Long-term clinical course in three patients with _MAMLD1_ mutations

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Abstract. Although _MAMLD1_ on chromosome Xq28 is known as a causative gene for 46,XY disorders of sex development, clinical information is virtually limited in patients of infancy to early childhood. Here, we report long-term genital and hormonal findings in three previously described Japanese patients with _MAMLD1_ mutations, i.e., patients 1 and 2 with p.E197X and patient 3 with p.R726X. As previously reported, patients 1–3 exhibited penoscrotal hypospadias with chordee, microphallus, bifid/hypoplastic scrotum, and/or bilateral cryptorchidism/retractile testes, in the presence of sufficiently high serum basal or hCG-stimulated testosterone values in the mini-pubertal period to early childhood. Subsequently, patient 1 had low serum hCG-stimulated testosterone value (126 ng/dL) at 13 11/12 years of age, and manifested microphallus (4.5 cm), relatively small testes (left 8 mL and right 10 mL), Tanner stage 3 genitalia and pubic hair development at 18 3/12 years of age. Similarly, patients 2 and 3 showed mild hypergonadotropic hypogonadism at 7 0/12 and 9 9/12 years of age, respectively, with serum GnRH-stimulated LH values of 5.5 and 7.2 mIU/mL and FSH values of 10.3 and 19.8 mIU/mL and hCG-stimulated testosterone values of 70 and 80 ng/dL, respectively. Testis ultrasound studies delineated microlithiasis in patients 1 and 3. These results imply for the first time deterioration of testicular function with age in patients with pathologic _MAMLD1_ mutations.

Keywords: _MAMLD1_, 46,XY DSD, Clinical course, Testicular function, Deterioration

_MAMLD1_ (mastermind like domain containing 1) on chromosome Xq28 is a causative gene for 46,XY disorders of sex development (DSD) [1]. This is based on the identification of several non-functional hemizygous nonsense, frameshift, and splice site _MAMLD1_ mutations (e.g., p.S143X, p.E197X, p.L210X, p.Q270X, p.R726X, c.544delG, and g.IVS4–2A>G) in 46,XY DSD patients with relatively severe hypospadias as the salient feature [1–5]. Furthermore, although such patients with pathologic mutations reported to date invariably have apparently normal testosterone (T) and/or LH/FSH values in infancy to early childhood (< 3 years of age) [1–5], recent studies argue for _MAMLD1_/Mamld1 being involved in the molecular network for T production: (i) _MAMLD1_/Mamld1 expression is up-regulated by _NR5A1_ (alias, _SF-1_ and _AD4BP_) [6] that functions as the master regulator for multiple sex development and steroidogenic genes [7]; (ii) the wildtype mouse _Mamld1_ is clearly co-expressed with _Nr5a1_ in Leydig and Sertoli cells around the critical period for fetal sex development [1], and its intra-testicular expression level is gradually increased during the fetal life in parallel with the intra-testicular testosterone level [8]; (iii) transient _Mamld1_ knockdown using small interfering RNAs significantly reduces _Cyp17a1_ expression and T production in cultured mouse Leydig tumor cells [9]; and (iv) _Mamld1_ knockout male mice, though they are free from genital and reproductive abnormalities, show significantly lower expression levels of multiple fetal Leydig cell-specific genes includ-
ing Star, Cyp11a1, Cyp17a1, Hsd3b1, and Insl3 [8]. Thus, it has been inferred that \textit{MAMLD1} mutations result in 46,XY DSD probably because of transiently compromised T production around the critical period for fetal sex development [1].

To our knowledge, however, there is no report on clinical findings in patients with \textit{MAMLD1} mutations after early childhood. Here, we report long-term genital and hormonal findings in three patients with \textit{MAMLD1} mutations.

\section*{Case Reports}

We studied three previously reported Japanese patients with \textit{MAMLD1} mutations [1]. This study has been approved by the Institutional Review Board Committees of Hamamatsu University School of Medicine and National Center for Child Health and Development, and molecular studies were performed after obtaining written informed consent from the parents of the three patients. Patients 1 and 2 were maternally related half-brothers with a maternally inherited p.E197X (previously described as p.E124X), and patient 3 was a sporadic case with p.R726X (previously described as p.R653X) of maternal origin. Transactivation function for the \textit{Hes3}-promoter was abolished in the p.E197X protein but was normal in the p.R726X protein [6]. However, the p.R726X mutation was shown to undergo nonsense mediated mRNA decay, as was the p.E197X mutation [1, 6]. Thus, both mutations were assessed as amorphic mutations.

Genital findings in early infancy of patients 1–3, and endocrine findings up to 2 5/12 years in patients 1 and 2 and 6 3/12 years of age in patient 3, have been described previously [1]. In brief, patients 1–3 exhibited penoscrotal hypospadias with chordee, microphallus, and bifid scrotum, and patients 1 and 3 also had bilateral cryptorchidism and retractile testes, respectively. Thus, they received urethroplasty and/or orchiopexy. Basal and/or GnRH- or hCG-stimulated LH, FSH, T, and dihydrotestosterone values were apparently normal. In particular, hCG-stimulated serum T was 250 ng/dL in patient 1 at 2 6/12 years of age (normal range [NR] > 200 ng/dL) [10], and basal T was 260 ng/dL in patient 2 at one month of age (NR, 59 – 408 ng/dL) [11] and 270 ng/dL in patient 3 at three months of age (NR, 3 – 349 ng/dL) [11].

However, patients 1–3 manifested primary hypogonadism in later ages (Table 1). Patient 1 exhibited incomplete secondary sexual development with microphallus and relatively small testes in the pubertal period. Patients 2 and 3, though they were still in their prepubertal period, showed borderline to definite microphallus. The GnRH-stimulated LH and/or FSH values were mildly elevated in patients 2 and 3, and the hCG-stimulated T values were obviously low in patients 1–3. Furthermore, testis ultrasound studies delineated microlithiasis in patients 1 and 3, but not in patient 2 (Fig. 1).

\begin{table}[h]
\centering
\caption{Genital and endocrine findings in three male patients with \textit{MAMLD1} nonsense mutations}
\begin{tabular}{|c|c|c|}
\hline
\textbf{\textit{MAMLD1} mutation} & \textbf{Patient 1} & \textbf{Patient 2} & \textbf{Patient 3} \\
\hline
\textbf{<Genital findings>} & & & \\
\hline
\textbf{Age at exam. (y:m)} & 13:11 & 7:00 & 6:03 \\
\hline
\textbf{Tanner stage} & Genitalia 1, Pubic hair 1 & Genitalia 1, Pubic hair 1 & Genitalia 1, Pubic hair 1 \\
\hline
\textbf{Penile length (cm)} & Not examined & 3.5 (3.4 – 5.8) & 3.2 (3.4 – 5.7) \\
\hline
\textbf{Testis size (mL)} & 3 (bilateral) (8 – 20) & 2 (bilateral) (1 – 2) & 1.5 (bilateral) (1 – 2) \\
\hline
\textbf{Age at exam. (y:m)} & 18:03 & ... & 9:09 \\
\hline
\textbf{Tanner stage} & Genitalia 3, Pubic hair 3 & ... & Genitalia 1, Pubic hair 1 \\
\hline
\textbf{Penile length (cm)} & 4.5 (no reference data) & ... & 3.2 (3.4 – 5.8 at 7 yr) \\
\hline
\textbf{Testis size (mL)} & 8 (left), 10 (right) (13 – 20 at 16 yr) & ... & 1.5 (bilateral) (1 – 4.5) \\
\hline
\textbf{<Serum hormone values>}
\hline
\textbf{Age at exam. (y:m)} & 13:11 & 7:00 & 9:09 \\
\hline
\textbf{LH (mIU/mL)} & Not examined & 0.2 (0.2 – 1.9) → 5.5 (1.1 – 6.0) \textsuperscript{a} & 0.5 (0.2 – 1.9) → 7.2 (1.1 – 6.0) \textsuperscript{a} \\
\hline
\textbf{FSH (mIU/mL)} & Not examined & 1.3 (<0.3 – 2.4) → 10.3 (1.9 – 7.6) \textsuperscript{a} & 5.0 (<0.3 – 2.4) → 19.8 (1.9 – 7.6) \textsuperscript{a} \\
\hline
\textbf{T (ng/dL)} & 12 (10 – 96) → 126 (> 200) \textsuperscript{b} & 12 (3 – 13) → 70 (> 200) \textsuperscript{b} & 28 (3 – 13) → 80 (> 200) \textsuperscript{b} \\
\hline
\end{tabular}

\textsuperscript{a} Basal and peak values during a GnRH test (100 µg/m\textsuperscript{2} [max. 100 µg] bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 min). \textsuperscript{b} Basal and stimulated values in an hCG test (3,000 IU/m\textsuperscript{2}/dose [max. 5,000 IU] i.m. for three consecutive days; blood sampling on days 1 and 4). The values in parentheses represent the age-matched normal range [10–13].
\end{table}
Discussion

This study showed for the first time deterioration of testicular function with age in patients with pathologic MAML1 mutations. Indeed, small testes during the pubertal period in patient 1 would imply spermatogenic impairment [14], and poor T responses to hCG stimulation and/or mild hypergonadotropism in patients 1–3 would argue for adult Leydig cell dysfunction [14, 15]. In addition, testicular microlithiasis in patients 1 and 3 may also imply the presence of non-specific testicular dysfunction, because it is often found in subjects with testicular tumors and spermatogenic failure as well as in patients with hypogonadism-associated disorders such as Down syndrome and Klinefelter syndrome [16–19]. Thus, the present data, in conjunction with the previous findings [1], suggest that MAML1 deficiency causes 46,XY DSD during the fetal life and, while it permits apparently normal T production in infancy to early childhood, results in deterioration of testicular function with compromised T production from mid-childhood.

Several findings are worth pointing out with respect to the biological function of MAML1/Mamld1. They include: (i) clear mouse Mamld1 expression in Sertoli and Leydig cells during the critical period for fetal sex development and weak Mamld1 expression in postnatal testes [1]; (ii) significantly reduced expression levels of Leydig cell-specific, but not Sertoli-cell specific, genes in the late fetal life of Mamld1 knockout mice [8]; (iii) compromised T production (~50%) by Mamld1 knockdown [9]; and (iv) positive human MAML1 expression in fetal and adult testes [2].

These findings would postulate several possibilities. First, MAML1 deficiency may compromise fetal Leydig cell function around the critical period for sex development, leading to 46,XY DSD with hypospadias because of reduced but not abolished T production, as has been proposed previously [1]. In this regard, recent mouse studies have indicated that prenatal T biosynthesis requires both fetal Leydig cells that produce Δ4-androstenedione and Sertoli cells that express Hsd17b3 for the conversion of Δ4-androstenedione into T [20], although such Sertoli cell-specific HSD17B3 expression has not been demonstrated in human fetuses. Thus, in contrast to Mamld1 knockout mice [8], if Sertoli cell function is also compromised in affected patients, this would also contribute to defective T production during fetal life of affected patients. Second, long-term MAML1 deficiency may gradually affect adult Leydig cell function, resulting in compromised T production with age. This notion
assumes that MAMLD1 plays a critical role in the functional maintenance rather than the development of adult Leydig cells [15], and would explain why T production can be preserved in the mini-pubertal period and in the early childhood. Spermatogenesis requires sufficient T production and normal Sertoli cell function [14]. If Sertoli cell function is also compromised with age in patients with MAMLD1 mutations, this factor, together with reduced T production, would contribute to defective spermatogenesis.

Two points should be made with regard to the present study. First, the apparently normal T values in infancy to early childhood (< 3 years of age) may not necessarily argue against the presence of hypogonadism. Indeed, there are no objective clinical indicators for hypogonadism in such a period, and serum T values may overlap between control boys and patients with incomplete/mild testicular dysfunction. In support of this notion, apparently normal serum T values in infancy to early childhood and declined serum T values in later ages have occasionally been reported in patients with incomplete/mild hypogonadism-associated disorders such as Prader-Willi syndrome, Down syndrome, and Klinefelter syndrome [21–23]. Second, while Mamld1 knockout male mice have been produced [8], they would not serve as good models to examine the age-dependent deterioration of testicular function. Although Mamld1 knockout male mice have reduced expression levels of Leydig cell-specific genes in the late fetal life, they have morphologically normal internal and external genitalia in the late fetal life, normal intra-testicular T values in the late fetal life and at 8 weeks of age, and normal reproductive capacity in adulthood [8 and our unpublished data]. Thus, further longitudinal clinical studies are required to determine whether the testicular function deteriorates with age in patients with MAMLD1 mutations.

Despite such caveats, the present study provides useful information for the testicular function in MAMLD1 mutation positive patients. Although the number of patients observed for a long time is quite limited, this study suggests age-dependent deterioration of testicular function in patients with MAMLD1 mutations.

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