

# Evidence to exclude SOX9 as a candidate gene for XY sex reversal without skeletal malformation

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## Abstract

**The skeletal malformation syndrome campomelic dysplasia (CMD1) is caused by mutations within the SOX9 gene or chromosomal rearrangement breakpoints outside SOX9. Approximately three quarters of cases of CMD1 in XY subjects show complete or partial sex reversal. As some mutations cause CMD1 alone and others cause CMD1 and sex reversal, it is conceivable that some mutations might cause sex reversal in the absence of CMD1. In this study, we have investigated this possibility by screening the entire coding region of SOX9 in 30 patients with a spectrum of XY sex reversal phenotypes. No mutations were identified, suggesting that SOX9 should not be considered a candidate gene for XY sex reversal without skeletal malformation.**

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Key words: SOX9; XY sex reversal; campomelic dysplasia.

CMD1 is a rare and usually lethal congenital skeletal malformation syndrome.<sup>1</sup> Approximately three quarters of the affected cases with a normal male karyotype show some degree of sex reversal. In such cases the gonads may show partial testis development or apparent complete failure of testis development.<sup>2</sup> SOX9, an SRY related gene, was identified close to the translocation breakpoints in several sex reversed CMD1 patients.<sup>3,4</sup> The finding of de novo SOX9 mutations in sex reversed CMD1 patients indicates that mutations in SOX9 cause both CMD1 and autosomal sex reversal and suggests that SOX9 has roles in both bone and testis development.<sup>3-5</sup> The mutations in SOX9 appear to affect only one allele. This, together with data from the translocation patients, implies that the CMD1 sex reversal phenotype is caused by haploinsufficiency of SOX9 rather than by dominant negative mutation.<sup>4,5</sup> Expression studies of SOX9 in fetal and adult tissues of man and mouse show a widespread distribution of expression. Significantly, expression is detected in the embryo at sites of chondrogenesis before the development of the skeleton and also in the developing male gonad.<sup>3,4</sup>

The predicted SOX9 protein has two potential functional domains: a DNA binding domain (the HMG box) and an activation domain (the polyproline/glutamine tract). Only one missense mutation has been identified in the HMG box region of SOX9 in an XY

subject. Interestingly, this mutation does not cause sex reversal.<sup>5</sup> If this mutation abolishes SOX9 DNA binding activity, it would suggest that the DNA binding activity of SOX9 may not be required for testis determining function. Alternatively, the mutation may not alter the protein structure significantly enough to impair the function of testis development. In all of the seven cases of CMD1 with sex reversal in which the mutation has been characterised, the C-terminal region of SOX9, including the polyproline/glutamine tract, is either lost or grossly altered. This suggests that the putative activation domain may be important for testis development. It is thus conceivable that the different domains mediate the different roles. If this hypothesis is true, some XY females without CMD1 should result from mutations in SOX9.

Mutations in SRY, the Y linked testis determining gene, cause total failure of testis development and hence result in 46,XY sex reversal.<sup>6</sup> However, approximately 80% of patients with XY gonadal dysgenesis do not have mutations in SRY and it is probable that some of these patients have mutations in other (as yet unknown) genes involved in the sex determination pathway or have mutations affecting the regulation of SRY.<sup>7</sup> We have therefore investigated whether mutation of SOX9 can cause sex reversal without affecting bone development, and to what extent SOX9 mutations might account for the majority of cases of XY gonadal dysgenesis not mutant for SRY.

DNA samples from a cohort of 30 46,XY patients with various aberrations of sexual development were screened for mutations in the SOX9 ORF by the single strand conformation polymorphism assay (SSCP). Some of these patients have other abnormalities associated with sex reversal, but none has skeletal malformation. Nine patients are classified as having gonadal dysgenesis (GD). In the absence of histological data, formal diagnoses of complete gonadal dysgenesis were not possible.<sup>8</sup> Three patients have gonadal dysgenesis with additional abnormalities: two patients have Turner stigmata and one has polycystic kidneys and visual problems as well as learning and behavioural difficulties. Ten patients have partial gonadal dysgenesis, a further three also have testicular regression, and one has Turner stigmata. Four patients do not have dysgenic gonads, but are incompletely masculinised with low testosterone levels (table 1).

Exons 1, 2, and 3 of SOX9 were amplified using primers int1 + D, F + int4, and int5 + W, respectively. Sixteen pairs of primers

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Table 1 Clinical features

Patient	Clinical phenotype	Additional anomalies
TOU186	GD	
CHM088	GD	
PF	GD	
CHM194	GD	
JA	GD	
B1	GD	
Z1	GD	
Cam185	GD	
Cl	GD	
Ou	GD	Turner stigmata
AM	GD	Polycystic kidneys, visual problems, learning and behavioural difficulties
922SSQ	GD	Mental retardation
B3	Partial GD	
B5	Partial GD	
B6	Partial GD	
Cam174	Partial GD	
Cam169	Partial GD	
Cam41	Partial GD	Dysplastic testes, fallopian tubes present
D16	Partial GD	Dysplastic testis on left, normal testis on right
B16	Partial GD	Dysplastic testis on left, streak gonad on right, Müllerian and Wolffian structures present
L16	Partial GD	Dysgenetic testes, Müllerian and Wolffian structures present
D10	Partial GD	Normal testis on right, streak ovary on left
Cam44	Partial GD	Dysplastic testis on right, normal testis on left, uterus present, fallopian tubes absent
B7	Partial GD	Turner stigmata
Z2	Partial GD	Testicular regression
F8	Partial GD	Testicular regression
U10	Incompletely masculinised	No Leydig cells
H15	Incompletely masculinised	
H6	Incompletely masculinised	
Cam301	Incompletely masculinised	

were used to reamplify short overlapping DNA fragments for SSCP analysis from these three primary PCR products. Additional genomic amplifications were required to amplify small fragments from the intron-exon junctions of exons 1 and 2 using primers 696 + int2nn and int3 + G, respectively. The primer sequences, PCR fragment sizes, PCR amplification, and SSCP conditions have been described previously.<sup>5</sup> These conditions were shown to give a high mutation detection efficacy. Apart from a cytosine to thymidine single base polymorphism at nucleotide position 507 described previously, no other SSCP shifts were detected in these patient samples.<sup>5</sup>

This would suggest that there are no discrete functional domains for the testis and bone formation properties. The lack of clear correlation between the type of mutation (or its location) with the presence or absence of sex reversal in CMD1 patients is consistent with this proposal. Furthermore, a frameshift mutation in the polyproline/glutamine region is shared between one 46,XY sex reversed CMD1 patient and a male 46,XY CMD1 patient.<sup>5</sup> These findings suggest that the presence or absence of the sex reversal syndrome associated with CMD1 is the result of variable penetrance or expressivity or both, rather than mutations affecting a specific domain of the SOX9 protein.

The two distinct functions of SOX9 are therefore probably dependent on the different cellular environments in which the gene is expressed. It is apparent from our data that SOX9 function in bone development is more

sensitive to disruption than the function in testis development. This implies a key role for SOX9 in skeletal formation, and hence mutations in SOX9 may always result in the distinct phenotype of CMD1. The finding of more mutations in non-sex reversed CMD1 patients, a more detailed spatial and temporal expression profile of SOX9 in the developing gonad, as well as investigating the effects of different types of SOX9 mutations in transgenic mice will yield more clues to the role of SOX9 in testis formation.

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