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Clinical Research Article

Familial Male-limited Precocious Puberty (FMPP) and Testicular Germ Cell Tumors

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Abstract

Objective: The purpose of this study is to report development of a malignant testicular germ cell tumor (GCT) in 2 young adult males with familial male-limited precocious puberty (FMPP) because of *LHCGR* pathogenic variants in 2 families. Secondarily, to study the possible relation between FMPP and testicular tumors and to investigate whether FMPP might predispose to development of malignant testicular tumors in adulthood a literature review is conducted.

Methods: Data on 6 cases in 2 families are obtained from the available medical records. In addition, a database search is performed in Cochrane, PubMed, and Embase for studies that report on a possible link between FMPP and testicular tumors.

Results: The characteristics of 6 males with FMPP based on activating LH receptor (*LHCGR)* germline pathogenic variants are described, as are details of the testicular GCTs. Furthermore, a literature review identified 4 more patients with signs of FMPP and a (precursor of) testicular GCT in adolescence or adulthood (age 15-35 years). Additionally, 12 patients with signs of precocious puberty and, simultaneously, occurrence of a Leydig cell adenoma or Leydig cell hyperplasia are reported.

Conclusion: There is a strong suggestion that FMPP might increase the risk of development of testicular GCTs in early adulthood compared with the risk in the general population. Therefore, prolonged patient monitoring from mid-pubertal age onward including instruction for self-examination and periodic testicular ultrasound investigation in patients with a germline *LHCGR* pathogenic variant might contribute to early detection and thus early treatment of testicular GCT.

Key Words: testotoxicosis, familial male limited precocious puberty, activating luteinizing hormone receptor, testicular tumors, germ cell tumors, peripheral precocious puberty

Abbreviations: FMPP, familial male-limited precocious puberty; GCT, germ cell tumor; *LHCGR*, LH/choriogonadotropin receptor; LCT, Leydig cell tumor; MAS, McCune Albright syndrome; SRY, Sex-determining Region Y.

Familial male-limited precocious puberty (FMPP), also referred to as testotoxicosis, is characterized by LH-independent precocious puberty (1). This disease is caused by an autosomal dominant inherited activating germline pathogenic variant of the gene coding for the LH/choriogonadotropin receptor (*LHCGR*), which belongs to the G protein-coupled receptor 1 family. In males, activating pathogenic variants of the *LHCGR* gene cause excessive production of testosterone, which triggers early peripheral (precocious) puberty, whereas the overactive receptor does not cause clinically relevant symptoms in girls (2, 3). Activating pathogenic variants of the *LHCGR* gene have been identified and described since 1993 (1, 4). Pathogenic variants in the sixth transmembrane segment and the third intracellular loop of the G protein-coupled receptor are described as the hot spot of amino acid alterations; however, changes in other transmembrane segments also have been reported (5). Kremer et al have reported a total of 12 *LHCGR* gene pathogenic variants in 68 independent patients and families, and their findings suggested that only pathogenic variants of specific amino acids are responsible for constitutive activation, which can also be transmitted to consecutive generations (2).

Normally, at puberty, the *LHCGR* mediates action of LH on the testosterone synthesis by Leydig cells. As a result of the activated *LHCGR* status in FMPP, Leydig cell hyperplasia occurs at a young age (6). Boys, typically between 2 and 6 years of age, present with precocious pubertal development, growth of the penis, and enlargement of the scrotum and

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testes, as well as linear growth acceleration, progressive bone age advancement, and growth of pubic and facial hair (2, 7). Consequently, this eventually may lead to compromised adult height as well as psychological effects associated with precocious puberty. Final height modestly below sex-adjusted mid-parental height and within the range for adult males in the general population has been reported (8). Current treatment consists of a combination therapy of a potent nonsteroidal antiandrogen agent (bicalutamide) and a third-generation aromatase inhibitor (7, 9). This combination treatment appears to be a safe and effective approach, with only minor adverse events having been described. However, data on long-term outcome of final height and fertility remain scarce; therefore, close follow-up of these patients remains important (7).

In this study, we report 2 families with FMPP. All affected males have a pathogenic variant in the *LHCGR* gene; c.1624A > C p.(Ile542Leu). This specific variant has previously been described to be pathogenic in 3 familial cases and 1 sporadic case (10). Because 2 of 3 fathers developed a malignant testicular germ cell tumor (GCT) in young adulthood, we wondered whether this might be related to the FMPP and if follow-up in FMPP patients after puberty is necessary for early detection of these tumors. To investigate whether FMPP might predispose to development of malignant testicular GCTs in adulthood, we conducted a literature search.

Methods

Case Series

In this study, 3 father-and-son pairs in 2 families with FMPP are described, of whom 2 fathers were diagnosed with a malignant testicular GCT in young adulthood. In both families, the c.1624A > C p.(Ile542Leu) pathogenic variant in the *LHCGR* gene was found. Data on all 6 cases were obtained from the available medical records. Pathology reports and specimens of both tumors were revised by an expert pathologist (R.R.K.). The study was approved by the institutional review board of the University Medical Center Utrecht and informed consent was obtained from all affected individuals.

Genetic Analysis

Genomic DNA was extracted from EDTA blood of each patient by standard methods. The coding region and exon–intron boundaries of the *LHCGR* gene (GenBank: NM_000233) were amplified from genomic DNA. Sanger sequencing was performed using the BigDye Terminator Sequencing Kit (Thermo Scientific) and ABI 3730 XL (Applied Biosystems). Coding sequences and flanking intron sequences were analyzed with SequencePilot (SeqPatient, JSI Medical Systems GmbH, Ettenheim, Germany). PCR and sequencing primers are available on request.

Literature Review

Review of the literature was based on a search for all available published articles in the databases Cochrane, PubMed, and Embase. The databases were searched until November 2020 for published studies that report or describe a possible link between FMPP and testicular tumors. The checklist of the PRISMA Statement was used to systematically search and include literature. However, because of the scarcity of literature describing both conditions, not all requirements of the checklist could be met (11). The search terms and their synonyms are listed in Supplementary Table 1 (12). After exclusion of duplicates, the remaining studies were screened for title and abstract by 2 authors (C.K. and A.M.) independently. The full texts of the articles that, after consensus, were labeled as potentially eligible were then assessed and included in the review. Subsequently, the information/data described in the included articles were manually searched according to the Guidelines for Snowballing in Systematic Literature (13).

To determine the level of evidence of the included articles, we used the Levels of Evidence (2011) of the Oxford Centre for Evidence Based Medicine (14).

Table 1. Clinical characteristics of the 2 families with FMPP

		FMPP			Testicular GCT		
		Age, y[a]	Treatment	DNA	Age, y[b]	Side	Histopathology
Family A[c]	Father (**A:** IV-10)	5	Ketoconazole	c.1624A > C (p.Ile542Leu)[d]	27	Left	Nonseminoma
	Patient (**A:** V-3)	5	Letrozole + bicalutamide	c.1624A > C (p.Ile542Leu)[d]	—	—	—
	Father (**A:** IV-3)	6	Ketoconazole; spironolactone + testolactone; cyproterone	c.1624A > C (p.Ile542Leu)[d]	—	—	—
	Patient (**A:** V-1)	4	Letrozole + bicalutamide	c.1624A > C (p.Ile542Leu)[c]	—	—	—
Family B[e]	Father (**B:** I-1)	2	Cyproterone acetate	c.1624A > C (p.Ile542Leu)[c]	25 years	Right	Nonseminoma
	Patient (**B:** II-1)	4	Letrozole + bicalutamide	c.1624A > C (p.Ile542Leu)[c]	—	—	—

Abbreviations: FMPP, familial male-limited precocious puberty; GCT, germ cell tumor.
[a]Age of onset FMPP in years.
[b]Age at diagnosis of testicular tumor.
[c]Family as depicted in Fig. 1.
[d]Substitution of isoleucine for leucine at position 542.
[e]Family as depicted in Fig. 3.

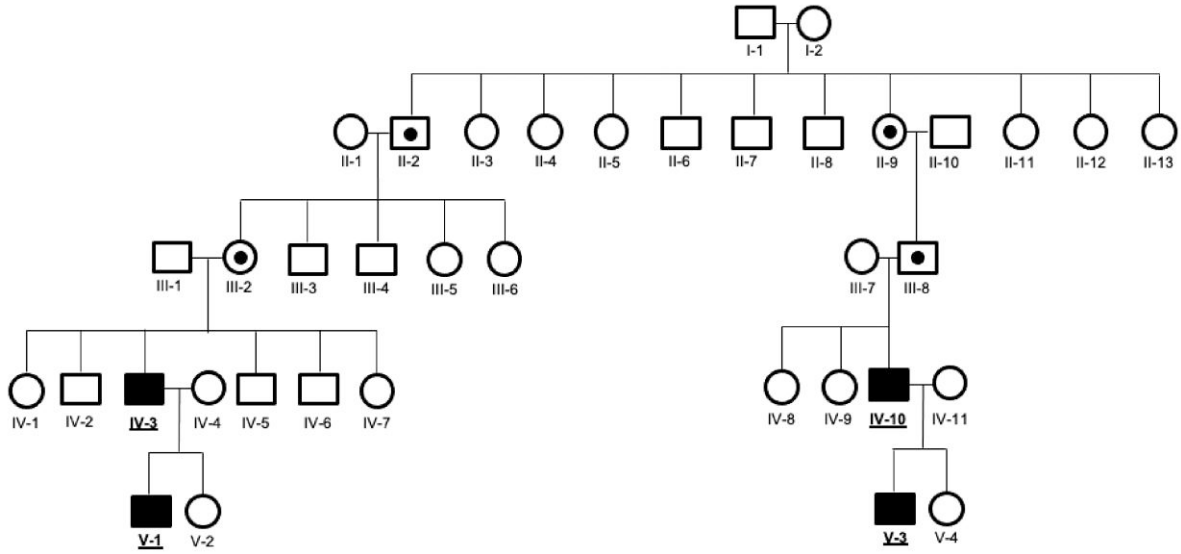

Figure 1. Family tree of family A. Precocious male puberty and a germline mutation in the LH/choriogonadotropin receptor (*LHCGR*) gene indicated in black; • = obligate carrier. Data on mutation analysis of the (*LHCGR*) gene was only available for the individuals who are underlined and depicted in bold numbers.

Results

Case Series

The clinical characteristics of the 3 boys (V-1 and V-3 of family A; II-1 of family B) and their fathers (IV-3 and IV-10 of family A; I-1 of family B) are summarized in Table 1.

Family A

At the age of 27 years, individual IV-10 was diagnosed with a malignant testicular GCT on the left side. He had presented at the age of 5 years with signs of precocious puberty and a positive family history for FMPP, as depicted in Fig. 1. Until the age of 12 years, he was treated with ketoconazole and progression of puberty was monitored thereafter. Radical orchidectomy followed presentation with a testicular mass. Histopathology revealed an embryonal carcinoma (being a type of nonseminoma) limited to the testis (alpha fetoprotein and beta human chorionic gonadotropin negative), and germ cell neoplasia in situ. A few months after the initial orchidectomy, a recurrence of the nonseminoma with tumor-positive para-aortic lymph nodes was diagnosed. After chemotherapy with bleomycin, etoposide, and platinum, individual IV-10 remained in complete remission. There was no family history of testicular tumors.

As far as other family members in this large family tree are concerned, we do not have information on possible precocious puberty or genetic analysis.

The son of IV-10, V-3, presented at the age of 4 years with suspected FMPP based on growth of facial and pubic hair, aggressive behavior, and an increased growth rate. At physical examination, testicular volume was 8 mL on both sides, which in the Dutch population usually matches pubertal development in boys aged 12 to 15 years. Serum testosterone was markedly elevated (16.1 nmol/L, normal value at age 4 years <0.5 nmol/L); with low LH and FSH (both <0.1 IU/L).

In the same family A, but not closely related (Fig. 1), IV-3 also presented at the age of 6 years with signs of precocious puberty. He was clinically diagnosed with FMPP and received treatment with multiple (combinations of) therapies up to the normal age of pubertal onset. During follow-up thereafter and confirmed by recent testicular ultrasound, no testicular abnormalities have developed.

V-1, the son of IV-3, also presented with pubic hair development and linear growth acceleration at the age of 4 years. During physical examination, his testes had an estimated volume of 6 to 8 mL. Elevated serum testosterone (7.7 nmol/L) and low LH and FSH (both <1.0 IU/L) were found. No scrotal ultrasound was performed at presentation. The increased testicular volume can be related to the effect of the LH receptor activation; this patient underwent an LHRH test at first presentation that did not show central precocious puberty.

DNA analysis from peripheral blood of both V-3 and V-1, performed after initial presentation with precocious puberty, confirmed a heterozygous pathogenic variant at c.1624A > C p.(Ile542Leu) in the *LHCGR* gene in both cases, in line with the clinical diagnosis of FMPP.

Family B

At the age of 25 years, patient I-1 presented with a suspicious scrotal mass in his right testicle; ultrasound revealed a hypoechoic solid mass and radical orchidectomy was performed. The family history for testicular tumors was negative. Histological evaluation reported an embryonal carcinoma (nonseminoma) with angio-invasion (alpha fetoprotein and beta human chorionic gonadotropin negative) (Fig. 2). The testicular parenchyma did not show Leydig cell hyperplasia. He was treated with adjuvant chemotherapy because lymph node involvement was found, and he has remained in complete remission since. The medical history of this man had also started with signs of precocious puberty at the age of only 2 years, similar to the males (fathers) of family A. Years of extensive diagnostic investigation followed, and he was treated with cyproterone acetate up to puberty. When genetic testing became available in the 1990s, DNA analysis confirmed FMPP based on a *LHCGR* pathogenic variant.

II-1, the son of I-1, presented at age 3 years with testicular enlargement, growth of pubic hair, acne, and accelerated

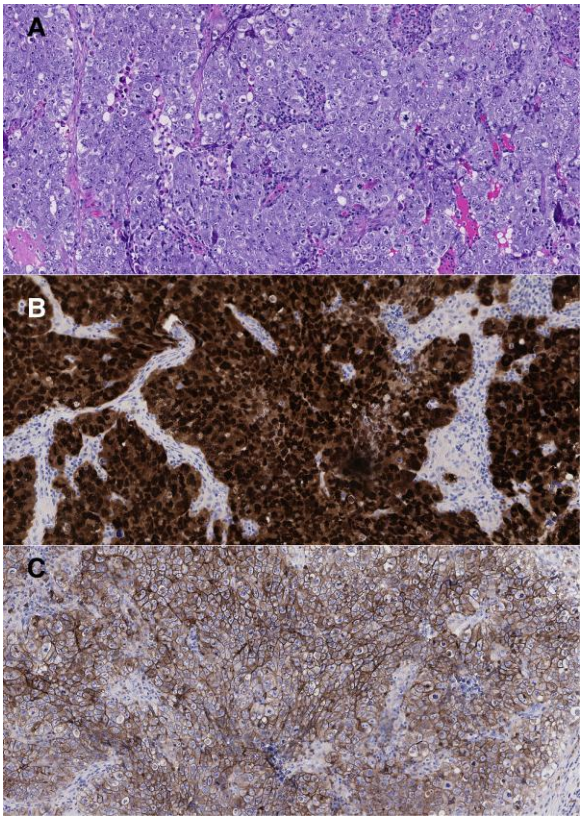

Figure 2. (A) Hematoxylin and eosin staining of embryonal carcinoma of patient I-1, showing typical morphology with sheets of undifferentiated cells with atypical nuclei and prominent nucleoli. (B) Immunohistochemistry with OCT3/4 showing strong nuclear staining of all tumor cells. (C) Immunohistochemistry with CD30 showing characteristic membranous staining of tumor cells.

growth. His testes had an estimated volume of 6 mL (normally <4 mL at this age). Serum testosterone was highly elevated (15 nmol/L, normal <0.5 nmol/L), and gonadotrophins were low (LH 0.87 IU/L; FSH <0.50 IU/L). Scrotal ultrasound showed no evidence of a testicular tumor. Suspicion of FMPP was confirmed by the presence of the same pathogenic variant in the *LHCGR* gene as was found in family A. The family tree of family B is presented in Fig. 3.

Pregnancies of V-3, V-1, and II-1 were all achieved without the help of assisted reproductive technologies. The option of genetic counseling either before or during pregnancy or after birth as well as the option of genetic testing in male offspring had not been brought to attention to the affected fathers.

Genetic Result

DNA-sequencing analysis of the *LHCGR* gene showed a heterozygous missense variant c.1624A > C p.(Ile542Leu) in all members with FMPP in family A (IV-3, V-1, IV-10, V-3). The same variant in the *LHCGR* gene was also detected in males with FMPP in family B (I-1 and II-1). The missense variant p.Ile542Leu is located in the fifth transmembrane helix and has been reported previously to be an activating pathogenic variant in 3 familial cases and 1 sporadic FMPP case (10). This variant is not present in the Genome Aggregation Database (gnomAD v2.1.1, https://gnomad.broadinstitute.org) (15). This variant can be considered pathogenic

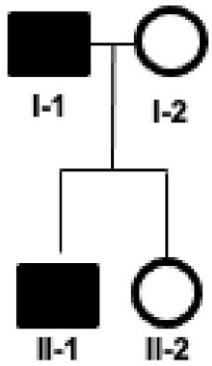

Figure 3. Family tree of family B. Precocious male puberty and a germline mutation in the LH/choriogonadotropin receptor (*LHCGR*) gene indicated in black.

according to American College of Medical Genetics and Genomics guidelines (PS3 PS4 PM1 PM2 PP1 PP4) (16).

Further genetic testing in both affected families has been a matter of attention but lies outside the scope of this report.

Literature Review

Literature search

The literature search in Cochrane, PubMed, and Embase provided a total of 65 studies that report or describe a possible link between FMPP and testicular tumors, of which 22 articles were duplicates. Thirty-five of the 43 remaining articles were excluded based on title and abstract screening. Of the 8 potentially eligible studies, only 2 consisted of an abstract and 1 did not match the focus of this study; 1 further study consisted of an overview of the literature. Manual searching lead to inclusion of three more studies, providing a total of 8 included full-text articles. Figure 4 gives an overview of the search process.

Characteristics of the included studies

Tables 2 and 3 show the (clinical) characteristics of the 8 included studies. Five studies describe a single case (17–21) and 3 reports include 2 or more patients (6, 22, 23), resulting in a total of 16 patients.

Table 2 summarizes 4 case reports that describe patients with FMPP who later in adolescence or adulthood developed a testicular (germ cell) tumor (age at FMPP presentation: range, 10 months to 8 years; age at diagnosis of testicular tumor: range, 15-35 years) (17, 19–21). Three patients were diagnosed with a malignant testicular GCT (including an embryonal carcinoma, a seminoma, and a mixed GCT) (17, 19, 21). The fourth patient was diagnosed with a Leydig cell adenoma and germ cell neoplasia in situ, the latter being the precursor lesion for all malignant testicular GCTs, both seminoma and nonseminoma (20). An *LHCGR* pathogenic variant had been identified in 3 of the 4 patients in whom DNA analysis had been performed.

On the other hand, Table 3 summarizes 4 studies that report on in total 12 patients who presented simultaneously with peripheral precocious puberty and (suspicion of) a unilateral testicular mass or testicular asymmetry on ultrasound (median age, 6 years; range, 2-8 years) (6, 18, 22, 23). Eleven of these 12 patients have been treated with an orchidectomy, except for 1 boy who underwent testis-sparing surgery. The majority

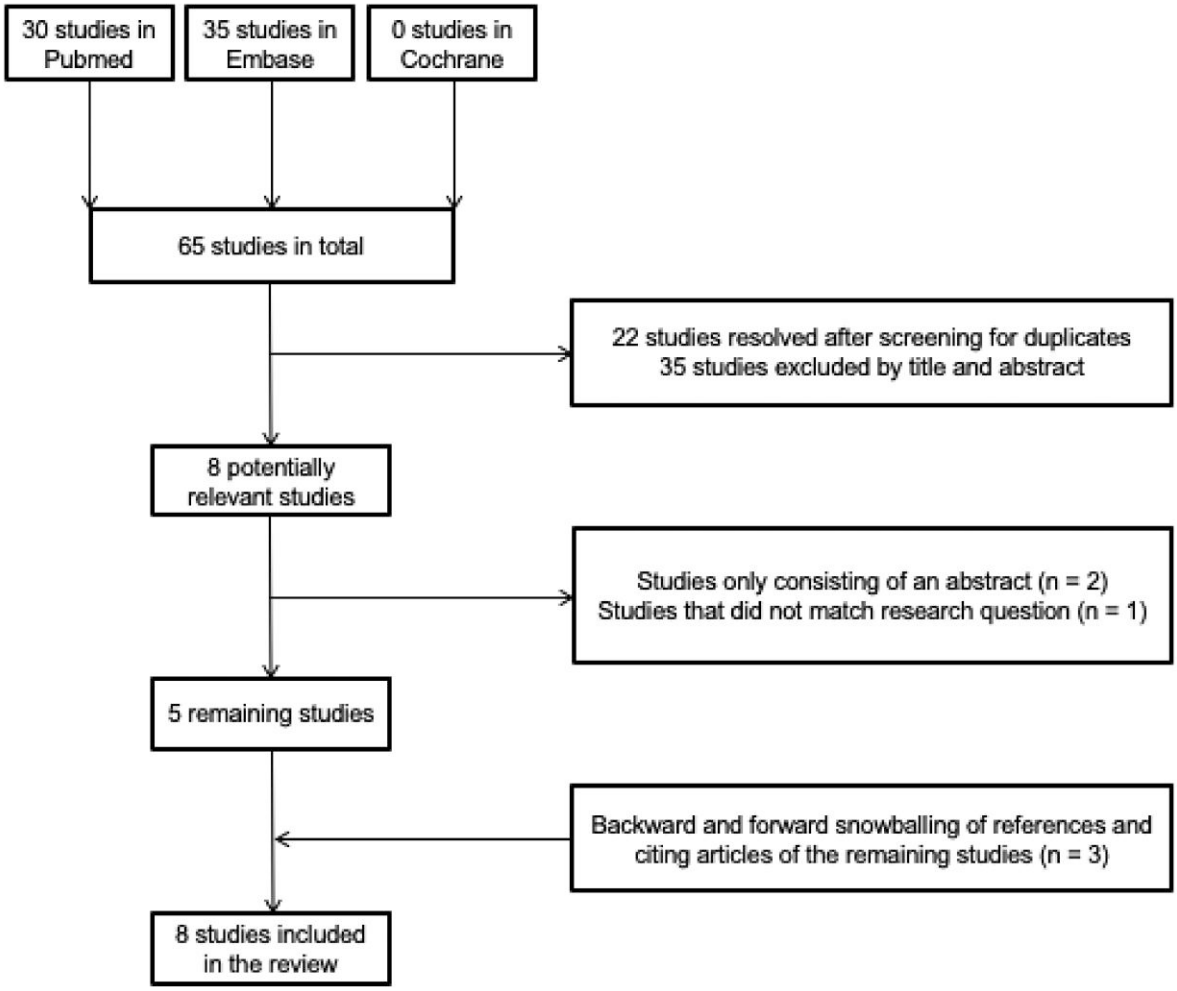

Figure 4. Flowchart of included and excluded articles during the systematic literature search.

(10) of the tumors appeared to be Leydig cell adenomas (6, 22, 23); 2 were classified as nodular Leydig cell hyperplasia (6, 18). Ten boys showed a somatic *LHCGR* gene pathogenic variant in the removed testicular tissue without a germline pathogenic variant. Furthermore, in 2 boys, *LHCGR* pathogenic variants were identified in both peripheral blood as well as the removed testicular tissue; examination of the testicular tissue in these 2 boys showed nodular Leydig cell hyperplasia in 1 boy and a Leydig cell adenoma in the other boy.

Discussion

This study reports on 2 males with FMPP who developed a testicular GCT and contains an analysis of the existing literature on the occurrence of testicular tumors in patients with FMPP, with the aim to analyze whether FMPP might predispose to development of malignant testicular tumors in adulthood.

First, the observation in our clinic that in 2 families men with FMPP had developed a testicular tumor in adulthood raised the question whether their sons, and boys with FMPP in general, are at an increased risk for development of testicular malignancies.

Literature research revealed 4 additional cases of men with FMPP that developed (the precursor of) a testicular malignancy.

The prevalence of both FMPP and, to a slightly lesser extent, malignant testicular GCT is rare. Making the simultaneous occurrence of these conditions remarkable. The incidence of a testicular carcinoma is approximately 6/100 000 men per year, with a small annual increase for no clearly documented reason, and mortality is 0.3/100 000 men per year (24, 25). Furthermore, prevalence of testicular tumors is most common in adolescents, comprising 12% of cancers in the 15- to 19-year of age range (26).

The cooccurrence of FMPP and a testicular malignancy in adulthood in at least 6 cases is remarkable and might indeed suggest an increased risk for development of malignant testicular tumors in males with FMPP. Quantification in patients with FMPP of the risk for development of a testicular malignancy later in life is not possible because the small number of FMPP patients, with an estimated prevalence of <1/1 000 000 (27). Of note, predisposition does not appear to be tied to 1 pathogenic variant because the pathogenic variant described in both families in this report is different from those reported for the cases in literature (Table 3). However, all are germline-activating pathogenic variants within exon 11 of the *LHCGR* gene.

Both benign adenomas such as we report in Table 3 as well as malignant tumors have also been reported for other diseases with activating receptor pathogenic variants. For example, in McCune Albright syndrome (MAS), which is

Table 2. Included articles that described patients with first peripheral precocious puberty and later in life, during adolescence or adulthood, development of a testicular tumor

Study	Oxford level[a]	n[b]	Age[c]	FMPP				Testicular tumor		
				Symptoms at presentation	Suspicion testicular tumor	Treatment	DNA (*LHCGR* gene) PB/TT	Age[d]	Histopathology	DNA (*LHCGR* gene) tumor
Mortensen 2017 (20)	4	1	10 mo	Pubic hair, growth of genitals, hands, and feet	No; bilateral testicular biopsies (age 1.5 y) showed Leydig cell hyperplasia	Ketoconazole	c.1732G > T p.(Asp578Tyr) (PB) (de novo)	25 y	Leydig cell adenoma with GCNIS	NP
Senniappan 2014 (17)	4	1	Before the age of 8 y[d]	Pubic and facial hair, growth spurt, pubertal staging G4P4A2	No; testicular ultrasound revealed no evidence of tumor	Anastrozole, spironolactone	No mutation (PB + TT)	14.8 y	Embryonal carcinoma	No mutation
Winston 2014 (19)	4	1	8 y	Tanner stage 4 genitalia, pubic hair, body odor, acne, and linear growth acceleration	No; scrotal ultrasound showed normal, symmetrical testes	Anastrozole, bicalutamide	NP	15 y	Mixed germ cell tumor (predominantly embryonal carcinoma and elements of mature teratoma and yolk sac tumor)	NP
Martin 1998 (21)	4	1	27 mo	Enlargements of genitals, appearance of pubic hair	No; testicular biopsy showed interstitial cell hyperplasia and evidence of early spermatogenesis	Medroxyprogesterone acetate	c.1733A > G p.(Asp578Gly) (PB)	35 y	Seminoma	NP

Abbreviations: GCNIS, germ cell neoplasia in situ; hLHR, human LH/CG receptor; *LHCGR*, LH/choriogonadotropin receptor; NP, not performed; PB, peripheral blood; TT, testicular tissue.
[a]Level of evidence based on the Oxford Centre for Evidence-Based Medicine: Levels of Evidence (2011) (14).
[b]Number of patients with a testicular tumor included in study.
[c]Age at diagnosis.
[d]Age at onset.

Table 3. Included articles that described patients with simultaneous presentation of peripheral precocious puberty and a testicular tumor

Study	Oxford level[b]	n[a]	Age[c]	Symptoms at presentation	Suspicion testicular tumor	Treatment until surgery	Surgical treatment	Histopathology	DNA (*LHCGR* gene) PB/TT	DNA (*LHCGR* gene) tumor
Boot 2011 (6)	4	7	4 y	Linear growth acceleration, frequent erections	Yes; ultrasound of the testis showed a tumor in the right testis	None	Inguinal orchidectomy right	Leydig cell adenoma	No mutation (PB)	c.1732G > C p.(Asp578His)
			2 y	Linear growth acceleration and early pubertal development	Yes; ultrasound showed an oval process in the right testis	None	Inguinal orchidectomy right; ketoconazole, letrozole, and bicalutamide	Nodular hyperplasia (both testes)	No mutation (PB + TT)	c.1732G > C p.(Asp578His)
			3.5 y	Severe form of precocious puberty	Yes; ultrasound examination using high resolution showed small tumors in 1 testis	Spironolactone and testolactone	Orchidectomy left	Leydig cell adenoma	c.1732G > C p.(Asp578His) (PB) AND Y133N change in exon 4 (PB)	c.1732G > C p.(Asp578His) AND Y133N change in exon 4
			5.5 y	Severe form of precocious puberty	Yes; ultrasound examination using high resolution showed small tumors in 1 testis	Spironolactone and testolactone	Orchidectomy right	Leydig cell adenoma	No mutation (PB)	c.1732G > C p.(Asp578His)
			6.5 y	Severe facial acne and pubarche	Yes; high-resolution ultrasound examination showed a small tumor in the right testis	None	Orchidectomy right	Leydig cell adenoma	No mutation (PB)	c.1732G > C p.(Asp578His)
			7.4 y	Precocious puberty (not specified)	Yes; ultrasound showed a tumor in the right testis	None	Orchidectomy right	Leydig cell adenoma	No mutation (PB)	c.1732G > C p.(Asp578His)
Canto 2002 (23)	4	2	4 y	Early pubertal development (not specified)	Yes; a discrete testicular mass was palpable and testicular ultrasound showed a unilateral left mass	None	Orchidectomy left	Leydig cell adenoma	No mutation (PB + TT)	p.(Asp578His)
			7 y	Early pubertal development (not specified)	Yes; unilateral ultrasound showed a unilateral mass right	None	Orchidectomy right	Leydig cell adenoma	No mutation (PB + TT)	c.1732G > C p.(Asp578His)
Leschek 2001 (18)	4	1	4 y	Precocious puberty (not specified)	Yes; scrotal ultrasound showed a 0.6-cm, solid, hypoechoic mass at the upper pole of the right testis	Spironolactone, testolactone, and deslorelin	Excisional biopsy right testis	Nodular Leydig cell hyperplasia	c.1691A > G p.(Asp564Gly) (PB)	c.1691A > G p.(Asp564Gly)
Liu 1999 (22)	4	3	8.3 y	Precocious puberty that had begun 1-2 y previously	Yes; unilateral mass on scrotal ultrasound	None	Orchidectomy right	Leydig cell adenoma	No mutation (PB + TT)	c.1732G > C p.(Asp578His)
			7.5 y	Precocious puberty that had begun 1-2 y previously	Yes; unilateral mass on scrotal ultrasound, mass also palpable	None	Testis-sparing surgery right	Leydig cell adenoma	No mutation (PB + TT)	c.1732G > C p.(Asp578His)
			7.8 y	Precocious puberty that had begun 1-2 y previously	Yes; unilateral mass on scrotal ultrasound	None	Orchidectomy left	Leydig cell adenoma	No mutation (PB + TT)	c.1732G > C p.(Asp578His)

Abbreviations: *LHCGR*, LH/choriogonadotropin receptor; PB, peripheral blood; TT, testicular tissue.
[a]Number of patients with a testicular tumor included in study.
[b]Level of evidence based on the Oxford Centre for Evidence-Based Medicine: Levels of Evidence (2011) (14).
[c]Age at diagnosis.

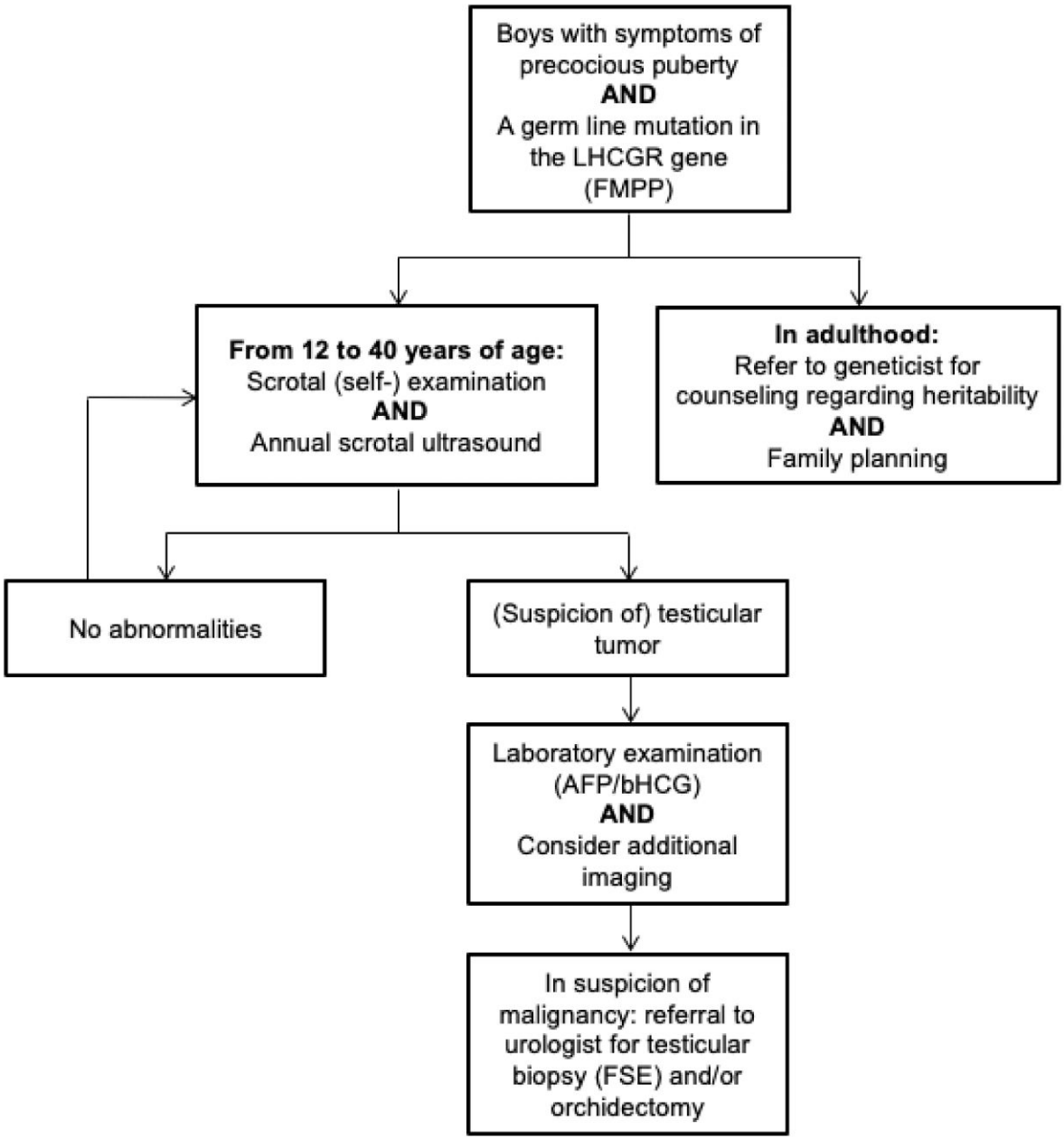

Figure 5. Flowchart depicting suggested follow-up in boys presenting with symptoms of precocious puberty and a germline mutation in the LH/choriogonadotropin receptor (*LHCGR*) gene. Abbreviations: FMPP , familial male-limited precocious puberty; FSE, frozen section examination.

caused by activating pathogenic variants in G protein-coupled receptors, testicular tumors, predominantly Leydig cell tumors (LCT) and Sertoli cell tumors have been repeatedly described. Moreover, Boyce et al also suspect MAS to be a predisposing factor for testicular malignancy after description of a patient with MAS who had both an embryonal carcinoma and a seminoma without predisposing exposures or a positive family history (28). This analogy in occurrence of testicular abnormalities in individuals with an activating G protein-coupled receptor mutation could support the hypothesis that activating *LHCGR* pathogenic variants may well increase the risk of development of a testicular GCT.

Second, the question was raised whether an increased malignancy risk also exists for testicular tumors identified in boys who present with asymmetrical testicular enlargement or testicular mass and simultaneous signs of peripheral precocious puberty. Interestingly, in all boys reported in Table 3, testicular histology was found to be benign in nature. Of note, it is unknown how these lesions might develop when left untreated; 1 of the cases reported in literature actually showed seminoma in an adult male at age 35 years, whereas testicular biopsy early in childhood had shown interstitial cell hyperplasia (21). Of note, an additional article has been published after we performed the systematic search of the literature. The authors report 2 additional patients who presented with simultaneous signs of precocious puberty and a testicular mass, whereas pathology assessment revealed a benign LCT in 1 boy and a malignant LCT in the other. Genetic analysis on the *LHCGR* gene or other genes was not reported for these 2 cases (29). We need to point out

that 1 of these 2 additional cases had a malignant testicular lesion in contrast to all previous patients reported and summarized here, who all had benign lesions.

Recent literature offers hypotheses that might explain the suggested increased risk for development of testicular malignancies in males with a germline pathogenic variant of the *LHCGR* gene. Abnormally increased testosterone levels, assumed to be present since fetal development, are thought to cause an imbalance in steroidogenesis and accelerated puberty, which might lead to disturbed gonocyte differentiation and promotion of neoplastic changes. However, no clear evidence of the underlying mechanism has yet been provided (17, 20, 21). Gonadal stromal cells during early development express both transcription factor Sex-determining Region Y (SRY) as well as its target gene SOX9, in the presence of a Y-chromosome. This leads to formation of Sertoli cells, which create a specific microenvironment that allows the differentiation of gonocytes into spermatogonia (30). SRY gene pathogenic variants are present in 10% to 15% of the 46XY disorders of sex development patients. These patients have an increased risk of development of a malignant gonadal GCT, which is related to prolonged existence of the embryonic phenotype of the germ cells, exemplified by the continuous expression of OCT3/4. One of the suggested candidate genes located within the so-called GonadoBlastoma on the Y-chromosome region is Testis Specific Protein on the Y-chromosome. This is suggested to be explained by its survival and proliferation inducing effect on embryonic germ cells (31–33). A similar mechanism, although now indirect because of the effect of the pathogenic variant in the supporting cell compartment (ie, Leydig cell), might inhibit the maturation process of the gonocyte toward spermatogonia, and because of such a result in an increased risk of development of testicular GCTs in case of a *LHCGR* germline pathogenic variant.

This study does have some limitations. First, because FMPP does not occur frequently, numbers are very low and there is a fair amount of uncertainty around the magnitude of the increased risk of testicular malignancies in these patients. Additionally, the current literature only consists of case reports. There also remains uncertainty about the pathophysiology of tumor development in both patients with a germline as well as a somatic pathogenic variant.

In view of the results described, we suggest increased awareness for the risk of development of testicular malignancy in patients with FMPP. Therefore, prolonged follow-up of these patients is advised during and after puberty, when the precocious puberty is no longer an issue and initial increased testosterone production does not require further follow-up or treatment as it had become age appropriate. Instruction of these patients to perform monthly scrotal (self-) examination and annual scrotal ultrasound might be justified from 12 to 40 years of age, to allow early detection of testicular malignancies (34). Therefore, we suggest a flowchart to assist clinicians in their considerations regarding follow-up in boys with symptoms of precocious puberty and a germline *LHCGR* pathogenic variants (Fig. 5).

In conclusion, there is a suggestion that the likelihood of development of testicular malignancy in (early) adulthood is increased in patients with FMPP based on a germline pathogenic variant in the *LHCGR* gene. Therefore, self-examination and ultrasound follow-up from 12 to 40 years of age might contribute to early detection and thus early treatment of testicular tumors.

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Disclosures

The authors declare no conflict of interest.

Data Availability

The dataset generated and analyzed during the current study is not publicly available but is available from the corresponding author on reasonable request.

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