Individual serum levels of anti-Müllerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study

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BACKGROUND: In adult women, the circulating level of anti-Müllerian hormone (AMH) is a novel marker of ovarian function, as it reflects the number of remaining ovarian follicles. Therefore, AMH has gained widespread attention in fertility clinics, and a low AMH is believed to predict impaired fertility and imminent menopause. However, the natural course of circulating AMH levels during female childhood and adolescence is not known.

METHODS: Serum levels of AMH and FSH were measured in girls participating in The COPENHAGEN Puberty Study. Longitudinal part: 85 healthy girls and adolescents were examined, and blood samples were drawn every 6 months for an average of 3 years: median (range) number of samples per girl was 6 (2–10), age at baseline was 9.2 (5.9–12.9) years. Cross-sectional part: 224 prepubertal girls (age 8.3, 5.6–11.7 years) were examined and each girl had one blood sample drawn.

RESULTS: The individual mean AMH levels in girls followed longitudinally ranged from 5 to 54 pmol/l (median 18 pmol/l). The mean intra-individual coefficient of variation of AMH was 22% (range 0–54%). Overall, each girl maintained her AMH level throughout childhood and adolescence although minor, but significant, changes occurred during pubertal transition. In prepubertal girls, AMH was negatively correlated with FSH ($r = -0.31$, $P < 0.001$). Twelve per cent (10/85) had mean AMH below a cut-off value of 8 pmol/l, indicating that the interpretation of low AMH as a marker of approaching menopause may not apply to pre- and peri-pubertal girls.

CONCLUSIONS: Circulating AMH exhibits only minor fluctuations during childhood and adolescence, and a random AMH measurement seems representative for a given girl. The negative AMH–FSH correlation in prepubertal girls supports the notion that AMH is a quantitative marker of ovarian follicles even in young girls.

Key words: ovary / ovarian reserve / AMH / MIS / female reproduction

Introduction

Primordial follicles in the human ovary are only formed during fetal life, and the potential of female reproductive capacity is therefore already established at birth (Baker, 1963). Even during childhood, the number of ovarian follicles declines with increasing age. When the follicle number falls below a critical threshold of a few thousand, the menstrual cycle becomes irregular as menopause approaches (Richardson et al., 1987).

Anti-Müllerian hormone (AMH) is produced by granulosa cells surrounding follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance (pre-antral and early antral follicles) (Andersen et al., 2010). In adult women, serum AMH level is considered to be a predictor of the follicle reserve.
High AMH levels are associated with high antral follicle count (de Vet et al., 2002), and with a high number of resting primordial follicles (Hansen et al., 2011). Unlike FSH, LH and estradiol, AMH appears to be relatively stable through the menstrual cycle (Hehenkamp et al., 2006), and levels are not affected by hormonal contraceptive treatment (Streuli et al., 2008). Thus, the evaluation of AMH is straightforward and has gained widespread attention and clinical use among gynaecologists and fertility doctors.

Patients with Turner syndrome (TS) experience primary ovarian insufficiency due to highly accelerated loss of follicles (Singh and Carr, 1966). Recently, we reported that a random serum AMH level below a cut-off value of 8 pmol/l was a highly specific and sensitive marker of ovarian failure in young TS patients. In the same study, we reported a stable but wide reference range of circulating AMH levels during childhood and adolescence in healthy girls (4.5–62.0 pmol/l) (Hagen et al., 2010a). The observed high prevalence of low AMH (<8 pmol/l) among otherwise healthy girls caused concern. However, due to the cross-sectional design of that study, it was not possible to discern whether the range of individual AMH levels reflected large inter-individual differences or, alternatively, intra-individual fluctuations.

FSH is measurable in all healthy prepubertal girls. Levels range from 0.3 to 6.8 IU/l (Sehested et al., 2000). It is not known if inter-individual FSH variation in healthy girls reflects different levels of ovarian activity.

We hypothesized that AMH is a marker of ovarian follicles even in young girls. By longitudinal evaluation of circulating AMH levels in a large contemporary group of healthy Danish girls, we expected to find slightly declining individual AMH levels during childhood and adolescence. A negative correlation of AMH and FSH levels in prepubertal girls would suggest an individual pituitary-ovarian set-point. This would support the notion that AMH is a quantitative marker of ovarian function even in young girls.

Longitudinal study population

To evaluate the inter- and intra-individual variation of hormone levels in serum during childhood, 108 girls and adolescents were examined every 6 months. The girls had no history of gynaecological diseases. Twenty-three girls were excluded from the present data set because one or both parents originated from a non-European country (n = 11), because none or only one AMH value was available (n = 11), and due to previous cytostatic treatment (n = 1). The remaining 85 healthy girls were included in the present study. They were followed for an average of 3 years (range: 0.5–4 years). Mean (range) age at baseline was 9.2 (5.9–12.9) years. AMH and FSH were evaluated in a total of 504 samples (median 6, range 2–10 per girl) and 502 (6, 1–10), respectively.

The age of pubertal onset was approximated using the date exactly between two visits where the girl advanced from B1 to B2 (or more). Thirty-nine girls entered puberty and 9 had menarche during the follow-up period.

Cross-sectional study population

In order to establish normative data of reproductive hormone levels during childhood and adolescence, blood samples were drawn from 995 healthy girls and adolescents. To evaluate the correlation between AMH and FSH levels in prepubertal girls, we included all 230 girls (age 5.6–11.7 years) without breast development (Tanner stage B1 on both sides) participating in the cross-sectional cohort. Six girls were excluded from the present study because one or both parents originated from a non-European country. Other aspects have previously been published (Hagen et al., 2010a).

Laboratory analyses

All blood samples were drawn between 8:00 a.m. and 1:00 p.m. from an antecubital vein, clotted, centrifuged and serum was stored at −20°C until hormone analyses were performed. Blood samples were analysed after a maximum of 4 years of storage in the freezer at −20°C. All samples were analysed in the same laboratory blinded for the technician for age and pubertal stage.

Reproductive hormone assays

Serum AMH levels were determined using the Beckman Coulter enzyme immunometric assay (Immunootech, Beckman Coulter Ltd., Marseilles, France) with a detection limit of 2.0 pmol/l. The intra-assay coefficients of variation (CVs) were <7.8 and 5.4% at 13 and 123 pmol/l, respectively. On the basis of results from the first 154 assays (corresponding to three batches), the inter-assay CVs were <11.3 and 9.2% at 18 and 99 pmol/l, respectively. In the following batches, several of the low and medium controls were above +2 SD. According to standard practice, all samples were adjusted using a batch-specific correction factor. Adjustments were blinded for age and stage of pubertal development. After adjustment of internal controls, the inter-assay CVs were 10.8 and 9.2% at 18 and 99 pmol/l, respectively.

Our AMH results were compared with those of other studies using another assay and other units. At present, two different AMH assays are commercially available: Immunootech, Beckman Coulter (BC) and Diagnostic System Laboratories (DSL). The recorded value of a specific serum AMH level is higher when measured on BC compared with DSL, and the detection limit of DSL is approximately five times lower than BC. To compare levels measured on different assays, the following conversion was used: AMH(BC) pmol/l = AMH(DSL) μg/l × 2.0 × 7.14 pmol/μg (Hehenkamp et al., 2006).
Serum FSH was measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer, Boston, MA, USA) with detection limits of 0.06 IU/l. Intra- and inter-assay CV were <5%.

Statistical analyses
To evaluate the individual fluctuation of AMH levels according to age, we compared intra-individual CV with the AMH inter-assay CV (CV% = SD/mean × 100). To evaluate the progress of AMH levels as a function of time from pubertal onset, we used a variance component model allowing each girl to have her own general AMH level. The time from B2 (numeric variable) was grouped into a categorized variable (i.e. 0.5 ≤ 0 year, 0.5 ≤ 1 year < 1.5, etc.). In case of multiple AMH values per girl in a given category, the mean AMH was used. To compensate for a skewed distribution of AMH, we transformed AMH values with the natural logarithm before analysis.

To enable visual evaluation of the association between longitudinal AMH and FSH levels as a function of chronological age, longitudinal FSH levels were grouped in tertiles, according to the mean AMH level of the girl.

To assess the correlations between AMH and FSH levels in prepubertal girls (cross-sectional study), Spearman’s correlation was used.

Ethical considerations
The Copenhagen Puberty Study (ClinicalTrials.gov ID: NCT01411527) was approved by the local ethical committee (KF 01 282214 and V200.1996/90) and the Danish Data Protection Agency (2010-41-5042). All children and parents received written information, and they were invited to an information meeting. All participants and their parents gave informed consent.

Results
Serum AMH was detectable (>2 pmol/l) in all samples. The individual mean AMH ranged from 5 to 54 pmol/l (median: 18 pmol/l). Overall, each girl maintained her AMH level throughout childhood and adolescence (Fig. 1). The mean intra-individual CV of AMH was 22.0% (range 0.1–53.7%). A total of 10 girls (12%) had a mean AMH level below a cut-off value of 8 pmol/l. Six of these 10 girls demonstrated ongoing pubertal development, whereas the remaining four did not enter puberty during follow-up. In the prepubertal samples from the girls with AMH <8 pmol/l, FSH levels ranged from 0.8 to 4.9 IU/l.

From 3 years prior to pubertal onset until 4 years after pubertal onset, individual AMH levels did not change significantly (from an average of 20–17 pmol/l, P = 0.082). During this period, AMH levels increased by 17% from 3 years prior to time of pubertal onset until start of puberty (from an average of 20–24 pmol/l, P = 0.023). After pubertal onset, AMH decreased 30% during the first 2 years (from an average of 24–17 pmol/l, P < 0.001). Subsequently, AMH levels were constant during the last 2 years of follow-up.

We found no correlation between individual AMH level (the mean level of AMH prior to pubertal onset) and age at entering puberty (Spearman: r = 0.14, P = 0.39).

Longitudinal FSH levels are shown according to AMH tertile groups as a function of chronological age (Fig. 2). Individual AMH levels were negatively associated with FSH levels; girls in the high AMH tertile (blue lines) having clearly lower FSH levels compared with girls in the low AMH tertile (black lines). The association was confirmed by a significant negative correlation between AMH and FSH serum levels in 224 prepubertal girls from the cross-sectional cohort (Spearman: r = −0.31, P < 0.001).

Discussion
To our knowledge, longitudinal AMH has not previously been evaluated in girls and adolescents, and this is the most comprehensive
study of circulating AMH levels evaluated longitudinally in healthy females at all ages. We found that circulating AMH showed only minor fluctuations during childhood and adolescence. Thus, a random AMH measurement seems representative for a given girl. Furthermore, AMH was negatively correlated with FSH prior to pubertal onset. Thus, each girl seems to have a prepubertal set-point of ovarian-pituitary activity, which may be determined by the number of ovarian follicles. Follicles large enough to produce AMH are present in prepubertal girls (Holm et al., 1995). Our findings support the notion of a random AMH value being a marker of pre-antral and early antral follicles even in girls. Inter-individual variation of AMH levels may also be affected by the presence of polycystic ovarian syndrome and insulin levels (Codner et al., 2011; Hart et al., 2010). Unfortunately, such data are not available from our present study. FSH may indirectly affect circulating AMH levels by inducing follicle growth, which reduces the number of AMH-producing follicles (La Marca et al., 2004). We speculate that the observed minor changes of AMH at pubertal onset are caused by redistribution of the follicle pool under the influence of the pubertal FSH surge.

Our finding of an individual prepubertal pituitary-ovarian set-point suggests that FSH is a marker of ovarian function in healthy girls during childhood. However, central inhibition of gonadotrophin secretion during mid-childhood makes elevated FSH an insensitive marker of ovarian failure in young patients with ovarian dysgenesis (Hagen et al., 2010b). In mammals, the onset of puberty is believed to be centrally regulated by pulsatile hypothalamic secretion of GnRH, which is largely unaffected by ovarian function (Pohl et al., 1995). In patients with TS, the age of the pubertal gonadotrophin surge does not depend on the remaining ovarian function (Hagen et al., 2010b). Thus, we expected to find that time of pubertal onset was not correlated with the prepubertal AMH level.

Serum AMH concentration has gained widespread attention as a marker of ovarian reserve in adult women, and AMH measurement in a single spot sample is now clinically used among fertility doctors worldwide. Longitudinal studies of AMH in adult women suggest that individual AMH levels decline over time, reflecting the continuous loss of follicles with age (van Rooij et al., 2005). Furthermore, low AMH in healthy adults predicts early time of menopause (Broer et al., 2011; Tehrani et al., 2011). Using the exact same AMH assay as reported in our present study, we have previously found that an AMH level <8 pmol/l was a specific and sensitive marker of premature ovarian failure in young patients with TS (Hagen et al., 2010a). Others have found that 0.56 mg/l (corresponding to 8 pmol/l) equals the median AMH level in 44-year-old women (van Disseldorp et al., 2008). Although circulating AMH levels may reflect different physiological conditions in this cohort compared with adult women or TS patients, we are concerned by the high prevalence (12%) of apparently healthy girls maintaining AMH levels <8 pmol/l. In the 1980’s, the incidence of primary ovarian insufficiency was 1% (Coulam et al., 1986). At this time, there is no fertility outcome on this study population available. Continuous longitudinal follow up is essential to evaluate if low AMH is predictive of reduced fertility and premature ovarian insufficiency in this cohort. Theoretically, low pre- and peripubertal AMH levels may have a different clinical implication than later in life, and the interpretation of low AMH as a marker of approaching menopause may not apply to pre- and peri-pubertal girls. One study suggests that AMH declines rapidly 5 years prior to time of menopause (Sowers et al., 2008). Longitudinal tracking of the steep premenopausal decline of AMH may be a more sensitive marker of approaching menopause in healthy females compared with consecutive low, but stable, AMH values.

As in any observational study, our findings are susceptible to confounding. The longitudinal study was conducted at two primary
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schools in Copenhagen, thus we cannot exclude possible sociodemographic selection bias. All pupils were invited to participate, and we consider the participants in this study as representative of age-matched healthy Danish girls. In the final data-analysis, we have only included Caucasian girls, as racial differences in AMH levels have been suggested (Seifer et al., 2009). None of the included girls had a history of gynaecological diseases or surgery, and they had not received gonadotoxic treatment or radiotherapy.

In conclusion, circulating AMH exhibits only minor fluctuations at the time of pubertal onset, and a random AMH measurement seems representative for a given girl during childhood and adolescence. The negative AMH–FSH correlation in prepubertal girls supports the notion that AMH is a quantitative marker of ovarian follicles even in young girls.

Authors’ roles


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Conflict of interest

None declared.

References


