ABNORMALITIES OF FEMALE PUBERTAL DEVELOPMENT

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ABSTRACT

Puberty is the period of growth that bridges childhood to adulthood and results in physical and sexual maturity as well as the capacity for reproduction. Over half of pubertal timing is considered heritable. Significant pathology can result in both advanced and delayed puberty and can result in altered attainment of adult height, secondary sexual characteristics and reproductive capacity. The age for evaluation of precocious puberty has changed in the recent past due to greater understanding of the timing of pubertal development and important racial differences. The early detection of significant intracranial pathology underscores the importance of the workup in young girls with true precocious puberty, and the close follow up of girls in whom a brain MRI is not initially indicated. GNRH agonists have become a mainstay of therapy in girls with precocious puberty. The optimal method of delivery and age of cessation is not known, but increases in adult height and no obvious reproductive sequelae have been demonstrated. Unlike precocious puberty, the definition of delayed puberty has not changed in recent years, and large studies suggest that the most common diagnosis after evaluation is constitutional delay, however, this is more common in boys presenting with delayed puberty than girls. The most common diagnosis in girls with delayed puberty is gonadal failure. Advances in reproductive technologies have allowed women with Turner's syndrome and MRKH to build their families. Among phenotypic women with all or part of a Y chromosome, gonadal extirpation is recommended, the timing of which varies with their genetic analysis which is the greatest predictor of risk for germ cell tumors. Molecular research and newer techniques of genetic analysis such as genome wide association studies and next generation sequencing have allowed the identification of genetic mutations that may be responsible for some of the complex diseases that cause both delayed and precocious puberty.

INTRODUCTION

The pubertal process is the period of transitional growth bridging the childhood years and adulthood. The genetic blueprint housed within the genome of the individual has long before set in motion a number of critical processes. The end result is the maturation of a multitude of endocrine axes necessary for (1) **secondary sexual development** and, (2) the attainment of the immediate **capacity for reproduction**. Intrinsic to this reproductive maturation is yet another important process of puberty: (3) a secondary wave of skeletal growth and the **attainment of adult stature**. Abnormal puberty, whether premature or delayed, may adversely

influence each of these events resulting in an untimely or altered ability for spontaneous secondary sexual development and spontaneous reproduction or abnormal growth.

In recent years numerous advances have been made in molecular medicine and the assisted reproductive technologies. The impact of these advances has had a tremendous effect on the care of patients with abnormal puberty by: changing the initial counseling provided to our patients; allowing for new treatments during the time of altered pubertal growth; and, providing reproductive options to individuals previously known to be infertile and some considered sterile. In addition, new insight about the physiology of puberty and the genetics of these disorders has accumulated. The focus of this chapter will be on our expanded knowledge of both the genotypes and phenotypes of the disorders presenting as abnormal puberty.

NORMAL PUBERTY FOR GIRLS (IT'S OCCURRING EARLIER!): A BASIS FOR THE DEFINITION OF ABNORMAL PUBERTY

Onset of Normal Pubertal Landmarks

The first somatic change associated with the initiation of puberty in girls is an **increase in growth velocity**. It is during the initial increment in growth velocity that the first sexual sign of puberty occurs. The initial standards of puberty were published in approximately 1970 by Marshall and Tanner. These standards reported that in British girls **thelarche** (breast budding) developed at an average age of 11 years, followed by **adrenarche**, the appearance of pubic hair. After thelarche and adrenarche, growth velocity continues to increase and peak, a landmark termed the adolescent growth spurt. A peak height velocity of 9 cm/year is attained at that time. Subsequently, with near closure of the epiphyses there is a deceleration phase for growth. It is in this deceleration phase of growth that **menarche** occurs. It is often at least 5 years after menarche until most of menstrual cycles are ovulatory; clinicians cannot consider that puberty is normal until this reproductive mechanism is established as it represents the final step in maturation of the HPO axis.

The sequence and timing of pubertal development may vary by ethnicity. The classic description of the normal sequence of pubertal signs as published by Marshall and Tanner was taken from studies of British Caucasian girls not long after WW II.(1,2) They noted that breast development was the first sign of puberty occurring on average at 11 years of age in the British girls. In contrast, a study of African girls in the 1970s noted that for the majority of them adrenarche preceded thelarche.

Several larger studies conducted in the United States have given further insight regarding the timing of pubertal events and suggest that the age of puberty may be decreasing. (3,4) The Pediatric Research in Office Settings (PROS) data were taken from a cross-sectional study of 17,077 American girls of whom 9.5% were African-American and 90.4% were Caucasian. It should be noted that Hispanic girls were included in both African-American and Caucasian groups. Surprisingly, nearly 30% of the African-American girls had evidence of breast and/or pubic hair development at age 7 years and nearly 50% by age 8 years. For Caucasian girls, 15% had started puberty by age 8 years and nearly 40% by age 9 years. The mean ages for

breast and pubic hair growth were 10.0 and 10.5 years for Caucasian girls, respectively, and 8.9 and 8.8 years for African-Americans, respectively. The average age of menarche for Caucasian girls remained unchanged at approximately 12.8 years with the African-American girls starting menstruation earlier and at a mean age of 12.16 years. (3) The PROS results may have been skewed slightly given the fact that inspection rather than palpation was utilized to determine thelarche. The NHANES studies did not collect onset of pubic hair or breast data in girls prior to the age of 12 years, centering their analyses on the timing of menarche and the attainment of completed puberty. (4)

In most studies taken from the US, an earlier time of menarche was reported when compared to the older data with ranges from 2 to nearly 5 months earlier depending on the ethnic group studied. While it is reasonable to consider that the original British normatives published by Marshall and Tanner are likely different from the heterogeneous American population at the end of the 20th century, tremendous debate about the shortcomings and interpretations of these American data has continued over the last 10 years in a number of different forums. An expert panel overall agreed that the weight of the evidence supports a secular trend toward earlier breast development and menarche but not for other female pubertal markers. (5) Some evidence exists that malnutrition in certain socioeconomic groups of US children may currently be reversing this trend. (5)

Determinants of Normal Pubertal Growth

From conception to the fusion of epiphyses during the later stages of puberty, a number of maturational processes occur for formation and modeling of the skeleton. Intrinsic to somatic growth is the initial mesenchymal cell condensation and differentiation into cartilage that serves as a template for subsequent bone formation. Osteoblast differentiation occurs on the surface of this cartilaginous template and endochondral bone formation results when such differentiation occurs on calcified cartilage at the growth plate.

Genetic, environmental (i.e., nutrition), and hormonal determinants exist which are critical for the attainment of adult stature. The long held tenets that adult height is polygenic have been supported by genome-wide association studies for height (6). It has been estimated that 50 or more loci are associated with final adult stature (6-8). If all of these genes are functional, these parental-inherited growth genes determine the final adult height attained by an individual. Minimal changes by any number of these genes may result in height variation within the predicted height distribution. One can estimate this height by a calculation of **mid-parental height**. For females this is determined by subtracting 13 cm from the father's height, adding this to the mother's height in cm and then dividing by 2.

Under pathophysiologic situations, an individual may be taller or shorter than would be dictated by parental height determinants. Sometimes these differences are genetically determined and in other situations abnormal hormonal influences alter an otherwise intact genetic predisposition, and in other cases environmental factors play a role.

Genetic Influences of Growth

Some statural genes are present on both X and Y-chromosomes with Y individuals being taller than X individuals. From tallest to shortest one can generalize the following: XYY > (taller than) XY > (taller than) XXX > (taller than) XX > (taller than) X individuals. A few genes have been implicated in these differences. One set of genes, the SHOX genes, exist on the distal X chromosome. (9-12) Mutations have resulted in short stature and deletion of this locus is associated with short stature in Turner syndrome (45,X). (9)

Hormonal Determinants of Growth (Some gene mediated)

No doubt, a normal endocrine environment critically influences bone growth. For example it is essential that intact and normal growth hormone and thyroid hormone production, among others, be present. This is demonstrated by the fact that growth hormone and thyroid hormone deficiency separately result in short stature until corrected. (13) Growth hormone excess results in such conditions as a gigantism and acromegaly.

In addition to these known growth-promoting hormones, sex steroids are essential for mediating the pubertal growth spurt and attainment of final adult stature. Premature sex hormone production in children with congenital adrenal hyperplasia causes premature epiphyseal growth and fusion: thus, tall as children and short as adults. Early onset precocious puberty similarly causes premature pubertal growth with the risk of short adult stature unless corrected. The lack of pubertal development (delayed puberty) allows for continued long bone growth since the epiphyseal centers remain open longer than normal. Usually, in these situations, growth is normal until the expected age onset of puberty and the growth spurt is not noticed; however, linear growth continues in the absence of epiphyseal closure. This results in eunuchoid body proportions: an arm span which exceeds the height by more than 6 cm and disproportionately long legs.

While it had always been accepted that estrogen mediates pubertal bone growth in females, it was not until this era of molecular medicine that it was determined that estrogen and not testosterone mediates the same function for males. Inactivating mutations in either the estrogen receptor gene or the aromatase gene (preventing conversion from androgens to estrogens) in males have resulted in lack of normal bone growth at puberty and lack of epiphyseal closure with resultant tall stature (i.e., taller than predicted). (14-17) These findings establish that estrogen is essential for initiation of pubertal growth, closure of the growth plate, and augmentation accrual of bone during puberty. The presence of both alpha and beta estrogen receptors have been identified in the growth plate and studies are underway to understand the exact mechanism of estrogen action. (18)

DEFINITION OF ABNORMAL PUBERTY

The classic definitions of abnormal puberty, whether premature or delayed, are based on timing that is considered to be 2.5 standard deviations removed from the mean. Previously, the definition of precocious development for girls was the appearance of secondary sexual development before the age of 8 years**,** an age felt to represent 2.5 standard deviations earlier than the mean.

Revised recommendations have been made based on the findings of the PROS Network. (19) These guidelines propose that **precocious puberty be defined by the presence of breast or pubic hair development before age 6 years in African-American girls and age 7 years in Caucasian girls**.

However some experts disagree with the PROS recommendations. A few girls with puberty starting between 6 and 8 years of age for African Americans and between 7 and 8 for Caucasians were initially reported with endocrine or CNS pathologic etiologies of early puberty. As a result, concerns emerged that the PROS definitions may miss significant pathology and that strict enforcement of the new guidelines will lead to missed diagnoses. (20-22) Data suggest that between 2-9% of girls in this age group will have underlying pathology and 1% will have a tumor. (23,24) Missed diagnoses have included CNS tumors, neurofibromatosis, hypothyroidism, congenital adrenal hyperplasia, and hyperinsulinism. Thus, for such children beyond the recommended age of evaluation with presenting symptoms of precocious puberty, a complete history and physical exam are warranted to ensure that a serious underlying condition is not missed. Some experts recommend a bone age evaluation and careful longitudinal follow-up for girls younger than age 8 years that do not fall into the PROS guidelines for evaluation for precocious puberty. (22)

Recommendations based on the findings of the PROS Network have not been made for revising the definition of delayed puberty in girls as they have for precocious. As such, **the absence of thelarche by age 13 years for girls signifies an abnormality, and remains the definition of pubertal delay**. The classic definition for delayed menarche, i.e., primary amenorrhea, has been the **absence of menarche by age 15 or 16 years, which is approximately 2.5 to 3 standard deviations from the mean**, respectively.

While some patients present strictly with the absence of the onset of pubertal development, others have abnormalities in the tempo and sequence of puberty that has seemingly begun on time. Menarche usually occurs within 3 years of thelarche, when most girls have tanner stage 4 breast development. The American Academy of Pediatrics and the American College of Obstetricians and Gynecologists have jointly published guidelines that recommend evaluation of delayed puberty if menarche does not occur within 3 years of thelarche. (25)
These guidelines also recommend evaluation of girls with the following characteristics:

- No breast development by age 13 years (delayed puberty)
- Absence of menarche by age 14 years in the presence of hirsutism or history or exam suggestive of eating disorder or excessive exercise or an outflow abnormality
- Absence of menarche by age 15 years.

Age definitions should be seen only as general guidelines. Rather than require a young woman meet the strict definitions of menarche by age 15 or 16 years to initiate an evaluation for delayed puberty, it has been suggested that all adolescents be followed annually throughout the pubertal process. (26)

For example, if a young woman presents concerned because of no menses at age 14 years, some of the major etiologies of primary amenorrhea could be recognized at an office visit without adding any significant costs. Screening at an age prior to 15 years should, as discussed in the previous paragraph, include screening for eating disorders and consideration of an excessive androgen disorder such as polycystic ovary disease. Exclusion of outflow tract disorders such as vaginal agenesis or imperforate hymen / transverse vaginal septum would require gentle pelvic examination. The physical exam should also be directed to identify findings that are typical of some associated endocrinopathies or syndromes such as gonadal dysgenesis. It would be better to begin a partial evaluation (i.e., FSH level and use of growth velocity curve) during earlier adolescent years at the time that abnormalities are first suspected than it would to wait until these young women are significantly different from their peers. No doubt, adolescence is one of the most difficult time periods in growth and development. It is potentially very harmful for an individual's psychosexual development to allow significant delays in secondary sexual development or onset of menses to continue without evaluation, treatment and appropriate counseling. Young women are particularly likely to be worried about delayed breast development.

PRECOCIOUS PUBERTY

Overview

The overall incidence of sexual precocity among American children has been estimated to be between 1:5,000 to 1:10,000. (27) The female to male ratio is approximately 10:1. Early activation of pulsatile gonadotropin-releasing hormone (GnRH) secretion is the most common mechanism of precocious puberty; usually it is idiopathic but it can be from serious conditions such as hypothalamic tumors. While the classic definition of sexual precocity is the appearance of secondary sexual characteristics before the age of 8 years in girls, newer guidelines as discussed above suggest that puberty is not considered precocious unless it occurs prior to age 6 years for African-American girls or age 7 years for Caucasian girls. (19) However, many pediatric endocrinologists in the United States routinely evaluate all girls with precocious development prior to the cutoff at age 8 years (28). As discussed above, even when puberty occurs between ages 6-7 and 8 years, it is important to consider evaluation of all children. (20-22) The child may be suffering from a serious CNS disorder associated with precocious puberty. (21)

Long term implications of early puberty include an increased risk of breast cancer, metabolic diseases (e.g., type 2 diabetes, obesity), endometrial cancer and cardiovascular disease. (29,30) In addition, psychosexual maturation remains concordant with chronological age, and unfortunately early physical sexual maturation at any age places these young girls at a high risk for sexual abuse. Clinicians should routinely screen children with early development for sexual abuse. Direct questioning in age appropriate language should be used and the history should include questions about behavioral markers including new onset bedwetting, nightmares, or other behavioral issues. It is thus important not only to make a reasoned judgment as to when to initiate an evaluation, but also to institute the appropriate therapy and support to prevent these potential long-term sequelae, even in selected girls who fall outside the new recommendations. It is also prudent to remember that early maturing girls, who may not "fit" the criteria of having

premature puberty, may elect to engage sooner in coitus and other risk taking behaviors such as drugs than later maturing girls.(31,32)

Precocious puberty represents the appearance of the secondary sexual characteristics from increased sex steroid production. This increase may be secondary to aberrant gonadotropin stimulation or intrinsic disease of the ovary or adrenals. Many terms have been used to describe the types of precocious puberty, and some are less used in contemporary literature.

True precocious puberty, also known as complete precocious puberty, refers to puberty that appears early and either progresses through each of the pubertal landmarks including menarche or, in the absence of treatment, would likely progress through each of these stages. In the majority of children presenting for precocious development this early evidence of puberty is not the result of true precocious puberty and will halt or even regress; treatment is unnecessary (33). Classically a GnRH challenge test that demonstrated a pubertal response of gonadotropins (i.e., LH response > FSH response) was the hallmark of this diagnosis. The usual ability to suppress pubertal development with GnRH agonists remains the hallmark of treatment.

Incomplete precocious puberty refers to the appearance of one phase of the pubertal process: thelarche, adrenarche, or menarche. **Isolated precocious thelarche, isolated precocious adrenarche, and isolated menarche** are the three forms of incomplete precocious puberty.

Sexual precocity has been further categorized according to whether the pubertal signs are concordant or discordant with the sex of the individual: **isosexual precocity referring to early sexual development consistent with the sex of the individual** (i.e., feminization of a female); **heterosexual or contrasexual precocity indicating precocious pubertal development that is limited to those physical signs not characteristic for the sex of the individual when presenting as isolated findings** (i.e., virilization of a female). **GnRH dependent and GnRH independent precocious puberty (GIPP)** refer to those causes of precocity that are or are not secondary to GnRH production. Central **precocious puberty (CPP)** refers to precocity of CNS origin.

A summary of the causes of sexual precocity is presented in Table I below, followed by a numeric breakdown of the frequency of occurrence of these disorders in Table II.

Table I. Classification of Female Precocious Puberty

I. Complete isosexual precocity (true precocious puberty: gonadotropin dependent)

A. Idiopathic
B. CNS lesions: Hamartomas, Craniopharyngioma, etc
C. Primary hypothyroidism
D. Post treatment for CAH
E. Genetic

II. Incomplete isosexual precocity (GnRH independent)

A. Isolated precocious thelarche
B. Isolated precocious menarche
C. Estrogen-secreting tumors of the ovary or adrenals in girls
D. Ovarian cysts
E. McCune-Albright syndrome
F. Peutz-Jeghers syndrome
G. Iatrogenic

III. Contrasexual precocity (Isolated virilization)

A. Isolated precocious adrenarche
B. Congenital adrenal hyperplasia
C. Androgen-secreting ovarian or adrenal neoplasm
D. Iatrogenic

Table II. Numeric breakdown of etiologies for precocious puberty in a large series of girls (N=438) evaluated from 1988-1999 by the classic definition (pubertal onset < 8 years) (24)

I. Central Precocious Puberty	**428 (97.7%)**		
Incompletely Evaluated	**124**		
Completely Evaluated	**304**		
Idiopathic		**226 (74.4%)**	
CNS Pathology		**56 (18.4%)**	
Hydrocephalus			11 (19.6%)
Encephalocele			2 (3.6%)
Neurofibromatosis			3 (5.4%)
Encephalitis			1 (1.7%)
Intracranial hemorrhage			1 (1.7%)
Hypothalamic hamartoma			7 (12.5%)
Pituitary microadenoma			5 (8.95%)
Optic chiasma astrocytomas			3 (5.4%)
Optic chiasm glioma			1 (1.7%)
CNS Vascular Malformation			1 (1.7%)
Other miscellaneous CNS disorders/lesions			21 (37.5%)
			(100%)
Coincidental/Associated Disorders		**22 (7.2%)**	
		(100%)	
II. GnRH Independent (GIPP)	**10 (2.3%)**		
McCune Albright syndrome	**3 (30%)**		
Ovarian "hyperfunction"/ follicular cyst	**4 (40%)**		
Ovarian tumors	**3 (30%)**		
Juvenile granulose cell tumor	(2)		
Theca-granulosa cell tumor	(1)		

In this review of 438 girls examined between 1988-1998, prior to the newer PROS definitions, the incidence of central precocious puberty (CPP) was noted to be 97.7% and GnRH independent precocious puberty (GIPP) was 2.3%. (24) Neurogenic abnormalities were noted in 18.4%, and idiopathic CPP in 74% of the girls in this study. The frequency of neurogenic CPP tended to be higher in the youngest girls (i.e., those under age 4 years) and the frequency of idiopathic CPP tended to be higher in girls presenting at older ages (i.e., between ages 7-

7.9). Those girls identified with idiopathic precocious puberty after age 7 may, in fact, represent the recent observations of earlier onset of normal puberty by Herman-Giddens. (3)

Central Precocious Puberty

Central precocious puberty results from early maturation of the hypothalamic- pituitary-gonadal axis. Serum gonadotropins, gonadal pulsatility and sex steroid concentrations are in the normal postpubertal range. As mentioned previously, **idiopathic precocious puberty** seems to be the most common cause of CPP. Neurogenic CPP seems to be found more frequently in extremely young girls with the earliest onset of puberty. **CNS lesions** identified include neoplasms, trauma, hydrocephalus, post infectious encephalitis, congenital brain defects, and such genetic disorders as neurofibromatosis type 1 and tuberous sclerosis, and granulomas of tuberculous origin. The most commonly identified neurogenic neoplasms found in CPP include hamartomas, astrocytomas, and pituitary microadenomas. (24) Hamartomas are congenital hypothalamic malformations that histologically contain fiber bundles, glial cells, and GnRH-secreting neurons and often act as a mini-hypothalamus. Less frequently identified tumors include epipendymomas, gliomas, and pinealomas. While the craniopharyngioma has usually been associated with delayed puberty, it can rarely cause precocity as well.

Known genetic causes of CPP are rare and are currently limited to the KISS1 and the MKRN3 genes. The former gene produces a peptide that many currently believe to be the primary stimulatory signal of puberty and the later gene seems to be related to an inhibitor of GnRH secretion that exists prior to puberty, Activating mutations have been found in the genes encoding kisspeptin 1 (KISS1) and its receptor (KISS1Rr1.(34-37) In addition, as in normal puberty, higher levels of kisspeptin 1 have been identified in children with CPP compared to controls.(38) Recent research on 15 families with central precocious puberty utilizing whole exome sequencing identified loss of function mutations in MKRN3 genes which encode the makorin RING-finger protein 3 in 5 of the 15 families. The gene is maternally imprinted and likely plays a vital role in developing cells, particularly in the central nervous system. Interestingly, a larger deletion of 15q11-q13 which contributes to Prader-Willi syndrome encompasses the MKRN3 gene. The protein, makorin RING finger protein 3, is involved with RNA binding and ubiquination and degradation. Further research in 215 unrelated children with sporadic CPP identified 8 children with mutations in MKRN3, all on the paternal allele. (39) While these mutations are rarely a cause of CPP, this research does suggest an inhibitory role of MKRN3 in GnRH secretion. (40)

Other chromosomal abnormalities associated with CPP have also been described, such as 9p deletion, Williams-Beuren syndrome (1q11.23 microdeletion), and 1p36 deletion. Also, maternal uniparental disomy of chromosome 14 (Temple syndrome) and 7 (Silver-Russell syndrome) have been identified. The latter two genomic imprinting disorders, taken into consideration with MKRN3 mutations and Prader Willi, suggest the importance of epigenetic alterations in the pathogenesis of precocious puberty. (41)

Girls with severe primary hypothyroidism can develop true precocious puberty. These girls have elevated gonadotropins in addition to high TSH levels. The associated precocity may result

from cross-activation of the FSH receptor by the high circulating TSH or from direct stimulation of the ovary by the gonadotropins. Large ovarian cysts are not uncommon in patients with primary hypothyroidism and precocious puberty. These girls will have the atypical finding for precocious puberty of delayed bone maturation.

Occasionally, treatment and correction of long standing virilizing congenital adrenal hyperplasia will be followed by the development of true precocious puberty. It has been hypothesized that GnRH secretion and gonadotropin stimulation of the ovary may ensue in these patients after the removal of hypothalamic androgenic suppression.

Contemporary Issues for Management of CPP

The evaluation of true precocious puberty requires confirmation of true puberty, a careful physical examination with attention to growth charts, and evaluation for a central lesion. If a CNS lesion is present, the child will typically have a pubertal gonadotropin response to GnRH that is usually associated with idiopathic true precocious puberty and occasionally with a hamartoma. The mainstay of CNS evaluation is imaging of the CNS.

In addition, bone age X-rays are helpful to identify the advanced physiologic age associated with true precocious puberty. Precocious development that continues to progress is almost always associated with a marked increase in growth velocity and sometimes this rapid growth occurs prior to the presentation of precocious development (42).

The long standing gold standard in the diagnosis of central precocious puberty has been the GnRH stimulation test. Peak levels of LH greater than 3 - 5 mIU/ml 30 – 40 minutes following stimulation are highly suggestive of central precocious puberty. (43) After GnRH was no longer available, in the United States, a GnRH-agonist was substituted as the stimulus. Either test remains today the gold standard for diagnosis. The measurement of a single LH value 30 - 60 minutes after administration of a GnRH agonist (leuprolide acetate at 20 mcg/hg) was considered adequate for diagnosing CPP; an LH value greater than 9.2 mIU/ml at 30 minutes was diagnostic in one study. (43,44). Today, however, with the use of ultrasensitive LH assays, it has become standard to use basal LH serum levels as the routine for diagnosis, saving the gold standard stimulation test for those patients with inconclusive unstimulated basal results. (39) Generally speaking, LH values are unmeasurable before pulsatile GnRH is secreted in the prepubertal period. Random LH levels greater than or equal to 0.3 mIU/ml were 100% specific in one study for distinguishing CPP. (45) An unstimulated LH value of 1.1 IU/L or greater has been considered sufficient to assume that endogenous GnRH is being secreted and diagnostic for CPP in another study. (46,47) One should remember that exclusion of central precocious puberty does not rule out gonadotropin independent puberty.

Ovarian imaging and thyroid testing may also complement the evaluation. Estradiol levels are not really helpful in the diagnosis of precocious puberty with one exception. Levels vary tremendously and estradiol levels may be in age appropriate normal ranges in girls with central precocious puberty. If, however, levels are markedly elevated (above 100 pg/ml) then it is likely

that the patient either has an ovarian cyst or an ovarian steroid producing tumor such as a granulosa cell tumor.

While some CNS lesions will need treatment (often surgery), the majority of remaining causes of true precocious puberty (i.e., idiopathic) respond to GnRH analogues. It has also been demonstrated that precocity associated with hamartomas, which may intrinsically produce GnRH, may be effectively treated with GnRH agonists. (48) Analogues work by desensitizing the pituitary and decreasing the release of luteinizing hormone and follicle stimulating hormone. (49)

GnRH agonist therapy initially increases circulating gonadotropin and estradiol concentrations for short periods of time. Chronic therapy is associated with suppression of pulsatile gonadotropin secretion and a blockade to the LH response of endogenous GnRH. Suppression is best monitored with GnRH challenge tests although basal LH values may be substituted when there is no doubt about suppression. Some children who are initially suppressed will escape suppression and require increased dosages. Additionally, measurement of serum estradiol (if elevated on prior analysis), height, bone age, and assessment of secondary sexual characteristics may be helpful. Evaluation of ovarian morphology and uterine size by pelvic ultrasonography may, in some cases, provide additional evidence of such suppression. Cessation of menses, regression in physical pubertal signs (i.e., breast size and pubic hair), and a diminution of uterine and ovarian size usually occur within the first 6 months of therapy. (50) Optimal time for discontinuation of treatment has not been established, however, discontinuation at age 11 appears to result in optimal height outcomes.(51) Pubertal changes reappear within months after cessation of therapy with a mean time to menarche of 16 months.(52)

Analogues can be given in Depot formulations (IM or SC injections q4-12 weeks), as an implant (q4week to 12 month) or as a nasal spray (1-3 times daily). Leuprolide intramuscular injection is the only available depot preparation in the United States, and no studies have documented greater adherence to the multi-monthly dose compared to monthly dose. Injection site reactions occur in 10-15% of patients.(53) Histrelin, the once yearly subcutaneous implant, can suppress gonadotropin secretion for up to 2 years. (54) A minor surgical procedure is necessary for implantation and some site reactions have been reported, even a risk of infection. A recently published open label phase 3 multicenter histrelin study documented the efficacy in sustained gonadotropin suppression with yearly histrelin implants for up to 6 years of use. 52.8% of participants experienced site reactions, all of which were mild to moderate in sequelae. Additional difficulties with implant breakage (22%) at removal were noted. Gonadotropin levels returned to puberty levels within 6 months of implant removal.(50)

The literature does not include randomized controlled trials of long term outcomes for children with central precocious puberty treated by GnRH analogues. Predicted height has been shown to often improve after long-term GnRH agonist therapy; the absence of treatment has been associated with reductions of these height predictions (51,55). In one large study mean gains ranged from 3-10 cm in girls treated up to age 11 years after treatment with GnRH therapy (56).

In comparison, one small study of children followed for 12 years with slowly progressive precocious puberty did not demonstrate a loss of adult height without treatment. However, these studies often have flaws such as the calculations of gained height based on unreliable predicted heights.

A consensus document of 30 experts from Europe, the US, and Canada concluded that: "The efficacy of GnRH analogs in increasing adult height is undisputed in early-onset (i.e., girls under age 6 years) precocious puberty" (57). Those children who do not benefit may have the following characteristics: slowly progressive puberty, the precocity of which does not adversely affect the child; a normal predicted height prognosis; and a lack of evidence for gonadal activation (58). While consideration should be given to withholding treatment for these children, studies consistently demonstrate that girls presenting under age 6 years are able to subsequently achieve normal adult height because of the GnRH agonist therapy (59,60). Two of the most difficult decisions in the treatment of central precocious puberty are whether to initiate treatment in girls between ages 6-8 years and to decide what age to stop treatment (61). Since GnRH agonists decrease the aberrantly increased GH secretion seen in precocious puberty, some have suggested that these analogues may significantly suppress growth velocity enough to compromise the predicted improvement in height which could explain the ambiguity in studies regarding analogue impact on adult height. Some studies have evaluated the benefit of GnRH agonists with growth hormone (GH) and a recent meta-analysis suggested greater final height and predicted adult height with combination therapy, but no difference in final height standard deviation scores. (62) A prospective cohort study evaluated GnRH agonist alone (n= 17) vs GnRH agonist and GH (n=23) and followed subjects until final adult height was achieved. Final adult height was significantly greater than target adult height in the combination treatment group (4.86 +/-0.9cm vs 1.51 +/- 1.0cm, $p<0.05$) suggesting benefit to the addition of growth hormone to GnRH analogues in CPP. (63)

The psychological effects of central precocious puberty have not been adequately studied (57). Therefore, decisions regarding whether and when to initiate treatment or stop treatment based on psychosexual concerns rely on clinical expertise and expert opinion.

Incomplete, Isosexual, or Gonadotropic Independent Precocious Puberty (GIPP)

GIPP can originate from the gonads, the adrenals, from extragonadal or intragonadal sources of human chorionic gonadotropin, or from exogenous sources. In girls, **functionally autonomous ovarian cysts** are the most common cause of GIPP. An ovarian follicle up to 8 mm in diameter are common in normal prepubertal girls and may appear or regress spontaneously, but rarely secretes significant amounts of estrogen (64,65). An intriguing finding of the somatic cell mutation associated with McCune-Albright syndrome in the cells of one such cyst sheds light on this occurrence (66). GnRH agonists are not effective in treating autonomous cysts.

Juvenile granulosa cell tumors or theca cell tumors of the ovary are a rare cause of GIPP. Tumor markers for granulosa cell tumors include Inhibin B and müllerian inhibiting substance. Other ovarian neoplasms even more rarely seen in this age group that may also secrete either estrogens and/or androgens include gonadoblastomas, lipoid tumors, cystadenomas, and

ovarian carcinomas (67). Peutz-Jeghers syndrome has been associated with GIPP; the mucocutaneous pigmentation and gastrointestinal polyposis seen in this disorder has been rarely associated with gonadal sex-cord tumors (68).

McCune-Albright syndrome (MAS) classically includes the triad of hyperpigmented café-au-lait spots, progressive polyostc fibrous dysplasia of the bones and GnRH-independent sexual precocity (69). Some girls will present with vaginal bleeding preceding thelarche. Bone lesions and café-au-lait spots may increase over time. The actual clinical phenotypes vary markedly.

This disorder is caused by postzygotic somatic cell mutations of the gene encoding the alpha-subunit of the stimulatory guanine nucleotide binding protein Gs. These activating mutations stimulate constitutive G protein activation in affected cells with aberrant cyclic AMP production (70). The mutations may occur at various times in fetal development with a patchy tissue distribution of affected cells. Each of the associated findings is affected by these mutations: granulosa cells in the ovary, melanocytes of the skin (71), and the dysplastic bone cells (72,73). In addition to the classic triad, other endocrine cells may also be similarly affected and associated with their autonomous hyperfunction: pituitary adenomas, usually growth hormone secreting, hyperthyroid goiters (74), and rarely adrenal hyperplasia (75). Another recent finding is the presence of these same somatic cell mutations in cells from isolated hyperfunctioning ovarian cysts of GIPP patients who do not exhibit other findings of McCune-Albright Syndrome (66). This may account for the findings of “ovarian hyperfunction” in patients with GIPP as reported in the series of Table II above (24).

The sexual precocity of McCune-Albright syndrome is due to autonomously functioning follicular cysts. These patients can progress from GnRH independent to GnRH dependent puberty; when their bone age reaches the physiologic age of the normal time-onset for puberty, awakening of the arcuate nucleus for pulsatile GnRH secretion may occur and progress to the establishment of ovulatory cycles.

Approaches to treatment have included aromatase inhibitors such as Testolactone and selective estrogen-receptor modulators. Studies evaluating the efficacy have been uncontrolled. One study with Testolactone showed only early effectiveness, with loss of efficacy over time (76). Another study showed success with Tamoxifen in reducing vaginal bleeding (77). However the effect of Tamoxifen on height has not been adequately evaluated. One international multicenter trial evaluated the efficacy of monthly fulvesterant in 30 girls with MAS and followed them for a year. Days of vaginal bleeding, and bone advancement were less in the treatment patients. However, there were no changes in predicted adult height or frequency of ovarian cysts.(78) An open label study evaluating the effectiveness of letrazole on 9 girls with MAS for 12-36 months demonstrated reduction in rates of growth, vaginal bleeding and bone age. Ovarian volume, estradiol, and bone metabolism indices which showed initial improvement, began to rise after 24-36months of treatment. (79) When the shift from gonadotropin independent to gonadotropin dependent puberty takes place, GnRH analog therapy then becomes the first line therapy.

Iatrogenic sexual precocity

In prepubertal children, exogenous intake of estrogen has been shown to cause precocious pubertal development. Estrogen containing products may include variety of health or nutritional supplements and personal products such as hair products, lotions, and creams. Ingestion of estrogen containing meat has also been implicated although controversial. In actuality, these causes of precocious development appear to be extremely rare.

Premature Thelarche

Isolated precocious thelarche is a common entity and is associated with unilateral or bilateral breast enlargement without other signs of sexual maturation. It generally occurs at early ages up to 4 years, with approximately 80% presenting prior to age 2 years. The thelarche regresses spontaneously after diagnosis in over half of girls (80).

In all girls gonadotropin levels rise in the newborns after delivery and remain elevated for up to 4 years of age. While most newborns rarely exhibit a dramatic ovarian response to these elevated levels, it is likely that isolated precocious thelarche is a result of this physiologic process. The uterus remains prepubertal in size during this time, however, the ovaries may develop temporary follicular activity, and estradiol levels will be slightly higher than is seen in control girls. This is usually a benign self-limiting disorder not associated with bone age progression. However, clinical consideration should be given that the breast development could be the first sign of precocious puberty. A careful history and physical assessing for neurological symptoms and signs and assessment for growth by growth charts and a bone age should usually be performed.

Premature Menarche

Premature menarche has been reported as periodic vaginal bleeding without other signs of secondary sexual development (81). While this entity has been repeatedly yet rarely reported, pediatric vaginal bleeding can occur as the first manifestation of sexual precocity in most causes of GIPP listed above. These etiologies should be excluded before one considers premature menarche as the diagnosis. The differential diagnosis of vaginal bleeding in a child without other signs of sexual maturation is quite different than precocious development and includes foreign objects in the vagina (common) and vaginal tumors (rare).

Contrasexual precocity

Virilizing precocious puberty in girls and isolated precocious adrenarche

Most girls with contrasexual precocious puberty present with early appearance of pubic hair or hirsutism. The most common cause is a mild form of 21-hydroxylase deficiency, which is present in 0.1-1.0% of the population. Other more rare forms of congenital adrenal hyperplasia have also been identified in these patients. Virilizing adrenal (occasionally malignant) and ovarian tumors (e.g., Leydig or Sertoli cell tumors) in young girls can similarly present with virilizing precocious puberty. In actuality, most girls with appearance of pubic hair likely have **isolated precocious adrenarche**. While many of them have only early yet normal pubertal development (3), evidence exists that the prevalence of ovarian hyperandrogenism,

hyperinsulinism and dyslipidemia is increased in this population (82). These findings suggest that premature pubarche in some girls may be a childhood marker for insulin resistance and polycystic ovary syndrome.

DELAYED PUBERTY

An Overview of Delays within the H-P-O Circuit (Delays of Secondary Sexual Development and Menarche)

Several large descriptive studies have been published which have categorized the causes of pubertal/ menarchal delay. In 1981, a series of 252 female adolescents evaluated over 20 years at the Medical College of Georgia from a large referral area in Georgia was published (83). It included all patients seen with either delay of the onset of puberty or menarchal delay. The series was subsequently expanded to include 326 patients. In this series the most common causes of abnormal puberty were: **(1) ovarian failure (now called ovarian insufficiency) (42%); (2) congenital absence of the uterus and vagina (14%), and (3) constitutional delay of puberty (10%)**. While these 3 disorders comprised two-thirds of all patients seen, a host of less frequent disorders was also diagnosed (see Table III below); the most common of these included PCOD and idiopathic hypogonadotropic hypogonadism (IHH), both at 7% each.

Table III. Etiologic breakdown of 326 patients with abnormal puberty (pubertal and menarchal delay) (Medical College of Georgia Series) (84)

	Group total	No.	%
Hypogonadism (Pubertal Delay)			
Hypergonadotropic hypogonadism:			
Turner Syndrome	84		26
Chromosomally Normal	57		16
46,XX		48	15
46,XY		9	2
Total	**141**	**57**	**43**
Hypo(eu)gonadotropic hypogonadism:			
Reversible	62		18
Constitutional delay		32	10
Systemic illness		7	2
Eating disorders		9	3
Primary hypothyroidism		4	1
CAH		3	1
Cushing syndrome		1	0.5
Pseudopseudohypoparathyroidism		1	0.5
Hyperprolactinemia		5	1.5
Irreversible	37		13
Congenital Deficiency Syndromes			
Isolated GnRH deficiency		23	7
Forms of hypopituitarism		6	2
Congenital CNS defects		2	0.5
Acquired anatomic lesions			
Unclassified pituitary adenoma		2	0.5
Craniopharyngioma		3	1
Unclassified malignant tumor		1	0.5
Total	**99**		**31**
Eugonadism: (Menarchal Delay)			
Anatomic	59		18
Müllerian aplasia		45	14
Outlet obstruction			
Transverse vaginal septum		10	3
Imperforate hymen		2	0.5
Cervical atresia		1	0.5
Inappropriate feedback	22		7
Intersex disorders	5		1.5
Androgen insensitivity		4	1
17-ketoreductase deficiency		1	0.5
Total	**86**		**26**

In April of 2002, a more recent series of both male and female patients evaluated for delayed

puberty at Children's Hospital in Boston between January 1996 and July 1999 was published (85). This study, like the MCG study, included patients with delayed onset of puberty; it, however, did not include patients with menarchal delay. For the females reported (N=74), the 3 most common causes were: (1) constitutional delay of puberty (30%); (2) ovarian failure now called ovarian insufficiency (26%); and permanent hypogonadotropic hypogonadism (20%). Over 20 other numerically less frequently reported disorders were identified and listed below (see Table IV).

Table IV. Etiologic breakdown of 74 females with delayed puberty (Children's Hospital Series, 2002) Revised from Sedlmeyer, et al. (85).

	Group total	No.	%
Hypogonadism (Pubertal Delay)			
Hypergonadotropic hypogonadism:			
Turner Syndrome	5		7
Chromosomally Normal	14		19
46,XX		13	17
46,XY		1	2
Total	**19**	**14**	**26**
Hypo (eu) gonadotropic hypogonadism:			
Reversible (Functional)			
Constitutional delay		22	10
Systemic illnes			
Giardiasis		1	
Rheumatoid Arthritis		1	
Systemic lupus erythematosis		1	
Sickle cell disease		1	
Congenital heart disease		1	
Isolated seizure disorder		1	
Eating disorders			
Endocrine disorders		2	
Growth hormone deficiency		1	
Hyperprolactinemia		1	
Irreversible (Permanent)		15	20
Congenital/ Genetic Syndromes			
Kallmann syndrome		1	
Idiopathic Hypo Hypo		2	
CHARGE syndrome		2	
Forms of hypopituitarism			
Rathke's pouch		2	
Hypophysitis		1	
Hypopituitarism		1	
Panhypopituitarism with hearing loss		1	
Acquired anatomic lesions			
Craniopharyngioma		3	
Germinoma		1	
Ologodenrdroglioma		1	
Total	**51**		**67**
Other	**4**		**5**

Numerical and physical clues to the disorders presenting with delays in pubertal development: organizing the approach to the patient.

The **numerical findings** in these series point out several useful facts. First, most practitioners confronted with females presenting with pubertal delay can identify a few disorders that present in the majority of patients: ovarian insufficiency, constitutional delay, and permanent hypogonadotropic hypogonadism (as frequent causes of delayed onset of puberty) and vaginal

agenesis (as the most frequent cause of menarchal delay). Rather than wait until the ages defining female pubertal or menarchal delay (ages 13 and 15 or 16 years, respectively), a physical examination with inspection of the introitus, plotting the patients on growth charts (longitudinal and velocity), and obtaining gonadotropins values will identify many of these disorders even before these age definitions are met. Idiopathic hypogonadotropic hypogonadism (IHH), however, is the exception being more difficult to diagnose in the younger patients. It is often a diagnosis of exclusion in the late teenage years. Secondly, constitutional delay occurs in less than one-third of patients in any series. While constitutional delay is a frequent cause of delayed puberty it occurs with higher frequency in males, and less frequently in females. Two thirds of all females presenting with delayed puberty historically have had underlying pathology. Lastly, pubertal delay can be an ascertainment for the identification of a rare disorder (See Table II). Similarly, should any diagnosis be made during childhood years and in advance of the time for normal puberty, plans can be made prior to the pubertal years to begin treatment and to allow for the most normal pubertal progression as is possible. At least in the Children's Hospital setting, this appears to be the case for Turner syndrome for which the frequency of presentation with delayed puberty was decreased from the earlier MCG series.

The **physical findings** of the patients in these series also provide clues for helping us to form a differential diagnosis and organize our diagnostic approach. **First,** classification according to estrogen as in the MCG series allows for a separation of major etiologies.

Table V. Classification of Pubertal Abnormalities

I. Hypoestrogenism/ Hypogonadism (Delayed Onset of Puberty)
- A. Ovarian failure/ insufficiency (Hypergonadotropic)
- B. Hypothalamic-Pituitary Immaturity or Suppression (Hypogonadotropic)

II. Normal estrogen milieu/ Eugonadism (Delayed Menarche)
- A. Congenital absence of uterus and vagina (CAUV)
- B. Chronic Anovulation (e.g., PCOD and endocrine disorders)
- C. Intersex Disorders (e.g., Androgen Insensitivity)

The **absence of breast development** suggests a cause of hypogonadism: ovarian failure / ovarian insufficiency or a hypothalamic-pituitary problem. The practitioner can further narrow these possible etiologies by obtaining an FSH level; high levels suggest ovarian insufficiency and low normal values direct one to etiologies that have their effect at the level of the hypothalamus or pituitary. The **presence of breast development** usually directs one towards causes of menarchal delay suggesting the ongoing production of estrogen. One should remember, however, that some patients may have initiated puberty only then to have this process (and estrogen production) suppressed. Historically, biological evidence for estrogen or its lack has been more helpful than a single estradiol assay. A vaginal smear which demonstrates greater than 15% superficial cells, a positive progestin challenge test, the presence of endometrium on ultrasound measuring 1.5 mm or greater, or the presence of copious cervical mucus will usually confirm the suspicion of ongoing estrogen production.(86) There are currently no available studies for which evidence supports or refutes any one best method of determining the presence of sufficient ongoing estrogen production. Patients demonstrating breast development in the absence of evidence of ongoing estrogen production by any of these methods should be treated like any other hypogonadal patient.

Second, absence of pubic hair after age 13 years is a very significant clue of several specific abnormalities. Pubic hair growth results from both adrenal and gonadal androgen production. One should remember that even when the H-P-O circuit appears delayed, the H-P-A (adrenal) circuit should still be functioning and providing adrenal androgens. For most disorders of delayed onset of puberty, at least some pubic hair should be present because this H-P-A circuit is unaffected by the defect (ovarian insufficiency and IHH). When pubic hair is absent after 13 years, it suggests a defect of: (1) pituitary function (i.e., the inability to stimulate both ovarian and adrenal androgen production as in pituitary insufficiency); (2) steroidogenesis (i.e., the inability to convert cholesterol to androgens as in 17-hydroxylase deficiency); or (3) androgen receptors (i.e., the inability to translate the hormone signal into end organ androgenization as in Androgen Insensitivity Syndrome (AIS)). The first two of these disorders occur in the 46,XX

hypogonadal patients (Tables III and IV) and demonstrate defects within both H-P-O and H-P-A circuits, the common denominator being pituitary insufficiency or a steroid enzyme block. When examined they are found to have a normal müllerian system. 46,XY patients with 17-hydroxylase deficiency will present with absence of: (1) pubic hair; (2) breast development; and (3) a müllerian system. Androgen receptor defects are found in patients with normal breast development and absence of the vagina (i.e., AIS). Thus, for the patient with absent pubic hair after age 13 years, the most critical portions of the examination include the breasts and introitus.

Third, the apparent **absence of a müllerian system** (i.e., vaginal agenesis) can occur for either 46,XX or 46,XY patients. However, an examination, not a karyotype, is the most cost effective initial screen. Patients may present with absence of the vagina yet also demonstrate normal pubertal breast and pubic hair development. If a rectal examination is unrevealing for them, the likely diagnosis is congenital absence of the uterus and vagina (CAUV) also known as müllerian aplasia or Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome. If, instead, a bulging midline mass is identified just above the "absent vagina," the patient likely has either a transverse vaginal septum (TVS) or imperforate hymen. None of these findings warrant chromosomal studies as they clinically suggest the presence of a 46,XX karyotype. The patient found to have breast development and absence of both pubic hair and a müllerian system likely has AIS. These latter findings alone warrant a karyotype to confirm the 46,XY compliment and the need for gondadal extirpation. As stated above, the patient with absence of the müllerian system as well as thelarche and adrenarche likely has 46,XY 17-hydroxylase deficiency.

Fourth, identification of **stature significantly shorter** than one would expect for an individual whose growth was interrupted only by the delayed onset of puberty often reveals a genetic cause for both short stature and delayed puberty (e.g., Turner syndrome). Alternatively these findings could be the result of an endocrine cause which stopped growth several years earlier than the usual time onset for puberty in addition to preventing or slowing the onset of secondary sexual development (i.e., growth hormone deficiency, thyroid deficiency, or pituitary insufficiency).

DISORDERS IDENTIFIED IN PATIENTS WITH EITHER DELAYED PUBERTY OR MENARCHE

The remainder of this chapter will address specific concerns of the most common causes of the pubertal abnormalities identified in the two series described above. It will primarily refer to the data of the MCG updated series of 326 patients presenting with either delayed pubertal onset or delayed menarche tabulated in Table III and classified according to Table V above (84). In addition to discussing the common findings associated with these etiologies it will point out recent findings from molecular medicine and summarize contemporary treatment strategies.

Hypogonadism

Hypergonadotropic Hypogonadism

The single most common cause of delayed puberty in all prior delayed puberty series has been primary ovarian insufficiency (83,84). Forty-three percent of all patients seen in the MCG series had hypergonadotropic hypogonadism. The fact that ovarian insufficiency presenting at puberty

was numerically less frequent (i.e., 26%) in the recent Children's Hospital series suggests that more children are being diagnosed with Turner syndrome and other forms of ovarian insufficiency before the adolescent years and that treatment may be presently initiated at an earlier age (85). In future series of delayed puberty, primary ovarian insufficiency may all but disappear as an etiology; ideally these patients being diagnosed before the usual time onset of puberty with earlier initiation of treatment.

Turner Syndrome

Numerically, more patients with ovarian insufficiency and delayed puberty have had a form of Turner syndrome than were diagnosed with either 46,XX or 46,XY gonadal dysgenesis. Approximately 30% of the Turner patients have the classic 45,X karyotype with the remainder of patients having mosaic forms of Turner syndrome (Table VI below). Mosaicism refers to the presence of two or more cell lines, both of which originated from a single cell line. Patients with mosaic forms of Turner syndrome usually have a 45,X cell line associated with another cell line such as 46,XX or 46,XY. Other cell lines exist which represent structural abnormalities of the X chromosome such as isochromosome for the long arm of X, i.e., [i(Xq)] ; they may occur either as single cell lines or as mosaicism in association with 45,X.

Table VI. Karyotypes of patients with CIOF.

Reproduced with permission (83)

Classical Turner Syndrome (45,X)		**28***
Y Cell Lines		**16**
46,XY	1*	
45,X/46,XY	12	
45,X/47,XY	1	
45,X/46,X?del(Y)	1	
45,X/46,X,i dic(Y)/47,XY,i dic(Y)/ 46,XY/47,XYY	1	
Structural abnormalities of X		**31**
Isochromosome		
46,X,i(Xq)	7*	
45.X/46,X,i(Xq)	10	
45,X/46,X,i dic(Xq)	2	
45,X/46,X,i (Xq)/46,i (Xq),i (Xq)	1	
45,X/46,X,i (Xq)/47,X,i (Xq),i (Xq)	2	
Other		
46,X,t (X;X)qter-p22	1*	
45,X/46,X,del X (q13)	2	
46,X,Xq+	1*	
45,X/46,X,Xq+	1	
45,X/46,X,r(X)	1	
45,X/46,XX/46,X,r (X)/ 47,X,r (X),R (X)	1	
45,X/46,X,r	1	
46,X,del X (q25)	1*	
Other X mosaic cell lines		**9**
45, X/46, XX	8	
45,X/47,XXX	1	
Total	**84**	

* Single cell lines.

\+ Turner phenotype with intra-abdominal streak gonad and contra-lateral intra-abdominal testis.

All of the chromosomal findings in mosaic and non-mosaic patients with Turner syndrome have a common denominator: privation of either the entire X chromosome or a portion of the X chromosome. Fetuses with Turner syndrome have as many germ cells at mid gestation as do 46,XX fetuses. It is commonly believed that the loss of critical X chromosome-linked ovarian determinant gene(s) (87-89) is the cause of accelerated loss of germ cells (90) due to a defect of follicular development as noted by Jirasek et al. Many of these individuals lose all of their follicles with associated germ cells before birth. Some of them lose the remaining germ cells during childhood years and before puberty. Less than 15% of patients with Turner syndrome

will lose their follicles (with germ cells) either during or after the pubertal process (83). Five percent of patients with Turner syndrome will have enough follicles (i.e., germ cells and surrounding granulosa cells) remaining at puberty to not only initiate the pubertal process but also to allow them to have regular, cyclic menses during at least a portion of their adolescent or adult years; 2-5% may spontaneously become pregnant.(91,92)

Once the germ cells are prematurely depleted from the ovaries, the only remaining tissue present is the connective stroma of the gonads. It usually appears as a ribbon of white connective tissue located beneath the fallopian tubes and along the pelvic sidewalls (90). These residual gonads have the appearance of "streaks" and are referred to as streak gonads. The presence of a Y cell line in a patient with Turner syndrome brings with it a 15-25% risk of developing malignant germ cell tumors within those streak gonads. In those particular patients the streaks need to be surgically removed as soon as a diagnosis is made. For all patients with Turner syndrome, privation of X chromosomal material is associated with the variable Turner stigmata, cardiovascular and renal abnormalities, and the development of a number of specific medical problems. Turner stigmata include short stature, high arched palate, low hair line and webbed neck, multiple pigmented nevi, short fourth metacarpals, shield chest, increased carrying angle of the arms (cubitis valgus), and lymphadema of ankles, to name a few.

These stigmata related to loss of X-chromosomal material are variably present in Turner patients. Furthermore, reports of phenotypic-karyotypic correlations have been inconsistent (83,93). Several observations and hypotheses have been made that help understand these relationships or lack thereof. First, it has often been felt that the presence of physical findings associated with Turner syndrome is dose dependent, i.e., the higher the percentage of 45,X cells the greater the likelihood of such abnormalities. While this makes the greatest sense intuitively, not all studies have been able to demonstrate a relationship between karyotype and phenotype (83). Recently, when ascertainment was considered, better correlations were made dependent on the degree of mosaicism. Patients found incidentally by prenatal karyotyping had fewer phenotypic features of Turner syndrome than those diagnosed after birth because of a clinical suspicion (94). Another explanation suggests that X chromosome gene imprinting exists and that some of the findings of Turner syndrome are related to the parental origin of the missing X chromosome in Turner patients (95).

Short stature is the one consistent phenotypic finding of Turner syndrome (83). The MCG series was reported prior to the treatment of Turner patients with growth hormone. The fact that none of the patients in that series was taller than 63 inches (160 cm) in height supported the tenet that statural genes are located on both arms of the X chromosome. The knowledge of consistent short adult stature, often under 5 feet (152 cm), and the potential psychological effect it has in combination with other features of Turner syndrome, provided impetus for identifying therapies independent from estrogen treatment for these patients. Many hundreds of Turner patients have now been treated with growth hormone pushing the final adult stature beyond this 63-inch (160 cm) mark for some and certainly past the predicted final height for many other Turner women.

The most serious somatic abnormalities found in patients with Turner syndrome are those involving the heart and great vessels. Cardiovascular disease is the primary cause of early mortality in women with TS with standard mortality ratios of 3.5 (CVD) to 24 (congenital anomalies).(96) Most of the mortality results from cardiac malformations, which have been reported in up to 50% of patients and include coarctation, pseudocoarctation, bicuspid aortic valves (separately between 30 and 45% incidence), and a host of other anatomic variants of the vascular tree, especially in the area of the ascending aorta. The high prevalence of these abnormalities has been reported in the years following the NIH consensus panel as has the recommendations for routine MRI screening (97-99). 1.4% of Turner patients have been estimated to develop dilation of the ascending aorta with subsequent dissection and rupture; most have died after being misdiagnosed with another cause of the chest or epigastric pain (97-101). Most patients with dissection and rupture of the ascending aorta have had a cardiac congenital malformation, hypertension, or pre-existing dilation. At least 10%, however, have had neither an identifiable risk factor including aortic dilation nor an aorta diameter above the previously held risk size (i.e., > 5 cm) (101). Several explanations have been given for dissection and rupture in patients not felt to be at risk. First, this occurrence has been associated with the pathohistologic entity of cystic medical necrosis of the vessel wall, the culprit of similar clinical outcomes in patients with Marfan syndrome. This suggests that there is an inherent defect of the vessel wall that predisposes all Turner women, with or without risk factors, for this occurrence (100). Second, prior measurements have not taken into account the fact that women with Turner syndrome are smaller and thus should have smaller size aortas. When the aorta size was normalized to body surface area in a study of 166 adult Turner patients and compared to a control population (n=26), over 30% of the Turner women had an ascending aorta measurement that was larger than that of 95% of the control population (99,101). As a result, new guidelines have been suggested for those aorta measurements above which significant risk for rupture exists (99,101,102).

Pregnancy may be the largest single risk factor for dissection and rupture of the aorta in Turner patients. There are nearly a dozen reports in the literature of death occurring during, immediately after or even more remotely removed from pregnancy in Turner patients who became pregnant from oocyte donation and embryo transfer. This gathering body of literature supports the fact that the cardiovascular (i.e., increased blood volume and stroke volume) and potential hormonal changes of pregnancy (perhaps remodeling of vessel wall by estrogen or progesterone) place these patients at a high risk of dissection, rupture of the ascending aorta, and death (101,103-105). A conservative estimate of a 2% maternal mortality rate has been reported from a US national survey and is 100 fold greater than the death rate for all causes during pregnancy (103). Similarly, a French study demonstrated a maternal mortality of 2.2%.(106) While death usually occurs during pregnancy, some evidence suggests that changes of the aorta during pregnancy may increase the risk of rupture in future years as well. The report of a more recent Nordic cohort study of pregnant Turner women did not find maternal deaths but did report: 35% hypertensive disorders; 20% of patients with pre-eclampsia; and a 3.3% potentially life threatening problem, (107) Further prospective longitudinal data are needed to understand the absolute risk to these women during pregnancy. It would be ideal if IVF registries included this information.

A number of other medical conditions may also be found in Turner patients. Horseshoe kidney is the most common renal abnormality observed and a number of autoimmune disorders, commonly Hashimoto thyroiditis, are diagnosed. Given the higher incidence of specific medical conditions for women with Turner syndrome than the general population, the NIH study group guidelines recommend continued monitoring of hearing and thyroid function, screening for hypertension, diabetes, and dyslipidemia as well as aortic enlargement (98).

Normal Chromosomes

The second largest group of young women with primary ovarian insufficiency has a 46,XX karyotype **(46,XX gonadal dysgenesis).** For them, some have a genetic etiology. An autosomal recessive form of this disorder was previously suggested by the presence of sibships reported in which several non-twin sisters are affected with ovarian insufficiency (83). The reports of mutations in candidate autosomal genes of affected patients provides support for the belief that autosomal etiologies exist for patients with 46,XX gonadal dysgenesis and premature ovarian insufficiency (POI). However, the more consistent finding that approximately 2% of sporadic and 14% of familial cases of 46,XX ovarian insufficiency have premutations for the fragile X syndrome makes this the current most likely explanation for the presence of 2 or more sisters with ovarian insufficiency (108). In addition, a number of other known **genetic disorders** have also been associated with POI including myotonia dystrophica, ataxia telangectesia, galactosemia, blepharophimosis-ptosis-epicanthus inversus syndrome, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome, and proximal symphalangism. In addition, **infiltrative diseases** such as mucopolysaccharidoses and **environmental etiologies** such as childhood viral illnesses may also cause premature depletion of oocytes from the ovaries. This is suspected in identical twins reported to be discordant for ovarian insufficiency (83). While mumps can cause orchitis in males, it is suspected that viruses such as mumps may cause oophoritis and loss of oocytes as well. Patients previously treated for childhood malignancies such as Wilms tumor, may develop germ cell depletion as a result of **radiation therapy or chemotherapy** (e.g., alkylating agents).

Probably the most common cause of premature primary ovarian insufficiency in women with a 46,XX karyotype is **autoimmune**. For the group of patients for whom an abnormality is not identified, autoimmune is considered the most likely cause. These patients have an increased risk for developing other autoimmune endocrine abnormalities such as thyroiditis with thyroid dysfunction, hypoparathyroidism, and adrenal insufficiency. In addition, pernicious anemia has been reported in some of these patients. They should be screened on a routine basis for thyroid dysfunction and the other endocrinopathies, if symptomatic. Previous recommendations for patients with 46,XX POI included annual screening with a.m. cortisol levels followed by an ACTH stimulation test in those whose a.m. cortisol levels measured less than 17 – 20 mcg%. Subsequently, given the low prevalence of adrenal insufficiency in these patients, it was suggested that such screening be contemplated only when Addisonian symptoms presented. The NIH has a high referral ascertainment of POI patients with adrenal insufficiency. Studies of these patients have now shown that routine screening for the presence of adrenal steroid or 21-

hydroxylase antibodies is effective to identify patients at-risk for adrenal insufficiency and, once identified, ACTH stimulation testing can follow (109).

As one would suspect, in the absence of an identifiable genetic etiology for depletion of the oocytes, more of the 46,XX gonadal dysgenesis patients present at puberty with residual germ cells after the initial insult than do those with Turner syndrome. In the MCG series of patients, nearly 40% of them had enough follicles at puberty to mount a pubertal response before presenting with amenorrhea and ovarian insufficiency (83). A number of patients with 46,XX gonadal dysgenesis will actually go through the pubertal process and have cyclic menses before developing ovarian insufficiency and amenorrhea in their late teens or 20's. Some of these patients who spontaneously go through puberty will also have reversal of ovarian insufficiency for indeterminate periods of time and rarely become pregnant during these times of spontaneous menstrual function. It is because of this natural history of POI that includes the reversal of the disease process in some patients that the term previously used for this condition by Fuller Albright, *ovarian insufficiency,* has been revived by some current authorities (109,110).

It is difficult to understand accurately the numeric breakdown of the different etiologies of ovarian insufficiency in pubertal delay patients. Reports of large series of such patients exhaustively studied to determine cause do not currently exist. There is, however, information regarding the breakdown of different etiologies in a large French cohort (N=357) of ovarian insufficiency patients spanning the ages of 11 to 39 years from which inferences may be made for the younger population (111). In that series, 7.8% of patients with POI had an identifiable genetic cause including chromosomal abnormalities (not Turner syndrome) (2%), FMR1 pre-mutations (2%), molecular alterations of genes thought to be etiologic (i.e., FSHR, GDF9, BMP15) (2%), congenital disorders of glycosylation (0.2%), and autoimmune polyglandular syndrome (APS) type 2 and multiple autoimmune disease (0.8%). In addition, 10% of the patients presented with an autoimmune disorder not identified as genetic. Ovarian insufficiency in the remainder of women was considered idiopathic.

Rare patients present with **46,XY gonadal dysgenesis**. These are patients who likely have mutations in a gene controlling testicular morphogenesis such as the SRY gene, often referred to as the master switch for testicular development. While only approximately 15% have SRY mutations, there are now a number of genes both upstream and downstream in expression of SRY for which mutations may alter testicular development. As a result, the germ cells that arrive at the genital ridge will organize in the cortical, rather than medullary region of the undifferentiated gonad. For these classic patients with 46,XY gonadal dysgenesis, however, germ cell loss is complete before birth. Since they never develop testes, they will not produce müllerian inhibiting substance to ablate the developing müllerian system. They will also not produce androgens to allow for masculinization of the external genitalia.

Historically these 46,XY individuals were labeled with Swyer syndrome; at birth they have a normal female phenotype with a normal vagina, uterus and fallopian tubes., i.e., complete 46,XY gonadal dysgenesis or sex reversal (112). At puberty, they do not initiate pubertal development and are found to have elevated gonadotropin levels. They do not have other phenotypic

abnormalities like the patients with Turner syndrome. They are often tall because of the presence of a Y chromosome. 46,XY individuals with gonadal dysgenesis have the highest risk for developing germ cell tumors of their streak gonads of any individuals with gonadal dysgenesis and a Y chromosome cell line. The streaks must be removed as soon after diagnosis as is reasonable. Less frequently, partial forms of this syndrome have been found to exist often in association with other systemic anatomic or medical conditions such as polyneuropathy, adrenal insufficiency, and even sudden infant death syndrome (113,114).

Molecular Findings

Turner syndrome. While Turner syndrome is considered to result from haploinsufficiency of critical loci or regions of the X chromosome and a number of putative genes have been identified, a molecular understanding of mechanisms involved is far from understood. A number of the stigmata and malformations of Turner syndrome have been thought to be caused by edema present during development because of an abnormal lymphatic vascular system and thus abnormal lymphatic drainage. As such, the abnormalities are actually deformations. For example, edema of the nail beds causes nail hypoplasia, edema of the neck causes cystic hygromas and webbed neck, and edema of the kidneys prevents them from migrating around the aortic bifurcation and results in horseshoe kidney. The presence of cystic hygromas during fetal life is also associated with coarctation of the aorta; lymphatic drainage back to the heart is sufficiently abnormal during development to cause this cardiac malformation and likely some of the other anatomic variations of the vascular tree that have been found in these patients.

One region of the X chromosome, Xp11.2-p22.1, has been thought to include “Turner syndrome loci”, as a number of associated features including ovarian insufficiency, short stature, high-arched palate, and autoimmune disease have been mapped here (115). Deletions of the X-chromosome linked SHOX gene has explained many of the dysmorphic skeletal features of Turner syndrome including the short stature (11). While not consistently reported, it has generally been thought that the number of phenotypic findings of Turner syndrome are related to the percentage of cells that are 45,X; the implication being that mosaic patients have fewer findings than do those with a single 45,X cell line. As stated above, a recent correlation between some of the findings associated with Turner syndrome suggested an imprinting effect with the variation in phenotype at least partially explained by the parent of origin of the remaining X chromosome. Renal abnormalities, for example, were exclusively found in patients retaining their maternal X chromosome (95).

Prior karyotypic/phenotype correlations have suggested that the proximal regions of both the p and q arms of the X chromosomes are most critical for maintenance of the germ cell compliment (93). However, terminal deletions at the telomeric regions of these arms are also associated with oocyte depletion, although to a lesser degree. Deletion of these regions are more likely to result in POI after some period of ovarian function rather than a complete loss of germ cells evident at the start of the teenage years as is more commonly seen with the proximal deletions.

Early molecular studies of patients with POI and translocations between the X- chromosome and autosomes identified 2 regions of the long arm of the X chromosome within the

translocation breakpoints which were felt to harbor important ovarian determinant genes. POF1 (Xq26-q28) (116) contains several candidate genes (HS6ST2, TDPF3, GPC3) (116) and one known to be associated with POI, the Fragile site Mental Retardation 1 (FMR1) gene. POF2 (116,117) (Xq13.3-q22), the human homologue of the *Drosophila melanogaster* diaphanous gene, contains several candidate genes for which one, (DIAPH2), has been disrupted in POI (118,119). Other loci on the X chromosome have also been identified as important in maintenance of a normal oocyte compliment. Members of the Transforming Growth Factor-β (TGF-β) superfamily proteins are known to have key functions within the oocytes and granulosa cells. Of them, Bone Morphogenetic Protein 15 (BMP15 or GDF9) is produced by a gene (BMP15) mapped to Xp11.2 (120). Mutations within this gene have been associated with POI (121-123). While the list of X-chromosome candidate genes for ovarian determinants is ever growing, 2 genes known to be important in drosophila ovarian development or oogenesis are the DEAD-box 3 (DBX) and the Ubiquitin-Specific Protease 9 (USP9X) genes. Both of these genes, are located within the human Xp11.4, an area known to escape X inactivation. It would appear that a double dosage of all of these genes, especially DBX and USP9X, is required for normal ovarian function. Mutations, interruption, or loss of one of these genes results in premature loss of germ cells from the ovaries. It is possible that mutations within these loci are responsible for ovarian insufficiency in women with intact X chromosomes as they likely are in patients with Turner syndrome. All in all, there appear to be numerous gene loci on both arms of the X-chromosome responsible for ovarian development and function. It is no wonder that all of the Turner variant chromosomes, each with different portions of the X chromosome missing, result in POI.

The most studied of the X-chromosome genes associated with POI is the FMR1 gene. When mutated by a CGG triple nucleotide repeat expansion the result is fragile X syndrome. As in many triple nucleotide repeat disorders, areas of normal repeat sequence may be predisposed to expansion during or before meiosis. Function of the gene is maintained within a given number of these triple repeats but when a certain threshold is reached gene function may be adversely altered. For the fragile X gene (FMR1), a CGG repeat sequence occurs with up to 60 such repeats being normal. Expansion to over 200 such repeats leads to fragile X syndrome; the high level of repeats causing hypermethylation of the promoter and silencing of the gene. Interesting observations were made that female carriers of the premutation of this locus with an unstable intermediary level of repeats (i.e., 60-199), often had POI. Best evidence suggests that this premutation is associated with a 21 fold greater chance of developing POI and that 2% of sporadic and 14% of familial ovarian insufficiency patients harbor this unstable intermediate trinucleotide repeat. Similarly, microdeletions of the FMR2 gene are associated with the same predisposition to POI (124).

46,XX Gonadal Dysgenesis.

The list of genes involved in ovarian development and maintenance of the germ cell compliment has continued to expand as molecular analysis of patients with 46,XX gonadal dysgenesis and POI has revealed etiologic mutations. Some patients have mutations within one of the X-chromosome loci. For others, mutations have been found within autosomal genes, some that are associated with **syndromic POI** and others with **nonsyndromic forms**. Additionally, many,

but not all POI or gonadal dysgenesis etiologies are associated specifically with the premature loss of germ cells. Examples of known genes for which mutations have been shown to cause **syndromic forms** of premature loss of germ cells include the Autoimmune Regulator (AIRE) gene causing autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or APECED (125), the Forkhead-Transcription-Factor-Like 2 (FOXL2) gene causing blepharophimosis-ptosis-epicanthus inversus syndrome (126), and the Galactose-1-Phosphate Uridylytransferase (GALT) gene causing galactosemia (127) located on chromosomes 21, 3, and 9, respectively. Myotonic dystrophy is an autosomal triple repeat disorder, like the fragile X premutation carrier state, that is similarly associated with premature loss of germ cells from the ovary. Autosomal genes for which mutations have been associated with **nonsyndromic** premature loss of germ cells include Inhibin A (INHA) (another member of the TGF-β family), NR5A1 (SF1), and NOBOX (128-131). Of these, mutations in SF1 have been most commonly found, first in 46,XY gonadal dysgenesis patients, and more recently in patients with primary and secondary amenorrhea with 46,XX ovarian insufficiency (130). Other autosomal candidate genes are currently under study (e.g., DAZL). It would appear that all of these mutations cause loss of germ cells.

Most previous studies have focused on single gene mutations and POI. However, it is likely that some etiologies of POI are multifactorial in nature with synergism between different genes and other epigenetic factors, particularly in complex diseases such as POI. After a recent GWAS study found associations between ADAMTS19 gene mutations and POI, further interest in the role of ADAMTS genes in ovarian development and function spurred additional genetic studies. (132) ADAMTS is expressed in the embryonic phase of gonadal development being important for angiogenesis and organ morphogenesis. SNP analysis found significant epistasis between SNPS in IGF2R and specific diplotypes for ADAMTS19 in women with POI. The authors hypothesized that since IGF2R is important in steroidogenesis and ADAMTS genes are regulated by progesterone, women with SNPs and diplotypes for these two genes are at higher risk of POI.

Our molecular understanding of **hypergonadotropic hypogonadal** patients has revealed a number of patients with seemingly **normal ovarian development** for whom germ cell depletion is not the cause of the elevation of gonadotropins. Rather, in these patients, the **inability for steroid production** is usually the cause of the hypergonadotropic state; and, hence the classification of ovarian insufficiency. The first such classic syndrome, Savage syndrome, was originally described as gonadotropin resistance. Initially, a number of families were identified in Finland in which 46,XX individuals with gonadotropin resistance were found to be homozygous for a single mutation of the FSH receptor gene (133-135). Reports of additional mutations have since accumulated throughout other parts of the world (136,137). Subsequently, other 46,XX hypergonadotropic patients have been identified with mutations in the LH receptor (138-142), the FSH β (143,144), and the LH β genes. Overall the result of these disorders is a lack of estrogen production and variable hypergonadotropic states. 46,XY individuals with homozygous or compound heterozygous mutations of the LH receptor gene do not masculinize in-utero and present during adolescence with a female phenotype, delayed puberty, and hypergonadotropic hypogonadism. Their gonads, however, are testes not ovaries.

The second classic hypergonadotropic state that has long been described in association with otherwise normal gonads is 17 α-hydroxylase deficiency. Both 46,XX and 46,XY individuals present with delayed puberty and a female phenotype, and ovaries and testes, respectively. Mutations have been also found in this gene (145,146). Similarly, mutations of the aromatase gene in 46,XX individuals have been identified and associated with delayed puberty and hypergonadotropism in these individuals (14,147-149). In contradistinction to the other hypoestrogenic syndromes, aromatase deficiency, however, is associated with elevations of androgens in-utero and at puberty and the predictable but variable degrees of masculinization in these otherwise phenotypic females. Finally, the fascinating report of a 46,XY patient identified with a mutation in the CBX2 gene suggests a new syndrome for which hypergonadotropism is associated with seemingly a normal ovarian architecture (150). When reported, this child was under 5 years of age. A more recent cohort series of 47 patients with disorders of sexual development did not find any pathogenic mutations in CBX2 mutations within their subjects. It is likely that mutations in CBX2 are a rare cause of gonadal disorders of sexual development.(151)

46,XY Gonadal Dysgenesis.

Our understanding of Swyer syndrome (46,XY gonadal dysgenesis/sex reversal) together with 46,XX sex reversal helped to identify the SRY gene on the Y chromosome short arm (152). Common thought has held that SRY expression is the essential signal in the process of testicular morphogenesis. Hence, SRY has been seen as the master switch for this process. However, only 15% of women with 46,XY gonadal dysgenesis have been found to harbor mutations in this gene (153,154). The fact that the remaining 46,XY gonadal dysgenesis patients have intact Y chromosomes and that most 46,XX true hermaphrodites studied have not been found to harbor SRY sequences provides evidence that other genes are present and necessary for testicular development either upstream or downstream in expression to SRY. Such conjecture has been replaced with an ever growing list of now known genes operative in this pathway of testicular morphogenesis. Mutations of the WT1 (155,156), SOX9 (155,157-160), DSS (161), SF-1 (114,162), DAX-1 (160), Desert Hedgehog (DHH) (113,163), TSPYL1 (164), and CBX2 (150) genes have all been associated with specific syndromes and 46,XY sex reversal. Of these, the most frequently reported and best characterized involves the SOX-9 gene and the accompanying syndrome of Campomelic dwarfism.

Contemporary Issues for Management

Patients identified with ovarian insufficiency will need evaluation for associated medical disorders. For Turner syndrome, the most commonly identified acquired medical condition is thyroiditis. For them, the most dangerous abnormalities involve cardiovascular malformations. While previously it has been well known that coarctation of the aorta occurs more frequently for these patients as does bicuspid aortic valves, it is now evident that these patients are also at increased risk of developing dilation of the ascending aorta (and less commonly at other vascular sites) with subsequent dissection and, if undiagnosed and untreated, rupture. Like patients with Marfan syndrome, they appear to have cystic medial necrosis as the predisposing vascular histopathology. Similar to Marfan syndrome, the increased cardiovascular demands of pregnancy also appear to increase significantly this risk. The NIH consensus panel has

suggested that all Turner patients have a baseline echocardiogram and, if normal, then a cardiac MRI (98). Additional evidence suggests that the MRI measurements of the aorta should be normalized for body surface area (99). Subsequent studies should be repeated every 3-5 years and perhaps during each trimester of pregnancy if patients are willing to take a risk estimated to be at least 2% for maternal mortality and, for those who survive a potentially increased risk after exposure to pregnancy.

All Turner patients should be counseled about their increased risk of dilation, dissection and rupture of the ascending aorta that is increased with pregnancy. Since most previous deaths occurred after misdiagnosis, Turner patients should be counseled to make health care providers aware of this possible diagnosis when being evaluated for disproportionate symptoms of indigestion and upper abdominal or chest pain. During dissection, the patient may have abnormal phonation and experience unusual coldness and sensations in their legs. It is possible that most deaths could have been avoided with timely diagnosis and surgical repair. Turner syndrome patients need evaluation for horseshoe kidney and for other less frequently diagnosed autoimmune disorders such as diabetes, hypertension, dyslipidemia, and hearing impairment (98).

Treatment of patients with Turner syndrome includes not only hormone replacement for pubertal progression and health maintenance at least through age 50 years, but an even earlier consideration for growth hormone treatment. While there were some initial conflicting reports, general consensus is that the use of growth hormone for enhancing adult stature is a worthwhile endeavor (165-178). The initiation of estrogen therapy at an age concordant with normal endogenous ovarian production (i.e., at least by ages 9 to 11 years) has always been considered important for normal psychosexual development of the adolescent. However, it is also believed that such early estrogen replacement might also result in an earlier closure of epiphyses and a potential limitation of final adult stature. The use of growth hormone therapy initiated during the childhood years may allow a more normal childhood stature (concordant with mid parental height) and the earlier initiation of estrogen therapy obviating these concerns (168,179,180). Synergistic benefits of low dose estradiol and GH treatment for these patients when begun as early as 5 years of age can add 2.1 cm to adult height, beyond the 5cm gain expected from GH therapy when combined with estradiol at 12 years of age. However, inappropriate feminization at a young age and unknown long term consequences of early estradiol supplementation limit the widespread use of adding estradiol to GH therapy in these young patients.(181) Other techniques to increase adult height include the delay of pubertal induction or the addition of oxandrolone until 15 years of age. Studies suggest that this technique can add up to 4cm of adult height but also raise concerns about effects of delayed puberty on bone health and the psychologic impact of delayed secondary sex characteristics.(174,182-184) Oxandrolone, an anabolic steroid, has significant side effects such as virilization and liver dysfunction, limiting its use.(185)

Most women with TS build their families utilizing oocyte donation due to premature oocyte depletion and ovarian insufficiency; an increasing number of them with gestational carriers due to the risk of death from aortic dissection (2%) during pregnancy. (186) A Nordic cohort of 106

women with TS who had a live birth after donor oocyte IVF reported 20% risk of preeclampsia, and potential life threatening complications in 3.3%, however no deaths occurred. The one woman with an aortic dissection had normal pre-pregnancy imaging. 9% of this cohort had a known cardiac defect before pregnancy. (107)

Due to the potential delayed depletion of oocytes in some TS women, there may exist a potential for fertility preservation in those women with regular menses. Case reports document the feasibility of oocyte cryopreservation in post pubertal girls (ages 13-15) with Turners syndrome who already had evidence of diminished ovarian reserve. A range of 4-13 oocytes have been cryopreserved. (187-189)

Given all of these considerations for natural reproduction in these women with TS, counseling is critical to provide them the most accurate information regarding risks and benefits. Turner syndrome remains a relative contraindication to pregnancy, and if risk factors are present it becomes an absolute contraindication. Until better data are available or prophylactic treatment of the aorta is developed that provides protection for pregnancy, counseling should be provided that includes alternatives such as use of a gestational carrier or adoption.

Patients with 46,XX gonadal dysgenesis should be evaluated for premutations of the fragile X (FMRI) gene. This finding should prompt counseling for themselves and other family members and prohibit use of their similarly affected sisters as oocytes donors. In addition, 46,XX ovarian insufficiency patients should be screened regularly for the development of Hashimoto thyroiditis and at least at baseline for adrenal steroid cell or 21-hydroxylase antibodies. Continued surveillance should be considered for the presence of hypoparathyroidism, adrenal insufficiency, and other autoimmune disorders such as pernicious anemia. All gonadal dysgenesis patients with a Y cell line need extirpation of their gonads including Turner patients with 45,X/46,XY (or those with a Y chromosome fragment) gonadal dysgenesis and the 46,XY gonadal dysgenesis patients. One should remember that rare Turner patients with seeming a single 45,X cell line might have undetected mosaicism for a Y cell line. Screening 45,X single cell line patients and those individuals with an unidentified chromosomal fragment with Y-DNA centromeric probes may be prudent to uncover those additional individuals at-risk for gonadal malignancies.

All patients with premature gonadal failure need estrogen therapy for initiation and completion of pubertal progression and subsequently for the maintenance of a multitude of health processes. While the continued accrual and remodeling of bone is of utmost importance, it remains likely that numerous other physiologic processes are dependent on normal estrogen status as well, at least through 50 years of age. The findings and concerns for long term hormone replacement of the Women's Health Initiative do not apply to these or any other patient prior to the age of 50 years and should not be used to prematurely stop their hormone replacement.

Counseling is of utmost importance for these individuals and should cover expectations for all aspects of these young women's lives including alternatives for reproduction. While the use of

donor oocytes and IVF has proven safe for 46,XX and 46,XY gonadal dysgenesis patients, an estimated maternal death rate of at least 2% exists for Turner syndrome patients and pregnancy may increase the risk for rupture in future years. While it is often easier to include pregnancy by donor oocyte as an alternative during counseling, until more information is available such discussions should be framed with the above concerns. One should also turn to patient guidelines of national organizations such as the American Society for Reproductive Medicine (ASRM) and the American College of Obstetricians and Gynecologists (ACOG) as they are developed about these issues. The use of "buddy programs" in which these patients are paired with others who have previously confronted the same issues during adolescence and support groups (e.g., Turner Syndrome Society) is an excellent complement to this counseling.

Hypogonadotropic Hypogonadism

A number of young women will present with delay of the onset of pubertal development who have no evidence of ongoing estrogen production, because something has interrupted either GnRH or gonadotropin secretion from the hypothalamus/pituitary. Patients with constitutional delay of puberty represent the most common of these disorders. Other disorders are clearly congenital or acquired.

Constitutional delay

Constitutional delay of puberty refers to a common condition for which patients will go through puberty but at a time that is more than 2.5 standard deviations delayed from the mean (Tables III and IV) (83-85). A number of these patients often have a family history of delayed puberty (85). Their physiologic age (i.e., bone age) lags behind that of their peers and is manifested by a delay in the adolescent growth spurt and temporary short stature. Most of these patients present between 13 and 16 years of age and at that time have very early signs of thelarche. Their gonadotropin levels are in the low to normal range and their workup is otherwise unrevealing.

Until recently, no specific mutations had been identified as causing constitutional delayed puberty, despite the observation that 50-80% of those with this disorder have a positive family history. A recent proband study evaluated families with delayed puberty for some of the common mutations found in idiopathic hypogonadotropic hypogonadism (IHH). They found that subjects with constitutional delayed puberty more commonly shared the same mutation with affected family members compared to non-affected family members (53% vs 12%, $p = 0.03$). They even found that subjects with delayed puberty without a similar family history were more likely than controls to carry mutations commonly seen in IHH (14.3% vs 5.6%, $p = 0.01$)(190).

In males, 60% of pubertal delay is constitutional. In females, however, no more than 30% have this benign reproductive condition. While constitutional delay represents a leading cause of female pubertal delay, prior emphasis on this statistic has led to the false diagnosis for many young women and the misguided reassurance that they were simply "late bloomers." As many as two-thirds of females presenting with delayed puberty will have an irreversible etiology for reproductive failure, not constitutional delay (83). For this reason, any patient presenting with delayed puberty and given the label of constitutional delay should be scrutinized very carefully

for other etiologies, especially if they are beyond age 16 years and have yet to initiate pubertal development.

It can be challenging to differentiate constitutional delay from IHH. Given the finding of similar mutations observed in some of both groups of patients, these disorders may, in fact, fall in the same spectrum, one being reversible and the other not. Numerous tests have been proposed to help distinguish the two; however, none have been particularly helpful. When previously performed, an intravenous GnRH challenge test usually confirmed early awakening of the hypothalamic-pituitary-ovarian circuit by demonstrating a pubertal gonadotropin response, i.e., a greater release of LH than FSH. Such a response is seen only after endogenous GnRH secretion occurs and puberty is at or beyond its very early stages. At the same time, this early gonadotropin release produces the multifollicular ovarian appearance of early puberty; the ultrasound appearance of which is likely as reassuring that puberty is marching onward as is the LH response of a GnRH challenge. The most helpful distinction between IHH and delayed puberty is the failure to enter puberty by the age of 18 years. However, many patients and their parents may not readily adopt the wait and see tactic and instead may prefer additional periodic assessments. One option is to follow with pelvic ultrasound studies looking for the appearance of the multifollicular ovary associated with the early stages of pubertal progression. It would be ideal that no adolescent would reach mid teenage years without spontaneous or exogenously-induced pubertal development!

Acquired Abnormalities

A number of acquired medical conditions may interfere with either the production of GnRH and/or gonadotropin secretion producing a hypogonadotropic hypogonadal state (Tables III and IV) (83,85). The Children's Hospital series refers to many of these as functional disorders (85). Endocrine disorders such as hypothyroidism, congenital adrenal hyperplasia, Cushing syndrome, and hyperprolactinemia that begin before or during the early pubertal process may interfere with gonadotropin secretion. While only some cases of growth hormone deficiency are acquired, this disorder is included here with the other endocrinopathies. Patients with unusually short stature, pubertal delay, and low gonadotropin levels should be considered as having one of the endocrinopathies that also affects growth (i.e., hypothyroidism and growth hormone deficiency). Treatment of these disorders will allow the resumption of puberty. Systemic illnesses including malabsorption states, eating disorders, active autoimmune diseases, and the rare hypoxemic states related to congenital heart malformations or severe anemias (i.e., sickle cell) are also occasionally etiologic for hypogonadotropism and pubertal delays. Most of these conditions are similarly reversible. Finally, pituitary tumors are consistently reported in rare patients of all descriptive delayed puberty series (83). The craniopharyngioma occurs usually between the ages of 6 and14 years prior to the usual time onset of puberty. It is an aggressive tumor that causes early destruction of the pituitary and suprasellar regions and usually delays any pubertal development. On the other hand, it can also be an indolent tumor not becoming apparent until the late teenage years or even the mid 20's. The typical calcification of these tumors makes them easily diagnosed radiologically. Unlike the craniopharyngioma, the prolactinoma usually does not develop until after puberty is initiated. Estrogen is known to increase messenger RNA for prolactin and its increase at

puberty is seemingly associated with the development of prolactinomas in at-risk individuals. For these patients, the prolactinoma usually arrests a pubertal process that has begun on time. These tumors are extremely slow growing and rarely interfere with other pituitary functions, if at all. If a dopamine agonist is given to lower the prolactin levels, puberty or menstrual function will usually proceed normally. The prolactinoma now outnumbers the craniopharyngioma as a cause of hypogonadotropic hypogonadism (83).

Congenital Abnormalities

A number of disorders classically felt to be irreversible are found in patients with hypogonadotropic hypogonadism. Some of these patients present with fractional or complete pituitary insufficiency. The majority of patients have been historically categorized with idiopathic hypogonadotropic hypogonadism (IHH) and, despite the fact that specific causes have now been identified for as many as 30% of them, the label of IHH has persisted. Such patients have absence of spontaneous pubertal development that persists beyond age 18 years; hypogonadotropism is usually the isolated pituitary deficiency for them. Specifically they have functional GnRH deficiency. Numerous studies involving frequent blood sampling have demonstrated 4 different aberrant patterns of gonadotropin secretion. The majority of patients with IHH demonstrated apulsatile secretion and the remainder were divided between sleep entrained pulsitility, decreased pulse frequency, and decreased pulse amplitude (191).

Both **syndromic and nonsyndromic etiologies** exist. Kallmann syndrome (KS) refers to IHH with anosmia or hyposmia. The association of IHH with anosmia is not surprising given that the GnRH secretory neurons originate within the olfactory placode and then migrate to the hypothalamus extending their axons to the median eminence. Normosmic IHH (nIHH) refers to those IHH patients with a normal sense of smell. A number of genes have been identified that regulate development and migration of GnRH neurons, the production, processing and secretion of GnRH, and its expression at the receptor. (192) Mutations have been identified within these genes which result in both KS and IHH and will be discussed further in this chapter. X-linked KS and some of the patients with mutations in these other genes may have unilateral renal agenesis (KAL1 mutations in males), midline facial defects, or neurologic and skeletal abnormalities (193,194).

It has always been intriguing that variable phenotypes have existed within families harboring the same IHH mutation (193-195). Perhaps more intriguing have been the reports that 10% of males with IHH, some with mutations within genes regulating GnRH neuronal development or secretion, have reversal of their disorder and spontaneous continued reproductive function after discontinuation of treatment that may have been given for months or years (196). Recent studies of adult onset hypogonadotropic hypogonadism in males with prior reproductive function have also reported finding the same mutations shared by those men who never initiated puberty. A series of 32 male patients with hypogonadotropic hypogonadism were assessed after treatment withdrawal and 6% had recovery of gonadal function.(197) Similarly, reversible hypogonadotropic hypogonadism has been reported after years of treatment in women, one who also had anosmia (personal patient, reported only in abstract form, Goldstein, Fertil Steril 2011,96: S116,), and an adult onset form has been identified in women with hypothalamic

amenorrhea sharing similar mutations.(198) Taken together, with the prior information about similar mutations in some patients with constitutional delay of puberty, what was previously labeled as IHH appears often to be a part of a spectrum disorder with overlap between constitutional delay of puberty (spontaneous early resolution), irreversible forms in both males and females, late onset forms in individuals who first established reproductive function, and late reversible forms in patients with prior longstanding hypogonadotropic hypogonadism. All of these forms of hypogonadotropic hypogonadism have been shown to have some patients with mutations in the same genes.

A number of other genetic defects have been found to cause hypogonadotropic hypogonadism such as leptin deficiency and adrenal hypoplasia congenital (193,199-206). Besides IHH, forms of hypopituitarism also exist and result in delayed puberty with hypogonadotropism. Included are septo-optic dysplasia (SOD) (207,208), combined pituitary hormone deficiency (CPHD) (209-212), CHARGE syndrome (213,214), Prader-Willi Syndrome, and Laurence-Moon-Bidel-Bardet Syndromes. Finally, other forms of hypopituitarism exist, some of which are associated with anatomic abnormalities such as Rathke's pouch cysts, anterior encephalocele, and hydrocephalus (83).

Molecular Findings

As in the patients with hypergonadotropic hypogonadism, molecular research has provided new insight into the clinical findings of a number of patients with hypogonadotropism. In particular, these studies have helped to better understand the variation of clinical presentation and gonadotropin levels, and the different responses to exogenous GnRH reported in these patients. For men with Kallmann syndrome, the first mutations found were those involving a cell surface adhesive gene, the KAL1 gene (215-217). The initial identification of these mutations began our understanding of the anosmia and hypogonadotropic state for KS patients; such mutations prevent normal development of the neurologic tract responsible for transport of GnRH to the median eminence and the olfactory bulb (193,218-221). Subsequently, a number of these men were also found to have unilateral renal agenesis. While similar mutations have not yet been identified in anosmic females, it is likely that a few will ultimately be uncovered. The second molecular finding involved nIHH patients and was the identification of mutations in the GnRH receptor gene (222-225). Since then, a host of mutations have been identified in hypogonadotropic patients; genes involved generally have their adverse effects in the hypothalamus, pituitary, or both.

Hypothalamic defects that are etiologic for KS and/ or nIHH involve mutations in genes responsible for GnRH production (GNRH1 gene) (226), GnRH processing (PCSK1 gene) (227-229), GnRH neuronal development that prevents subsequent normal transport through the neuronal pathways to the median eminence [FGFR1 (215,230-235), FGF8 (236), PROK2, PROKR2 (237) and CHD7 (238) genes in addition to the KAL1 gene], and GnRH secretion (GPR54 or KISS1 and receptor genes) (33,34,190,239,240) into the portal circulation.

Those genes for which mutations have been identified as a cause of IHH primarily at the level of the **pituitary** include the GNRHR, HESX1 (207,208), PROP1 (209,210,241), SOX2 (242),

SOX3 (243), LHX3 (211,212), LHX4 (244,245), LHβ (246), and FSHβ (144) genes. Except for GNRHR or gonadotropin β gene mutations, the other mutations produce a host of phenotypic findings that often include other pituitary or endocrine deficiencies. Mutations within the leptin (201,202), leptin receptor (204,206), and NROB1 (DAX1) (247,248) genes appear to cause IHH within both the **hypothalamus and pituitary**. The former mutations are associated with extreme obesity (201,202). Finally, additional mutations yet to be fully understood have been found in IHH patients in the TAC3, TACR3, and nasal embryonic LHRH factor (NELF) genes (249). Numerically, the most commonly found mutations among IHH patients are those within KAL1 (men only), the FGFR1, CHD7 (CHARGE syndrome), and GNRHR genes. Interestingly, the least common and last to be identified are the mutations in the GNRH gene.

The identification of all of these mutations gives us tremendous insight into the requirements and signals for normal pubertal development It appears that the pubertal process is well orchestrated between a number of different genes and a mutation in any one of them may result in the absence of pubertal development. Given the findings of KISS1 and KISS1R mutations in a few patients with central precocious puberty, if there is a single signal for the pubertal process among all of the genes identified it is likely kisspeptin. The other genes identified in these patients with hypogonadotropic hypogonadism appear to provide the framework within which the reproductive system works. We now know that a number of genes are involved in laying down the normal neuronal transport pathway for GnRH. Some are sufficiently tightly involved with the optic bulb development (KAL1) that all patients with mutations have anosmia. Mutations in others (FGFR1) may result in either anosmic or normosmic IHH. It also appears that if a mutation exists in one of the genes that prevents normal neuronal development (e.g., FGFR1), rarely sufficient development may ultimately occur in the absence of this seemingly critical protein either with time or induced from hormone therapy such that reversal of this disorder may occur in a few patients (196). There seems to be overlap between these genes as well, given that patients may be compound heterozygotes with two mutations and each in a different gene (249). In addition, several patients have presented with a KS-like phenotype and found to have mutations in CHD7 gene, usually etiologic for the CHARGE syndrome (238).

Contemporary Issues for Management

As has been elaborated, numerous different disorders exist for patients presenting with hypogonadotropic hypogonadism. Many of these are rare and best managed by specialists who treat the specific disorder, each disorder having very specific individual clinical concerns. It should be determined early whether treatment of the disorder will allow subsequent pubertal progression or whether a form of hypogonadotropism exists for which puberty will not progress without sex steroid replacement. Early hormone therapy is critical for the management of such patients. Similarly important is the individual counseling about expectations for pubertal development, associated problems, reproductive options, and chance of recurrence or reversal. No doubt, this may require a multidisciplinary team approach. An interesting finding of the Children's Hospital study was that it provided evidence that there may be an association between hypogonadotropic hypogonadal causes of delayed puberty and attention deficit disorder with or without hyperactivity (85). Finally, as more and more gene mutations are identified in IHH patients, an understanding of minor phenotypic findings associated with them

may make earlier diagnosis possible. When seen, for example, in an extremely obese adolescent, leptin or leptin receptor mutations should be considered.

Eugonadism

The MCG series presented a third group of females with pubertal abnormalities and evidence of ongoing estrogen production. These patients primarily present with delayed menarche.

Anatomic Abnormalities

Congenital absence of the uterus and vagina (CAUV), also known as müllerian aplasia or Meyer-Rokitansky-Kuster-Hauser-syndrome (MRKH), is the second most common cause of pubertal aberrancy in the MCG series (84). In particular, these patients present with delayed menarche. They have fusion failure of the two müllerian anlagen during embryogenesis. The normal fusion process is usually followed by canalization of the vagina. In its absence, small uterine remnants and their attached normal fallopian tubes remain; the vaginal plate and uterine remnant(s) are uncannalized. Rare patients will have a variable degree of uterine fusion and/or variable foci of functional endometrium (250). These patients progress through puberty at the normal time. They present with normal pubertal development and delayed menarche and on examination are found to have isolated absence of the vagina. They have normal ovarian function. Nearly 30% of these patients have concomitant renal abnormalities, including unilateral renal agenesis, horseshoe kidneys and urethral duplication. From 12 to 50% of these patients will have associated skeletal abnormalities, scoliosis being the most common and limb defects such as lobster claw hand deformity and phocomelia rarely present (83). Other abnormalities may also occur.

Another group of patients who may present with an anatomic cause of delayed menarche are those with an imperforate hymen or rarely a transverse vaginal septum (TVS). Given the average age of menarche, most girls with an imperforate hymen will present several years before the age of 15 years and thus may not be “labeled” as presenting with primary amenorrhea. While a complete TVS causes a presentation similar to imperforate hymen, the majority of patients with a TVS will have perforations in their septum and will not present with absence of menses.

Patients with an imperforate hymen or complete TVS initiate puberty at the normal time and present with cyclic pain, on average, within 1 to 2 years after menarche. Being obstructed, they develop an hematocolpos with or without an hematometra. On examination they are found to have an obstructing membrane, the thin imperforate hymen often bulging on valsalva maneuver or a thicker TVS. The latter is usually located at the junction of the upper one-third of the vagina but can be at lower levels as well and because of its thickness usually does not bulge on valsalva. Once these obstructing membranes are surgically excised normal menstrual function usually follows. In contrast to patients with outlet obstruction, those with vaginal agenesis will usually have normal hymeneal tissue and either an absent vagina or a small pouch created by attempts at coitus. For them, there is never a midline mass on rectal exam.

Molecular Findings

Because patients with CAUV were never previously able to have children, the inheritance pattern for most of them has been generally unknown and clues for potential candidate genes have remained elusive. The majority of these patients are sporadic occurrences within their family. Rare sibships with several non-twin sisters affected have been reported and twins both concordant and discordant for CAUV also exist (83). A report of the outcome of pregnancy for these patients who were able to have their own biological children through IVF utilizing a gestational host suggests that this condition is not commonly autosomal dominant; none of the female babies were found to be similarly affected (251).

A number of genes have been proposed as candidate for harboring germ-line mutations etiologic for the syndrome of CAUV. The anti-Müllerian hormone (AMH), anti-Müllerian hormone receptor (AMHR), and other genes involved in the pathway of AMH directed müllerian regression (e.g., the β-catenin gene) have been considered likely candidates. Since a number of somatic systems are involved in this syndrome, studies have centered around developmental genes and in particular, the HOX family of genes. In addition, HOXA10 is expressed in the developing paramesonephric ducts. Mutations in HOXA13 have been associated with the hand-foot-genital syndrome and in HOXD13 have caused synpolydactyly in humans. Furthermore, the PBX1 gene protein is thought to be a HOX cofactor during müllerian and renal development. Other developmental gene candidates considered have included the PAX2, Wilms tumor transcription factor (WT1), and WNT4 genes as well as genes controlling the synthesis of retinoic acid receptors, the RAR-gamma and RXR-alpha genes. The latter 3 of these genes, when mutated in mice, have produced müllerian abnormalities. Finally, given that cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations cause congenital absence of the vas in men and that the early wolffian anlagen seemingly direct müllerian development in females, this gene too has entered the list of suspects.

Our laboratory has performed mutation analyses for a number of these candidate genes in müllerian aplasia patients including CFTR (252), WNT7, AMH (253), AMHR (253), HOXA10 (254,255), HOXA13 (256), galactose-1-phosphate uridyl transferase (GALT), PAX2 (257), WT1 (258), and WNT4 (259). Studies by others have not found mutations in HOXA7, HOXA13, PBX1 (260), β-catenin (261), RXR-α, and RXR-γ genes (262). To date worldwide, excepting WNT4, none of these analyses have revealed a convincing association.

Several patients with congenital absence of the uterus and vagina have now been identified with mutations in the WNT4 gene (263-265). These patients all seem to have a variation of the classic presentation of congenital absence of the uterus known as Mayer-Rokitansky-Kuster-Hauser syndrome. In addition to müllerian aplasia, these patients have signs or biochemical evidence of androgen excess and either modified location of their ovaries (in two patients) or seemingly hypoplastic ovaries (in the third patient). Their phenotype is very similar to that of the WNT4 knockout female mouse: absence of the müllerian system associated with aberrant androgen overproduction and premature loss of follicles (266). Studies of these 3 patients have given further insight into the role of WNT4 in human reproductive development and steroidogenesis. Given the infrequency of these mutations in patients with congenital absence

of the uterus and vagina, however, some have proposed that it is, in fact, a specific entity (264,265,267). A study of ovarian steroidogenesis and oocyte number in patients with müllerian agenesis undergoing IVF for transfer of embryos to a gestational carrier did not find impairment in either of these parameters (268). This further supports that WNT4 mutations are rare and a specific entity.

With the development of next generation sequencing and its ability to investigate genetically heterogeneous diseases, whole exome sequencing is utilized for diseases for which causative genes have not yet been identified. A recent whole exome sequencing and copy number variation case series in women with MRKH showed high frequency in loss of function variants of the OR4M2 and PDE1 1A genes and deletions in 15q11.2, 19 q13.31, 1pq36.21, 1q44, suggesting new candidate genes in the development of MRKH. (269)

One may question why, except in a rare phenotype that seems to be a different entity (i.e., patients with WNT4 mutations), no individuals with classic Mayer-Rokitansky-Kuster-Hauser syndrome have been found to harbor a mutation in a host of very likely candidate genes? Explanations might include: (1) the presence of mutations in yet-to-be-studied candidate genes; (2) multifactorial inheritance; or, (3) the presence of nonconventional genetic mechanisms. The latter seems to be an attractive explanation. In particular, this condition has the characteristics of disorders such as McCune-Albright Syndrome that are caused by somatic cell rather than germ-line mutations; somatic cell mutations occur at some point after fertilization in the dividing somatic cells of the embryo or in stable somatic cells later in life. They are almost never present in the germ cells. As a result the patient is usually a random occurrence within a family and neither inherits nor passes this condition on to the next generation. If this occurs during development (such as seen in McCune-Albright syndrome), the mutated somatic cells will migrate to various areas of the fetus; the phenotype always being consistent, but often with some variation dependent on the final location of the affected cells. The vast majority of patients with Mayer-Rokitansky-Kuster-Hauser syndrome are the only such affected member of a family. The consistent phenotypic findings in these patients all involve the loss of structural integrity (müllerian aplasia, renal agenesis, and bone defects) and some degree of variability exists with which specific system is involved. Patients with scoliosis, lobster claw defects and congenital amputations represent the extreme variation. Somatic cell mutations would easily explain each of these occurrences. The report of identical twins, one with isolated vaginal agenesis and the other with bilateral tibial longitudinal deficiency (congenital leg amputations) (270) makes a strong case that somatic cell mutations beginning in the initial embryo migrated to the bones in one twin and to the developing müllerian system of the other, after the process of identical twinning. Unfortunately, if, in fact, somatic cell mutations are etiologic for most cases of müllerian aplasia and involve genes that cause loss of structural integrity, the cells with the culprit mutations may no longer be present for analysis. They may have been in the original cells of the now absent uterus, vagina, kidney or bone. A recent comparative study of different tissues (blood, saliva, rudimentary uterus) in 5 pairs of discordant monozygotic twins found differences in copy number variations utilizing SNP microarray technology in the affected twin compared to non-affected twin in the following genes: MMP-14, LRP- 10, ECM, and neoangiogenesis genes. There were no differences between the mutation analyses in saliva,

but similar differences in the blood and uterine tissue, mesodermal derivatives, suggesting a tissue specific mosaicism.(271)

For the transverse vaginal septum and imperforate hymen patients, molecular analysis has been essentially nonexistent.

Contemporary Issues for Management

The diagnosis of CAUV is essentially clinical. The classic finding of absence of the vagina or a vaginal pouch (usually developed through prior coital attempts) associated with otherwise Tanner stage 5 breast and pubic hair development is unlikely anything else but CAUV. A search for associated physical findings of bony malformations (commonly scoliosis) and rarely inguinal hernias or scars from prior repair should be conducted. The inguinal hernias occur because the round ligaments can pull the unconnected uterine remnants and associated fallopian tubes and ovaries into the inguinal canals. The diagnosis of CAUV can be confirmed simply by a pelvic ultrasound study that demonstrates the presence of ovaries with follicular activity. The midline uterus will not be seen. **Neither a karyotype nor laparoscopy is necessary for the diagnosis in the majority of CAUV patients.** The prepubertal patient could be misdiagnosed with AIS. However, post-pubertal the clinical findings for CAUV and AIS are sufficiently different that diagnosis of each is usually straightforward. If in doubt, a serum total testosterone level is the least expensive method of resolving the confusion; levels within the female and male ranges will differentiate the two conditions. One must now always consider, however, the WNT4 mutation syndrome for which patients with müllerian aplasia may manifest symptoms or biochemical evidence of androgen excess and reduced ovarian reserve.

Although not currently recommended as first-line management, treatment of this condition has previously been surgical; a number of different surgical techniques have been utilized for creation of the vagina. In the United States, the McIndoe vaginoplasty has been the most commonly performed surgery for neovaginal creation. This is the classic procedure in which a skin graft is sewn around a mold and inserted into a newly dissected vaginal space. After a skin graft takes, the patient wears a vaginal mold for an extended period of time and until regular coitus to prevent scarring down of the neovagina. In other parts of the world and some areas of the US, the Vecchietti procedure is more commonly performed. In this procedure an olive shaped instrument is placed at the perineal dimple and pulled inward under tension by attached wires, sutures, or threads stretching the perineal skin in the direction of the normal vaginal axis. The tension cords were originally placed by abdominal surgery and in more recent years have been placed by laparoscopy (272-276). Another procedure, the Davydov procedure, was developed in Russia and is gaining popularity worldwide including the US (277,278). In this procedure, laparoscopic assistance is used to bring peritoneum from the pouch of Douglas into the space created for the neovagina. A purse-string suture is placed at the top and the neovagina is created. Results of both of these alternatives have been overall very encouraging (279-281).

The majority of patients, however, can avoid surgery altogether and should be encouraged to attempt creation of a neovagina first by the Ingram dilation technique (282,283). Experts have

agreed that the nonsurgical approach should be the first line approach because it is successful in approximately 90% of patients, is less morbid than surgery, and is not associated with possible contracture (284,285). A vaginal dilator is held in place at the vaginal dimple with athletic underwear. The patient then sits on a bicycle seat of a stationary bicycle or a specially designed chair for regular periods of time. The size of the mold is increased over time and until a normal sized vagina is created or coitus can be initiated. With motivated patients and careful instructions and follow-up the majority of patients will succeed. When new patients are paired up with prior successful CAUV patients for support, this method rarely fails. Patient pairing is particularly helpful for the emotional support and personal advice that only women who have weathered the various challenges of this condition can provide.

The assisted reproductive technologies have provided these women a means of having their own biological children. The use of gestational carriers with IVF after oocyte retrieval and fertilization has made this possible. Given that the CAUV patient and her husband are the biological parents of these children, legal issues involving the gestational carriers are certainly better delineated and problems arising from them much less likely than were the initial uses of surrogacy.

Recent advances in uterine transplantation have led to the first live born infant and several additional pregnancies from a transplanted uterus to patients with MRKH. The Swedish team spent years of preparation and experimentation beginning with animal models understanding basics of the surgeries involved as well as immunosuppression. They developed separate teams for removing and implanting the uteri. In some parts of the world, including Sweden, the use of gestational carriers is banned. As a result, uterine transplantation is the only hope in these countries for having a biological child for these women with MRKH. Since this therapy remains highly experimental and fraught with both medical and ethical concerns regarding potential surgical complications as well as issues from immunosuppression, it should only be performed by teams as well prepared as the Swedish team, who has completed this remarkable feat.

Counseling patients with vaginal agenesis and other disorders of sexual development (DSD) requires special skills and sensitivities and is covered briefly at the end of this chapter.

The imperforate hymen and the transverse vaginal septum are surgically treated by one of a number of procedures described in most gynecologic textbooks. These procedures are usually straightforward. Occasionally the transverse vaginal septum is difficult and requires more involved surgery including an abdominal approach, a Z-plasty or skin graft. None-the-less, only an experienced surgeon should perform all of these procedures.

CHRONIC ANOVULATION

Polycystic ovarian syndrome (PCOS) and a number of other endocrine abnormalities may result in chronic anovulation and may present as delayed menarche as reported in the MCG series (83). Although most patients with PCOS present in adolescence with menstrual irregularity, occasionally a patient will present with primary amenorrhea. If patients are

androgenzied and have not menstruated they should be evaluated by at least age 14 years as covered above. These patients may not have their first menses until given a progestin challenge. While most of them have classic PCOS, other endocrinopathies and hypothalamic dysfunction need to be ruled out. The contemporary management of PCOS and its associated gynecologic and metabolic disorders includes evaluation for diabetes and hyperlipidemias and consideration for treatment of it as an insulin resistant state in addition to the classic management considerations of ovarian suppression, endometrial protection, as well as androgen targeted treatments. This topic is covered in greater detail elsewhere in this text.

DISORDERS OF SEXUAL DEVELOPMENT

Patients with **androgen insensitivity** present at puberty with normal onset of breast development, absent pubic hair, and delayed menarche. These 46,XY women have been found to harbor mutations in their androgen receptor genes that render their androgen receptors nonfunctional. Despite normal testes development and normal male testosterone production, they are unable to convert the testosterone signal into the end organ events of masculinization of the external genitalia in-utero or at puberty. They present with a normal female phenotype and a small blind vaginal pouch. At puberty, their androgens are converted to estrogens with normal breast development. They are usually taller than predicted by mid-parental height for females because of the presence of the Y chromosome and its associated statural genes. The presence of the Y chromosome places them at risk for developing malignancies of their gonads and dictates removal. Unlike gonadal dysgenesis patients, the risk does not increase until after puberty; additionally, these tumors are usually seminomas rather than the gonadoblastomas or germ cell tumors. Unless the testes are located within the inguinal canals, they are usually left in place until after breast development is complete.

Molecular Findings

Androgen insensitivity syndrome has been extensively studied by molecular analysis (286,287). A number of intriguing and frustrating findings have been made. First, mutations have been found in virtually every portion of the androgen receptor (AR) gene (288). Mutations in the hormone binding region of the AR gene have explained those classic patients previously determined to have nonfunctional androgen receptors. Mutations in the DNA binding domain helped explain why other AIS patients with the same classic phenotype had normally binding androgen receptors. Second, many families studied have mutations unique to their specific family (286). Until gene sequencing is routine, this precludes studying patients with a suspicious AIS phenotype for a specific AR mutation. Third, identification of mutations in this gene has widened the spectrum of incomplete AIS phenotypes to include phenotypic females with genital ambiguity, phenotypic males separately with undermasculinization (289), gynecomastia, breast cancer (290), prostatic cancer, or azoospermia/severe oligospermia (291). Fourth, individuals with the same mutations have exhibited varying phenotypes (288,292,293). Finally, clinical correlations have been made between specific mutations and the ability to masculinize further with exogenous androgens for those individuals with a male sex of rearing and not presenting as delayed female puberty (294,295).

Contemporary Issues for Management

For the classic patient with AIS who presents with delayed menarche, absent pubic hair, and a vaginal pouch, an expedient evaluation and diagnosis is necessary. Unlike the CAUV patients, once the diagnosis of AIS is suspected, chromosomal analysis is necessary to document a 46,XY karyotype. It is necessary to remove the gonads in patients with AIS (296). This can be done after puberty to allow spontaneous breast development. Support for this includes the fact that the earliest reported malignancy in patients with AIS is 14 years of age.

No doubt, one of the most critical issues related to this syndrome is counseling. No longer is it possible or advisable to hide the presence of the 46,XY finding from these patients. However, a multidisciplinary and well thought out approach and close follow-up is needed for such counseling. The psychosexual transition during adolescence is difficult and patients with intersex disorders/disorders of sexual development will face an even more difficult transition. Patients and their family require support and should be actively involved in the decision processes. Links to a variety of support groups for specific disorders can be found on the Disorders of Sexual Development website (296,297).

Many of these patients have a vaginal pouch, the embryonic remnant of the prostatic utricle. For them, coital attempts will enlarge the vagina and surgery is not needed. For others a similar, although somewhat different, approach can be utilized as was described for the patients with CAUV. Furthermore, once gonadectomy is performed, estrogen replacement therapy is essential for all of the obvious reasons.

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