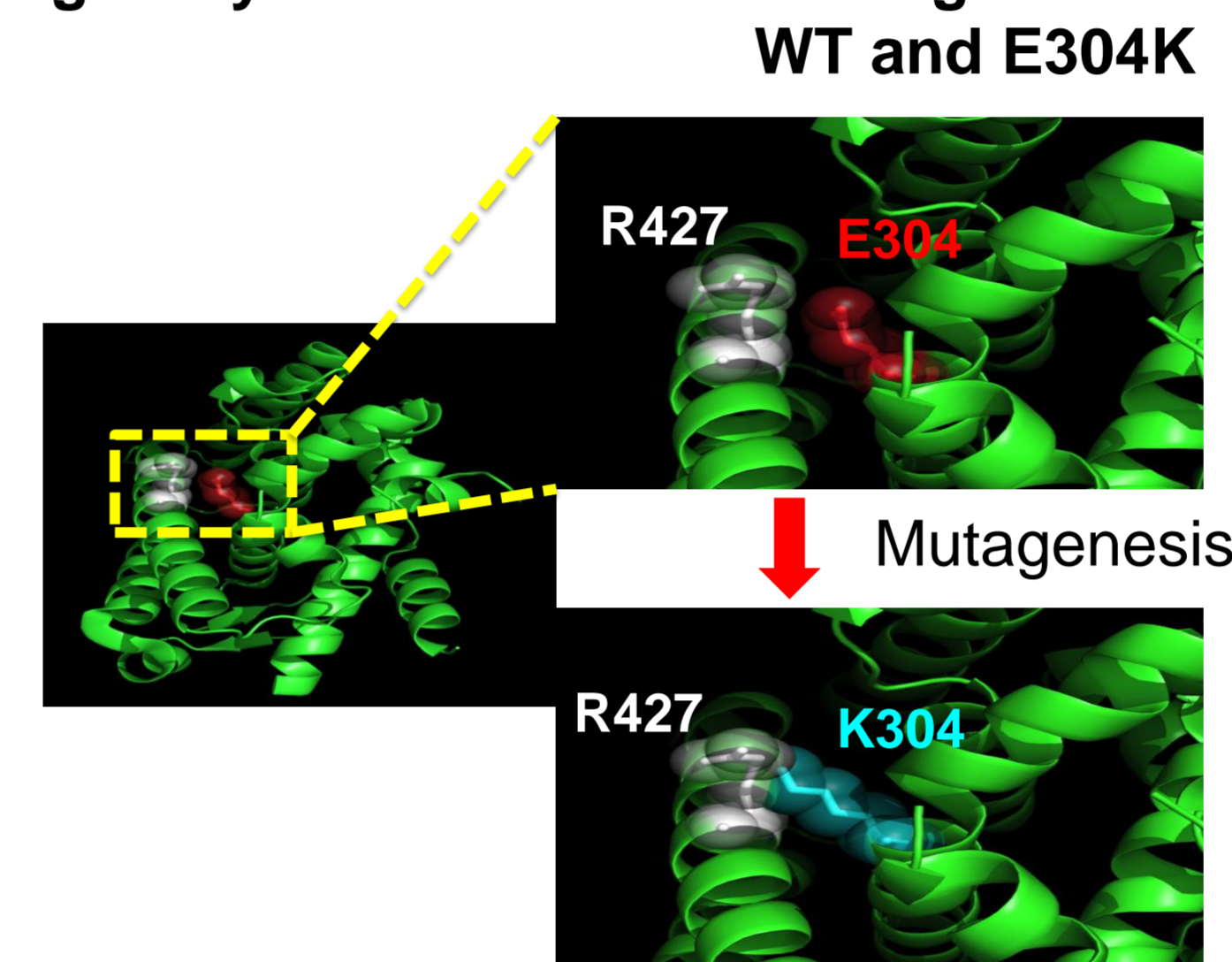


Fig.5 Western blot analysis

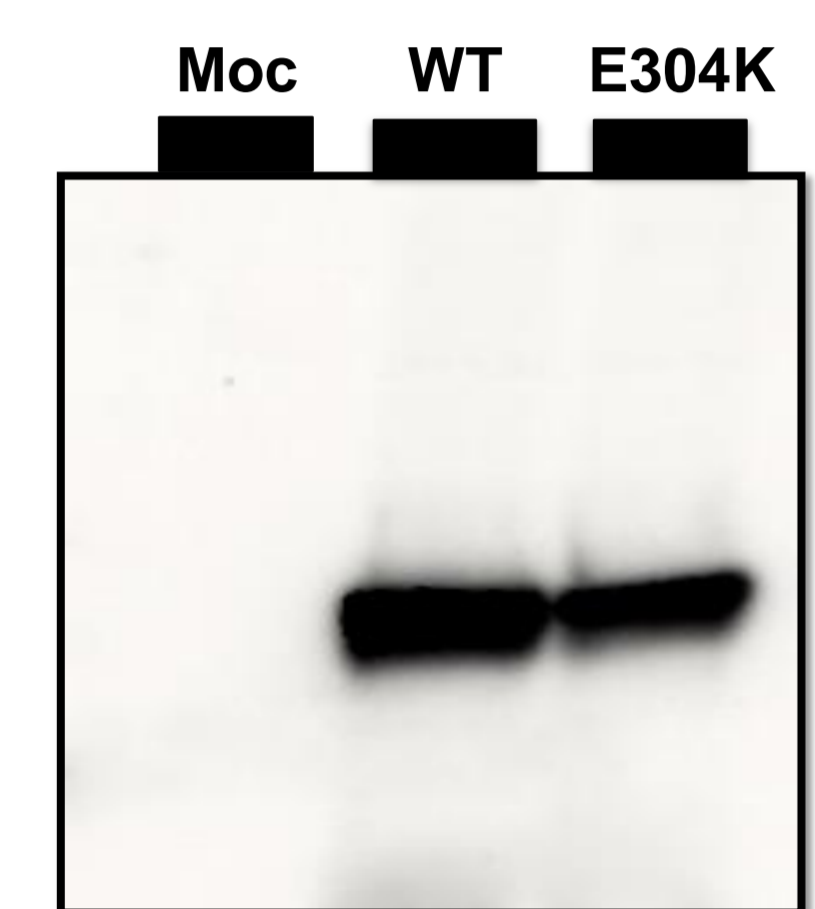


Fig.6 Immunofluorescence assays

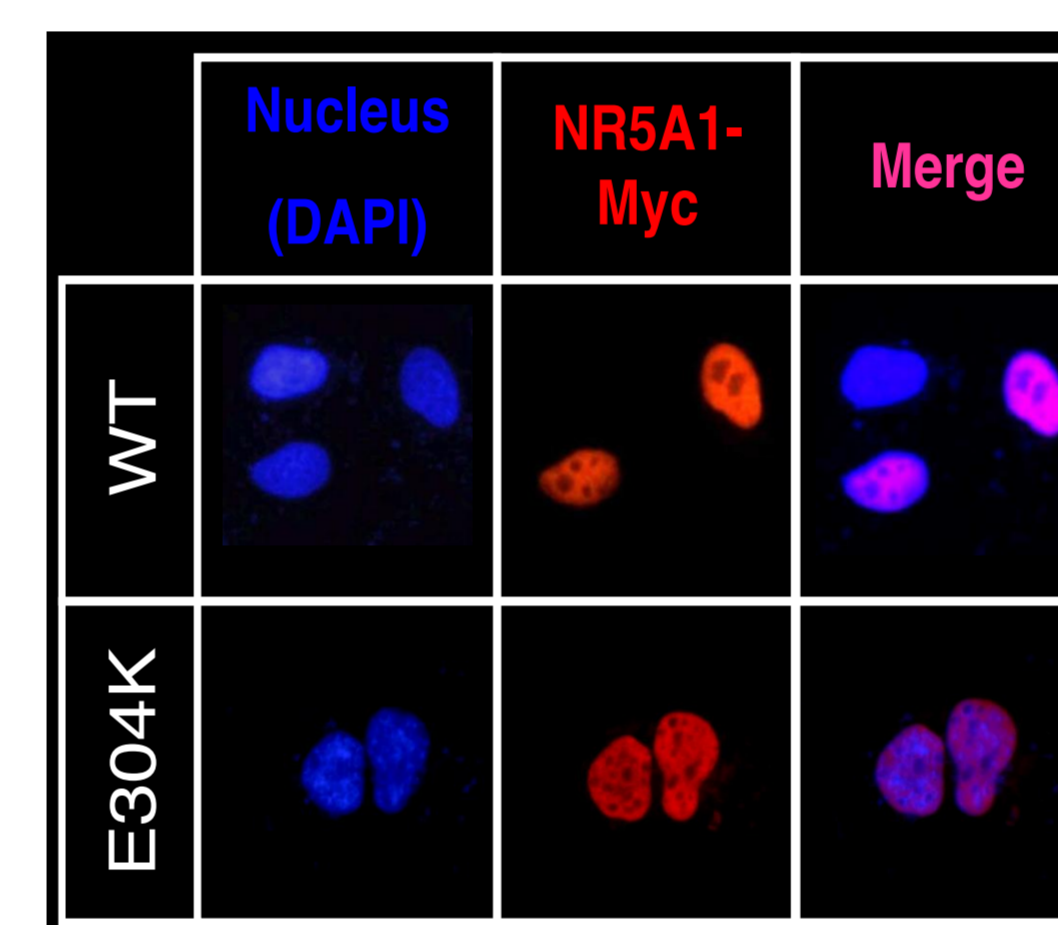


Fig.7 EMSA experiments

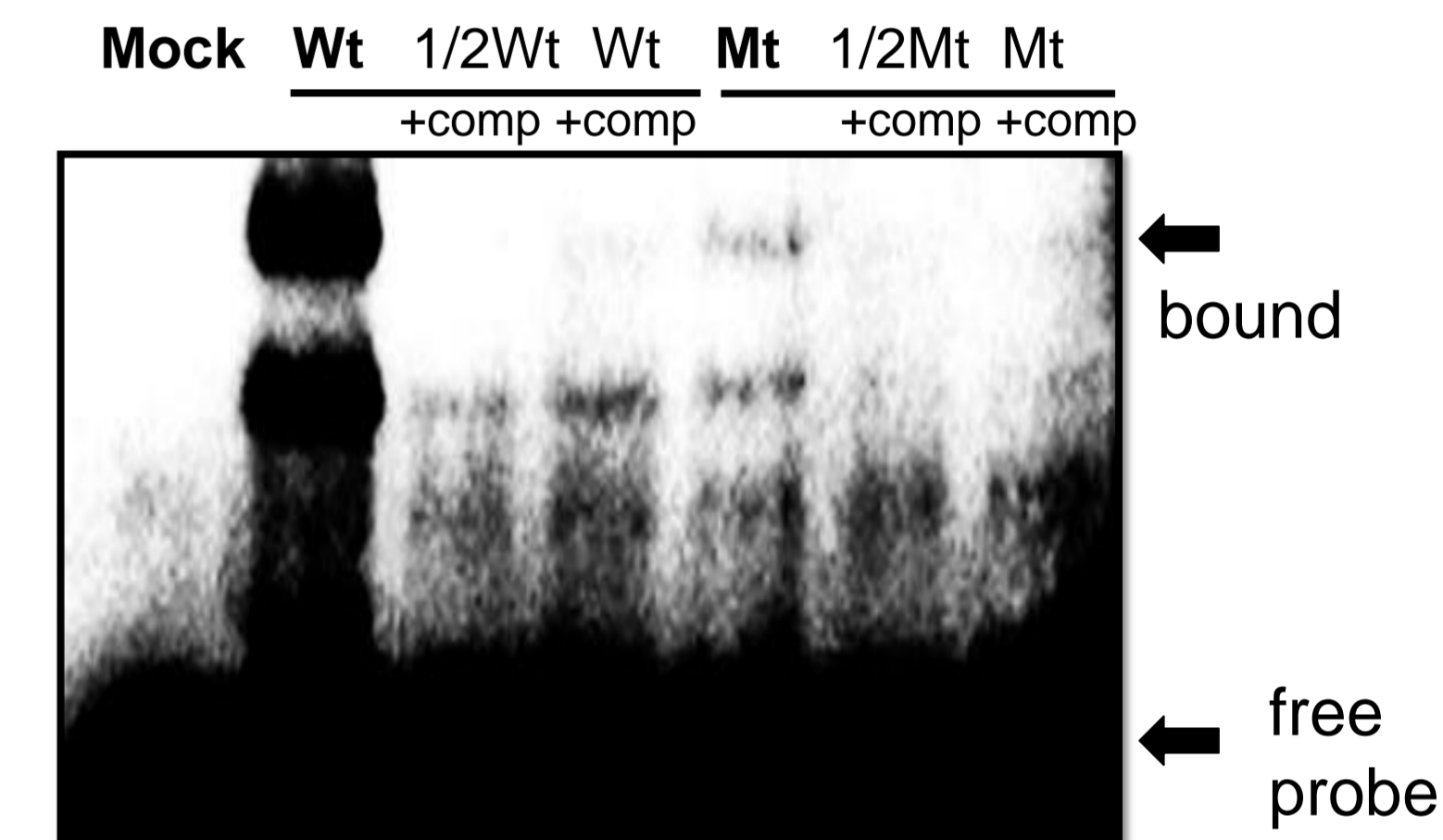
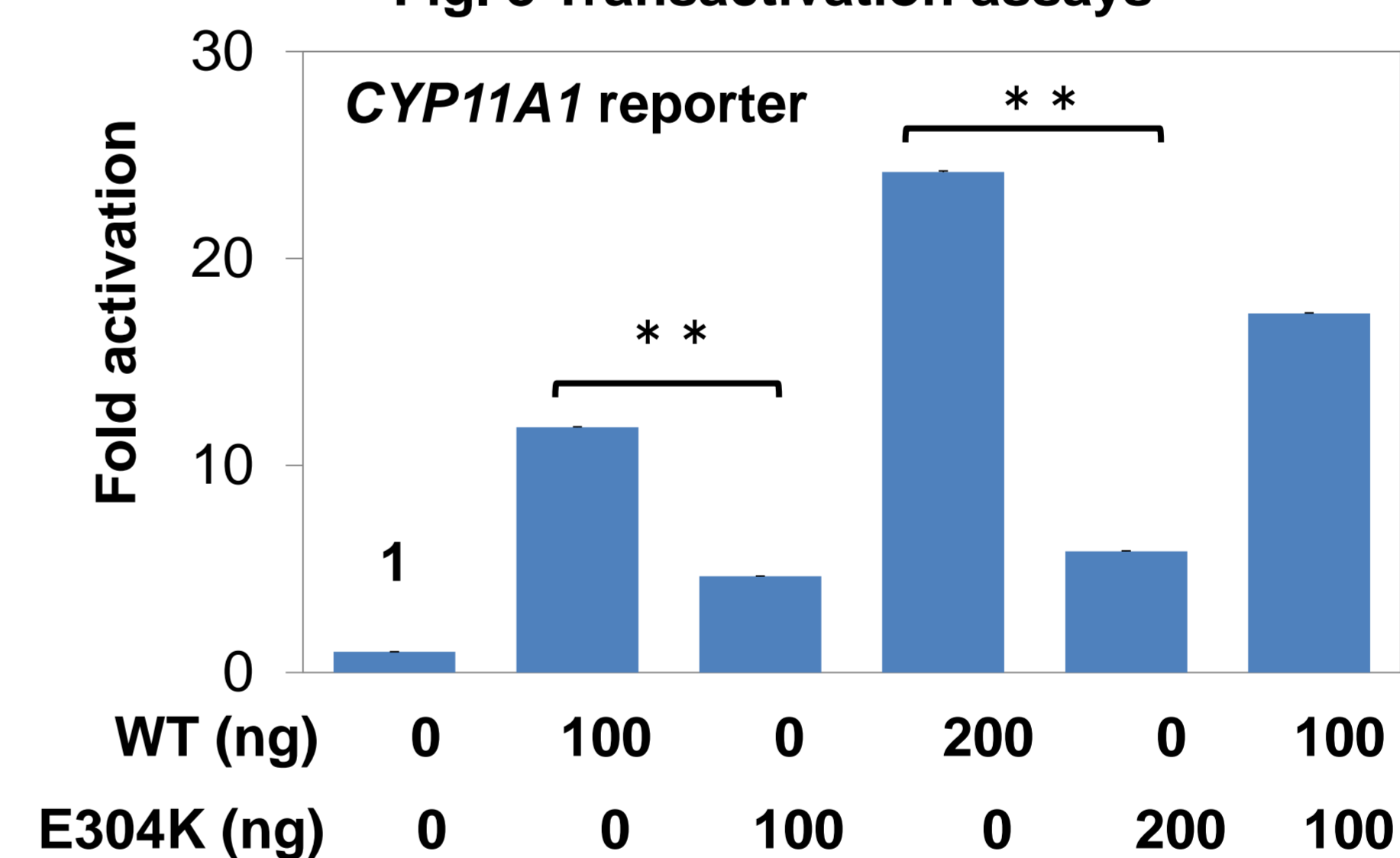


Fig. 8 Transactivation assays



• A novel heterozygous missense mutation of c.910G>A, p.E304K in *NR5A1* gene was identified in both siblings (Fig. 2). E304K was located in the ligand binding domain, which is highly conserved among *NR5A1* proteins (Fig. 3). Their asymptomatic father carried the same heterozygous mutation, which we revealed in two tissues.

• K304 *NR5A1* was predicted to bind to R427 (Fig.4). WT and E304K proteins were expressed in comparable amounts and localized exclusively to the nucleus (Fig. 5,6). The DNA-binding affinity of E304K was lower than that of WT (Fig. 7). The transcriptional activity of E304K was 35% less than that of WT. No dominant negative effect was observed (Fig. 8).

## Discussion

• We assume that the residual function of E304K may be related to the preserved fertility of the father in this family.

• The mechanism underlying the effect of LBD mutation (E304K) on DNA binding remains to be established.

• Similar to the previously reported familial case of *NR5A1* mutations with preserved fertility, the phenotype of the external genitalia differed between the father and his sons in this family.

• Little is known about the prognosis of gonadal functions of 46,XY patients with mild undervirilization due to heterozygous *NR5A1* mutations. It is important to follow up with these male siblings to confirm the fertility preservation.