University of Helsinki
Pediatric Graduate School
Hospital for Children and Adolescents
Helsinki University Hospital
Helsinki, Finland

TIMING OF PUBERTY

Genetic Regulation

KAROLIINA WEHKALAMPI

Academic Dissertation

To be publicly discussed, with the permission of the Faculty of Medicine,
University of Helsinki, in the Niilo Hallman Auditorium,
Hospital for Children and Adolescents, Helsinki,
on December 5th, 2008, at 12 noon.

Helsinki 2008

Supervised by

Professor Leo Dunkel
Department of Pediatrics
Kuopio University Hospital and University of Kuopio
Kuopio, Finland

Docent Tiina Laine
Hospital for Children and Adolescents
Helsinki University Hospital and University of Helsinki
Helsinki, Finland

Reviewed by

Docent Jarmo Jääskeläinen
Department of Pediatrics
Kuopio University Hospital and University of Kuopio
Kuopio, Finland

Docent Kristiina Aittomäki
Departments of Clinical and Medical Genetics
Helsinki University Hospital and University of Helsinki
Helsinki, Finland

Official opponent

Professor Jorma Toppari
Departments of Pediatrics and Physiology
Turku University Hospital and University of Turku
Turku, Finland

ISBN 978-952-92-4787-5 (nid.)
ISBN 978-952-10-5124-1 (PDF)
http://ethesis.helsinki.fi
Helsinki University Print
Helsinki 2008

To my beloved

CONTENTS

ABSTRACT

In the general population, the timing of the onset of puberty is normally distributed. This variation is determined by genetic and environmental factors, but the exact mechanisms underlying these influences in the control of pubertal onset remain elusive. The purpose of this study was to gain insight into genetic regulation of pubertal timing. Contributions of genetic versus environmental factors to the normal variation of pubertal timing were explored in twins. Familial occurrence and inheritance patterns of constitutional delay of growth and puberty, CDGP (a variant of normal pubertal timing), were studied in pedigrees of patients with this condition. To ultimately detect genes involved in the regulation of pubertal timing, genetic loci conferring susceptibility to CDGP were mapped by linkage analysis in the same family cohort.

To decompose the overall phenotypic variance of pubertal timing into genetic and environmental components, genetic modeling based on monozygous twins sharing 100% and dizygous twins sharing 50% of their genes was used in 2309 girls and 1828 boys from the FinnTwin 12-17 study. The timing of puberty was estimated from height growth, i.e. change in the relative height between the age when pubertal growth velocity peaks in the general population and adulthood. This reflects the percentage of adult height achieved at the average peak height velocity age, and thus, pubertal timing.

Boys and girls diagnosed with CDGP were gathered through medical records from six pediatric clinics in Finland. First-degree relatives of the probands were invited to participate by letter; altogether, 286 families were recruited. When possible, families were extended to include also second-, third-, or fourth-degree relatives. The timing of puberty in all family members was primarily assessed from longitudinal growth data. This enabled objective and similar estimation of pubertal timing in family members of different ages, and of both genders. Delayed puberty was defined by onset of pubertal growth spurt or peak height velocity taking place 1.5 (relaxed criterion) or 2 SD (strict criterion) beyond the mean. If growth data were unavailable, pubertal timing was based on interviews. In this case, CDGP criteria were set as having undergone pubertal development more than 2 (strict criterion) or 1.5 years (relaxed criterion) later than their peers, or menarche after 15 (strict criterion) or 14 years (relaxed criterion). In first-degree relatives, the timing of puberty was mostly assessed from growth charts (80%), and in second-degree relatives, by interviews (60%). Familial occurrence of strict CDGP was explored in families of 124 patients (95 males and 29 females) investigated in two hospitals from Southern Finland. In linkage

analysis, we used relaxed CDGP criteria; 52 families with solely growth data-based CDGP diagnoses were selected from all clinics.

Based on twin data, genetic factors explain 86% and 82% of the variance of pubertal timing in girls and boys, respectively. In families, 80% of male and 76% of female probands had affected first-degree relatives, in whom CDGP was 15 times more common than the expected 2.5% prevalence in the general population. In 74% (17 of 23) of the extended families with only one affected parent, familial patterns were consistent with autosomal dominant inheritance (affected pedigree members of both sexes transmitting CDGP to roughly 50% of their offspring). By using 383 multiallelic markers and subsequently fine-mapping with 25 additional markers, significant linkage for CDGP was detected to the pericentromeric region of chromosome 2, to 2p13-2q13 (multipoint HLOD 4.44, α 0.41).

The findings of the large twin study imply that the vast majority of the normal variation of pubertal timing is attributed to inter-individual differences in genetic factors. Moreover, the high frequency of dominant inheritance patterns and the large number of affected relatives of CDGP patients suggest that genetic effects also markedly contribute to constitutional delay of puberty. Detection of the locus 2p13-2q13 in the pericentromeric region of chromosome 2 associating with CDGP is one step towards unraveling the genes that determine pubertal timing.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by Roman numerals I-IV:

I) WEHKALAMPI K, VANGONEN K, LAINE T, DUNKEL L. Progressive reduction of relative height in childhood predicts adult stature below target height in boys with constitutional delay of growth and puberty. *Hormone Research* 2007; 68(2):99-104.

II) WEHKALAMPI K, SILVENTOINEN K, KAPRIO J, DICK DM, ROSE RJ, PULKKINEN L, DUNKEL L. Genetic and environmental influences on pubertal timing assessed by height growth. *American Journal of Human Biology* 2008; 20(4):417-423.

III) WEHKALAMPI K, WIDÉN E, LAINE T, PALOTIE A, DUNKEL L. Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care. *Journal of Clinical Endocrinology & Metabolism* 2008; 93(3):723-728.

IV) WEHKALAMPI K*, WIDÉN E*, LAINE T, PALOTIE A, DUNKEL L. Association of the timing of puberty with a chromosome 2 locus. *Journal of Clinical Endocrinology & Metabolism* 2008 [epub ahead of print].

*Joint first authors.

ABBREVIATIONS

ACE	additive genetic/common environment/specific environment
ADE	additive genetic/dominant genetic/specific environment
AE	additive genetic/specific environment
Arc	arcuate nucleus
AVPV	anteroventral periventricular nucleus
B2	Tanner breast stage 2
BMI	body mass index
CDGP	constitutional delay of growth and puberty
DZ	dizygous
EDC	endocrine-disrupting chemical
FGFR1	fibroblast growth factor receptor 1
FH	final adult height
FSH	follicle-stimulating hormone
G2	Tanner genital stage 2
GABA	gamma amino butyric acid
GH	growth hormone
GnRH	gonadotropin-releasing hormone
GnRHR	gonadotropin-releasing hormone receptor
GPR	G-protein-coupled receptor
HD:SDS	height difference in standard deviation scores
HH	hypogonadotropic hypogonadism
HPG axis	hypothalamic-pituitary-gonadal axis
IGF-I	insulin-like growth factor I
IPP	idiopathic precocious puberty
IUGR	intrauterine growth retardation
KS	Kallmann syndrome
LH	luteinizing hormone
minSD	minimum relative height at puberty
MZ	monozygous
NELF	nasal embryonic LH releasing hormone factor
NHANES	National Health and Nutrition Examination Survey
NPY	neuropeptide Y
OSDZ	opposite-sex dizygous
phv	peak height velocity

PROK2	prokineticin 2
PROKR2	prokineticin receptor 2
PROS	Pediatric Research in Office Settings
SD	standard deviation
SSDZ	same-sex dizygous
take-off	onset of pubertal growth spurt
TH	parent-specific target height

INTRODUCTION

The timing of the onset of puberty varies greatly in the general population (Tanner 1962). Based on concordant pubertal timing between monozygotic twins (Fischbein 1977, Sharma 1983, Treloar and Martin 1990, Kaprio et al. 1995, Beunen et al. 2000, Towne et al. 2005, van den Berg and Boomsma 2007, Silventoinen et al. 2008) and similarities in the ages at pubertal onset between family members (Sedlmeyer et al. 2002, de Vries et al. 2004, Ersoy et al. 2005, van den Berg and Boomsma 2007), the timing of puberty appears to be largely regulated by genetic factors. In addition, the timing of puberty seems to be influenced by signals from peripheral tissues (Fernandez-Fernandez et al. 2006, Gamba and Pralong 2006) and the environment (Magee et al. 1970, Albright et al. 1990). The exact genes and mechanisms initiating puberty and controlling its timing, however, are poorly characterized.

A global secular trend towards earlier puberty observed for more than a century reflects improved nutrition and health (Roche 1979, Wyshak and Frisch 1982). Novel environmental influences have been suggested to explain more recent observations of exceptionally early pubertal signs in some female populations (Herman-Giddens et al. 1997, Huen et al. 1997, Papadimitriou 2001). The increasing problem of childhood obesity is associated with early puberty and may be a factor underlying advanced maturation (Guo et al. 1997, Biro et al. 2001, He and Karlberg 2001, Kaplowitz et al. 2001, Juul et al. 2006, Kindblom et al. 2006, Sandhu et al. 2006, Silventoinen et al. 2008). In addition, environmental endocrine-disrupting chemicals have been suggested to explain early pubertal maturation, e.g. in internationally adopted children (Krstevska-Konstantinova et al. 2001, Mul et al. 2002, Teilmann et al. 2006). Early pubertal development may cause long-term adverse health effects, such as increased risk of breast cancer in genetically susceptible individuals (Hamilton and Mack 2003, Ahlgren et al. 2004), and reduced adult height (Biro et al. 2001, Llop-Viñolas et al. 2004). Furthermore, advanced maturation is associated with adult obesity and cardiovascular risk factors (Remsberg et al. 2005, Kindblom et al. 2006, Sandhu et al. 2006), and may predict psychosocial problems in adolescence (Kaltiala-Heino et al. 2003, Patton et al. 2004). Thus, the possibility that trends towards early maturation affect not only certain subgroups but whole populations is a major public health concern.

Unraveling the exact mechanisms of the complex neuroendocrine network controlling pubertal onset, and understanding the interplay between this hypothalamic system and the environment, would undoubtedly be facilitated by more precise knowledge of the genetic factors involved in the regulation of pubertal timing.

REVIEW OF THE LITERATURE

1. TIMING OF PUBERTAL ONSET

1.1. PUBERTY AS A MATURATIONAL PROCESS

Puberty is a maturational process of the hypothalamic-pituitary-gonadal (HPG) axis. It is initiated through reactivation of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, which are hormonally active in the fetus (Kaplan et al. 1976) and newborn infant (Winter et al. 1975), but thereafter become relatively dormant (Terasawa and Fernandez 2001). Robust release of GnRH at the onset of puberty stimulates gonadotropes of the anterior pituitary gland to secrete gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) (Terasawa and Fernandez 2001, Grumbach 2002). LH and FSH induce the final development of genital organs, leading to production of mature gametes and sex steroids (gonadarche) (Grumbach 2002). Small amounts of gonadal estrogen and testosterone are secreted already in childhood by the prepubertal gonads (Mitamura et al. 1999, 2000), but only a sufficient increase in their plasma concentrations induces the development of secondary sex characteristics. During puberty, also body height, weight, and composition change in a sexually dimorphic pattern, resulting in attainment of adult stature and body proportions (Tanner 1962, 1989).

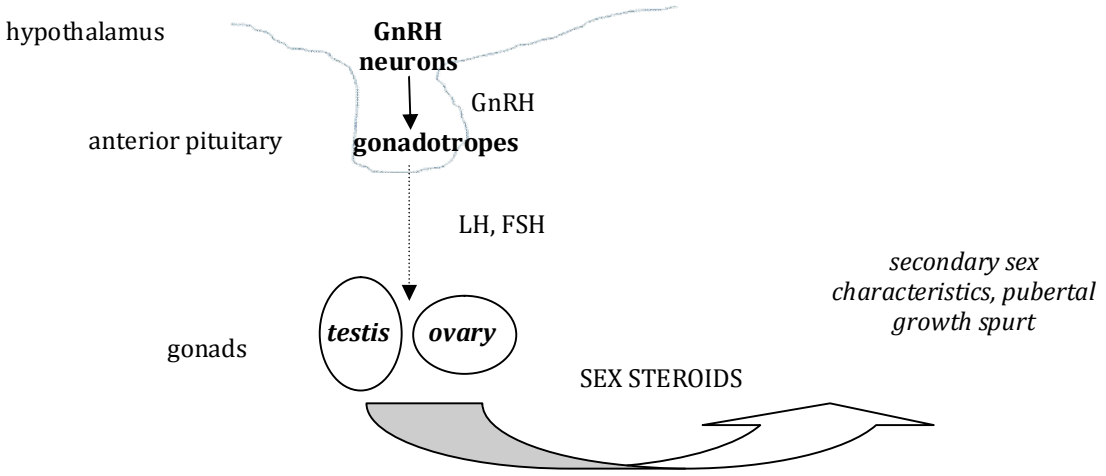

Figure 1. Maturation of the HPG axis. Hypothalamic GnRH stimulates secretion of pituitary gonadotropins, LH and FSH, which induce gonadal maturation and production of sex steroids (testosterone and estrogen), leading to development of secondary sex characteristics and pubertal growth spurt.

1.2. CLINICAL MARKERS AND NORMAL VARIATION OF PUBERTAL TIMING

Clinically, activation of the HPG axis, i.e. onset of puberty, can be assessed only indirectly by recording different physical changes related to pubertal maturation. These appear at different ages in healthy adolescents, with the timing following a normal distribution (Tanner 1962). The cut-off age limits for normal pubertal timing are based on statistical considerations; ±2 standard deviations (SD) of the mean age at pubertal onset in the population. Puberty beginning earlier or later than this range in an otherwise healthy adolescent is called idiopathic precocious puberty (IPP) or constitutional delay of growth and puberty (CDGP), respectively. Originally, the 2 SD age limits were based on photographed breast, genital, and pubic hair stages of 420 institutionalized Caucasian British children from the 1960s (Marshall and Tanner 1969, 1970). However, the ages at pubertal onset change over time (Roche 1979, Wyshak and Frisch 1982) and vary between populations and races/ethnic groups (Parent et al. 2003). Distinct pubertal events as markers of pubertal timing and examples of their worldwide variation are reviewed below.

1.2.1. SECONDARY SEX CHARACTERISTICS

The development of secondary sex characteristics usually occurs in a certain sequence, classically described by Tanner stages 1 through 5 for breast, genital, and pubic hair growth (Marshall and Tanner 1969, 1970). Appearance of breast tissue (Tanner breast stage 2, B2) is the first sign of puberty in girls (Marshall and Tanner 1969). However, premature breast tissue growth, thelarche, may sometimes occur as an isolated phenomenon, without true activation of the HPG axis (Diamantopoulos and Bao 2007). Exogenous environmental estrogens may also induce early breast development (Colón et al. 2000, Felner and White 2000). Furthermore, breast tissue may not be easily distinguished from fat in obese girls (Bonat et al. 2002). Thus, assessment of pubertal timing by recording breast buds only may be imprecise. Pubic hair growth (pubarche) is mainly activated independently of the gonads by adrenal androgens (adrenarche), especially in girls (Sklar et al. 1980). Adrenarche is probably a gradual process beginning in early childhood (Palmert et al. 2001). Pubic hair is therefore not a reliable marker for gonadarche, and thus, pubertal timing. In boys, the first physical sign of puberty is enlargement of the testes and reddening of the scrotal skin (Tanner genital stage 2, G2) (Marshall and Tanner 1970). Testicular volume of 3 ml or more may be used to demarcate pubertal onset (Zachmann et al. 1974, Biro et al. 1995). However, observation of this requires frequent longitudinal examinations and palpation since staging of genital development may be unreliable by visual inspection alone. Measuring of testicular size is, however, impractical and may cause considerable embarrassment to the adolescent. Overall, self-assessment of pubertal timing may be

inaccurate (Finkelstein et al. 1999, Hergenroeder et al. 1999, Bonat et al. 2002), but assessment by a physician also involves marked interobserver variation (Hergenroeder et al. 1999).

According to the British reference data, the mean age at achieving B2 is 10.8 years in girls (Marshall and Tanner 1969). Based on this, IPP is diagnosed if breast tissue appears before age 8.0 years, and CDGP if no breast development occurs by 13.0 years. The mean age at B2 has been reported to be 10.8 years also in other parts of Europe and Finland (Ojajärvi 1982, Mul et al. 2001, Juul et al. 2006). In the US, this age varies between 10.6 and 11.2 years (Nicolson and Hanley 1953, Foster et al. 1977, Tanner and Davies 1985, Roche et al. 1995), although the most recent studies report mean ages of 8.9-10.4 years (Herman-Giddens et al. 1997, Sun et al. 2002, 2005, Wu et al. 2002). Based on these, the mean B2 age among African Americans is earlier (8.9-9.5 years) than in Caucasian American girls (10.0-10.4 years). The mean age at G2 based on Marshall and Tanner's (1970) reference data is 11.6 years, approximately 0.8 years later than B2 in girls (Marshall and Tanner 1969). In IPP, testicular growth is observed before age 9.0, whereas CDGP is diagnosed if no testicular enlargement occurs before 13.5 years. The mean age at G2 in other Western European countries is 11.5 years (Lindgren 1996, Mul et al. 2001, Juul et al. 2006), similar to data from Great Britain. This age is later in Finland (12.2 years) (Ojajärvi 1982), and somewhat earlier in Greece (11.0 years) (Papadimitriou et al. 2002). In the US, the mean G2 age varies between 10.8 and 11.9 years (Reynolds and Wines 1951, Nicolson and Hanley 1953, Tanner and Davies 1985, Roche et al. 1995, Sun et al. 2002), being earlier in African American (10.8 years) than in Caucasian American boys (11.1 years) (Sun et al. 2002).

1.2.2. MENARCHE OR SPERMARCHE

Menarche, the occurrence of first menstruation, is a relatively late phenomenon in female puberty. Although it usually occurs approximately 2.3 years after achieving B2, it may sometimes be the first sign of puberty (Marshall and Tanner 1969, Tanner and Whitehouse 1976). The time interval between B2 and menarche is longer in those with earlier breast development than in those with later-onset B2 (Bourguignon 1988, de Ridder et al. 1992, Martí-Henneberg and Vizmanos 1997, Llop-Viñolas et al. 2004, Biro et al. 2006). Menarche has currently been shown to only moderately correlate with the onset of breast or pubic hair development (r=0.37-0.39) (de Ridder et al. 1992, Biro et al. 2006), although in earlier studies the correlation was 0.64 (Marshall and Tanner 1969). This suggests that menarche and breast or pubic hair development are not completely parallel events in female pubertal maturation. Appearance of B2 can be regarded a more reliable marker of pubertal onset than age at menarche. In addition, although recalled menarche after 30 years may correlate well with medical records (r=0.79) (Must et al. 2002), some studies report that less than half of girls recall their menarcheal age correctly after two years (Koo and Rohan 1997). Spermarche, the first emission of spermatozoa in boys corresponds to girls' menarche. Its

determination is based on observing spermatozoa in urine (spermaturia). This, however, may be ascertained at a wide range of testicular volumes (3-19.6 ml) (Nielsen et al. 1986, Nysom et al. 1994). Further limiting the use of spermaturia as an indicator for pubertal onset, subsequent urine samples after spermarche may be intermittently sperm-negative (Hirsch et al. 1985).

The mean menarcheal age varies between 13.0 and 13.5 years in Scandinavia and Western Europe (Ojajärvi 1982, Lindgren et al. 1991, Fredriks et al. 2000, Juul et al. 2006). This age is lower (12.3 years) in the Mediterranean countries (de la Puente et al. 1997, Papadimitriou et al. 1999) and in Asia (Chompootaweep et al. 1997, Huen et al. 1997). In the US, the mean age at menarche varies between 12.0 and 12.8 years, with the lowest ages recorded in African Americans (12.0-12.5 years) (Nicolson and Hanley 1953, Tanner and Davies 1985, Herman-Giddens et al. 1997, Biro et al. 2001, Wu et al. 2002, Anderson and Must 2005). Menarche in African American girls occurs approximately one year earlier than in black girls in Africa (Cameron and Wright 1990, Pasquet et al. 1999). In underprivileged countries, the mean menarcheal age is as late as 16.0 years (Simondon et al. 1997, Graham et al. 1999). The reported mean or median ages at spermarche vary between 13.4 and 15.0 years (Nielsen et al. 1986, Kulin et al. 1989, Bornman 1990, Schaefer et al. 1990, Ji 2001).

1.2.3. PUBERTAL GROWTH SPURT

The growth of a child can be divided into infancy, childhood, and pubertal growth phases (Karlberg 1989). Growth in infancy is initially fast but decelerates rapidly. Childhood growth somewhat overlaps with the infancy phase and slowly decelerates reaching a velocity of 5-6 cm/year in both genders (Tanner 1989). At the onset of puberty, growth velocity almost doubles (Tanner et al. 1976). In boys, this onset of pubertal growth spurt (take-off) most often occurs at Tanner genital stage 3, while in girls it is often, together with B2, the first sign of puberty (Tanner and Whitehouse 1976). Pubertal growth spurt usually reaches its highest velocity, peak height velocity (phv), at Tanner stage 3 and 4 in girls and boys, respectively (Tanner and Whitehouse 1976). After phv, growth velocity gradually declines, during which time menarche usually occurs in girls (Tanner and Whitehouse 1976). Pubertal growth spurt increases stature by approximately 25 (range 17-33) and 28 (range 21-36) cm in girls and boys, respectively (Tanner et al. 1976).

Multiple factors determine the process of growth, e.g. appropriate nutrition and normal endocrine function (Kerrigan and Rogol 1992). Fetal and infant growth is apparently mainly limited by metabolic substrate supply (Blizzard and Alberts 1956), whereas childhood growth involves regulation by growth hormone (GH) (Albertsson-Wikland and Rosberg 1988). Sex hormones, in turn, are responsible for the acceleration of growth during puberty (Kerrigan and Rogol 1992), although also GH is critical for normal pubertal height gain (Aynsley-Green et al. 1976). Of sex steroids, estrogen is

the main one regulating onset of pubertal growth in both genders (Cutler 1997). This is evidenced by the absence of a pubertal growth spurt in men with estrogen receptor defects or a deficiency in the aromatase enzyme, which converts androgens to estrogens (Smith et al. 1994, Morishima et al. 1995). Since the majority of estrogen in men is formed by peripheral aromatization (MacDonald et al. 1979), whereas in girls estrogens are mainly produced by the ovaries, sex differences in aromatase expression may explain the pubertal growth spurt occurring later in pubertal maturation of boys than girls (Tanner and Whitehouse 1976). Whereas low doses of estrogen stimulate acceleration of pubertal growth, high doses induce epiphyseal closure (Cutler 1997). With aromatase deficiency and estrogen receptor defects, growth continues exceptionally in adulthood (Smith et al. 1994, Morishima et al. 1995).

Occurrence of take-off and phv as well as attainment of adult height depend on the timing of puberty – they are delayed in late maturers (**Figure 2A**), and occur earlier than average in those maturing early (**Figure 3A**) (Tanner et al. 1976, Tanner and Davies 1985, Karlberg et al. 2003). The timing of puberty also characteristically influences relative height (height SD) during pubertal years. Reduction in height SD is observed in late maturers compared with those with average pubertal timing because of the longer prepubertal period of slow growth and later acceleration of growth in puberty (Tanner et al. 1976, Tanner and Davies 1985, Karlberg et al. 2003), and thus, these individuals may have short stature relative to age-matched adolescents (**Figure 2B**). However, in CDGP, the reduction in height SD may sometimes be observed well before puberty, in early childhood (Rensonnet et al. 1999, Du Caju et al. 2000). Conversely to pubertal height SD reduction in late maturers, early maturers' height SD during pubertal years is increased compared with those with average pubertal timing, and these adolescents are tall in relation to peers at adolescence (Tanner et al. 1976, Tanner and Davies 1985, Karlberg et al. 2003) (**Figure 3B**). Adult stature, however, tends to be reduced especially if puberty is precocious (Bourguignon 1988, Biro et al. 2001). In CDGP, adult height has been shown to range within the normal limits (Volta et al. 1988, Brämswig et al. 1990, Albanese and Stanhope 1993, Sperlich et al. 1995, Arrigo et al. 1996, Rensonnet et al. 1999). Final height is not expected to be affected, although late maturers have reduced duration and amplitude of the pubertal growth spurt, and consequently, reduced total pubertal height gain relative to those entering puberty earlier (Tanner and Davies 1985, Bourguignon 1988, Stanhope et al. 1988, Karlberg et al. 2003), because late maturers have longer than average prepubertal growing time (Tanner and Davies 1985, Bourguignon 1988, Stanhope et al. 1988). Final height in delayed puberty does not, however, always correspond to genetic height potential, i.e. target height (Brämswig et al. 1990, LaFranchi et al. 1991, Albanese and Stanhope 1993, 1995). In the average population, the correlation of final height and target height is 0.71 (Tanner et al. 1970), whereas this is significantly lower (0.40) for boys with CDGP (Rensonnet et al. 1999).

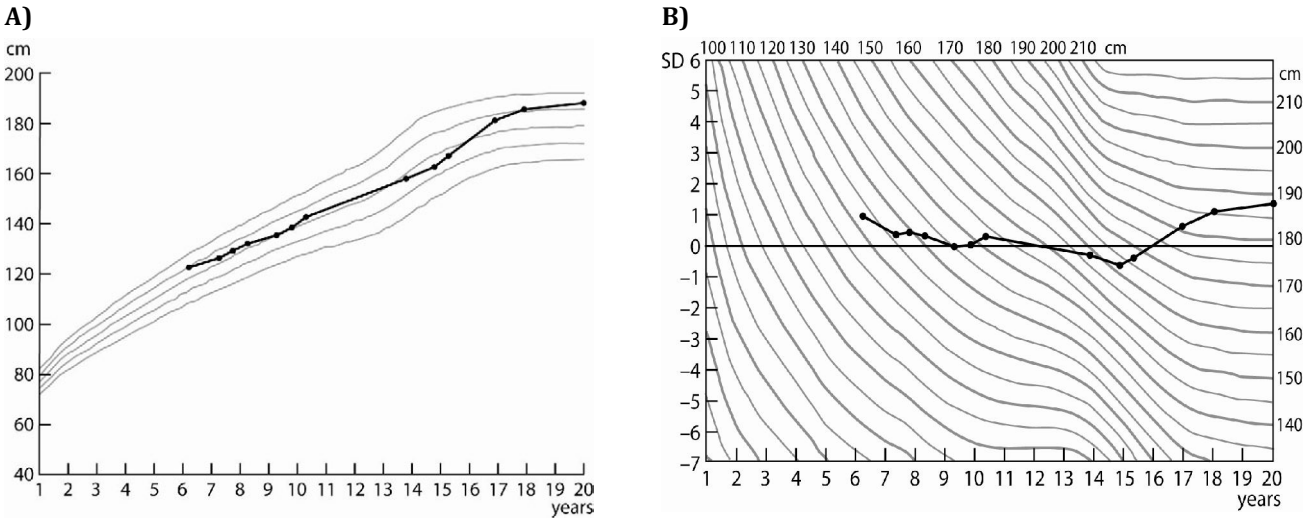

Figure 2. Longitudinal growth of a male with later than average pubertal maturation. **A)** Later than average onset of pubertal growth spurt, peak height velocity, and attainment of adult height. **B)** Typical reduction in relative height (height SD) at pubertal years when compared with age-matched peers.

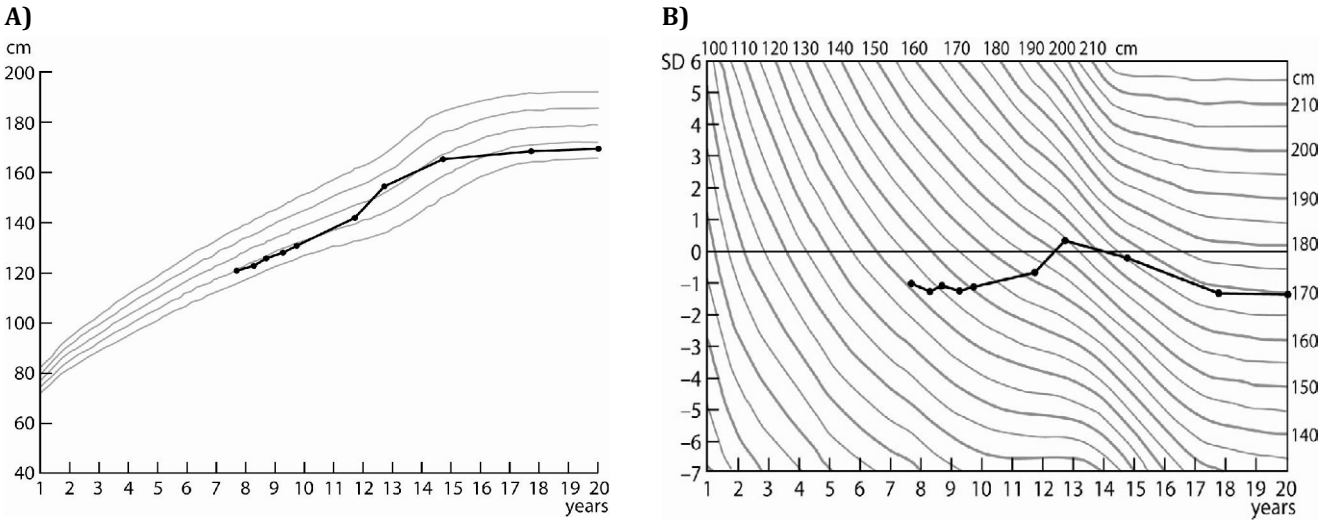

Figure 3. Longitudinal growth of a male with earlier than average pubertal maturation. **A)** Earlier than average onset of pubertal growth spurt, peak height velocity, and attainment of adult height. **B)** Typical increase in relative height (height SD) at pubertal years when compared with age-matched peers.

Based on the Harpenden Growth Study of longitudinal growth of 35 British girls and 55 boys from the 1960s, the mean ages at take-off are 10.3 and 12.1 years, respectively (Tanner et al. 1976) (**Table 1**). Few other studies reporting take-off ages exist. In one Swedish study investigating childhood growth patterns retrospectively from routine health visit measurements, take-off age was also 12.1 years in boys, but 11.1 years in girls (Persson et al. 1999). Gender difference in take-off age seems to be approximately 1.8 years (Tanner et al. 1976). In the Harpenden Growth Study, the mean age at phv was 11.9 in girls and 13.9 years in boys (Tanner et al. 1976). Swedish growth studies report mean ages at phv to vary between 11.6 and 12.0 for girls, and between 13.5 and 14.0 years for boys (Hägg and Taranger 1991, Liu et al. 2000, Kindblom et al. 2006). The median or mean value for phv age in boys varies from 13.5 to 14.0 years in other European countries (Hulanicka and Kotlarz 1983, Kemper et al. 1985, de la Puente et al. 1997), and is 11.5 years for Spanish girls (de la Puente et al. 1997). Based on US studies, the mean phv age is 11.1-11.9 years in girls (Nicolson and Hanley 1953, Berkey et al. 2000, Biro et al. 2006) and 13.8 years in boys (Nicolson and Hanley 1953). Phv age has been reported to be 11.9 years in Caucasian, and 11.5 years in African American girls (Biro et al. 2006). In Japan, the mean age at phv is 11.2 and 13.0 years in girls and boys, respectively (Tanaka et al. 1988). Based on Tanner et al. (1976), phv occurs 2.0 years later in boys than in girls.

Table 1. Reported mean (median) ages (years) at onset of pubertal growth spurt (take-off) and peak height velocity (phv) in girls and boys.

Girls		**Boys**		**Reference**
take-off	***phv***	***take-off***	***phv***	
10.3	11.9	12.1	13.9	*Tanner et al. 1976 (Great Britain)*
11.1		12.1		*Persson et al. 1999 (Sweden)*
	12.0		14.0	*Hägg and Taranger 1991 (Sweden)*
	11.6		13.5	*Liu et al. 2000 (Sweden)*
			13.6	*Kindblom et al. 2006 (Sweden)*
			13.9	*Hulanicka and Kotlarz 1983 (Poland)*
			(14.0)	*Kemper et al. 1985 (Netherlands)*
	11.5		13.5	*de la Puente et al. 1997 (Spain)*
	11.5		13.8	*Nicolson and Hanley 1953 (US)*
	11.1			*Berkey et al. 2000 (US)*
				Biro et al. 2006 (US)
	11.9			- Caucasian Americans
	11.5			- African Americans
	11.2		13.0	*Tanaka et al. 1988 (Japan)*

1.3. SECULAR TRENDS IN TIMING OF PUBERTY

A change in the timing of pubertal maturation towards an earlier age has been observed from the mid-19th to the mid-20th century. During this time, the age at menarche has advanced from 17 to under 14 years, and concurrently, the mean adult height has increased approximately 1-3 cm per decade, but not in synchrony with advancement in menarcheal age, suggesting different causal mechanisms (Roche 1979, Wyshak and Frisch 1982, Cole 2000). A secular trend in menarcheal age has even been observed during the latter half of the 20th century in developing parts of the world (Graham et al. 1999, Hwang et al. 2003) and in some parts of Europe (Papadimitriou et al. 1999, Chodick et al. 2005, Ersoy et al. 2005), whereas in most European countries the trend has halted (Fredriks et al. 2000, Mul et al. 2001, Juul et al. 2006). The mean ages at B2 and G2 have stabilized in Europe (Fredriks et al. 2000, de Muinck Keizer-Schrama and Mul 2001, Papadimitriou et al. 2002, Juul et al. 2006). In Sweden, however, a very modest decline (0.2-0.6 years) in these ages between 1970 and 1980 (Lindgren 1996), and a 0.5-year advancement in the mean age at phv have been noted in both genders (Karlberg 1989, Liu et al. 2000). Although the secular trend in pubertal maturation seems to have slowed down or stopped in industrialized countries, the secular trend in height seems to continue (Cole 2000, Fredriks et al. 2000, Juul et al. 2006), presumably through increased height in the first 2 years of life (Cole 2000).

In the US, in 1997, a collaborative pediatric interoffice research project (Pediatric Research in Office Settings, PROS) reported a striking observation that the mean age at B2 in girls was, compared with previous reports (Nicolson and Hanley 1953, Foster et al. 1977, Tanner and Davies 1985, Roche et al. 1995), approximately one year earlier in Caucasian (10.0 years), and two years earlier in African American girls (8.9 years) (Herman-Giddens et al. 1997). Methodological problems may, however, have influenced the findings. Multiple observers assessed the breast stages in most girls by visual inspection only, which may have caused overestimation of breast development. Moreover, the findings were probably not representative of the US general population since the data were collected from specialist offices. Compared with PROS, the data from national studies (National Health and Nutrition Examination Survey, NHANES III, 1988-1994), providing better coverage of the whole American population, evidenced less dramatic advancement in breast development, reflected by mean B2 age being 10.4 in Caucasian and 9.5 years in African American girls (Sun et al. 2002, 2005, Wu et al. 2002). The mean menarcheal age has not recently decreased in distinct racial groups in the US (Anderson et al. 2003, Anderson and Must 2005). Neither was advancement seen in the girls of the PROS study, despite earlier age at B2 (Herman-Giddens et al. 1997). The dissociation between B2 and menarche may be explained by separate early breast development, without full HPG axis activation, or by typical lengthening of the time interval between B2 and menarche in early maturers (Bourguignon 1988, de Ridder et al. 1992, Martí-Henneberg and Vizmanos 1997, Llop-Viñolas et al. 2004, Biro et al. 2006). Similar to

girls of the PROS study, especially breast development showed advancement in Chinese girls between the years 1962 (median age at B2 10.7 years) and 1993 (median age at B2 9.8 years), while advancement in the median age at menarche was only 0.5 years (Huen et al. 1997).

In the US, NHANES III (1988-1994) reported the median ages at G2 in boys to be 10.1 years in Caucasian Americans and only 9.3 years in African Americans (Herman-Giddens et al. 2001, Karpati et al. 2002, Sun et al. 2002). Later than these medians, but still somewhat earlier than reported previously (~11.9 years) (Reynolds and Wines 1951, Nicolson and Hanley 1953, Tanner and Davies 1985, Roche et al. 1995), were calculated mean values (11.1 and 10.8 years) (Sun et al. 2002). However, pubertal stage determination in these studies may have been inaccurate since testicular size was assessed by inspection alone. In addition, the use of different criteria for the onset of male genital development in different American studies hinders the evaluation of whether pubertal onset has truly advanced. Boys in more recent surveys may have been taller at younger ages (Herman-Giddens et al. 2001) and attained mature height earlier than in the past (Karpati et al. 2001), indirectly suggesting an advanced pubertal growth spurt. In Greece, however, more intense advancement in pubertal maturation has been suggested in girls than in boys (Papadimitriou et al. 1999, 2002, Papadimitriou 2001). Furthermore, although in Hong Kong the mean age at B2 in girls showed advancement (Huen et al. 1997), the median age at G2 in Chinese boys showed no comparable trend (Wong et al. 1996). Whether pubertal timing advances in girls but not in boys, and whether the phenomenon of a secular trend is global for entire (female) populations remain unanswered (Euling et al. 2008).

2. GENETIC REGULATION OF PUBERTAL TIMING

2.1. EVIDENCE FOR GENETIC EFFECTS

Within a population, much of the variation of pubertal timing is due to genetic factors. This is evidenced by demonstrating similarities in ages at pubertal onset within racial groups (Wu et al. 2002, Anderson and Must 2005) and between family members (Sedlmeyer et al. 2002, de Vries et al. 2004, Ersoy et al. 2005, van den Berg and Boomsma 2007). Both early and late variants of the normal spectrum of pubertal timing, i.e. IPP and CDGP, also tend to aggregate in families (Sedlmeyer et al. 2002, de Vries et al. 2004). The strongest evidence for genetic effects derives from twin studies; pubertal timing between monozygous (MZ) twins sharing 100% of their genes is much more similar than between dizygous (DZ) twins with only 50% of genes shared (Fischbein 1977, Sharma 1983, Treloar and Martin 1990, Kaprio et al. 1995, Beunen et al. 2000, Mustanski et al. 2004, Towne et al. 2005, van den Berg and Boomsma 2007, Silventoinen et al. 2008). The onset of puberty, estimated from breast or genital development in 48 twin pairs, has been shown to be concordant, i.e. occur within 3 months, in as many as 88-

100% of MZ twins compared with only 30-39% of DZ twins (Sharma 1983). By comparing MZ and DZ twin correlations, and more recently, by using twin genetic models (Neale 2003), the magnitude of genetic influences to the variation of pubertal timing can be estimated. The estimates are, however, influenced by the number of twins studied and the marker used for pubertal onset. Small studies may overestimate genetic factors due to selection effects or sampling variation. They enable, however, for practical reasons, the use of a precise marker, and thus, exact assessment of the onset of puberty. By using age at take-off as an indicator, the genetic contribution to the variation of timing of puberty was as much as 89-93% in a study of 99 twin pairs of both genders (Beunen et al. 2000). Considerably larger studies, encompassing over 1000 individuals and using age at menarche as a marker for pubertal timing for girls, have revealed a much lower contribution of genetic effects (50-75%) (Treloar and Martin 1990, Kaprio et al. 1995, Towne et al. 2005). Compared with pubertal growth or secondary sexual characteristics, probably more complex menarche is less precise as a marker for pubertal onset and provides information for only females (Marshall and Tanner 1969, Tanner and Whitehouse 1976, Tanner et al. 1976, de Ridder et al. 1992, Biro et al. 2006). Large studies having comparable numbers of both females and males are few (Mustanski et al. 2004). The exact contribution of inter-individual differences in genetic factors to the variation of pubertal timing remains somewhat unclear.

2.2. CURRENT CONCEPTS OF GENES RELATED TO PUBERTAL TIMING

Based on the Gaussian distribution of the timing of pubertal onset in the general population (Tanner 1962), genes regulating this process are multiple. However, these genes remain largely unknown. Although the genetic composition of a complex polygenic trait, such as pubertal timing, likely derives from modest additive effects of multiple genes, important insights may result from identification of single high-impact genes, mutations in which cause abnormalities in the onset of pubertal development. A number of genes important in the onset of puberty have been discovered by investigating individuals with hypogonadism due to variable congenital defect in GnRH neuron or gonadotrope structure or function causing congenital/idiopathic hypogonadotropic hypogonadism, HH. HH is familial in 30% of cases (Waldstreicher et al. 1996), and is currently known to be caused by several gene mutations (**Table 2**). The genes contributing to familial CDGP and IPP (Sedlmeyer et al. 2002, de Vries et al. 2004) would also provide clues to those genes that are important for the control of pubertal onset. To date, however, the vast majority of genes causing susceptibility to CDGP and IPP, as well as HH, are unknown. Current knowledge of genes and mechanisms involved in the onset of puberty is reviewed below.

2.2.1. GENES INVOLVED IN ONTOGENESIS OF GnRH NEURONS AND GONADOTROPES

GnRH neurons migrate during embryogenesis from outside the brain, the olfactory placode, along the olfactory guidance fibers, to the olfactory bulb, and then reside in the final destination in the medial basal hypothalamus (Schwanzel-Fukuda and Pfaff 1989). Mutations in genes, which are involved in this process, cause abnormal GnRH migration and underdeveloped or absent olfactory bulbs or tracts, and thus, HH with anosmia/hyposmia — Kallmann syndrome (KS). KS may be X chromosomal, in which case there is a deficiency in the *KAL1* gene encoding an axonal guidance factor, anosmin-1 (**Table 2**) (Franco et al. 1991, Legouis et al. 1991). In addition, KS may be autosomal dominant caused by mutations in fibroblast growth factor receptor 1 (*FGFR1*), for which anosmin-1 is a ligand (Dodé et al. 2003, Xu et al. 2007). Mutations in prokineticin 2 (*PROK2*) and its receptor (*PROKR2*) (Dodé et al. 2006, Pitteloud et al. 2007b, Abreu et al. 2008), and mutations in *FGF8* are also possible in the etiology of KS (Falardeau et al. 2008). One sporadic case had a mutation in nasal embryonic LH releasing hormone factor (*NELF*) (Miura et al. 2004), but there is currently no definitive link between *NELF* and KS. *CHD7* mutations causing a multisystem autosomal dominant disorder, CHARGE, may also cause KS phenotype (Kim et al. 2008). Especially with autosomal mutations, the phenotypes vary greatly; the sense of smell is not invariably affected (Pitteloud et al. 2007b, Xu et al. 2007, Falardeau et al. 2008, Kim et al. 2008), hypogonadism may be reversible or partial (Pitteloud et al. 2005, 2006, Raivio et al. 2007, Ribeiro et al. 2007), or it may not begin until in adulthood (Nachtigall et al. 1997). CDGP phenotype is also possible (Seminara et al. 2003, Pitteloud et al. 2005, 2006, Lin et al. 2006). Thus, neither disruption of the olfactory bulbs nor a defect in GnRH migration is always complete, or other mechanisms may be involved. The variation in phenotypes within families may be partly explained by the presence of modifier genes. Family members carrying two different mutations may have the full HH phenotype, whereas milder phenotypes are seen in those with a single gene mutation (Dodé et al. 2006, Pitteloud et al. 2007a). Polymorphic common variants of these genes causing variable HH could potentially determine pubertal timing in general. However, an extensive recent study exploring the association of common genetic variants in *KAL1*, *FGFR1*, *PROK2*, and *PROKR2* with early or late age at menarche, detected no association (Gajdos et al. 2008). This suggests that, although necessary for normal initiation of puberty, these genes don't play a substantial role in the normal variation of pubertal timing.

X chromosomal *DAX-1* gene is involved in the formation of the adrenal gland and, in a complex manner, also the GnRH region in the hypothalamus, as well as formation of the pituitary and gonads (**Table 2**). Mutations in this gene cause HH and congenital adrenal insufficiency (Muscatelli et al. 1994, Caron et al. 1999). Development of the highly specialized cells of the anterior pituitary gland, including gonadotropes, is regulated by a cascade of signaling molecules and developmental transcription factors – homeobox genes (Zhu et al. 2005). In

humans, mutations in a few of these genes, namely *HESX1* (Dattani et al. 1998, Thomas et al. 2001, Tajima et al. 2003), *PROP1* (Wu et al. 1998), *LHX3* (Netchine et al. 2000), and *LHX4* (Pfäffle et al. 2008), are known to cause, in addition to variable degrees of defects in other pituitary hormones, defective gonadotropin secretion. These genes functioning at the pituitary level may also provide clues to the genes involved in the control of pubertal onset. Very rare mutations in gonadotropin β-subunit genes affect the structure of the specific gonatropin. In females, mutations in *FSHB* encoding FSH-β subunit cause delayed breast development and menarche, as well as infertility (Layman et al. 1997). However, infertile males carrying these mutations have unaffected pubertal maturation (Phillip et al. 1998). One male with an inactivating mutation in *LHB*, encoding LH-β subunit, has been reported (Weiss et al. 1992). He presented with infertility and delayed puberty. The role of these pituitary genes or their polymorphisms have unknown role in the normal variation of pubertal maturation.

2.2.2. GENES INVOLVED IN GnRH NEURON AND GONADOTROPE FUNCTION

Upon migration, GnRH neurons extend axonal projections into the median eminence, where GnRH is released to the hypophyseal circulation. GnRH induces pituitary gonadotropes to release gonadotropins through its receptor, GnRHR. Mutations in the *GNRH* gene (8p11.2-p21) have not been identified in humans, but a number of autosomal recessive mutations in *GNRHR* are known to cause isolated HH, i.e. HH without associated anomalies (de Roux et al. 1997, Layman et al. 1998) (**Table 2**). To explore the significance of *GNRH* and *GNRHR* for normal variation of pubertal timing, Sedlmeyer et al. (2005) investigated the association of their sequence variations or haplotype structures in subjects with CDGP as well as in a multiethnic cohort recruited in Hawaii and California representing the full spectrum of pubertal timing. No association was found in either group. Nor were polymorphisms in *GNRHR* (Nanao and Hasegawa 2000), or *GNRHR* and *GNRH* (Gajdos et al. 2008) associated with the age at menarche in two other studies.

In addition to the pituitary, GnRH neurons also form many thousands of connections with other central neurons in the hypothalamus. These connections are crucial to the regulation of HPG axis maturation. Other hypothalamic neurons have been suggested to inhibit GnRH secretion during the childhood dormant period in GnRH acitivity, and to reactivate GnRH neurons at adolescence (Terasawa and Fernandez 2001). However, the biological background for this inhibition and reactivation has long remained unresolved. Activation of GnRH release at the onset of puberty has been suggested to be induced by, for example, glutamate (Goldsmith et al. 1994, Terasawa et al. 1999). Gamma amino butyric acid (GABA), in turn, has been proposed to mediate the downregulation of GnRH neurons during childhood (Mitsushima et al. 1994, Terasawa et al. 1999, Cottrell et al. 2006). An attempt to elucidate the relationship between IPP and functional mutations or polymorphisms in GABA receptor gene has been carried out, but no

association was found (Brito et al. 2006). The exact roles of glutamate and GABA at the onset of puberty remain obscure. In contrast, multiple studies in animals and humans have consistently demonstrated that kisspeptins, structurally related proteins encoded by the *KISS1* gene (1q32-q41), which was formerly known for involvement in tumor metastasis suppression (Lee et al. 1996), are direct and very potent activators of GnRH neurons (Gottsch et al. 2004, Irwig et al. 2004, Navarro et al. 2004, 2005, Dhillo et al. 2005, Messager et al. 2005, Shahab et al. 2005, Castellano et al. 2006, Plant et al. 2006). With reduced tone, they may also be largely responsible for the brake in pulsatile GnRH release in prepuberty. The important role of kisspeptins, and their G-protein coupled receptor GPR54 (Kotani et al. 2001, Ohtaki et al. 2001), in the control of pubertal onset was first discovered after an observation that familial isolated HH and CDGP were caused by mutations in *KISS1R* encoding GPR54 (de Roux et al. 2003, Seminara et al. 2003) (**Table 2**). *KISS1R* and *KISS1* genes are strong candidates for determining pubertal timing in general. In fact, an activating autosomal dominant mutation in *KISS1R* has been reported in a patient with IPP (Teles et al. 2008), and also two mutations in *KISS1* have been suggested, but not confirmed, to be associated with this condition (abstract by Silveira et al. 2008). However, two other studies evaluating the association of polymorphic variants of *KISS1R* and *KISS1* with IPP revealed no significant correlation (Luan et al. 2007a, 2007b). Gajdos et al. (2008) found no association between menarcheal age and common genetic variants in *KISS1R* or *KISS1*. Therefore, other genes and pathways are expected to be involved in the regulation of pubertal timing.

In addition to central neuronal stimulus, also modifications in glial inputs have been proposed to trigger GnRH release at the onset of puberty (Ojeda et al. 2003). Furthermore, both neural and glial cells controlling GnRH output have been suggested to be regulated by upper echelon transcription factors, such as *OCT2*, *TTF1*, and *EAP1* (Ojeda et al. 1999, Mastronardi et al. 2006, Heger et al. 2007). Based on animal studies, loss of function in these genes results in delayed pubertal maturation in females. Their role in the control of pubertal onset in humans is yet unknown.

At the hypothalamic level, mutations in leptin (*LEP*) and leptin receptor (*LEPR*) genes cause absence of pubertal maturation and obesity (Clément et al. 1998, Strobel et al. 1998) (**Table 2**). *LEP* or *LEPR* have not been shown to be associated with age at menarche (Gajdos et al. 2008), or delayed puberty (Banerjee et al. 2006), suggesting no major contribution to pubertal timing in general. The role of leptin in hypothalamic control of pubertal onset is more closely discussed in a later section (Signals integrating metabolism and pubertal onset, p. 31).

Table 2. Genes implicated in the etiology of hypogonadotropic hypogonadism (HH). Suggested pathogenic mechanisms and major phenotypes associated with mutations are also listed. CDGP=constitutional delay of growth and puberty, IPP=idiopathic precocious puberty.

Gene	*Locus*	*Pathogenic mechanism*	*Phenotype*
		Hypothalamus	
KAL1	Xp22.3	abnormal GnRH neuron migration	HH with anosmia/hyposmia
FGFR1 (KAL2)	8p12	abnormal GnRH neuron migration	HH with/without anosmia/hyposmia, CDGP
PROKR2 (*KAL3*)	20p13	abnormal GnRH neuron migration	HH with anosmia/hyposmia
PROK2 (*KAL4*)	3p13	abnormal GnRH neuron migration	HH with/without anosmia/hyposmia
FGF8	10q24	abnormal GnRH neuron migration	HH with/without anosmia/hyposmia, CDGP
NELF	9q34	abnormal GnRH neuron migration	HH with anosmia/hyposmia (?)
CHD7	8q12	abnormal GnRH neuron migration	CHARGE, HH with/without anosmia/hyposmia
KISS1R (*GPR54*)	19p13	abnormal GnRH neuron activation	HH, CDGP, IPP
LEP	7q31.33	abnormal appetite control and GnRH secretion	obesity, HH
LEPR	1q31.2	abnormal appetite control and GnRH secretion	obesity, HH
		Hypothalamus and pituitary	
DAX-1	Xp21	abnormal development of the adrenal cortex, hypothalamus, pituitary gonadotropes, and gonads	congenital adrenal insufficiency, hypogonadism due to combined hypothalamic-pituitary-gonadal defect
		Pituitary	
HESX1	3p21	abnormal develoment of the forebrain, midline, and the pituitary	septo-optic dyplasia, isolated or combined deficiency of pituitary hormones
PROP1	5q35	abnormal develoment of the pituitary, or specialization of its cells	combined deficiency of pituitary hormones
LHX3	9q34	abnormal develoment of the pituitary and extrapituitary structures	combined deficiency of pituitary hormones, rigid cervical spine
LHX4	1q25	abnormal develoment of the pituitary, or specialization of its cells	combined deficiency of pituitary hormones
GNRHR	4q13-q21	abnormal gonadotrope activation	HH, CDGP
LHB	19q13.32	abnormal Leydig cell stimulation in a male	infertility and delayed puberty in a male
FSHB	11p13	abnormal ovarian stimulation in females and Sertoli cell stimulation in males	infertility in both genders, delayed breast development and menarche in females, unaffected pubertal development in males

2.2.3. GENETIC LOCI RELATED TO PUBERTAL TIMING

Most studies attempting to identify genes determining the normal variation of pubertal timing have used candidate gene-based strategies (Nanao and Hasegawa 2000, Sedlmeyer et al. 2003, Banerjee et al. 2006, Luan et al. 2007a, 2007b, Gajdos et al. 2008). In contrast, the use of whole genome scans may allow ultimate identification of previously unknown genes. In humans, linkage scans aiming at detection of genetic loci associated with pubertal timing are few and have only used age at menarche as a marker for pubertal timing. A whole genome scan on age at menarche by Guo et al. (2006) detected a significant locus on chromosome 22q13 (LOD 3.70), and Rothenbuhler and collegues (Rothenbuhler et al. 2006) found a strongly suggestive locus on 16q12 (LOD 3.12) -

16q21 (LOD 3.33). A third genome-wide linkage analysis on age at menarche did not find evidence for common highly penetrant variants influencing this trait, however (Anderson et al. 2008). In mice, a quantitative trait locus on chromosome 6 has been demonstrated to be associated with vaginal opening (a marker of the initiation of puberty) (Nathan et al. 2006). Another work in these animals detected a significant locus on X chromosome (Zhu et al. 2008).

3. ENVIRONMENTAL MODIFIERS OF PUBERTAL TIMING

The neuroendocrine network initiating pubertal maturation is sensitive to environmental factors, which may thus affect pubertal timing. For instance, pathological conditions, such as chronic somatic illnesses (Pozo and Argente 2002), psychological distress (Tahirovic 1998), and intense physical training (Georgopoulos et al. 1999), are known to delay pubertal maturation. On the other hand, hypothalamic lesions, such as hamartomas or optic nerve gliomas (Chemaitilly et al. 2001) as well as other intracranial processes, e.g. hydrocephalus (Löppönen et al. 1996), may cause precocious pubertal development. Early or precocious puberty is also common in children having received low-dose cranial irradiation for malignancy, whereas high doses usually cause pubertal delay (Toogood 2004). Also in healthy adolescents, the normal variation of pubertal timing is apparently influenced by nongenetic factors. This is evidenced by, for example, social class and urban versus rural differences in pubertal timing, as well as by observing secular trends toward earlier maturation in conjunction with improved health and nutrition (Roche 1979, de Muinck Keizer-Schrama and Mul 2001). The worldwide variation of pubertal timing is largely explained by nutritional factors (Parent et al. 2003). Climate and the light-darkness cycle also have some influence on pubertal timing in a physiological situation (Magee et al. 1970, Albright et al. 1990). Based on twin studies, the contribution of environmental factors to the variation of pubertal timing may be, however, less than 50% (Fischbein 1977, Sharma 1983, Treloar and Martin 1990, Kaprio et al. 1995, Beunen et al. 2000, Towne et al. 2005, van den Berg and Boomsma 2007, Silventoinen et al. 2008). Genetic differences may well determine how an individual responds to nongenetic, environmental modifiers (van den Berg and Boomsma 2007). Environmental factors influencing hypothalamic control of pubertal onset directly or through changes in peripheral tissues, as well as effects of gonadal sex steroids at the hypothalamic level are discussed below.

3.1. HYPOTHALAMIC EFFECTS OF GONADAL SEX STEROIDS

Sex steroids induce maturation of secondary sex characteristics through their effects on peripheral target tissues, but they also have effects at the hypothalamic level (Moore and Price 1932). In females, positive feedback of estrogens induces the preovulatory LH surge required for ovulation (Karsch et al. 1997), whereas negative feedback of plasma levels of sex steroids from

both the testis and ovaries inhibits GnRH release. This negative feedback partly operates already in childhood, when plasma levels of sex steroids are very low (Mitamura et al. 1999, 2000). An early "gonadostat theory" of the onset of puberty by Hohlweg et al. (1931) states that at the initiation of pubertal maturation the sensitivity of the hypothalamic level to suppressive effects of sex steroids decreases, resulting in the initial pubertal GnRH release. This theory has, however, been contradicted by observations of similar patterns of gonadotropin concentrations in agonadal and healthy subjects (Conte et al. 1980). Since the gonadostat theory, GnRH have been known to be centrally regulated (Terasawa and Fernandez 2001), as reviewed above. The feedback effects of sex steroids are, however, important modifiers in the onset and during the progress of pubertal maturation, and are crucial for adult reproductive competence. Since GnRH neurons themselves do not express receptors for sex steroids (Herbison and Theodoris 1992), the feedback effects must be mediated by other neurons, such as those expressing *KISS1*. *KISS1* expressing neurons found in the anteroventral periventricular (AVPV) and arcuate nuclei (Arc) of the hypothalamus (Gottsch et al. 2004, Smith et al. 2005a,b), areas known to send projections to the area where GnRH cell bodies reside (Simerly 2002), are regulated by gonadal steroids (Irwig et al. 2004, Navarro et al. 2004, Smith et al. 2005a,b). In the Arc, *KISS1* expressing neurons possibly mediate the negative feedback of sex steroids since these neurons are inhibited by testosterone and estradiol replacement and upregulated by castration and ovariectomy in male and female rats, respectively (Irwig et al. 2004, Navarro et al. 2004, Smith et al. 2005a,b). The positive feedback of estrogen in females seems to be regulated by the hypothalamic AVPV, in which *KISS1* expression is stimulated in response to estrogen (Smith et al. 2005a, 2006).

3.1.1. ENDOCRINE-DISRUPTING CHEMICALS

In our environment, we are exposed (in intrauterine life or postnatally) to various hormone-like compounds, e.g. organochlorine pesticides, synthetic medical drugs, and phytoestrogens. These substances may have biological effects and disturb endocrine functions, such as reproductive health and pubertal maturation, and are thus called endocrine-disrupting chemicals (EDCs) (Teilmann et al. 2002, Rasier et al. 2006, Buck Louis et al. 2008). EDCs may sometimes have anti-estrogenic or anti-androgenic actions, in which case exposure has been shown to be associated with a slow tempo of pubertal maturation or a delay in onset of puberty (Staessen et al. 2001, Selevan et al. 2003). For instance, the probability of slow genital development in boys or breast development in girls living in a Belgian industrial suburb rises with higher serum concentrations of PCBs or dioxin-like compounds (Staessen et al. 2001). EDCs are, however, mostly estrogenic and may potentially advance appearance or pubertal indices or full maturation of the HPG axis. Food contamination or other accidental EDC exposure in childhood or in utero has been demonstrated to be associated with the early

appearance of breast tissue (Saenz de Rodriguez et al. 1985, Colón et al. 2000, Felner and White 2000), acceleration of growth (Felner and White 2000), and early menarche (Blanck et al. 2000, Vasiliu et al. 2004). A large epidemic of premature thelarche in children in Puerto Rico during 1978-1981 was suggested to be caused by exogenous estrogen contamination in the food ingested by the children and their mothers (Saenz de Rodriguez et al. 1985). Precocious puberty, observed much more often in children adopted from developing countries than in the natives (incidence 20-80 fold), has also been proposed to be caused by EDCs (Proos et al. 1991, Krstevska-Konstantinova et al. 2001, Mul et al. 2002, Teilmann et al. 2006), e.g. pesticides, which are not, in contrast to the Western world, banned in developing countries (Turusov et al. 2002). Supporting the role of an organochlorine pesticide DDT (metabolite p,p´-DDE) in a series of precocious pubertal girls from Belgium, those in ethnic minority groups had much higher serum levels of this compound than natives (Krstevska-Konstantinova et al. 2001). Furthermore, the risk of precocious puberty is increased in older adoptees expected to have had a longer EDC exposure than those adopted at younger ages (Teilmann et al. 2006).

Childhood exposure to EDCs with sex steroid-like effects or sex steroid antagonistic actions may affect pubertal development by peripheral, gonadotropin-independent mechanisms through interaction with steroid receptors, or through alterations in sex steroid metabolism (Buck Louis et al. 2008). As a consequence of estrogen excess, separate early breast development may occur without full HPG axis maturation (Saenz de Rodriguez et al. 1985, Colón et al. 2000). EDCs may, however, also have direct effects at the hypothalamic level. This is anticipated by observing the association of EDC exposure with early menarche and acceleration of pubertal growth, possibly reflecting full HPG axis maturation (Blanck et al. 2000, Felner and White 2000, Vasiliu et al. 2004). Furthermore, in vitro data have shown that certain mixtures of organochlorine pesticides increase GnRH mRNA expression in cultured immortalized GT1-7 cells, which have many similarities to GnRH neurons in vivo (Gore et al. 2002). In children adopted from developing countries, precocious puberty has been suggested to be induced by direct HPG axis activation, or indirect withdrawal of EDCs' sex hormone-like negative feedback effects, which take place when moving to a new home country (Krstevska-Konstantinova et al. 2001). Prenatal exposure to EDCs has been proposed to permanently alter endocrine "set-points" in later life, possibly by affecting size of the baby (Rylander et al. 2000) or altering expression of genes that regulate sex steroid activity and metabolism (Teilmann et al. 2002).

3.2. NUTRITION AS A REGULATOR OF PUBERTAL TIMING

Appropriate nutrition is an important prerequisite for the function of endocrine organs and reproduction. Excessive weight loss secondary to somatic or psychiatric illnesses is associated with hypogonadotropic hypogonadism (Vigersky et al. 1977). In chronic childhood

caloric deprivation, pubertal maturation — appearance of secondary sex characteristics, menarche, and growth — is delayed or absent (Satyanarayana and Naidu 1979, Kulin et al 1982, Leenstra et al. 2005), and also low blood levels of gonadotropins have been reported (Kulin et al. 1984). In addition to delay in the onset of puberty, slow progression (tempo) of maturation is also possible, evidenced by a more pronounced delay in menarche than in age at achieving B2 (Kulin et al. 1982). In Kenya, where the level of malnutrition is high in children, puberty begins 1.5-2 years later than in the US (Leenstra et al. 2005). With adequate energy supplies, however, specific diets and macro- or micronutrients mostly lack a correlation with pubertal timing (Moisan et al. 1990, Maclure et al. 1991, Koprowski et al. 1999). Earlier age at menarche and age at phv have been reported to correlate with consuming more animal than vegetable protein at the ages of 3-5 years and 6-8 years, respectively (Berkey et al. 2000), but mostly, pubertal timing has been shown to be influenced by the amount of adipose tissue (Maclure et al. 1991, Koprowski et al. 1999). In fact, in the 1970s, a certain body weight was hypothesized to trigger pubertal onset (Frisch and Revelle 1970, Frisch and McArthur 1974). This proposition was based on the association between menarcheal age and a certain body weight/body fat ratio calculated indirectly from height and weight measurements. This "critical weight theory" of pubertal onset is, however, controversial. Based on longitudinal studies and direct skin-fold thickness measurements, menarche occurs independently of changes in body composition as a result of maturation of the HPG axis and increased levels of pubertal hormones (de Ridder et al. 1992, Legro et al. 2000).

3.2.1. SIGNALS INTEGRATING METABOLISM AND PUBERTAL ONSET

Despite controversy regarding the "critical weight theory", nutritional factors (accumulation of fat) influence hypothalamic control of pubertal onset; the central regulation of energy balance and reproduction are tightly linked. Thus, the information from energy reserves must somehow be signaled to the hypothalamic GnRH region, which determines the onset of pubertal maturation. Several factors may serve as such peripheral signals. One of these is leptin, an adipose tissue-derived satiety hormone, the deficiency of which due to mutations in the leptin (Strobel et al. 1998) or leptin receptor gene (Clément et al. 1998) causes obesity, and in addition, absence of pubertal development because of hypogonadotropic hypogonadism. In fact, leptin was, in the late 1990s, proposed to be the initial trigger for pubertal onset. This was based on the observation that leptin induced pubertal pattern of LH release in a girl with congenital leptin deficiency (Farooqi et al. 1999), and reversed reproductive failure in leptin-deficient mice (Chebab et al. 1996). Some studies have shown that leptin treatment accelerates pubertal onset also in normal mice (Ahima et al. 1997, Chebab et al. 1997). However, others have found that, although leptin advanced puberty in food-restricted rodents with delayed puberty, advancement was not observed relative to untreated ad libitum-fed animals (Cheung et al. 1997). One longitudinal study in human males

reported a brief pulse of leptin preceding the onset of puberty (Mantzoros et al. 1997), but other studies have failed to repeat this finding (Ahmed et al. 1999). On the contrary, leptin levels were shown to be relatively constant in prepubertal boys and girls, and to rise in a dimorphic pattern relating to characteristic sex-specific gains in fat and fat-free mass only after the onset of puberty (Ahmed et al. 1999). This and the observation that leptin treatment advances puberty only in food-restricted rodents with delayed puberty (Cheung et al. 1997) contradicts leptin's role as the primary activator of pubertal maturation. However, leptin's permissive role in the onset of pubertal development is apparent.

Over the years, also fatty acids (Schneider and Wade 1989), glucose (He et al. 1999), and insulin-like growth factor I (IGF-I) (Hiney et al. 1996) have been proposed to facilitate GnRH secretion and link the HPG axis and metabolism. Other possible peripheral signals are gastrointestine-derived ghrelin and polypeptide YY, administration of which has been shown to suppress LH secretion in experimental animals (Fernandez-Fernandez et al. 2006). Recently, insulin has been found to serve as a peripheral signal integrating metabolism and hypothalamic control of pubertal onset. Knock-out mice harboring neuron-specific insulin receptor inactivation display hypogonadism secondary to impaired GnRH secretion (Brüning et al. 2000). In addition, increased levels of insulin stimulate LH release in rats (Burcelin et al. 2003). In girls with low birth weight and subsequent fast catch-up weight gain, obesity, and hyperinsulinemic insulin resistance, frequent early menarche is delayed by metformin treatment (Ibáñez et al. 2006b,c). This suggests that insulin is a major codeterminant of the progression of puberty in girls.

Peripheral messengers may require other cells to mediate their information to GnRH neurons. Insulin possibly has direct effects (Salvi et al. 2005), but leptin's signaling effects must occur through other cells, since GnRH neurons themselves do not express leptin receptors. Leptin's signaling effects may occur through pro-opiomelanocortin cells or neuropeptide Y (NPY) -producing neurons of the Arc (Finn et al. 1998). NPY has been shown to have, e.g. inhibitory effects on GnRH cells (El Majdoubi et al. 2000), and mediate starvation-induced inhibition of the HPG axis. This is evidenced by mice lacking NPY Y1 receptor proceeding normally into puberty even when energy stores are inadequate (Pralong et al. 2002). The existence of the leptin-NPY-GnRH pathway is reflected in dramatically accelerated puberty in these mice with blocked NPY effects when treated with leptin. Recently, hypothalamic *KISS1* expressing neurons have been shown to be sensitive to metabolic conditions, as *KISS1* expression is diminished during states of negative energy balance (Fernandez-Fernandez et al. 2006). In addition to direct activation of GnRH neurons at the onset of puberty (Gottsch et al. 2004, Irwig et al. 2004, Navarro et al. 2004, 2005, Dhillo et al. 2005, Messager et al. 2005, Shahab et al. 2005, Castellano et al. 2006, Plant et al. 2006), and involvement in negative and positive feedback of sex steroids (Irwig et al. 2004, Navarro et al. 2004, Smith et al. 2005a,b, Smith et al. 2006), *KISS1* neurons also seem to serve as central links between GnRH neurons and peripheral metabolic signals, such as leptin (Luque et al. 2007).

3.2.2. EFFECTS OF WEIGHT GAIN ON TIMING OF PUBERTY

In contrast to undernutrition, excess of nutrients and obesity have in many studies been shown to be associated with advanced pubertal development (Maclure et al. 1991, Kaprio et al. 1995, Guo et al. 1997, Koprowski et al. 1999, Biro et al. 2001, He and Karlberg 2001, Kaplowitz et al. 2001, Remsberg et al. 2005, Juul et al. 2006, Kindblom et al. 2006, Sandhu et al. 2006, Silventoinen et al. 2008). For instance, girls with a body mass index (BMI) above the median have a significantly lower age at B2 (10.4 vs. 11.2 years) and menarche (13.1 vs. 13.7 years) compared with those with BMI below the median (Juul et al. 2006). Although most studies have encompassed girls (Maclure et al. 1991, Kaprio et al. 1995, Koprowski et al. 1999, Biro et al. 2001, Kaplowitz et al. 2001, Remsberg et al. 2005, Juul et al. 2006), similar observations have been made also in boys (Guo et al. 1997, He and Karlberg 2001, Kindblom et al. 2006, Sandhu et al. 2006, Silventoinen et al. 2008). A study in Swedish male twins demonstrated that BMI at ages 1 through 10 years correlated negatively with age at onset and phv of the pubertal growth spurt (Silventoinen et al. 2008). Similarly, He and Karlberg (2001) showed that higher BMI gain in childhood between 2 and 8 years in both genders was related to earlier onset of pubertal growth. In addition to earlier pubertal growth spurt, childhood growth rates are increased in obese children, but subsequently, height gain during the pubertal growth spurt is reduced (He and Karlberg 2001). Thus, despite taller stature in childhood, adult height is not increased.

Whereas excessive postnatal weight gain is clearly associated with early maturation, advanced puberty relates to poor weight gain in prenatal life, i.e. to small weight relative to length at birth (Cooper et al. 1996, Adair 2001, Luo et al. 2003, Tam et al. 2006). Hypothetically, nutritional deprivation causing intrauterine growth retardation (IUGR) during the prenatal sensitive period could independently affect the subsequent maturation schedule through developmental plasticity in response to undernutrition (Barker 2004). From an evolutionary perspective, this would be beneficial when an adverse prenatal environment predicts short survival after birth (Gluckman and Hanson 2006). However, IUGR children with persistent short stature and absence of catch-up growth after birth mature late rather than early (Lienhardt et al. 2002). Fast postnatal catch-up growth during the first two years of life is often seen in IUGR children with small birth weight (Hokken-Koelega et al. 1995) and is positively associated with childhood obesity at age 2-5 years (Ong et al. 2000, Ibáñez et al. 2006). Thus, the effects of prenatal malnutrition on timing of maturation may be potentiated by fast postnatal catch-up weight gain (Persson et al. 1999, Adair 2001, Luo et al. 2003, Tam et al. 2006), or early obesity may independently contribute to advanced maturation (dos Santos Silva et al. 2002). In the study by dos Santos Silva et al. (2002), the initial finding that low birth weight was associated with early menarche was reversed after controlling for growth in infancy; menarche was reached later in girls who were light at birth. In foreign adopted children, early malnutrition (Miller et al. 1995) and subsequent fast catch-up growth after arrival to the new home country have been

suggested to explain their increased risk for advanced (precocious) maturation (Proos et al. 1991, Mul et al. 2002, Teilmann et al. 2006).

Obesity-related hormonal factors may contribute to the association between advanced pubertal maturation and obesity. Since percentage of body fat is correlated with serum leptin levels (Considine et al. 1996, Ahmed et al. 1999), adiposity-related increases in leptin concentrations may permit pubertal development at an early age. Abnormalities in sex hormone production and metabolism (Kirschner et al. 1982) may also play a role in advanced maturation. In obesity, levels of prehormones for both testosterone and estrogen are elevated, extragonadal aromatization of androgens to estrogens is increased, and bioavailability of sex steroids is heightened because of lowered levels of sex hormone-binding globulin (de Ridder et al. 1992). Enhanced adrenal gland function in obesity (Genazzani et al. 1978, de Ridder et al. 1992) may also contribute to earlier pubertal development. Such changes in hormonal milieu may accelerate first appearance of pubertal signs or the tempo of maturation, i.e. progression of puberty after its initiation (de Ridder et al. 1992, Biro et al. 2006), by peripheral effects (e.g. through estrogen receptors). Hormonal changes may also influence pubertal maturation at the hypothalamic level and advance full HPG axis maturation. This is evidenced by an accelerated pubertal growth spurt in association with obesity (He and Karlberg 2001, Kindblom et al. 2006, Silventoinen et al. 2008). In fetal growth retardation and subsequent fast catch-up weight gain, amplified adrenarche, functional ovarian hyperandrogenism (Francois and de Zegher 1997, Ibáñez et al. 1998), increased IGF-I levels (Ong et al. 2002), and hyperinsulinemia and insulin resistance related to rapid early weight gain (Bhargava et al. 2004, Ong et al. 2004, Ibáñez et al. 2006a, 2006b, 2006c) may be among the factors mediating the shift in pubertal timing.

Obesity may partly explain the likelihood of earlier puberty in African versus Caucasian Americans (Herman-Giddens et al. 1997, Biro et al. 2001, Sun et al. 2002, 2005, Wu et al. 2002, Anderson and Must 2005) since mean body fat (Kaplowitz et al. 2001), as well as leptin levels (Wong et al. 1998), are higher in African Americans. Increasing prevalence of childhood obesity in the US (Ogden et al. 2002, Hedley et al. 2004) may be one explanation for the exceptionally early pubertal maturation observed in some American girls, especially African Americans (Herman-Giddens et al. 1997, Kaplowitz et al. 2001). The prevalence of obesity is increasing also in Europe (Ong et al. 2000) and calls for careful recording of pubertal timing data in European populations. Secular changes in pubertal timing and obesity may, however, be independent phenomena, or the increase in obesity is not a determinant but rather a consequence of early maturation. Despite the association demonstrated in longitudinal cohorts (Guo et al. 1997, Biro et al. 2001, He and Karlberg 2001, Kindblom et al. 2006, Sandhu et al. 2006), a causal link between obesity and early maturation has not been substantiated. Both high BMI and early puberty may be consequences of accelerated maturation of the HPG axis, thus being manifestations of the same physiological process.

Supporting this, twin data have shown that both obesity and early puberty are regulated by a substantial proportion of shared genes (Kaprio et al. 1995, Silventoinen et al. 2008).

In conclusion, the normal variation of pubertal timing is influenced by multiple factors, including genes and the environment (**Figure 4**), but their exact contributions and mechanisms are yet largely unknown.

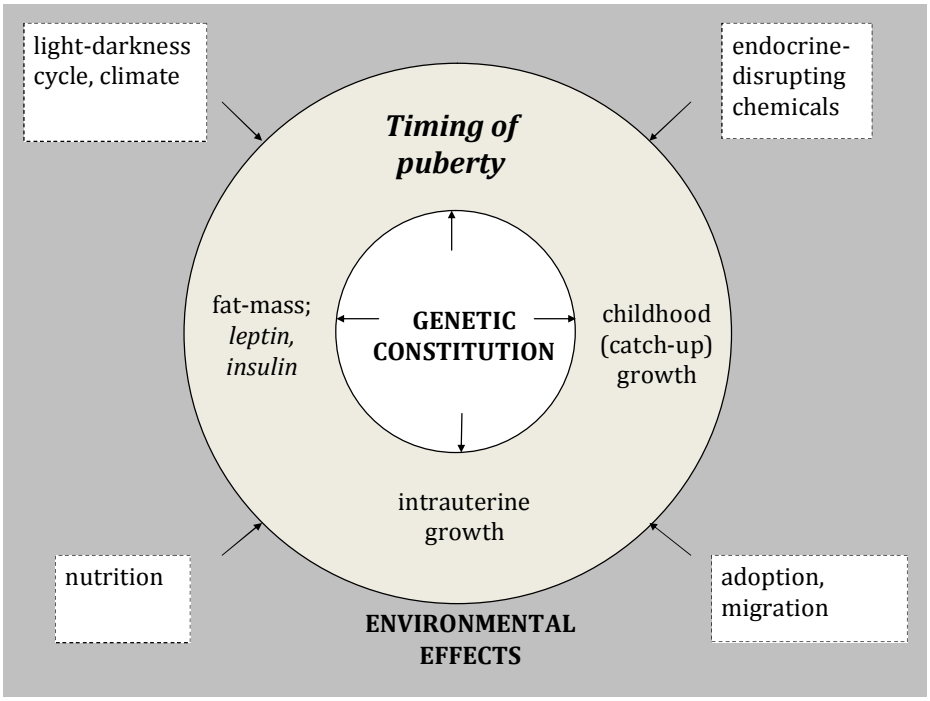

Figure 4. Normal variation in the timing of pubertal onset is influenced by genetic factors and superimposed by environmental hits, which may affect through changes in peripheral tissues or directly at the hypothalamic GnRH region. Modified from Parent et al. *The timing of normal puberty and the age limits of sexual precocity: variations around the world, secular trends, and changes after migration*. Endocr Rev, 2003;24(5):668-693 (Copyright © 2003 The Endocrine Society).

AIMS OF THE STUDY

Mechanisms controlling the timing of the onset of puberty remain elusive. The purpose of this study was to shed light on the genetic regulation of pubertal timing by:

1. investigating the use of growth pattern data in assessing the timing of puberty, e.g. height SD in puberty versus adult height SD. In addition, childhood growth patterns and adult height attainment in constitutional delay in growth and puberty (CDGP) were explored (I).

2. evaluating the contributions of genetic versus environmental factors to the variation of the timing of puberty assessed by growth data in twin boys and girls (II).

3. exploring familial clustering and inheritance patterns of CDGP, an extreme variant of normal pubertal timing, as diagnosed from longitudinal growth data in pedigrees of patients with this condition (III).

4. detecting genetic loci linked to CDGP, thus associating with pubertal timing, in multiply affected CDGP pedigrees by genome-wide scans (IV).

SUBJECTS

1. POPULATION OF TWINS (II)

Contributions of genetic and environmental factors to the variation of pubertal timing were investigated in twins. Data were derived from the FinnTwin12-17 study, which included five subsequent birth cohorts of Finnish twins born in 1983-1987 (Kaprio et al. 2002). Height data at the mean ages of 11.4 (SD 0.29) and 17.6 years (SD 0.27) in girls, and 14.1 (SD 0.08) and 17.6 years (SD 0.24) in boys were collected by questionnaires. Information on 2309 girls and 1828 boys, including 457 MZ and 465 same-sex dizygous (SSDZ) and 399 opposite-sex dizygous (OSDZ) complete twin pairs, was obtained. Zygosity was determined by a deterministic algorithm using questions on physical similarity at school age (Sarna et al. 1978).

2. FAMILIES OF PATIENTS WITH DELAYED PUBERTY

Boys and girls diagnosed with CDGP were gathered through medical records of adolescents referred to specialist pediatric care due to pubertal delay (ICD 8, 9 or 10 diagnoses 626.00, 2590A, or E30.00/E30.09) first at the Hospital for Children and Adolescents, Helsinki University Hospital, and at Jorvi Municipal Hospital, Espoo, Finland, between the years 1982 and 2004. Then the study was extended to encompass also patients investigated at Peijas Municipal Hospital, Vantaa, and at Kuopio, Tampere and Turku University Hospitals during the same years, as well as at the Hospital for Children and Adolescents in the beginning of 2005. Criteria for CDGP were more than 2 SD later than average age at G2, corresponding to a testicular volume of more than 3 ml in boys, and more than 2 SD later than average age at B2 in girls (Marshall and Tanner 1969, 1970), with no signs of chronic illnesses accounting for delayed puberty. These were excluded by medical history, clinical examination, and routine laboratory tests (blood cell count, thyroid, kidney and liver function tests, gonadotropin and sex steroid determination, and laboratory tests excluding chronic infections and celiac disease). Growth hormone stimulation tests were performed if growth pattern suggested GH deficiency. Hypogonadotropic hypogonadism, if suspected, was excluded by GnRH testing and by clinical follow-up ensuring spontaneous pubertal development in later adolescence. All subjects were born full-term and had normal birth weight.

All patients with CDGP were contacted by letter requesting their and primarily their parents' and siblings' (first-degree relatives) participation. Altogether, 286 families (146+23 from Helsinki, Espoo and Vantaa, and 117 families from Kuopio, Tampere and Turku participated (**Figure 5**). All members in participating families filled in a questionnaire and were interviewed by phone. Families were excluded if first-degree relatives had any chronic illnesses that could have affected growth or development. In addition, only families of Finnish origin were included because, as the inheritance pattern of CDGP is likely to be complex, the aim was to keep the study population as homogeneous as possible. Second-degree relatives, such as aunts, uncles, and grandparents, and third-degree relatives, such as cousins and grand-grandparents, and even fourth-degree relatives of the probands were recruited if the interview suggested CDGPs to also be present in these more remote relatives and if permission was obtained from the parents of the proband. Consanguinity was not suspected or verified in any of the families.

2.1. PEDIGREE ANALYSIS (III)

Clustering of CDGP in family members was explored in families of male and female patients investigated for pubertal delay in Helsinki and Espoo during 1982-2004 (**Figure 5**). Of 726 patients (566 males, 160 females), 68% (401 males, 91 females) fulfilled the diagnostic criteria for CDGP and had both parents available for contact. Of 146 families willing to participate after contact by letter, 22 were excluded because of parental illnesses (6), undeterminable pubertal timing in one or both parents (14), or parental origin being something other than Finnish (2). The analysis, thus, included 124 families (of 95 male and 29 female probands) representing 25% of families of all eligible subjects. The number of probands' first-degree relatives was 387, including 248 parents and 139 postpubertal siblings (58 brothers, 81 sisters). Second-degree or more remote relatives were successfully recruited in 30% (37/124) of the families. Total numbers of investigated male and female family members of the probands were 290 and 304. These families encompassed a total of 718 individuals, including the probands.

2.2. LINKAGE ANALYSIS (IV)

For linkage, a total of 52 families (37+4 families from Helsinki, Espoo and Vantaa, and 11 families from Kuopio, Tampere and Turku) with pubertal delay in only one of the parents of the proband and his/her second-degree or more remote relatives were selected from all participating families (**Figure 5**).

2.3. ANALYSIS OF GROWTH PATTERNS (I)

Of the participating male probands investigated for CDGP at all six clinics (**Figure 5**), 73 were aged 20 years or more, and for 70, also childhood growth data were available. In these men, childhood growth patterns and adult height attainment was investigated.

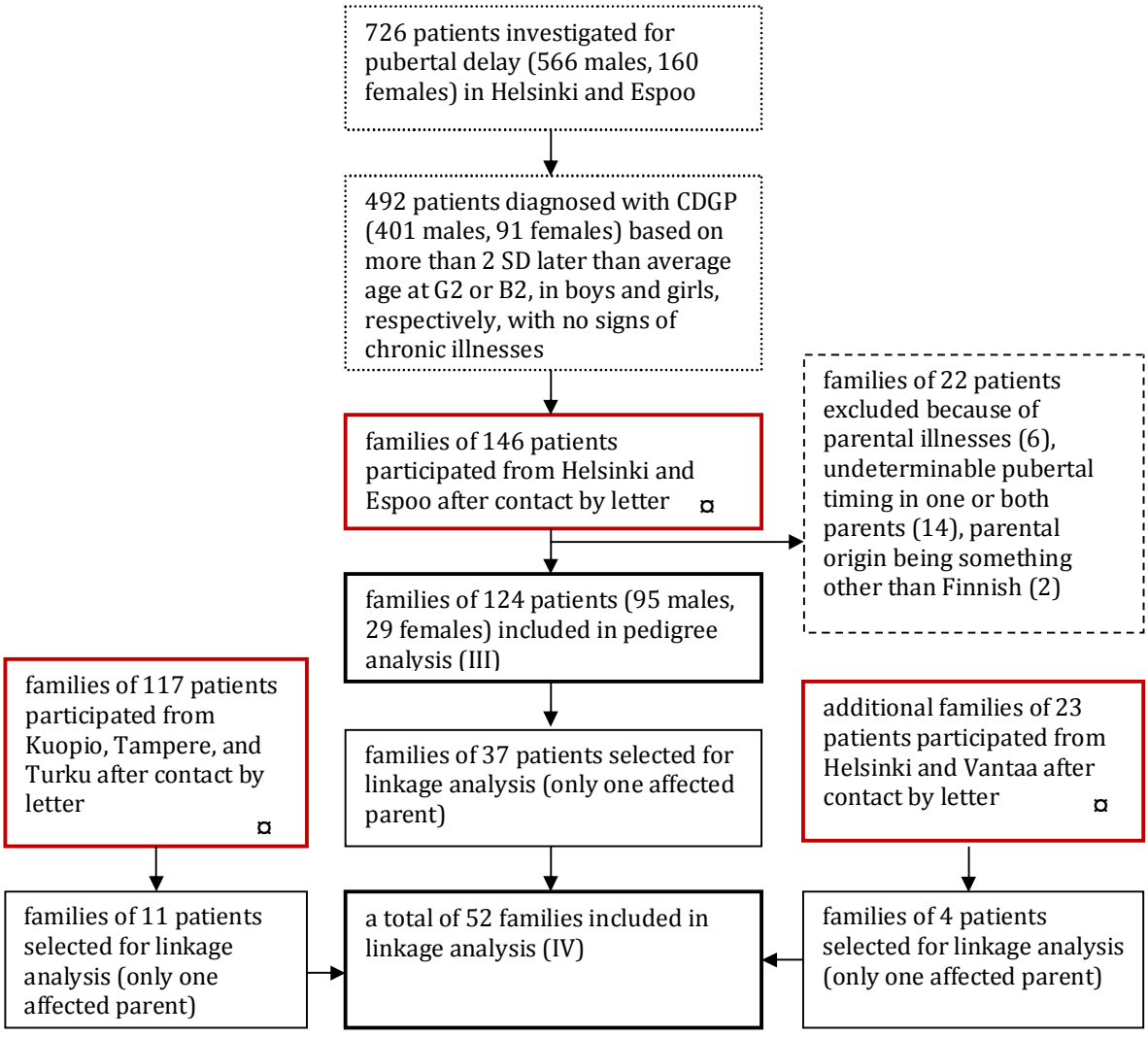

Figure 5. Collection of families of patients with constitutional delay of growth and puberty (CDGP) for pedigree (III) and linkage (IV) analyses. Seventy male CDGP subjects for growth pattern analysis (I) were selected from probands of all participating families (¤).

METHODS

1. ASSESSMENT OF PUBERTAL TIMING BY GROWTH DATA

The timing of puberty in all family members was primarily assessed from linear growth. Probands' growth data were retrieved from medical records, and family members' from archived well-baby clinic and school healthcare height and weight measurements. Final adult height (FH) was obtained mostly by interview. The timing of puberty was assessed by observing the chronological age at onset of pubertal growth (take-off) and pubertal peak height velocity (phv). The age at take-off was estimated from the point at which growth velocity increased after the slowest growth velocity at the beginning of the pubertal growth spurt, resulting in more than 0.3 SD increase in height SD (Karlberg et al. 2003). Age at phv was estimated from the point where growth velocity was the fastest, i.e. where the largest increment in standing height occurred during one year at adolescence (Nicolson and Hanley 1953, Liu et al. 2000). Criteria for CDGP were set by the age at take-off or phv occurring 2 SD (strict criterion) or 1.5 SD (relaxed criterion) beyond the mean (**Table 3**), based on the reference data by Tanner and colleagues (1976) (mean age at take-off 12.05 ± 0.85 and 10.30 ± 0.90, and mean age at phv 13.91 ± 0.84 and 11.89 ± 0.90 in males and females, respectively).

Based on these criteria, the age at take-off was 3.4 and 2.8 SD later than the average in 95 male probands (mean age at take-off 14.97±0.65 years) and in 29 female probands (mean age at take-off 12.96±0.84 years), respectively, included in the pedigree analysis. Delay in the appearance of Tanner stage 2 in these probands was comparable; the mean G2 age (15.10±0.83 years) was 3.4 SD later than average in Finnish boys (Ojajärvi 1982), and the mean age at B2 (13.75±0.71 years) more than 2.3 SD later than average for Finnish girls (Ojajärvi 1982). Thus, the set growth chart-based CDGP criteria were expected to identify family members with pubertal delay. Based on retardation of bone maturation determined by calculating the difference between bone age (Greulich and Pyle 1959) and chronological age at the initial evaluation (2.6±0.7 years in boys and 2.7±1.0 years in girls), the degree of pubertal delay was similar in both male and female probands.

Family members' growth data obtained from archived height measurement records were not always sufficiently complete to assess exact ages at take-off or phv. Therefore, other components of pubertal growth, such as the age at attaining adult height (growth velocity less than 2 cm/year), were used in growth chart-based assessment of pubertal timing (**Table 3**). Pubertal timing was based on growth data in 80% of the first-degree relatives. If

growth data were unavailable, the timing of puberty for family members was evaluated by structured interviews. Interview-based CDGP criteria were set as having undergone pubertal development more than 2 (strict criterion) or 1.5 years (relaxed criterion) later than their peers (both sexes), or menarche after 15 (strict criterion) or 14 years (relaxed criterion) (**Table 3**). Interviews were mostly used for second-degree or more remote relatives (60%).

Table 3. Applied growth chart- and recall-based age limits for CDGP.

Gender	***Marker of pubertal development***	***Relaxed criteria for CDGP (years)***	***Strict criteria for CDGP (years)***
Male	onset of pubertal growth spurt, take-off	>13.3	>13.8
	peak height velocity, phv	>15.2	>15.6
	adult height attainment	>18	>19
	recalled pubertal development	>1.5 later than peers	>2 later than peers
Female	onset of pubertal growth spurt, take-off	>11.7	>12.2
	peak height velocity, phv	>13.2	>13.7
	adult height attainment	>16	>17
	recalled pubertal development	>1.5 later than peers	>2 later than peers
	menarche	>14	>15

In addition to age at attaining adult height, relative height, i.e. height compared with same-aged peers (height SD), during pubertal years is influenced by pubertal timing. Height SD is increased if maturation is early and decreased if pubertal development occurs late compared with those with average pubertal maturation (Tanner et al. 1976, Tanner and Davies 1985, Karlberg et al. 2003). Especially at the age when height growth peaks in the general population, at approximately 14 and 12 years in boys and girls, respectively (Tanner et al. 1976), height SD is influenced by the timing of pubertal maturation, but also by genetic height potential (Tanner et al. 1976, Karlberg et al. 2003). By calculating the difference in height SD between 12 or 14 years and adulthood, the influence of genetic height potential can be excluded. Thus, the resulting change in the relative height between the age when height growth peaks in the general population and adulthood, HD:SDS (Height Difference in Standard Deviation Scores), reflects pubertal timing.

Validity of HD:SDS as a marker for the timing of puberty was tested by investigating its correlation with the age at take-off and phv in a subpopulation of 215 men and 211 women from CDGP families providing longitudinal growth data. The ages at take-off and phv, and height SD at the age 14 or 12 years were obtained from growth charts, and FH by phone. For men, the correlation of HD:SDS (change in height SD between 14 years and adulthood) with age at phv was 0.84 ($p<0.001$) (**Figure 6A**), and with the age at take-off, 0.75 ($p<0.001$). For women, the correlations of HD:SDS (change in height SD between 12 years and adulthood) with age at phv and take-off were 0.78 ($p<0.001$) (**Figure 6B**) and 0.71 ($p<0.001$), respectively. Significant correlations confirm that HD:SDS is a valid marker for pubertal timing in a population of boys or girls.

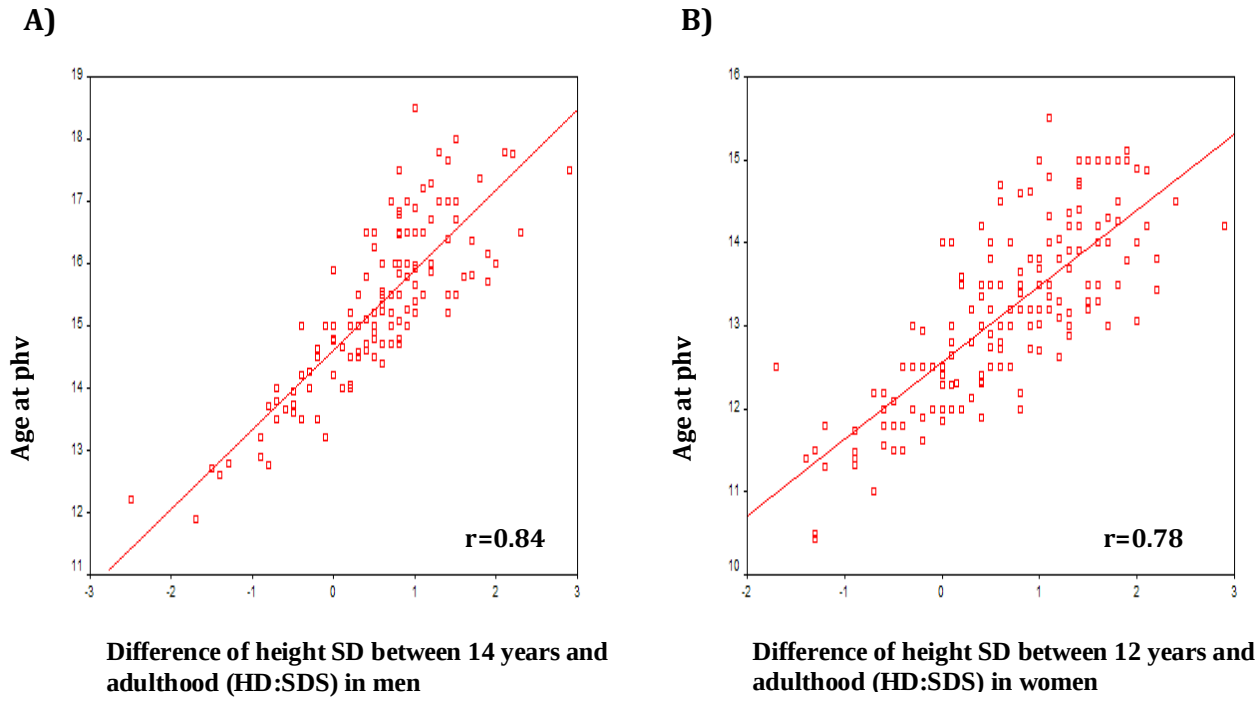

Figure 6. Correlation of HD:SDS with age at phv in **A)** 215 healthy men (r=0.84, p<0.001) and **B)** 211 healthy women (r=0.78, p<0.001). Modified from Wehkalampi et al. *Genetic and environmental influences on pubertal timing assessed by height growth.* Am J Hum Biol, 2008;20(4):417-423 (Copyright © 2008 Wiley-Liss, Inc., A Wiley Company).

Pubertal timing in twins was assessed by HD:SDS. Heights in centimeters reported by the twins were changed into standard deviations and adjusted by the exact age at the time of response by calculating regression residuals to account for small age differences (Cole and Green 1992, Cole et al. 1998). The age 11.5 years in girls is close to, and 14.0 years in boys is the average age at phv in the general population (Tanner et al. 1976). Height at age 17.5 years was considered to be near-final height since by this age 99.8% of girls and 99.1% of boys reach adult height (Nicolson and Hanley 1953). Height SD at age 11.5 in girls and 14.0 years in boys was subtracted from height SD at age 17.5 years, creating the difference HD:SDS. HD:SDS is negative for early and positive for late maturers.

1.1. GROWTH PATTERNS IN DELAYED PUBERTY (I)

For the 70 male CDGP probands included in the analysis of childhood growth patterns and adult height attainment from all six clinics (**Figure 5**), it was explored whether those boys who did not attain FH corresponding to their genetic target height displayed an identifiable growth pattern already in childhood.

The age at first observing G2 as well as bone maturation at the initial evaluation (Greulich and Pyle 1959) were obtained from medical records. Birth length, relative height at ages 3, 5, 7, and 9 years, age at take-off, minimum relative height at puberty (minSD, the lowest observed height SD at the beginning of pubertal growth spurt), age at minSD, and BMI at the initial evaluation were recorded from growth charts. FH was obtained by phone or from growth charts at the mean age of 23.9 years (range 20.0-35.5 years), by which age even late-maturing boys were expected to have reached adult height (Hägg and Taranger 1991). Heights of the parents were obtained from their growth charts or by interview, and parent-specific target height (TH) was calculated as follows: 0.0611 x father's height (cm) + 0.0703 x mother's height (cm) - 22.37 (Sorva et al. 1989). When asked about receiving testosterone treatment, 40 subjects (57.1%) had decided to wait for spontaneous progression of puberty without medication, whereas 30 (42.9%) had chosen the treatment. This was started between the ages of 14.2 and 17.0 years (mean 15.5 years), with a monthly dose of 50-100 mg (1-2 mg/kg/month) of testosterone enanthate (mean 62.6 mg) over 3-18 months (mean 8.3 months).

Before 9 years of age, 44% (31/70) of the subjects had a progressive reduction in height SD (difference in height SD between 3 and 9 years ranged from -0.40 to -1.90 SD). In 56% (39/70) of subjects, no such early reduction in height SD was seen (difference in height SD between 3 and 9 years ranged from -0.30 SDs to +0.90 SD). In the groups with and without early height SD reduction, 13 of 31 and 17 of 39 subjects, respectively, had been treated

with testosterone. The remaining subjects in both groups chose not to receive treatment. Pubertal height gain (FH-minSD), FH, and attainment of target height (FH-TH) were compared between those with early progressive height SD reduction present or absent in untreated subjects. Then, effects of testosterone treatment on FH-minSD, FH, and FH-TH in both groups were investigated.

2. TWIN GENETIC MODELING (II)

The classic twin study design is based on comparing the phenotype of genetically identical MZ twins with that of DZ twins, who share only half of their segregating genes (Boomsma et al. 2002). Based on this, phenotypic variation can be decomposed into genetic and environmental components. The estimated genetic component can be further divided into additive genetic effects and effects due to dominance, i.e. interaction between alleles in the same locus. Within MZ pairs the expected correlation for additive and dominant genetic effects is 1, while within DZ pairs the expected correlation is 0.5 for additive and 0.25 for dominant genetic effects. Environmental variation can further be divided into fractions of common and specific environment. Correlations for common environmental effects are assumed to be 1, and specific environmental effects 0, within both MZ and DZ twins. Based on these assumptions, it is possible to estimate values for each of these variance components treating them as latent standardized variables in linear structural equation models. However, since the twins in the present study were reared together, simultaneous estimation of common environmental and dominant genetic effects could not be carried out. Thus, the possible twin models were additive genetic/common environment/specific environment (ACE), additive genetic/dominant genetic/specific environment (ADE), and additive genetic/specific environment (AE). Of these, the best-fitting model for both genders was selected. Assumptions of the twin model, i.e. equal means and variances for MZ and DZ twins as well as for first-, and second-born co-twins were tested by comparing chi-square change between the twin model and the saturated model, which did not make any of these assumptions. By using twin genetic models, genetic versus environmental contribution to the variation of HD:SDS was explored in 457 MZ and 465 SSDZ twins.

3. PEDIGREE ANALYSIS (III)

In familial clustering and inheritance pattern analysis of 124 pedigrees (**Figure 5**), the applied CDGP criteria (both growth chart- and interview-based) were strict. Based on pubertal timing in first-degree relatives, the probands were grouped as familial (at least

one affected first-degree relative) or sporadic (no affected first-degree relatives). According to the number of affected parents and available data on second- or third-degree relatives, familial probands were further subdivided into three groups: 1) unilineal families (only one affected parent and no evidence of CDGP in the other parent or his/her first-degree relatives), 2) bilineal families (either both parents themselves affected or one parent affected and the other unaffected but having an affected sibling or parent), and 3) families with unaffected parents (one or more siblings affected). To further evaluate inheritance patterns of CDGP, extended pedigrees with unilineal background were explored. Bilineal extended pedigrees were not included in inheritance pattern analysis because the presence of two affected parents makes determination of the mode of inheritance difficult. In relatives of all probands with a positive family history of CDGP, occurrence of CDGP in males and females was calculated, first in first-degree relatives with pubertal timing assessed mostly by growth data, and then in second-degree relatives with mostly interview-based assessment of pubertal timing. Occurrence of CDGP was also calculated separately in parents and siblings of the male and female probands with unilineal background. In these families, the proportions of paternal and maternal inheritance of CDGP can be compared, and also the occurrence of CDGP in male versus female siblings evaluated.

4. GENOME-WIDE LINKAGE SCAN (IV)

From all clinics, 52 unilineal families with only growth chart-based CDGP diagnoses were selected for linkage (**Figure 5**). The criterion for CDGP in family members was relaxed. The families comprised a total of 410 subjects (213 males, 197 females). Of these, 179 individuals (97 males, 82 females) were classified as affected. The largest family contained nine affected pedigree members.

A blood sample was drawn for DNA extraction after obtaining signed consent from all individuals. Genome-wide mapping was carried out using ABI PRISM Linkage mapping Set MD10 (Applied Biosystems). Standard PCR protocols were used for amplification of DNA fragments using 10 ng of genomic DNA as a template. The fluorescently labeled PCR products were separated using an ABI3730 (Applied Biosystems) automated electrophoresis system, and the genotype calls were made by GeneMapper3.7 software. All allele calls were verified by two independent reviewers and any discrepancies were resolved. Genotypes were retrieved for 383 markers spanning all autosomes and the X chromosome. The program GRR (Graphical Representation of Relationships) (Abecasis et al. 2001), which calculates the average identical-by-state allele-sharing between all pairs in a dataset, was used to screen for inconsistencies in familial relationships. Genotypes were checked for

violation of Mendelian segregation of alleles using PEDCHECK (O'Connell and Weeks 1998). Marker locations were based on DeCode map distances. In case a marker had not been placed on the DeCode map, estimates of their genetic locations were based on linear interpolation by using the physical location and the genetic locations of the immediately flanking deCODE markers in the University of California Santa Cruz (UCSC) database using Cartographer (https://apps.bioinfo.helsinki.fi/software/cartographer.aspx). Fine-mapping markers were selected from the UCSC database. Markers were selected one every 1 million bases, preferentially picking markers with heterozygosity > 0.7.

STATISTICS

Statistical Package for Social Sciences (SPSS for Windows, release no 11.0. Inc., Chicago, IL) was used in analyzing growth patterns and familial occurrence of CDGP. Comparison of growth parameters between male probands with early height SD reduction present or absent was performed using independent samples t-test. Pearson χ^2 -test served to compare the numbers of subjects between these groups and between groups of individuals meeting distinct CDGP criteria in pedigrees. $p<0.05$ was considered significant.

Twin genetic models were created using Mx statistical package (version 1.4.06) designed for studies of twin and family data (Neale 2003). The significance of the twin correlations was evaluated by fitting nested models and examining the chi-square distributed change in the -2 log-likelihood values ($\Delta\chi^2$) between the models with and without the correlation parameter set to zero.

Parametric linkage analysis used CDGP as a dichotomized trait and was carried out using the program Merlin. All unaffected family members were scored as having an unknown phenotype, i.e. linkage analyses were run using the affected-only approach since expressivity of potential alleles co-segregating with CDGP is unknown. Three dominant models were tested. The penetrance for affected heterozygotes and homozygotes was kept at 0.9 in all models, whereas the disease allele frequency was set to 0.01, 0.001, and 0.0001 in models 1, 2, and 3, respectively.

ETHICS

The Ethics Committee of the Hospital District of Helsinki and Uusimaa and the Institutional Review Boards of Indiana University, Bloomington, Indiana, approved the study protocol and data collection on twins. Appropriate consent was obtained at the initiation of the study.

Data collection on CDGP patients and families, and protocols for growth and inheritance pattern analyses, as well as for DNA collection and linkage analysis were approved by the Ethics Committee of the Hospital for Children and Adolescents and the Department of Psychiatry, Hospital District of Helsinki and Uusimaa. The approval was extended to encompass the family and DNA sample collections at Kuopio, Tampere, and Turku University Hospitals. First-degree relatives of probands were initially contacted only after proband's permission. Subsequently, second-degree or more remote relatives were contacted after permission from the parents or proband. Confidentiality was maintained during the interviews. The study was conducted in accordance with the guidelines of the Declaration of Helsinki. All participants or their parents or guardians provided their written informed consent.

RESULTS

1. GROWTH PATTERNS IN DELAYED PUBERTY (I)

The mean FH in all 70 subjects included in the analysis of childhood growth patterns and adult height attainment was -0.13±1.0 SD, 178±6.8 cm. This was 0.23±0.75 SD below TH (range from 1.95 SD below to 1.77 SD above TH). Untreated subjects who had or those who did not have early progressive reduction in height SD did not differ significantly in birth length, height at age 3 years, TH, age at take-off, or delay of bone maturation or BMI at the initial evaluation (**Table 4**). Subjects with early height SD reduction had smaller minSD than subjects without such a reduction. Both groups gained height equally well during the pubertal growth spurt (FH-minSD). FH was ascertained at the mean age of 23 years in both groups. FH in subjects who did not have early reduction in height SD was significantly closer to their TH (FH-TH) than in those with early height SD reduction (**Figure 7**, gray boxes).

Table 4. Growth parameters (mean ± SD) in untreated male CDGP subjects with early reduction in relative height present or absent. Modified from Wehkalampi et al. *Progressive reduction of relative height in childhood predicts adult stature below target height in boys with constitutional delay of growth and puberty* Horm Res, 2007;68(2):99-104 (Copyright © 2007 S. Karger AG, Basel).

	Early reduction in relative height	
	Present	**Absent**
Birth length (cm)	50.9 ± 2.6 (N=14)	50.9 ± 2.1 (N=18)
Height at age 3 years (SD)	-0.52 ± 0.82 (N=18)	-0.52 ± 0.84 (N=22)
Minimum relative height at puberty, minSD (SD)	-2.19 ± 0.65 (N=18)	-1.34 ± 0.81 (N=22) *
Delay of bone maturation at initial evaluation (years)	2.51 ± 0.73 (N=16)	2.23 ± 0.63 (N=18)
BMI at initial evaluation (kg/m^2)	18.49 ± 2.27 (N=18)	19.47 ± 3.70 (N=22)
Age at onset of pubertal growth spurt, take-off (years)	14.93 ± 0.67 (N=16)	15.04 ± 0.62 (N=20)
Difference of final adult height from minSD, FH-minSD (SD)	1.54 ± 0.42 (N=18)	1.64 ± 0.64 (N=22)
Parent-specific target height, TH (SD)	-0.02 ± 0.49 (N=18)	0.25 ± 0.68 (N=22)
Final adult height, FH (SD)	-0.65 ± 0.69 (N=18)	0.30 ± 1.17 (N=22) *
Difference of final and target height, FH-TH (SD)	-0.63 ± 0.50 (N=18)	0.05 ± 0.94 (N=18) *

* $p<0.05$ between untreated subjects with early reduction in height SD present or absent.

In both groups with early height SD reduction present or absent, both treated and untreated subjects were similar in physical characteristics such as birth length, height at age 3 years, and TH (data not shown). In those without early reduction in height SD, FH-TH did not significantly differ between untreated and treated subjects (FH-TH 0.05±0.94 vs. -0.08±0.70, p=0.64) (**Figure 7**, right panel). Neither did FH-TH differ between untreated and treated subjects with early height SD reduction present (FH-TH -0.63±0.50 and -0.36±0.48, respectively, p=0.15) (**Figure 7**, left panel). These data show that testosterone treatment does not influence final height attainment. Thus, the groups of treated and untreated subjects were combined. Subjects who did not have early reduction in height SD reached FH closer to their TH than subjects who had this characteristic (FH-TH -0.01±0.83 SD vs. -0.52±0.50 SD, p=0.004). A significant (over 0.5 SD) shortfall from TH was observed in 55% (17/31) of those who had early height SD reduction, and in only 21% (8/39) of those without such a reduction (p<0.01 between groups). FH was smaller in subjects with than without early height SD reduction (FH -0.56±0.64 SD vs. 0.20±1.13 SD, p=0.001) (**Figure 8**).

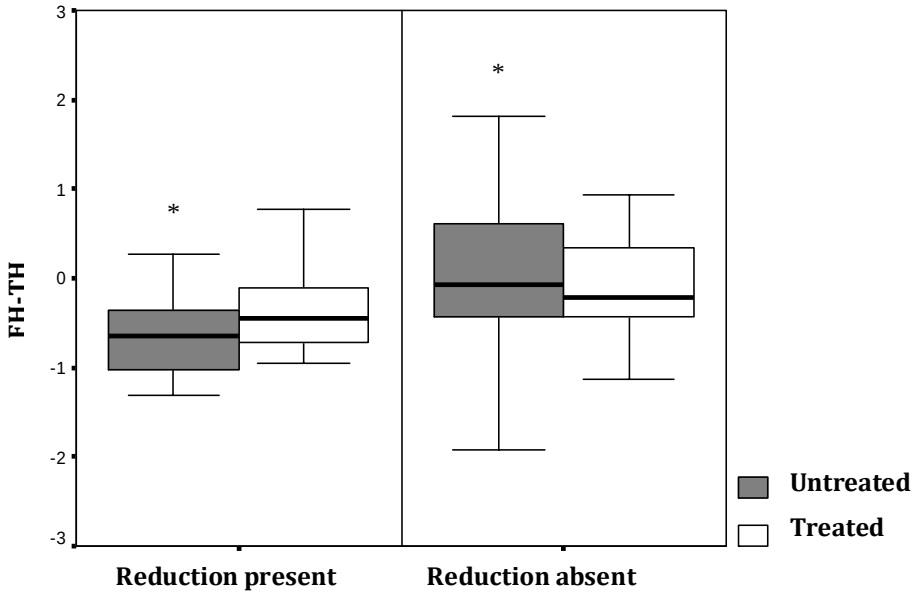

Figure 7. Difference between final adult height and parent-specific target height (FH-TH) in subjects with CDGP. Box plots summarizing the median and quartile values in subjects untreated and treated with testosterone, who had (left panel) and who did not have (right panel) an early reduction in relative height. FH-TH did not differ between treated and untreated subjects in either groups. Untreated subjects without early height SD reduction reached their TH better than untreated subjects with such reduction (*p=0.009). Modified from Wehkalampi et al. *Progressive reduction of relative height in childhood predicts adult stature below target height in boys with constitutional delay of growth and puberty.* Horm Res, 2007;68(2):99-104 (Copyright © 2007 S. Karger AG, Basel).

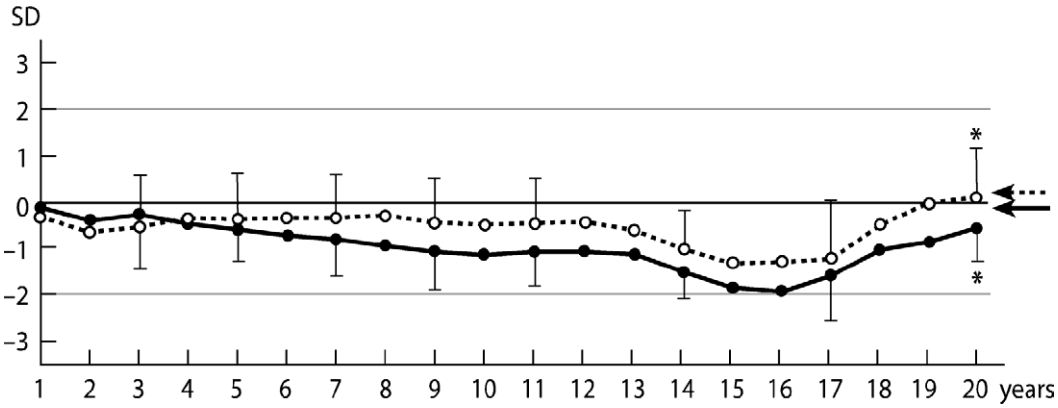

Figure 8. Height SD curves and mean parent-specific target heights (arrows) of all subjects divided by the presence (N=31) and absence (N=39) of early reduction in relative height. Heights (mean ±1 SD) at ages 1 through 20 years in subjects who had (solid line) and who did not have (dotted line) early reduction in relative height. *p=0.001 between final adult heights. Modified from Wehkalampi et al. *Progressive reduction of relative height in childhood predicts adult stature below target height in boys with constitutional delay of growth and puberty* Horm Res, 2007;68(2):99-104 (Copyright © 2007 S. Karger AG, Basel).

2. GENETIC VERSUS ENVIRONMENTAL INFLUENCES ON PUBERTAL TIMING (II)

Total number of twins and means of the traits (height SD, HD:SDS) appear in **Table 5**. DZ boys were taller than MZ boys at ages 14.0 (0.20 SD vs. 0.10 SD, p=0.036) and 17.5 years (0.19 SD vs. 0.07 SD, p=0.021), whereas no differences were seen in girls. Birth data (length, weight, and gestational age, data not shown) or HD:SDS did not differ by zygosity within genders. Age-adjusted intra-class correlation coefficients of height SD at different ages were higher within MZ pairs (0.89-0.92 in girls and 0.92 in boys) than DZ pairs (0.51 and 0.49-0.52, respectively) (**Table 6**). Similarly, correlations of HD:SDS were higher within MZ (0.82 in girls and 0.86 in boys) than DZ pairs (0.51 and 0.27, respectively).

Table 5. Mean ± SD of relative height (SD) at age 11.5 years in twin girls and 14.0 years in boys (Height 11.5/14.0), and at age 17.5 years for both girls and boys (Height 17.5), and difference in height SD between ages 11.5 and 17.5 years in girls, and 14.0 and 17.5 years in boys (HD:SDS) by sex and zygosity. p-values indicate the significance of the difference in means between MZ and DZ twins. Modified from Wehkalampi et al. *Genetic and environmental influences on pubertal timing assessed by height growth.* Am J Hum Biol, 2008;20(4):417-423 (Copyright © 2008 Wiley-Liss, Inc., A Wiley Company).

	Height 11.5/14.0	***Height 17.5***	***HD:SDS***
Girls			
MZ	0.08 ± 1.01 (N=778)	0.25 ± 1.00 (N=706)	0.18 ± 0.78 (N=673)
DZ	0.15 ± 1.04 (N=1515)	0.35 ± 0.99 (N=1292)	0.18 ± 0.80 (N=1244)
p	0.21	0.11	0.94
Boys			
MZ	0.10 ± 1.11 (N=666)	0.07 ± 0.91 (N=779)	-0.01 ± 0.79 (N=570)
DZ	0.20 ± 1.00 (N=1450)	0.19 ± 0.95 (N=1679)	-0.03 ± 0.79 (N=1247)
p	0.036	0.021	0.85

Table 6. Intra-class trait correlations of relative height at age 11.5 years in twin girls and 14.0 years in boys (Height 11.5/14.0), and at age 17.5 years for both girls and boys (Height 17.5), and difference in height SD between ages 11.5 and 17.5 years in girls, and 14.0 and 17.5 years in boys (HD:SDS). Modified from Wehkalampi et al. *Genetic and environmental influences on pubertal timing assessed by height growth.* Am J Hum Biol, 2008;20(4):417-423 (Copyright © 2008 Wiley-Liss, Inc., A Wiley Company).

	MZ		**SSDZ**		**OSDZ**
	Girls	***Boys***	***Girls***	***Boys***	
Height 11.5/14.0	0.92 (N=381)	0.92 (N=319)	0.51 (N=357)	0.52 (N=362)	0.46 (N=668)
Height 17.5	0.89 (N=344)	0.92 (N=284)	0.51 (N=313)	0.49 (N=321)	0.46 (N=604)
HD:SDS	0.86 (N=321)	0.82 (N=267)	0.51 (N=291)	0.27 (N=300)	0.31 (N=563)

Of the twin genetic models, the ACE model had the best fit for girls ($\Delta\chi^2_6$=6, p=0.44), and the ADE model for boys ($\Delta\chi^2_6$=4, p=0.68). Nonsignificant differences in χ^2-values between ACE or ADE models and saturated models suggest that the assumptions of twin modeling were not violated. Fit of the models is presented in **Table 7**. Using the ACE model in girls, 71% (95% CI 56-87%) of the variance of HD:SDS was attributed to additive genetic, 15% (95% CI 0-30%) to common environmental, and 14% (95% CI 12-17%) to specific environmental effects. Using the ADE model in boys, 24% (95% CI 00-62%) of the variance in HD:SDS was attributed to additive genetic, 58% (95% CI 20-85%) to dominant genetic, and the remaining 18% (95% CI 15-22%) to specific environmental effects. As the use of equal models in both genders allows better comparison between boys and girls, analyses were repeated using the AE model in both genders. This was permissible because also the AE model yielded an adequate fit to the data (p-values for χ^2-differences compared with the

saturated models were 0.28 and 0.06, respectively). In this case, 86% (95% CI 83-88%) and 82% (95% CI 78-85%) of the variance in HD:SDS was attributed to additive genetic effects in girls and boys, respectively. Specific environmental contributions were the same as in ACE and ADE models.

Table 7. Fit of the saturated twin genetic model compared with ACE, ADE, and AE models (most parsimonious model bolded). Modified from Wehkalampi et al. *Genetic and environmental influences on pubertal timing assessed by height growth.* Am J Hum Biol, 2008;20(4):417-423 (Copyright © 2008 Wiley-Liss, Inc., A Wiley Company).

	saturated model	***ACE model***		***ADE model***		***AE model***	
	-2LL (d.f.)	$\Delta\chi^2$ (Δd.f.)	p-value	$\Delta\chi^2$ (Δd.f.)	p-value	$\Delta\chi^2$ (Δd.f.)	p-value
Girls							
HD:SDS	2518 (1279)	**6 (6)**	**0.44**	9 (6)	0.17	9 (7)	0.28
Boys							
HD:SDS	2584 (1213)	13 (6)	0.04	**4 (6)**	**0.68**	13 (7)	0.06

3. FAMILIAL CLUSTERING OF DELAYED PUBERTY (III)

Positive family history of CDGP (strict criteria) was evident in 79% (98/124) of the probands (**Figure 9**). Male and female probands had affected first-degree relatives equally often: 76 of 95 males (80%) and 22 of 29 females (76%). Sporadic CDGP was found in 26 families (19 male and 7 female probands). In these, none of the 75 first-degree relatives had CDGP, and parental interviews revealed only one first cousin with CDGP among all second- or third-degree relatives.

Most commonly, the 98 families with multiple CDGP had one affected parent (unilineal families) (**Figure 10A**). This was found in 46% and 41% of the families of male and female probands, respectively. In 33% and 31% of the families of male and female probands, both parents were affected (bilineal families) (**Figure 10B**). Of these 40 families classified as bilineal, 9 (23%) were so by extension, i.e. the unaffected parent had a first-degree relative with CDGP (**Figure 10C**). Families with only siblings affected were rare (2%). Of the 23 extended unilineal families (**Figure 9**), CDGP was verified in three generations in 16 families (70%), and 17 families (74%) displayed an inheritance pattern of CDGP consistent with autosomal dominant inheritance (affected pedigree members of both sexes transmitting CDGP to roughly 50% of their offspring) (**Figure 10A**). In all 23 extended unilineal pedigrees, 49% (91/186) of the family members, including the probands, were affected.

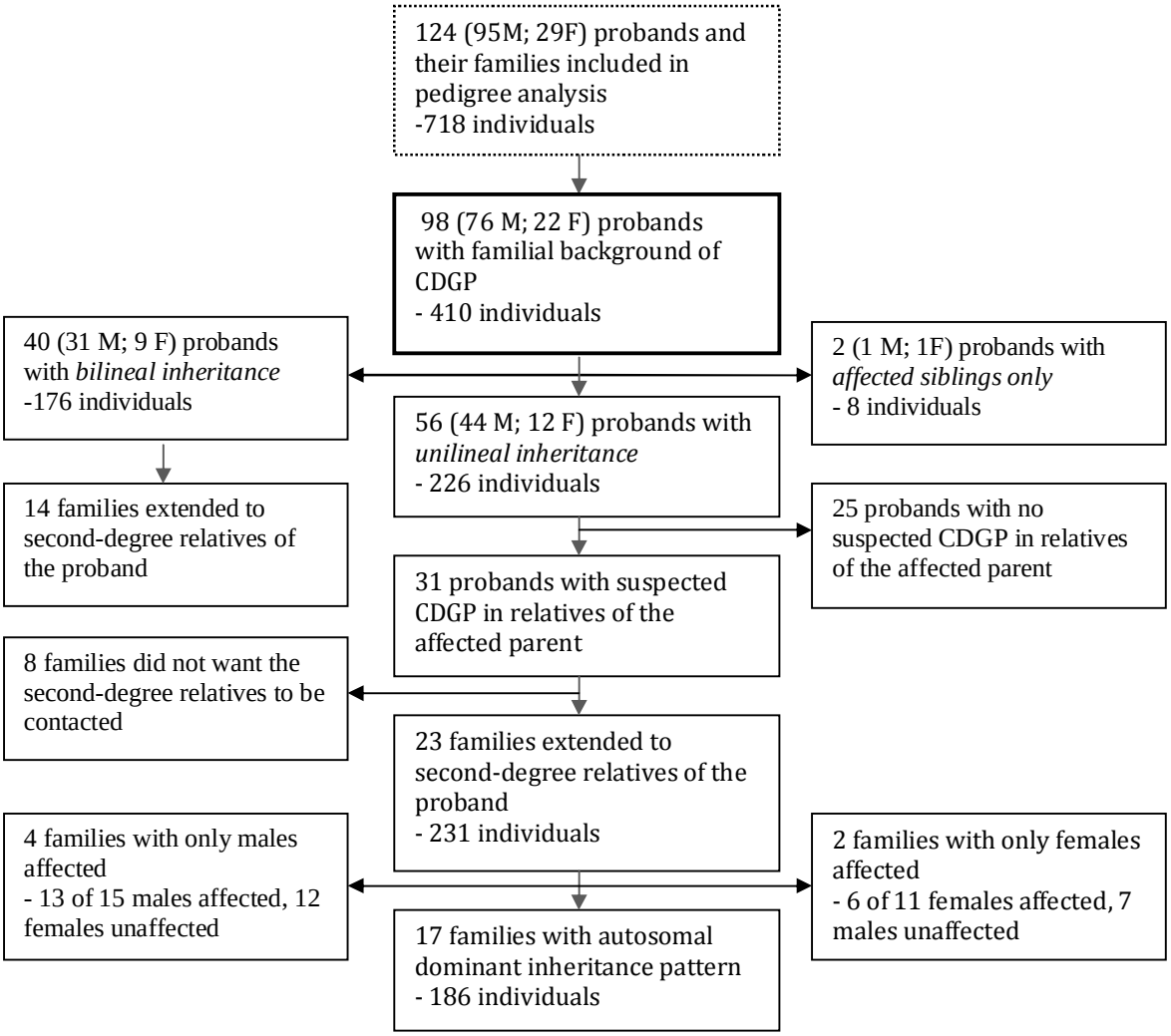

Figure 9. Aggregation of CDGP in families of 98 probands (M, male; F, female) with at least one affected first-degree relative, among the total of 124 families included in pedigree analysis (**Figure 5**). The figure shows proportions of probands and total number of investigated individuals in unilineal families (one affected parent and no evidence for CDGP in the other parent or his/her first-degree relatives), bilineal families (either two affected parents, or one affected parent and the other having an affected sibling or parent), and families with no affected parents (one or more siblings affected). Enrolment of second-, third-, and fourth-degree relatives and proportions of families with an autosomal dominant inheritance pattern, as evidenced by affected pedigree members of both sexes transmitting CDGP to roughly 50% of their offspring, are also presented. Modified from Wehkalampi et al. *Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care*. J Clin Endocrinol Metab, 2008;93(3):723-728 (Copyright © 2008 The Endocrine Society).

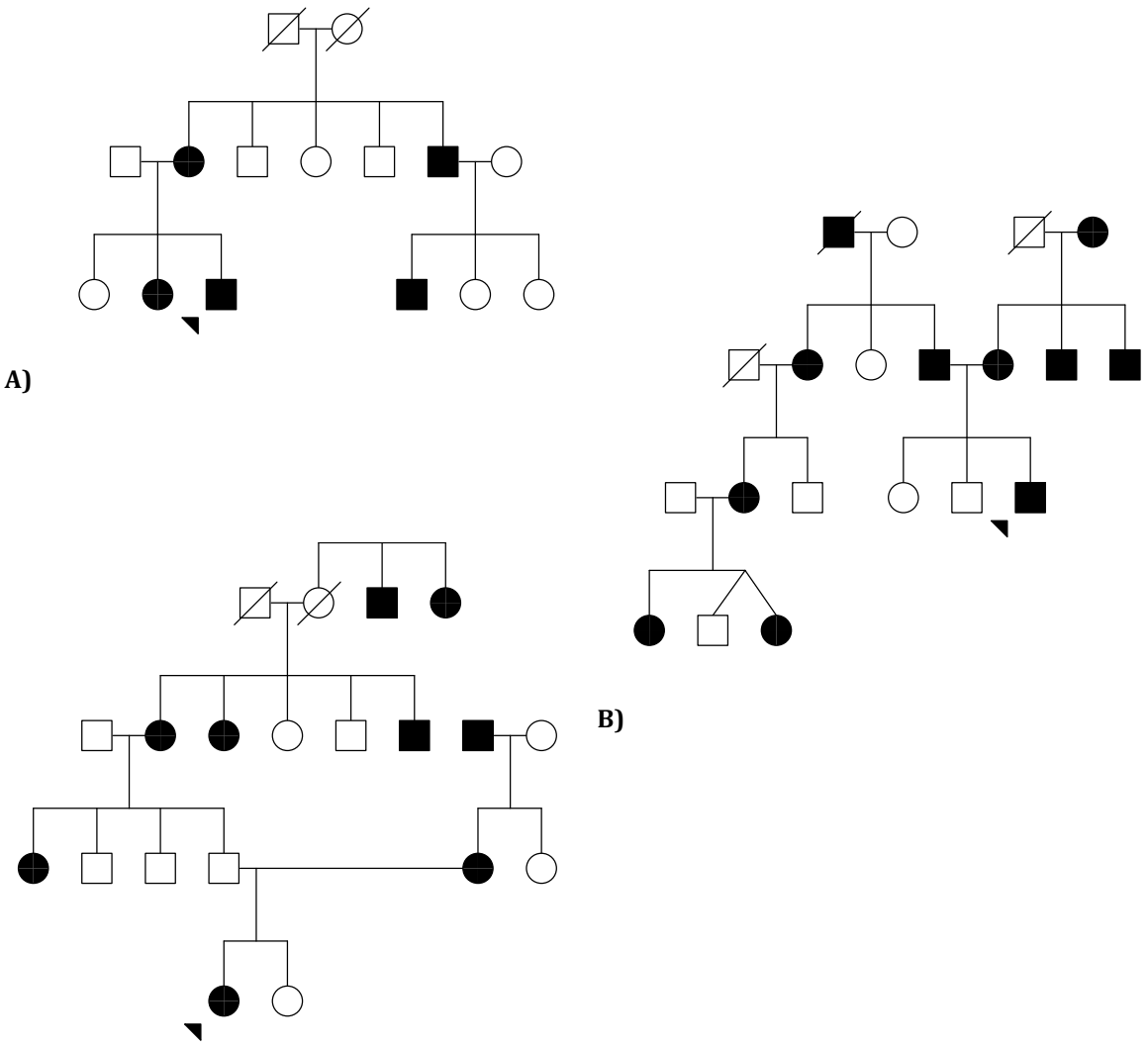

Figure 10. Examples of pedigrees of adolescents (proband, arrowhead) with CDGP. Affected individuals with more than 2 SD later than average pubertal growth spurt, or recalled 2 years later than average puberty (●/■), unaffected individuals (○/□). **A)** Male proband with one affected parent and affected relatives of both sexes transmitting CDGP to roughly half of their offspring (unilineal autosomal dominant inheritance). **B)** Male proband with two affected parents (bilineal inheritance). **C)** Female proband with one parent affected and the other parent unaffected but having relatives with CDGP (bilineal inheritance by extension). From Wehkalampi et al. *Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care*. J Clin Endocrinol Metab, 2008;93(3):723-728 (Copyright © 2008 The Endocrine Society).

Male first-degree relatives of all probands with a familial background of CDGP were only slightly more often affected than were females; 79 of 148 males (58%) versus 64 of 164 females (39%) (χ^2=6.46, p=0.01). The male-to-female ratio for affected first-degree relatives was 1.2:1. When second-, third-, and fourth-degree relatives with pubertal timing assessment mostly based on interviews were included in the analysis, an equal proportion of affected male and female relatives was found; 133 of 265 males (50%) versus 128 of 290 females (44%) (χ^2=0.73, p=0.39). Gender-specific occurrence of CDGP was then investigated separately in parents and siblings of all probands with unilineal background. CDGP tended to be more common in brothers than sisters, but this difference was not significant: 9 of 27 brothers (33%) versus 5 of 37 sisters (14%) (χ^2=3.59, p=0.06) (**Table 8**). Mothers were equally often affected with CDGP as were the fathers: 32 of 56 mothers (57%) versus 24 of 56 fathers (43%) (χ^2=2.29, p=0.13). Mothers were more often affected than sisters: 32 of 56 mothers (57%) versus 5 of 37 sisters (14%) (χ^2=17.7, p<0.01). CDGP criteria were met equally often in fathers and brothers: 24 of 56 fathers (43%) versus 9 of 27 brothers (33%) (χ^2=0.69, p=0.41). The occurrence of CDGP was similar in mothers and fathers also when unilineal families of male and female probands were investigated separately. No significant difference appeared between male and female probands in their numbers of affected brothers or sisters either, but the number of siblings in these families was small.

Table 8. Prevalence of CDGP in first-degree relatives of probands with a unilineal background of CDGP. From Wehkalampi et al. *Patterns of inheritance of constitutional delay of growth and puberty in families of adolescent girls and boys referred to specialist pediatric care*. J Clin Endocrinol Metab, 2008;93(3):723-728 (Copyright © 2008 The Endocrine Society).

	Female probands *(N=12)*	***Male probands*** *(N=44)*	***All probands*** *(N=56)*
Fathers	7 of 12 (58%)	18 of 44 (41%)	24 of 56 (43%)
Mothers	5 of 12 (42%)	26 of 44 (59%)	32 of 56 (57%)*
Male siblings	0 of 5 (0%)	6 of 19 (32%)	9 of 27 (33%)
Female siblings	2 of 10 (20%)	3 of 24 (13%)	5 of 37 (14%)*

* χ^2=17.7, p<0.01 between mothers and female siblings

4. GENETIC LOCI LINKED TO PUBERTAL DELAY (IV)

The genome was screened with 383 microsatellite markers in 52 families to identify chromosomal regions co-segregating with growth chart-based CDGP (relaxed criterion). The average marker success rate was 98%, and the average information content 0.87. The reported HLOD scores were obtained by setting disease allele frequency to 0.0001 (model 3). All HLOD scores obtained with parametric linkage analysis are presented in **Figure 11**, and a summary of loci yielding HLOD scores above 1.5 appears in **Table 9**. The two-point LOD scores exceeding 1.5 were from chromosomes 1, 2, 7, and 18. Of these, only the locus on chromosome 2 was supported by multipoint analysis, which at marker locus D2S2216 resulted in a HLOD score of 2.54 (α 0.31). Neighboring loci D2S2333 and D2S160 yielded HLOD scores of 1.79 and 2.18, respectively, supporting the linkage.

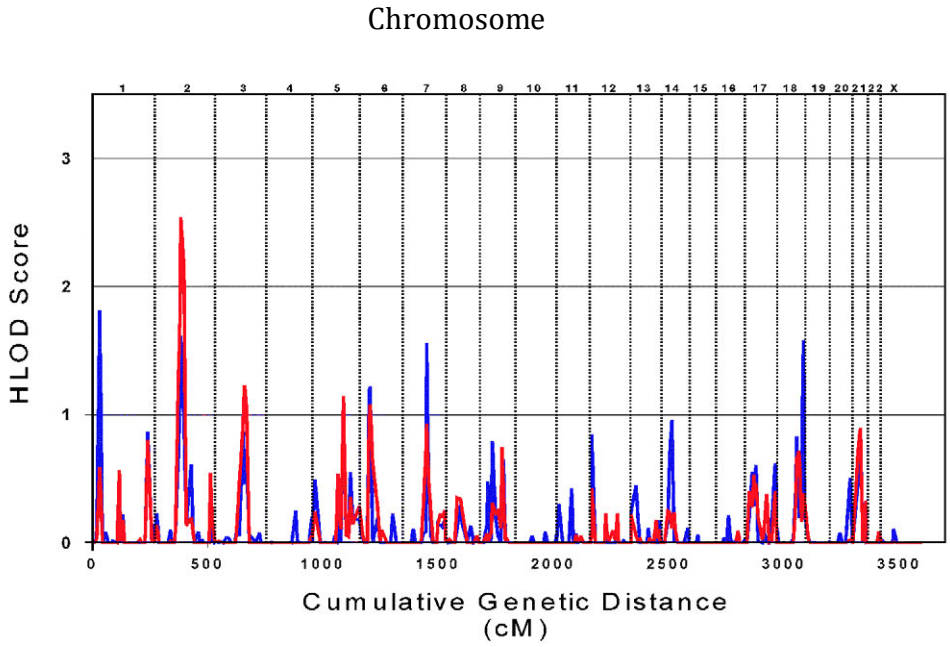

Figure 11. HLOD scores obtained by two- (blue line) and multipoint (red line) linkage analyses of CDGP. From Wehkalampi et al. *Association of the timing of puberty with a chromosome 2 locus*. J Clin Endocrinol Metab, in press (Copyright © 2008 The Endocrine Society).

Table 9. Two- and multipoint HLOD scores exceeding 1.5 in the genome-wide linkage of CDGP. From Wehkalampi et al. *Association of the timing of puberty with a chromosome 2 locus*. J Clin Endocrinol Metab, in press (Copyright © 2008 The Endocrine Society).

Locus	*Position(cM)*	***Two-point analysis***			***Multipoint analysis***		
		HLOD	α	Information	HLOD	α	Information
D1S2697	32.0	**1.82**	0.45	0.55	0.59	0.22	0.78
D2S2333	108.8	1.14	0.22	0.71	**1.79**	0.24	0.92
D2S2216	111.5	**1.62**	0.27	0.67	**2.54**	0.31	0.91
D2S160	124.9	0.97	0.20	0.67	**2.18**	0.26	0.86
D7S657	103.5	**1.56**	0.31	0.74	0.93	0.19	0.92
D18S462	116.4	**1.58**	0.47	0.47	0.39	0.19	0.88

To further test whether the initial linkage signal on chromosome 2 represents true co-segregation of the chromosomal region with CDGP or is a false-positive result associated with marker D2S2216, the region on chromosome 2p13-2q13 was fine-mapped. All family members were genotyped for 25 additional microsatellite markers covering the region at 0.97 cM intermarker distances, increasing the overall multipoint information content to 0.97. Adding these markers clearly supported the initial linkage finding. The highest two-point HLOD score was obtained at marker locus D2S2364 (HLOD 3.70; α 0.62) (**Table 10**). Six other loci yielded HLOD scores exceeding 2. The highest multipoint HLOD score in the region (HLOD 4.44; α 0.41) was obtained at marker locus D2S2229, at a distance of 119.0 cM (**Figure 12**).

Table 10. Two- and multipoint HLOD scores exceeding 2 in fine-mapping the locus 2p13-2q13. From Wehkalampi et al. *Association of the timing of puberty with a chromosome 2 locus*. J Clin Endocrinol Metab, in press (Copyright © 2008 The Endocrine Society).

Locus	*Position(cM)*	***Two-point analysis***			***Multipoint analysis***		
		HLOD	α	Information	HLOD	α	Information
D2S388	110.1	**2.15**	0.36	0.62	**2.01**	0.26	0.98
D2S2159	112.6	**2.12**	0.33	0.73	**2.62**	0.33	0.99
D2S2209	114.8	**2.77**	0.44	0.70	**2.70**	0.34	0.99
D2S2264	116.1	**2.17**	0.41	0.57	**3.94**	0.41	0.99
D2S2364	118.3	**3.70**	0.62	0.71	**4.08**	0.40	0.97
D2S2229	119.0	**3.38**	0.43	0.73	**4.44**	0.41	0.99
D2S293	119.8	**2.44**	0.65	0.76	**3.01**	0.37	0.99

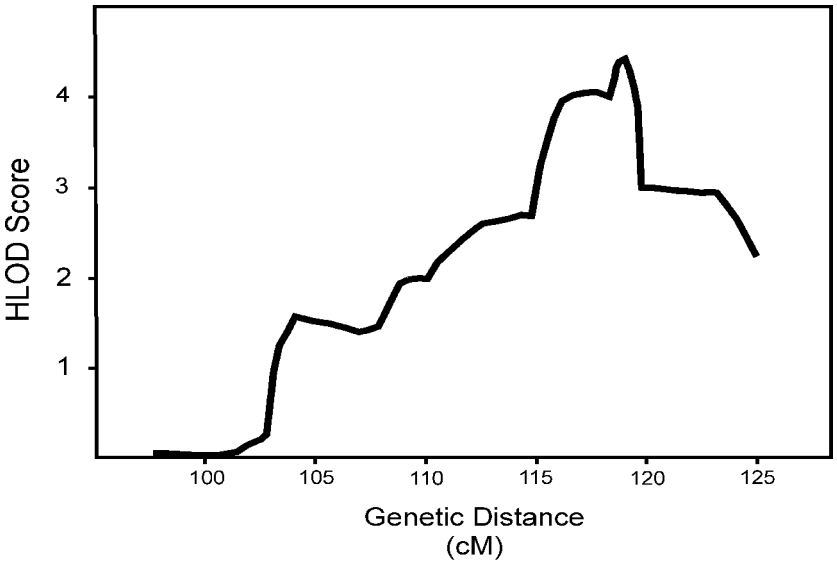

Figure 12. Multipoint HLOD scores after adding 25 microsatellite markers to the region 2p13-2q13. The highest multipoint HLOD score was obtained at a distance of 119 cM. From Wehkalampi et al. *Association of the timing of puberty with a chromosome 2 locus*. J Clin Endocrinol Metab, in press (Copyright © 2008 The Endocrine Society).

As indicated by the α-value, only 40% of the families contributed to the linkage signal at chromosome 2. To reduce allelic heterogeneity, the families were further stratified based on place of origin, and a set of 7 families originating from the central and eastern parts of Finland was reanalyzed. Running these families alone in the analysis showed that they contributed to a substantial proportion of the linkage signal at chromosome 2. Multipoint analysis of the 7 families for all markers on chromosome 2p13-2q13 yielded a HLOD score of 3.46 (α 0.75). The segregating haplotypes in the 5 families that contributed to this linkage signal were further examined. None of these families shared a haplotype pattern spanning more than 2 markers (data not shown).

DISCUSSION

By using growth chart-based assessment of the timing of puberty and twin genetic models, genetic contribution to the normal variation in pubertal timing was estimated to be about 85% in both genders. Of male and female patients with constitutional delay of growth and puberty, CDGP, approximately 80% had first-degree relatives with pubertal delay. Thus, this normal variant of pubertal timing is expected to be strongly influenced by genetic factors. A locus 2p13-2q13 was significantly linked to delayed puberty in CDGP pedigrees, and may thus harbor a gene, which is important in the regulation of pubertal timing.

1. GROWTH CHART-BASED ASSESSMENT OF PUBERTAL TIMING

The timing of puberty in both twin and family cohorts was assessed by using pubertal growth spurt as a clinical indicator. In families, the age at which pubertal growth spurt occurred was estimated from growth charts comprising archived height measurements. Retrospective assessment of pubertal timing was important since most family members had long since passed puberty. Assessing exact ages at appearance of secondary sex characteristics would have required prospective data collection, which is impractical in family studies. Objective assessment of the timing of puberty was, however, crucial for linkage analysis, which is very sensitive to misclassification of the phenotype. Misclassification is likely if the time of pubertal initiation is based on recall. Most recall-based data on pubertal timing have used age at menarche as a marker, which only moderately correlates with onset of breast development (r=0.37-0.39) (de Ridder et al. 1992, Biro et al. 2006). Menarche is also a late phenomenon in pubertal maturation and provides information only for females (Marshall and Tanner 1969, Tanner and Whitehouse 1976). In both genders, acceleration of growth velocity during adolescence reflects initiation of pubertal maturation, although pubertal growth spurt occurs later along somatic maturation in boys than in girls (Marshall and Tanner 1970, Tanner and Whitehouse 1976). The later growth spurt in boys compared with girls may be a consequence of sex differences in the expression of aromatase, which converts androgens to estrogens - the main sex hormones regulating pubertal growth (Cutler 1997).

The timing of pubertal growth spurt in family members of CDGP probands was mainly assessed by estimating ages at take-off and phv. These could not always be assessed, however, since archived height measurements were occasionally too infrequent. In addition to age at take-off and phv, also attainment of adult height reflects pubertal timing. This can

be affected by estrogen receptor defects or aromatase deficiency delaying epiphyseal closure (Smith et al. 1994, Morishima et al. 1995), although these conditions are extremely rare. Adult heights were mostly self-reported, but these usually correlate well with measured stature (r=0.75) (Brooks-Gunn et al. 1987, Sperlich et al. 1995). Obesity may interfere with assessment of pubertal timing from growth charts since it accelerates growth velocity in childhood and reduces pubertal height gain (He and Karlberg 2001). Undernutrition and chronic illnesses (Kulin et al. 1982, Pozo and Argente 2002) and hypogonadotropic hypogonadism, all of which may influence pubertal growth, were excluded by thorough interviews and assurance of spontaneous albeit later-than-average puberty.

Pubertal timing in twins was estimated by HD:SDS – change in relative height between the age when height growth peaks in the general population and adulthood, which reflects the percentage of final height achieved at population's average phv age. At the time of their highest increment in height per year during puberty, adolescents have usually reached about 92% of their final height (Beunen et al. 2000). At the population's average phv age, however, at age 14 years in boys and 12 years in girls, those maturing early may already be at their adult height, whereas late maturers may still have their whole growth spurt ahead. The validity of HD:SDS as a marker for pubertal timing was confirmed by observing a significant correlation (r=0.71-0.84) with take-off and phv age in CDGP family members for whom longitudinal growth data were available. HD:SDS calculated between only two height measurements was especially useful in the very large twin population. HD:SDS can be of value in future large-scale evaluations related to pubertal timing.

Examination of growth patterns in adult CDGP males revealed that approximately half had a reduction in relative height, i.e. slow growth velocity, already in childhood. During pubertal years, all subjects had a similar reduction in relative height, and subsequently, a similar amount of height gain during the pubertal growth spurt. Importantly, distinct patterns of childhood growth did not seem to affect estimation of the timing of the pubertal growth spurt by the chosen growth parameters. Final height in CDGP does not always correspond to genetic height potential (Brämswig et al. 1990, LaFranchi et al. 1991, Albanese and Stanhope 1993, 1995). Albanese and Stanhope (1995) observed that CDGP males who were taller and had faster growth rates at diagnosis attained their target height better than those without these characteristics. Accordingly, the present study revealed that those CDGP subjects who had early height SD reduction did not attain their target height as well as those with relatively unchanged relative height between 3 and 9 years of age. Importantly, testosterone treatment did not affect adult height attainment in either group.

2. IMPORTANCE OF GENETIC EFFECTS ON TIMING OF PUBERTY

Based on the present large twin study, the vast majority of the variance of pubertal timing is explained by inter-individual differences in genetic factors in both genders. This strengthens the observations from previous studies, which have, however, mostly assessed pubertal timing in either sex, used less reliable age at menarche as a marker, or assessed the timing of puberty only in a small twin population (Fischbein 1977, Sharma 1983, Treloar and Martin 1990, Kaprio et al. 1995, Beunen et al. 2000, Towne et al. 2005, van den Berg and Boomsma 2007, Silventoinen et al. 2008). Here, contributions of genetic factors to the variation of the timing of pubertal growth, 86% in girls and 82% in boys, obtained by using the AE model were similar in both genders. However, using the very best-fitting ACE and ADE models in girls and boys, respectively, the estimates for genetic contributions revealed a sex difference (71% for girls and 82% for boys), with common environmental component in girls, and dominant genetic effects in boys, being greater. A corresponding greater influence of the environmental component in girls than in boys has been reported in other traits, e.g. height (Silventoinen et al. 2003). In addition, a stronger influence of environmental factors on pubertal onset in girls than in boys may be suggested, based on recent observations of advanced pubertal signs in girls, but not convincingly in boys (Wong et al. 1996, Herman-Giddens et al. 1997, Huen et al. 1997, Herman-Giddens et al. 2001, Papadimitriou 2001). However, in the present study, the sex difference in model fits and the increased estimates of either dominant genetic or environmental effects in boys and girls, respectively, may have been caused by small sex differences in stages of sexual maturation at ages of response (Eaves and Silberg 2003). At the age of 17.5 years, some of the boys may not have ceased to grow yet (Hulanicka and Kotlarz 1983, Hägg and Taranger 1991), while even the latest maturing girls have. Furthermore, girls at the age of 11.5 years may have been less mature than boys aged 14.0 years. Thus, possible differences in the effects of genetic or environmental factors between genders cannot be ascertained based on the present data.

Clinicians have for long known that CDGP aggregates in families (Sperlich et al. 1995, Du Caju et al. 2000), but only one study has previously extensively addressed the scope of positive family histories in these patients (Sedlmeyer et al. 2002). The present study was not, however, based on histories only; pubertal timing in most family members was assessed from growth charts. In pedigree analysis, CDGP was diagnosed in first-degree relatives of 80% of male and 76% of female probands. The observed number of affected first-degree relatives was 15 times higher than the expected 2.5% prevalence of 2 SD later than average pubertal growth spurt in the general population. The observation that affected pedigree members of both genders transmitted the trait to roughly half of their offspring suggests the inheritance pattern of CDGP to be consistent with autosomal dominant inheritance. This was also common in CDGP pedigrees investigated by Sedlmeyer et al. (2002). In the present study, selection bias

possibly affected the frequency of dominant modes since parents with delayed puberty may have been more willing to participate than parents with average pubertal timing. On the other hand, the prevalence of sporadic CDGP may also have been overestimated since extensive interviews of relatives in these families were not carried out. Based on dominant inheritance patterns, linkage mapping for detecting loci conferring susceptibility to CDGP was assumed to be successful, provided that very careful pedigree selection was conducted.

A peculiarity of CDGP is that clinicians observe this condition more often in boys than in girls; the male-to-female ratio has been reported to range from 2:1 to 5:1 (Crowne et al. 1991, Sedlmeyer and Palmert 2002, Sedlmeyer et al. 2002). No physiological explanation exists for this, although it has been suggested that the sensitivity of pituitary gonadotropes to GnRH could differ between the genders (Stanhope et al. 1987). This would also explain girls more often having precocious puberty than boys (Chemaitilly et al. 2001, de Vries et al. 2004). However, defining CDGP based on statistical considerations, the criteria should be met by 2.5% of adolescents of both genders. The apparent contradiction of more frequent finding of CDGP in boys than in girls has only rarely been addressed in the literature (Papadimitriou and Chrousos 2005). Family members of patients with IPP have been shown to be mostly female, suggesting sex-dependent penetrance (de Vries et al. 2004). Sex-dependent penetrance cannot, however, be proposed in CDGP since female and male probands had affected mothers and fathers equally often. If CDGP truly was less frequent in girls than in boys, it would be expected that female probands had a stronger genetic predisposition to delayed puberty than boys. This was not the case since similar numbers of female and male CDGP probands had affected relatives. This and the fact that a comparable male-to-female ratio to that observed in CDGP probands (3:1) was not detected among affected first-degree relatives (1.2:1) suggest that the marked overrepresentation of boys with CDGP at pediatric clinics is biased. Skewing of referral practices generating the male predominance may be due to the pubertal growth spurt being later in boys (Tanner et al. 1976), and the first signs of puberty being more easily detected in girls (recording of B2 vs. G2). As a consequence, one would expect referred girls to show more severe delay of puberty than boys. This was noted in a previous study detecting more severe delay of bone maturation in CDGP girls than in CDGP boys (Sedlmeyer and Palmert 2002). Delay of bone maturation in the present study was not, however, different between genders (mean approximately 2.6 years in both boys and girls). Therefore, instead of skewed referral practices, the explanation for the higher ratio of boys to girls with CDGP may lie in boys requesting further investigations for their delayed puberty and growth more easily than girls. This may be due to boys' keener interest in athletic performance and physical characteristics, such as height, around puberty (Crocket and Petersen 1987).

Boys outnumbering girls with delayed puberty may also be explained by a selective secular trend towards earlier pubertal maturation in the female population. In this case, the use of 1960's cut-off age limits for normal pubertal maturation (Marshall and Tanner 1969, 1970) would not identify similar numbers of girls and boys, or daughters and mothers with CDGP. Supporting this, mothers of probands were more often affected than sisters in the present study, whereas the occurrence of CDGP was similar between fathers and brothers. Brothers, in turn, tended to have CDGP more often than sisters, but the difference was not statistically significant. Whether sex differences in secular trends exist, remains to be elucidated by future investigations.

3. PUBERTAL TIMING IS ASSOCIATED WITH A CHROMOSOME 2 LOCUS

By using a linkage mapping approach in multiply affected pedigrees, the present study detected a locus co-segregating with CDGP in the pericentromeric region of chromosome 2. This genomic area, predisposing to late variant of normal puberty, may harbor a gene, which is potentially important in the regulation of pubertal timing. Previous linkage scans of CDGP have not been reported, but a few studies have mapped loci for other pubertal timing phenotypes. Whole genome scans on age at menarche have detected loci on 22q13 (LOD 3.70) (Guo et al. 2006) and 16q21 (LOD 3.33)-16q12 (LOD 3.12) (Rothenbuhler et al. 2006). These loci differ from the one detected in the present study probably because menarche and pubertal growth spurt are not equal markers for pubertal timing (Marshall and Tanner 1969, Tanner and Whitehouse 1976, Tanner et al. 1976). In addition, the scans for menarcheal age used the phenotype as a quantitative trait, thus mapping common low-impact genes affecting the whole distribution of pubertal timing present in the (female) population. The present study population (of both genders) was selected from the extreme end of the continuous distribution of pubertal timing, representing less than 10% of the general population, probably possessing rare genes with high impact.

The locus 2p13-2q13 at the pericentromeric region of chromosome 2 does not harbor any previously known genes related to pubertal timing. However, a number of genes in the area have a potential connection to the control of pubertal onset. One of these is *GPR45*, a gene coding for a G-protein-coupled receptor, the family of which GPR54, GnRHR, and PROKR2 are examples of. Mutations in their encoding genes are known to cause HH (de Roux et al. 1997, 2003, Layman et al. 1998, Seminara et al. 2003, Dodé et al. 2006). The present locus also contains *POU3F3*, a member of the family of POU homeodomain transcription factors, which are involved in the development of the anterior pituitary gland (Zhu et al. 2005). Mutations in another member of this family, *POU1F1* (*PIT1*), cause deficiency in pituitary thyrotropin, growth hormone, and prolactin (Pfäffle et al. 1992, Tatsumi et al. 1992), and mutations in other developmental transcription

factors (*HESX1*, *PROP1*, *LHX3*, *LHX4*) also deficiency in gonadotropins (Dattani et al. 1998, Wu et al. 1998, Netchine et al. 2000, Thomas et al. 2001, Tajima et al. 2003, Pfäffle et al. 2008). *POU3F3* could potentially be involved in the development of central structures of the HPG axis crucial for normal initiation of pubertal maturation. Furthermore, the identified chromosomal region contains a neuronal PAS domain protein 2, *NPAS2* (Zhou et al. 1997), a central nervous system transcription factor functioning as a part of a time-keeping molecular clock mechanism that generates circadian rhythmic oscillations in gene expressions and physiologic functions (Reick et al. 2001). Such clock mechanisms regulate, in synchrony with the master pacemaker residing in the hypothalamic suprachiasmatic nucleus, e.g. rhythm of sleep and wakefullness, as well as ovulatory cycles (de la Iglesia and Schwartz 2006). *NPAS2* might be part of, or interact with, the developmental clock regulating the timing of pubertal onset.

SUMMARY AND CONCLUSIONS

Retrospective investigation of longitudinal childhood growth can be of value in estimating how well an individual will exploit his genetic height potential. If treatment to improve final height by, for instance, aromatase inhibitors (Wickman et al. 2003, Hero et al. 2006) is considered, it can be targeted to those CDGP boys with a reduction of relative height in childhood, who are at risk of not using their full genetic height potential. For those prepubertal 7-9-year-old boys investigated for short stature with progressive reduction in relative height after 3 years of age, careful follow-up through puberty and consideration of treatment to improve final height are warranted.

An approximately 85% genetic component to the variation of timing of pubertal growth spurt in the twin study indicates that inter-individual differences in genetic factors are strong determinants of the normal variation of pubertal timing. At the end of the normal spectrum, genetic influences are also important. This is evidenced by the approximately 80% occurrence of CDGP in relatives of subjects with this condition. Further supporting the strong impact of genes on the trait, dominant inheritance patterns in pedigrees segregating CDGP are frequent. This anticipates that genetic loci conferring susceptibility to CDGP may be detected by linkage. Using genome-wide scans and careful selection of only growth chart-based multiply affected families, a locus affecting CDGP was detected in the pericentromeric region of chromosome 2. The locus 2p13-2q13 potentially harbors a gene involved in the control of the onset of puberty. However, given the large size (~6-20 Mb) of the detected chromosomal region, further identification of the causative gene will be challenging. Saturation linkage mapping and linkage disequilibrium analyses with very dense single-nucleotide polymorphism markers will hopefully confine the locus to a smaller genomic region, which will then allow positional cloning. Evaluation of the impact of the identified locus in other CDGP samples and at the population level, also await further studies.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Murrosiän alkamisen ajankohta noudattaa terveessä väestössä normaalijakaumaa. Tätä vaihtelua selittävät vaikutusmekanismeiltaan vielä puutteellisesti tunnetut geneettiset ja ympäristötekijät. Tämän tutkimuksen tarkoituksena oli selvittää murrosiän aikataulun geneettistä säätelyä. Tervettä väestöä edustavilla kaksosilla tutkittiin kuinka yksilöiden väliset erot geneettisissä ja ympäristötekijöissä selittävät murrosiän aikataulun väestöllistä vaihtelua. Normaalia ääripäätä edustavan myöhäisen murrosiän esiintymistä suvuissa ja periytymismalleja tutkittiin perheaineistossa. Kytkentäanalyysillä kartoitettiin perheissä viivästyneeseen murrosikään kytkeytyviä geenialueita, mistä tulevaisuuden tavoitteena on tunnistaa murrosiän käynnistymistä sääteleviä geenejä.

Tutkimuksen kaksosaineisto käsitti FinnTwin 12-17 kohortin 2309 tyttöä ja 1828 poikaa. Murrosiän normaalivaihtelu jaettiin, perustuen samanmunaisten kaksosten geneettiseen identtisyyteen erimunaisten kaksosten jakaessa vain noin 50% geeneistään, geneettisten ja ympäristötekijöiden määräämiin osuuksiin. Murrosiän aikataulun mittarina käytettiin suhteellisen pituuden muutosta aikuisiän ja väestön keskimääräisen murrosiän kasvupyrähdyksen huippunopeusiän välillä, mikä heijastaa ko. ajankohdan jälkeen jäljellä olevaa kasvua ja siten murrosiän aikataulua. Murrosiän kasvu varhain murrosiässä kehittyvillä on väestön keskimääräisen kasvupyrähdyksen huippunopeusiän jälkeen vähäisempää kuin myöhään kehittyvillä, joilla koko kasvupyrähdys saattaa tuolloin olla vielä edessä.

Tutkimuksen toisen aineiston muodostivat potilaat ja heidän perheensä. Konstitutionaalisesti viivästyneen murrosiän vuoksi kuudessa suomalaisessa sairaalassa tutkittuja poikia ja tyttöjä vanhempineen, sisaruksineen ja mahdollisuuksien mukaan myös kaukaisempine sukulaisineen pyydettiin osallistumaan tutkimukseen. Osallistuvia perheitä oli 286. Murrosiän ajoitus perheenjäsenillä määritettiin kasvukäyriltä murrosiän kasvupyrähdyksen ajankohdan perusteella. Tämä mahdollisti tarkan murrosiän aikataulun määrittämisen eri-ikäisiltä henkilöiltä ja molemmilta sukupuolilta. Murrosikä todettiin viivästyneeksi, mikäli kasvupyrähdyksen alku tai huippunopeus ajoittui 1.5 tai 2 SD:ta keskimääräistä myöhäisempään ikään. Kaikki tutkittavat myös haastateltiin, mitä käytettiin hyväksi murrosiän ajankohdan määrittämisessä kasvutietojen puuttuessa. Ensimmäisen asteen sukulaisten murrosikä määritettiin pääasiassa kasvutietojen perusteella (80%), kun taas suurimman osan kaukaisempien sukulaisten murrosiän aikataulun määrityksestä perustui haastatteluun (60%). Viivästyneen murrosiän (2 SD kriteeri) periytymistä

tutkittiin 124 (95 pojan, 29 tytön) pääkaupunkiseudun potilaan suvussa, kun taas kytkentäanalyysiin valittiin 52 sukua koko Suomesta. Kytkentäanalyysiin sopiviksi katsottiin suvut, joissa kasvutietoihin perustuva viivästynyt murrosikä (1.5 SD kriteeri) todettiin vain toisella potilaan vanhemmista ja osalla tämän sukulaisista.

Kaksostutkimuksen perusteella, yksilöiden väliset geneettiset erot selittävät tytöillä 86% ja pojilla 82% murrosiän aikataulun väestöllisestä vaihtelusta. Viivästyneen murrosiän vuoksi tutkituista pojista 80%:lla ja tytöistä 76%:lla todettiin samanlainen ominaisuus myös vähintään yhdellä ensimmäisen asteen sukulaisella. Näillä viivästynyttä murrosiän kasvupyrähdystä oli 15 kertaisesti väestön keskimääräiseen 2 SD:tä myöhäisempään kasvupyrähdykseen nähden (2.5%). Viivästynyt murrosikä periytyi vallitsevasti, eli esiintyi molemmilla sukupuolilla ja noin 50%:lla jälkeläisistä, 74%:ssa (17/23) niistä kaukaisempiinkin sukulaisiin laajennetuista suvuista, joissa vain toinen potilaan vanhemmista oli murrosiässään viivästynyt. Koko genomin kartoituksella ja kytkentäanalyysillä hienokartoituksineen viivästyneeseen murrosikään kytkeytyi merkitsevästi kromosomialue 2p13-2q13 (*multipoint* HLOD 4.44; α 0.41).

Tutkimus osoittaa, että geneettiset tekijät määräävät suurelta osin murrosiän aikataulun normaalivaihtelua. Ominaisuus kehittyä murrosiässään keskimääräistä myöhäisemmin on vahvasti perinnöllinen. Viivästyneen murrosiän usein vallitseva periytymistaipumus kertoo geneettisten tekijöiden vahvasta vaikutuksesta ilmiasuun ja mahdollisuudesta paikantaa tätä ominaisuutta määrääviä geneettisiä tekijöitä kytkentäanalyysillä. Tutkimuksessa viivästyneeseen murrosikään kytkeytyikin merkitsevästi 2p13-2q13 kromosomialue. Tämä sisältänee murrosiän alkamisen säätelyssä merkityksellisen aiemmin vielä tuntemattoman geenin, jonka tunnistaminen tulee auttamaan murrosiän aikataulun säätelyn ja vielä osin tuntemattomien murrosiän käynnistysmekanismien selvittämisessä.

ACKNOWLEDGMENTS

This thesis was carried out at the Hospital for Children and Adolescents, Helsinki University Hospital, and at the University of Helsinki, during 2003-2008. My deepest gratitude is due to Professors Mikael Knip, Christer Holmberg and Erkki Savilahti from the Hospital for Children and Adolescents, and to Professor Markku Heikinheimo, Director of the Pediatric Graduate School, for providing excellent research facilities.

I am grateful to my supervisor, Professor Leo Dunkel, for guiding me through this research project. His vast knowledge in the fields of research and pediatric endocrinology was crucial to the completion of this work. Thank you, Leo! Docent Tiina Laine is also warmly thanked for generous support, assistance, and many insightful conversations.

I thank Docents Kristiina Aittomäki and Jarmo Jääskeläinen for their careful review of this thesis and for constructive criticism. Docent Outi Mäkitie and Professor Anna-Elina Lehesjoki are acknowledged for being members of my Thesis Committee. Thank you both for following up my thesis work and for help in all situations.

I am indebted to all of my collaborators. I thank Docent Elisabeth Widén and Professor Aarno Palotie for providing expertise in molecular genetics. I especially thank Elisabeth for her valuable contribution to the linkage analyses and preparation of two manuscripts. I warmly thank Professor Jaakko Kaprio and Docent Karri Silventoinen for introducing me to the interesting world of twin research and for their contribution to twin analyses and manuscript. Richard Rose, Danielle Dick, and Lea Pulkkinen are also thanked for their help. I warmly thank Katarina Päkkilä (neé Vangonen) for sharing with me the fascinating world of growth research. It's been wonderful working with you. All the best to you and your family!

I thank my colleaques and staff from the Division of Endocrinology of the Hospital for Children and Adolescents, Helsinki, for helping me recruiting patients for my study. Your help has been valuable! Doctors Hanna-Liisa Lenko, Jyrki Lähde, Pekka Ojajärvi, Eero Kaprio and Päivi Tapanainen from other clinics in Finland are also to be thanked for helping me with all the formalities required for patient and family collections. Sanna Kettunen and Tuuli Miettinen are entitled to special thanks for identifying patients and families in Kuopio.

I thank my research assistants Tarja Haajanen and Minna Savolainen for their invaluable help. Helena Schmidt is also to be thanked for her help with some figure preparations. I am

also deeply grateful to Carol Ann Pelli for her skillful editing of the English language of the manuscript of this thesis. The cover was skillfully designed by Päivi Talonpoika-Ukkonen.

Above all, I wish to acknowledge all participating families. Without your contribution, this project would still be unfinished.

I wish to thank all of my colleagues and friends at the Hospital for Children and Adolescents and at the Pediatric Graduate School. Special thanks to Helena Valta, Pirjo Tynjälä, Kaija Mikkola and Matti Hero for your friendship, and to Petteri Hovi for enduring my company on our long but sunny congress trip to Hawaii in May 2008. My deepest gratitude goes to Eero Kajantie, who has always been ready to help and with whom I will face new challenges in the future.

I thank my closest friends Sari Hammarén-Malmi, Minna Kalajoki, Tiina Vahti, and Pirita Mikkanen for bringing balance to my life and for sharing with me many of the best moments. Special thanks to Eija Kotiranta for friendship and for looking for spelling errors in the final version of this thesis.

My heartfelt thanks are owed to my mother Annuli, and sisters Epe and Siso.

I warmly thank my husband Jyrki, whose love, patience, and understanding have been instrumental in any successes in recent years. I owe my deepest gratitude to him and to the best and most loved children in the world, Ella and Kirmo, who always bring joy (and hullabaloo) to my life. (Äiti rakastaa teitä). Our new family member, lagotto Enzo, is to be thanked for finally dragging me in fresh air.

This study was financially supported by the European Union (PIONEER project), the Foundation for Pediatric Research, the Päivikki and Sakari Sohlberg Foundation, and the Paulo Foundation.

Helsinki, November 2008

Karoliina Wehkalampi

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