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MAMLD1 mutations seem not sufficient to explain a 46,XY DSD phenotype. What else?

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Background

The *MAMLD1* gene (Xp28) is thought to cause disorder of sex development (DSD) in 46,XY patients, mostly presenting with hypospadias, and, recently, also gonadal dysgenesis. However, there is some controversy about the role of *MAMLD1* in sex development.

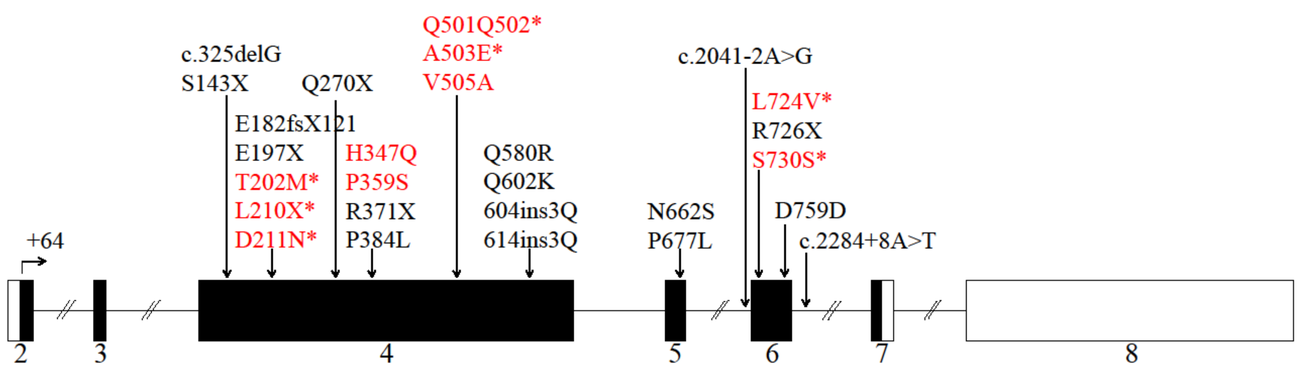
Aims and Objectives

We searched for *MAMLD1* sequence variations in 108 46,XY DSD individuals presenting with a wide spectrum of DSD phenotypes. Identified variations were functionally tested *in vitro*, and findings were compared with reported cases and the literature of MAMLD1 focusing on sex development.

Methods and Results I

Nine *MAMLD1* mutations (7 novel) in 9 46,XY DSD patients were detected by Sanger sequencing.

All the mutations and polymorphisms detected in the *MAMLD1* gene



In red: present study; *: novel

Characteristics of the 9 patients with *MAMLD1* mutations and polymorphisms

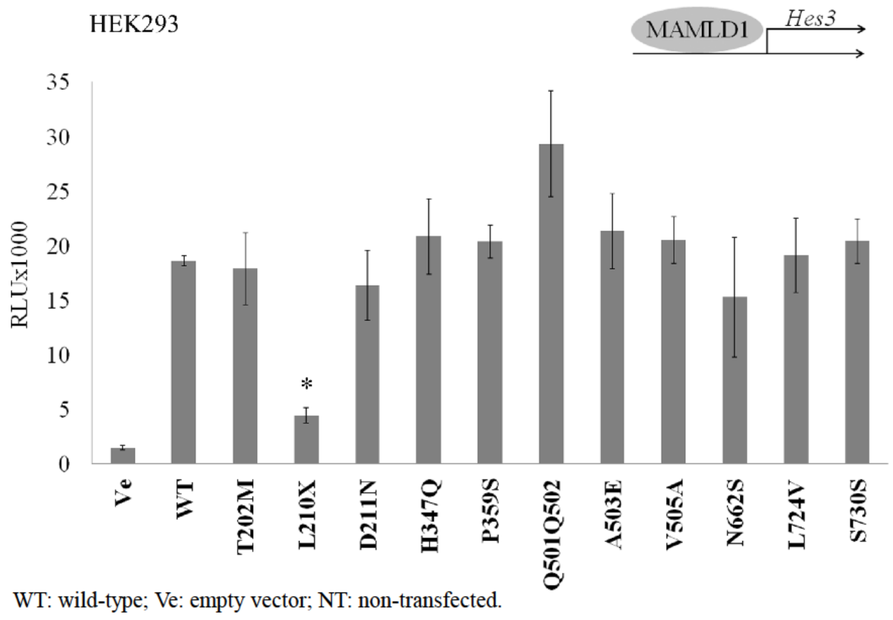
Pat.	Karyotype Assigned sex	MAMLD1 gene mutation	Genital anatomy. *Testes histology (age)*	Gonadal function (age)	Adrenal function (age)	Remarks
1	46,XY Male	T202M c.605C>T	Penoscrotal hypospadias. Small penis. Unilateral cryptorchidia.	Normal T (minipuberty). Normal gonadotropins. No hCG test.	ND	SGA. Short stature. Low implantation thumbs.
2	46,XY Female	L210X c.626delT D211N c.631G>A	Penoscrotal hypospadias. Small penis. Testes 0.5 ml. *Normal for age (15 d).*	Normal T (baseline) and hCG test (10 days).	Normal baseline (15 d).	Abnormal GGN repeat in AR. Mother non-carrier; norfloxacine treatment during pregnancy.
3	46,XY Female	H347Q c.1041C>A rs62641609	Female genitalia. Gonads in labia.	Normal hCG test (2 y).	Normal baseline (2 y).	
4	46,XY Male	H347Q c.1041C>A rs62641609	Penoscrotal hypospadias. Testes 2 ml.	Normal hCG test. Normal AMH (2.5 y).	Normal baseline (2.5 y).	
5	46,XY Male	Q501Q502 c.1503_1504dupCAGCAG	Hypospadias. Short penis. Delayed puberty. Testes 8 ml.	Baseline T and gonadotropins normal (70 y). Fathered a boy.	Normal baseline (70 y).	
6	46,XY Male	A503E c.1508C>A	Penoscrotal hypospadias. Small penis. Testes 2 ml.	Normal baseline T (3 m). Normal hCG test (9 m).	Normal baseline (3 d).	
7	46,XY Female	V505A c.1514T>C rs61740566	Penoscrotal hypospadias. Small penis. Unilateral cryptorchidia. *Normal for age (2 y).*	Normal hCG test.	Normal Synacthen test.	
8	46,XY Male	L724V c.2170C>G	Penoscrotal hypospadias. Small penis. Testes 1 ml.	Normal prepubertal baseline T (15 m). Normal AMH.	Normal baseline (15 m).	Esophageal atresia. Right aortic arch.
9	46,XY Female	S730S c.2190G>A	Penoscrotal hypospadias. Small penis. *Normal for age (nests of Normal Leydig cells; normal fertility index (1 y).*	Normal baseline (12 m). No hCG test.		Müllerian ducts.

ND: not done. d: day(s), m: month(s), y: year(s)

Methods and Results II

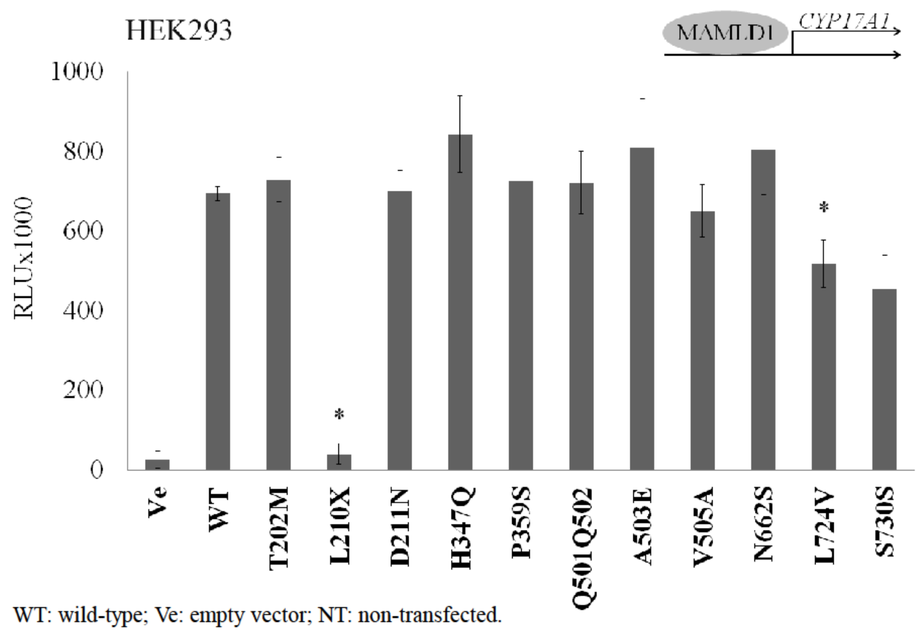
Functional studies were completed in non-steroidogenic HEK293, adrenal NCI-H295R and Leydig MA-10 cells.

MAMLD1 transcriptional activity tested on *Hes3* promoter by luc assays



WT: wild-type; Ve: empty vector; NT: non-transfected.

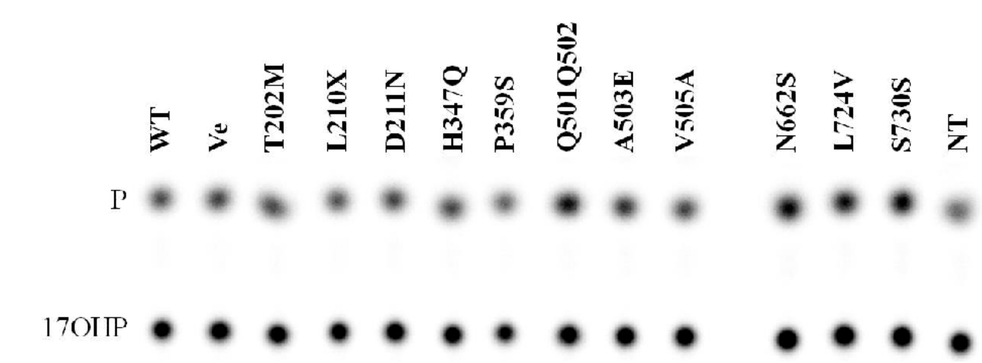
MAMLD1 transcriptional activity tested on *CYP17A1* promoter by luc assays



WT: wild-type; Ve: empty vector; NT: non-transfected.

Most MAMLD1 variants acted similarly to the WT.
Only the L210X mutation showed loss of function in all tests, while variants L724V and S730S showed a decrease in *CYP17A1* promoter activation.

Effect of MAMLD1 on CYP17A1 activity in NCI-H295R cells

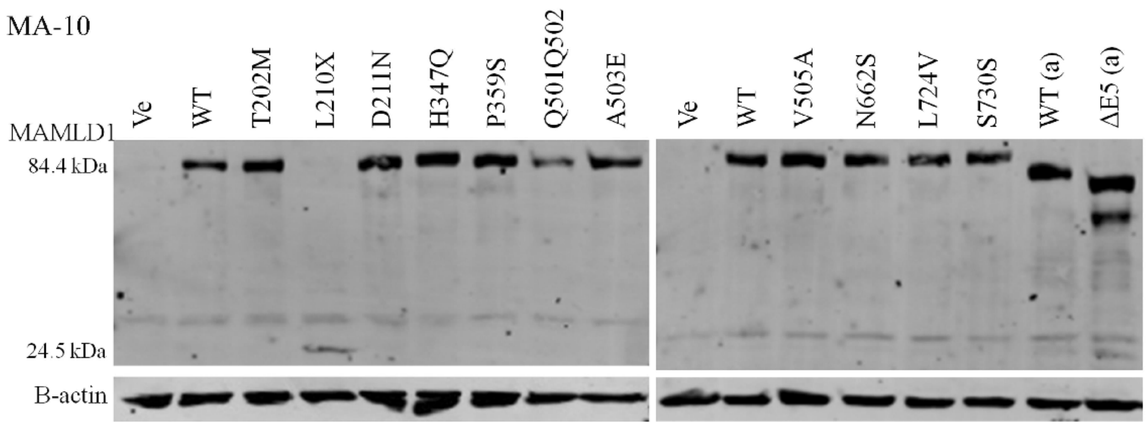


P: progesterone; 17OHP: 17-hydroxyprogesterone; WT: wild-type; Ve: empty vector; NT: non-transfected.

Effect of MAMLD1 on androgen production assessed by testing the CYP17A1 activity in the 3 cell lines. No effect of either WT or any MAMLD1 variant on CYP17A1 enzyme activity was found.

Methods and Results III

WT and mutant MAMLD1 expression was assessed in mouse Leydig MA-10 cells.



No difference for MAMLD1 protein expression was found, except for a shorter L210X.

Conclusion

Our data support the notion that *MAMLD1* sequence variations may not suffice to explain the DSD phenotype in carriers.

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