Hormone Phenotypes and the Timing of Pubertal Milestones in a Longitudinal Cohort of Girls

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https://www.menstrupedia.com/articles/puberty/physical-changes-girls
Hormones During Puberty

Hormone levels change throughout puberty.¹

1. Gonadotropin-releasing hormone (GnRH) is released at the beginning of puberty.
2. The follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are then released into the bloodstream.
3. LH and FSH stimulate the ovaries to produce estrogen (estradiol, estrone, and estriol) to initiate breast development.
4. The adrenal gland hormones, DHEA-S (dehydroepiandrosterone sulfate) and testosterone, stimulate pubic hair growth.²


Hormones Attributes
Mean hormone values across time related to thelarche (time=0)*

* Note the units of each hormones differ.
Individual Girl’s Hormones

**ODARAB BMI % = 27.7**

- Estradiol

**WEBTAD BMI % = 29.5**

- Estradiol

**ODARAB BMI % = 27.7**

- DHEA-S

**WEBTAD BMI % = 29.5**

- DHEA-S
Objective

Determine if the hormone levels in girls around the time of thelarche are the same for all girls or if girls have different patterns in increases and decreases in hormone levels.

• Identify peri-pubertal hormone phenotypes (or clusters) in young girls based on hormone levels around thelarche (e.g. estradiol at -6 and 0 or testosterone and estrone at 0).

• Determine if the phenotypes are associated with differences in the ages of pubarche, thelarche and/or menarche.
Study Design – Cincinnati Cohort

BCERP’s Puberty Cohort
• Three site, longitudinal, prospective cohort: East Harlem, New York; Greater Cincinnati Area; San Francisco Bay Area
• Recruited girls aged 6-8 from 2004 until 2006 (n=1,239)

Cincinnati Cohort -
• 379 girls were enrolled in the Cincinnati cohort.
• The girls were seen every six months for study visits from 2004-2014.
  • Anthropometric measurements
  • Blood for serum collected
  • Pubarche and thelarche staging
  • Answered questions regarding menarche, exercise, nutrition, etc.
• For this analysis, girls will be excluded if they report taking oral contraceptives or have an underlying hormone condition.
• This analysis included 269 girls with hormone measurements.
Unique Longitudinal Cohort

Most cohorts studying pubertal hormones are cross sectional and based on absolute age or pubertal status rather than timing related to puberty.

Cross sectional - looks at hormones of the girls who achieved thelarche vs those who did not or looks at hormones of 12 year olds versus 11 year olds versus 10 year olds regardless of pubertal status.

Longitudinal cohort - ability to determine age of thelarche and link other visits to time before or after thelarche.

<table>
<thead>
<tr>
<th>Visit</th>
<th>1A</th>
<th>1B</th>
<th>2A</th>
<th>2B</th>
<th>3A</th>
<th>3B</th>
<th>4A</th>
<th>4B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girl 1</td>
<td>-12</td>
<td>-6</td>
<td>Thelarche</td>
<td>+6</td>
<td></td>
<td></td>
<td></td>
<td>Menarche</td>
</tr>
<tr>
<td>Girl 2</td>
<td>-18</td>
<td>-12</td>
<td>-6</td>
<td>Thelarche</td>
<td>+6</td>
<td></td>
<td></td>
<td>Menarche</td>
</tr>
<tr>
<td>Girl 3</td>
<td>-18</td>
<td>-12</td>
<td>-6</td>
<td>Thelarche</td>
<td>+6</td>
<td></td>
<td></td>
<td>Menarche</td>
</tr>
<tr>
<td>Girl 4</td>
<td>-18</td>
<td>-12</td>
<td>-6</td>
<td>Thelarche</td>
<td>+6</td>
<td></td>
<td></td>
<td>Menarche</td>
</tr>
</tbody>
</table>

There were visits beyond 4B included in this analysis. Girls who entered at -6 were not included.
Pubertal Milestone Measurements

Thelarche and Pubarche were achieved when sexual maturation stage 2 or greater was reached.

Age of Pubarche in months—Used accessory light sources to determine the presence or absence of hair in the pubic area.

Age of Thelarche in months—Staff observed and palpated to determine breast stage.
  – Staff graded down if all criteria were not met for a stage.
  – Girls with inconsistent breast staging were considered the lower stage until consistently the higher stage.

Age of menarche in months - Self-reported from study participant’s and/or her caregiver’s answers to questions regarding their first menstrual cycle.

Figures attributed to Dr. Frank Biro, 1989
Demographic Information (n=269 girls)

Race
- Non-Hispanic White: 63%
- Black: 32%
- Asian Hispanic: 1%
- All Other: 0%

Caregiver’s Education
- Associate's or bachelor's degree: 45%
- More than a bachelor's degree: 26%
- High school degree or less: 29%
Methodology

Employ an objective and agnostic analysis using Principal Component Analysis followed by Cluster Analysis (PCA-CA) to define hormone phenotypes only looking at the hormones (estradiol, estrone, testosterone and DHEA-S at times -6, thelarche and +6) not any other variables suspected or known to influence puberty such as race or BMI.

1. Pearson Correlations – determine if hormones at the time periods are highly correlated

2. Principal Component Analysis – determine if variable reduction is possible given that hormones at certain time periods are redundant /highly correlated and they measure the same thing

3. Cluster Analysis – classify girls into phenotypes/clusters based on the 12 hormone data points such that girls in one cluster are more similar to each other than girls in another cluster

4. Survival Analysis – determine which phenotypes are associated with different ages of pubertal milestones
Methodology

Principal Components followed by Cluster Analysis

• PCA-CA is a validated statistical approach to identify subgroups or phenotypes. Previously patients were classified into “phenotypes” based on a few well characterized traits or thought to be homogenous.

• PCA-CA phenotypes have been well documented for cardiovascular risk, chronic obstructive pulmonary disease, asthma, and sleep apnea.
  – For each study, different sets of known disease symptoms presented in each of the disease phenotypes.

Correlations and Principal Components

- The lack of correlation of E2 at different time periods and the high degree of correlation among the other absolute hormones supports the inclusion of them into PCA-CA.

- PCA did not result in variable reduction of the absolute hormone values.
  - Should use all four absolute hormones measurements at -6, 0 and 6 as objective predictive variables in the cluster analysis.
Participant Clustering using CA based on estradiol, estrone, DHEA-S and testosterone at times =-6,0,6

CA on all girls (n=290)

CA on Cluster 3 (n=172)

Cluster 1 (n=42)
Cluster 2 (n=37)
Cluster 3 (n=172)

*Assigns each girl to only one cluster and identifies outliers.
Mean Hormone Values by Phenotype*

Phenotype 1

- Estradiol (pg/mL)
- Estrone (pg/mL)
- Testosterone (ng/dL)

N=42

Phenotype 2

- Estradiol (pg/mL)
- Estrone (pg/mL)
- Testosterone (ng/dL)

N=37

Phenotype 3a

- Estradiol (pg/mL)
- Estrone (pg/mL)
- Testosterone (ng/dL)

N=74

Phenotype 3b

- Estradiol (pg/mL)
- Estrone (pg/mL)
- Testosterone (ng/dL)

N=96

* Note the units of each hormone differ.

No statistically significant differences across the phenotypes.

Race and parental education

- Phenotype 1
  - Black: 35.71%
  - White and all other: 64.29%

- Phenotype 2
  - Black: 27.03%
  - White and all other: 72.97%

- Phenotype 3a
  - Black: 29.73%
  - White and all other: 70.27%

- Phenotype 3b
  - Black: 34.38%
  - White and all other: 65.63%

Education Levels:
- High school degree or less
- Associate’s or bachelor’s degree
- More than a bachelor’s degree
No statistically significant difference in BMI by phenotype but 3a and 3b have the largest BMI%.
Family History of Breast Cancer
First or Second degree maternal family member with a breast cancer diagnosis

No statistically significant difference in family history by phenotype.
Clustering of phenotypes

- Cluster analysis assigned each girl to a phenotype based on her estradiol, estrone, DHEA-S, and testosterone at each time period (-6,0,6).

- The hormone levels at the different time periods across the four phenotypes varied greatly indicating hormone levels relative to the timing of thelarche are not the same for all girls.

- No statistical difference in other variables including BMI, race/ethnicity, parental education level and family history of cancer existed among the phenotypes which confirms phenotypes should be based on the hormones at the three time periods.
Associations With Pubertal Milestones from Survival Analysis

Earlier ages of thelarche, pubarche and menarche differed by phenotypes, confirming heterogeneity of hormone phenotypes.

- Phenotype 2 much more likely to experience menarche earlier than all other phenotypes.
- Phenotype 3a and 3b more likely to have an earlier age at thelarche than 1.
- Phenotype 3a is more likely to have an earlier age of pubarche than 3b.

All analyses controlled for race, BMI nearest but before age of the pubertal milestone, and mother’s age of menarche. As expected:

- Black girls were twice as likely to reach puberty earlier than other girls.
- Heavier girls were more likely to reach puberty earlier than those with a lower BMI.
- Girls with mother’s ages of menarche younger than 12 years old are 50% more likely to reach all three milestones earlier than girls with mother’s ages at least 14 years old.
Conclusions

• Classifying hormone heterogeneity prior to puberty is highly informative in unveiling different pathways through puberty.

• The four distinct hormone phenotypes in girls indicate hormones levels relative to the age of thelarche are not the same in all girls and help to explain disparity in the age of onset.

• These findings underscore the need to better understand female sex hormones prior to puberty based on time related to puberty rather than chronological age or pubertal status.
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Are the phenotypes predictive of the differing ages of pubertal milestones?

Average age of pubertal events in months by phenotype:

- High DHEA-S (1)
- High estradiol (2)
- Not high hormones (3a)
- Low hormones (3b)

Thelarche, Pubarche, Menarche
Further Characteristics of the Phenotypes after being agnostically defined by the hormones

- Statistical difference exists between the age of thelarche and the age of pubarche among the four phenotypes.
- Girls in phenotypes 1 and 3b had an average age of menarche statistically later than girls in phenotype 2.
- The tempo of girls in phenotype 2 was statistically shorter than that for girls in phenotype 3b.
- Girls in phenotype 3b were more likely to enter puberty via pubarche rather than thelarche which is different than the other phenotypes.
- No differences between the phenotypes existed for the following characteristics:
  - BMI
  - ethnicity
  - family history of 1st or 2nd degree breast cancer
  - mother’s age of menarche
  - caregiver’s education
Phenotype 1

• Higher DHEA-S (over 100% higher than the cohort mean at each time period)
• Testosterone values over 50% higher than the cohort mean
• E1 values over 50% higher than the cohort mean
• Large increase in DHEA-S from -6 to 0 and 0 to 6
• Latest age of thelarche
• Latest age of menarche
• Less risk of early thelarche than 3a or 3b
Phenotype 2

- High E2 values across the time period from 30%-200% higher than the mean of the cohort
- E1 90% higher at +6
- Testosterone >50% higher at -6 and +6
- Huge increase in E2 from 0 to 6 (300%) 
- Decrease in testosterone, estrone and DHEA-S from -6 to 0, then larger increase in all hormones from 0 to 6
- Earliest to achieve menarche
- Shortest tempo
- 50% greater risk of earlier menarche than other phenotypes
- Less risk of early thelarche than 3b
Phenotype 3a

- Hormones lower than the cohort averages by about 10% except
  - 20% lower for DHEA-S at -6
  - >20% lower for all E2 time periods
- Large increase in DHEA-S from -6 to 0
- Earliest to pubarche
- More girls entering pubarche prior to thelarche than other phenotypes
- Greater risk of entering pubarche early than 3b
Phenotype 3b

- Over 30% lower levels for all hormones
  - DHEA-S was 50% lower at all 3 time periods
  - E1 was over 40% lower at 6 and
  - E2 at -6 and 6 and E1 at 0 (over 20% lower)
- Minimal changes in the hormones
- Earliest to thelarche
- Latest to pubarche
- Second to last to enter menarche
- Longest tempo
- Fewest girls entering pubarche prior to thelarche
Limitations
Limitations

• Breast tissue is sometimes confused with fat tissue making some question validity of breast maturation staging.
  – Our study staff was trained and certified to assess breast maturation.
  – Cohen’s Kappa (0.67) indicated ”substantial agreement”

• As with any study there is the potential for volunteer bias.

• One site study leads to lack of generalizability to the United States.
  – The cohort has a similar racial and social economic background to the United States (US).
  – BMI% of the cohort is similar to the NHANEs data making it more generalizable to the entire US.¹

Limitations

• Accuracy of recall of age of menarche could be questioned.
  – Questionnaire data on menarche was collected yearly.
  – Recall of menarche is typically high because it is not an arbitrary event.
  – Studies have shown age of menarche recall over 63% accurate after a year or more.¹

• Lack of age of menarche for 21% of the girls who either dropped out of the study or the study ended prior to them achieving menarche.

Strengths
Strengths

• The use of HPLC-MS enabled us to evaluate hormones measurements that are typically too low to measure in young girls with earlier methods.

• Esoterix Laboratories (now LabCorp) is qualified by the CDC.\(^1\)
  – Interassay Precision (% of coefficient variation for the low, medium and high control serum samples) for initial 252 girls were all less than the standard expectation of 15%. The interassay precisions follow:
    • Estradiol $\leq 4.4\%$, Estrone $\leq 4.9\%$, DHEAS $\leq 8.4\%$, Testosterone $\leq 9.9\%$\(^1\)
  – The average bias estimations from on-going proficiency studies are less than 2%.\(^1\)

Strengths

• Study staff was trained and certified to take standardized anthropometric measurements, with quality assurance procedures.

• A limited number were trained and certified to assess pubertal maturation.
  – Cohen’s Kappa = 0.67 for agreement between the examiner and master trainer during 127 dual examinations across the three sites.¹

Strengths

• Longitudinal analysis
  – First study to quantify hormones in a longitudinal way relative to time to thelarche rather than based on chronological age.
  – Examining hormones based on a chronological age would have diluted the differences in hormone levels.

• Agnostic, objective, and innovative statistical analysis
  – PCA-CA has been used to identify clinical phenotypes in other medical conditions (e.g. COPD, asthma, sleep apnea).
  – Each phenotype included some but not all of the hormones supporting the heterogeneity of the phenotypes.
Future Directions
Future Directions

• Identification of these phenotypes and their relationships to clinical characteristics should be repeated in a larger longitudinal study to replicate these findings and extend them to a more nationally representative population. This would also enable clinical references for the phenotypes to be defined.

• Associate these phenotypes with other outcomes such as migraine headaches and breast cancer.
“Windows of Susceptibility” are periods when the developing breast tissue is most susceptible to gene-environment interactions and environmental exposures that increase the risk of breast cancer.
Lifecycle of the Breast

In utero – breast ducts begin to form
Puberty – breast tissue proliferation: lengthening and branching of ducts and development of lobules
Pregnancy – terminal end bud differentiation
Lactation – the milk duct system grows as more lobules form
Menopause – reduction of glandular tissue in the breast

Pubertal Milestones

There are a number of pubertal milestones reached before the end of puberty and full sexual maturation.

Sex Characteristics

• Primary Sex Characteristics – sex organs responsible for reproduction e.g. ovaries, uterus

• Secondary Sex Characteristics – physical characteristics that are not responsible for reproduction e.g. pubic hair, enlarged breasts, increase height
Objective

• The objective of this research project is to use longitudinal cohort data and quantitative research methods to identify sex hormone phenotypes around the time of thelarche. These analyses will incorporate serum concentrations of up to four hormones (DHEA-S, estradiol, estrone and testosterone) at five different time periods measured in 6 month increments from 18 months prior to 6 months after the age of thelarche.

• A second objective of the research is to determine if the ages of pubertal milestones (thelarche, pubarche, and menarche) are associated with a hormone phenotype.
Study Measurements

BMI% - calculated as weight in kilograms/ height in meters\(^2\)

• Derived from the average of two measurements taken at each study visit by trained staff
• Determined using CDC growth charts from 2000
Study Measurements
BMI% vs BMlz score?

• BMlz score is the number of standard deviations away from the mean BMI for an age group. This score can be compared across age groups unlike BMI% and the measure is usually normal in distribution.

• BMI% is the percentage of people who fall below a certain value. However it is likely to not be normal in distribution.
# Hormone Attributes

Table 1 - Description of hormones for the study cohort girls across the 5 time periods (-18, -12, -6, 0, and 6)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>N</th>
<th>Median</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
<th>LOD</th>
<th># &lt; LOD</th>
<th>% &lt; LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S (ug/dL)</td>
<td>920</td>
<td>22.00</td>
<td>30.16</td>
<td>26.57</td>
<td>7.07</td>
<td>211.00</td>
<td>10.00</td>
<td>170</td>
<td>18.48</td>
</tr>
<tr>
<td>Estradiol (pg/mL)</td>
<td>856</td>
<td>1.80</td>
<td>3.42</td>
<td>6.26</td>
<td>0.71</td>
<td>114.00</td>
<td>1.00</td>
<td>207</td>
<td>24.18</td>
</tr>
<tr>
<td>Estrone (pg/mL)</td>
<td>858</td>
<td>3.60</td>
<td>4.36</td>
<td>3.41</td>
<td>1.77</td>
<td>51.00</td>
<td>2.50</td>
<td>254</td>
<td>29.60</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>859</td>
<td>4.10</td>
<td>4.89</td>
<td>3.59</td>
<td>1.77</td>
<td>50.00</td>
<td>3 or 2.5*</td>
<td>242</td>
<td>28.17</td>
</tr>
</tbody>
</table>

All values <LOD imputed with LOD/√2
* Two batches were sent off to assay.
Objective

Use principal component and cluster analysis (PCA-CA) to identify, for the first time, distinct hormone phenotypes of girls in a longitudinal cohort.

• Focus on patterns in the data, that could be predictors of risk of early or late pubertal maturation.

• Use an agnostic approach to define the phenotypes by completing the statistical analysis of the sex hormone data only and then looking at other variables to describe the phenotypes.
Correlations

- The lack of correlation of E2 at different time periods and the high degree of correlation among the other absolute hormones supports the inclusion of them into PCA-CA.

- The overall lack of correlation between the differences in the hormone values between the time periods (e.g. the difference in testosterone values between -18 and -12) does not support inclusion of these “change” variables into PCA.
Results from factor analysis will be used as a guide to determine which variables to be used in cluster analysis to determine a hormone phenotype.

1. PCA of absolute hormones at the time periods -6, 0, 6 – produced fairly consistent results in the sensitivity analysis (n=260 vs 67).

2. PCA of the differences in hormones between the time period produced one factor including estrone, testosterone and DHEA-S differences between -6 and 0 and the differences between 0 and 6 which is consistent with the lack of correlation

… seems to make sense not to combine the two ways of looking at the hormones (absolute vs differences) and focus on the absolute values for cluster analysis
Participant Clustering
Proc Fastclus – Hormone Values at time =-6,0,6

- Cluster analysis will classify the participants into phenotype groups that exhibit similar clinically relevant hormone values related to thelarche.
- Identifies disjoint clusters of observations by distance (nearest centroid).
- Assigns participants to a cluster where the other participants are more similar to them than participants in another cluster. Each participant belongs to only one cluster.
- Uses $k$-means method (least squares)
  - User defines the number of clusters ($k$)
  - Observations are divided in to the clusters
  - The first $k$ observations (no missing values) are selected as the initial seeds
  - Other observations are then assigned to the closest cluster
  - The cluster center is updated
  - Repeated in an iterative process until all observations are grouped into their closest cluster

- An adjusted distance is computed for missing data (Fastclus is the only cluster procedure that handles missing data)

- Good for datasets larger than 100
- Very sensitive to outliers and has a method to identify outliers in their own clusters (unlike other cluster procedures)
- Need to standardized the variables first because Fastclus uses an algorithm that emphasizes variables with larger variances.
Changing the number of Clusters

CA - n=260; clusters = 6

Outliers = 7
Cluster 1 n=49
Cluster 2 n=17
Cluster 3 n=105
Cluster 4 n=33
Cluster 5 n=24
Cluster 6 n=25

- cluster 1 and 6 overlap implying they not distinct
- cluster 2 is really small (perhaps should be outliers?)
- Too many clusters????
1 - Changing the number of Clusters

CA - n=260; 4 clusters and 5 clusters

Still seems to be overlap of some of the clusters and there is still one large cluster. What if we look at that large cluster only?
Creating 4-6 clusters of the entire cohort (n=260) did not produce distinct clusters. This reinforces the results from PCA, 3 components that represent 74% of the variance should result in 3 clusters. But are we missing something?

Therefore let’s try redoing the PCA-CA on only cluster 3 (N=172)
## Hormone Phenotype Objective Predictors

Characteristics of the 172: girls means reported unless noted.

<table>
<thead>
<tr>
<th></th>
<th>Cluster 3A</th>
<th>Cluster 3B</th>
<th>Significance (P value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort</td>
<td>N=269</td>
<td>N=74</td>
<td>n=96</td>
</tr>
<tr>
<td>Testosterone -6‡</td>
<td>4.48</td>
<td>3.79</td>
<td>2.97</td>
</tr>
<tr>
<td>Testosterone 0‡</td>
<td>6.16</td>
<td>5.72</td>
<td>3.44</td>
</tr>
<tr>
<td>Testosterone 6‡</td>
<td>4.00</td>
<td>3.54</td>
<td>2.65</td>
</tr>
<tr>
<td>Estrone -6‡</td>
<td>4.47</td>
<td>4.10</td>
<td>3.43</td>
</tr>
<tr>
<td>Estrone 0‡</td>
<td>6.09</td>
<td>5.45</td>
<td>3.53</td>
</tr>
<tr>
<td>Estrone +6‡</td>
<td>28.23</td>
<td>22.51</td>
<td>12.56</td>
</tr>
<tr>
<td>DHEA -6‡</td>
<td>30.79</td>
<td>31.00</td>
<td>13.63</td>
</tr>
<tr>
<td>DHEA 0‡</td>
<td>38.32</td>
<td>38.00</td>
<td>18.55</td>
</tr>
<tr>
<td>Estradiol -6</td>
<td>2.83</td>
<td>2.07</td>
<td>2.22</td>
</tr>
<tr>
<td>Estradiol 0‡</td>
<td>3.37</td>
<td>2.67</td>
<td>2.88</td>
</tr>
<tr>
<td>Estradiol +6‡</td>
<td>5.38</td>
<td>3.51</td>
<td>3.05</td>
</tr>
</tbody>
</table>

* Comparison between clusters using analysis of variance for continuous variables.

Cluster 3a – hormones lower than the cohort averages by about 10% except 20% lower for DHEA-S at -6 and >20% lower for all E2 time periods

Cluster 3b – hormones much lower than the cohort, over 30% lower for all hormones except E2 at -6 and 6 and estrone at 0 (over 20% lower). E2 was over 40% lower at 6 and DHEA-S was 50% lower at all 3 time periods.

Much lower values
Phenotypes

Initially….
Hormones were used to define 4 phenotypes of sex hormones in girls around the time of thelarche.

Then….
After phenotypes were formed, additional variables were then examined to further describe the participants in each of the phenotypes.
Phenotype Characteristics - from CA (mean values)

Sex hormone serum concentrations of the girls according to the four phenotypes identified using principal component based cluster analysis of DHEA-S (ng/dL), estrone and estradiol (pg/mL), testosterone (ng/dL)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cohort</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Testosterone -6: 4.48</td>
<td>2.69</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Testosterone 0: 4.96</td>
<td>2.76</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Testosterone 6: 6.16</td>
<td>3.64</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Estrone -6: 4.00</td>
<td>2.19</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Estrone 0: 4.47</td>
<td>2.46</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>Estrone +6: 6.09</td>
<td>4.46</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>DHEA-S -6: 28.23</td>
<td>24.99</td>
</tr>
<tr>
<td>Phenotype 1</td>
<td>N=269</td>
<td>DHEA-S 0: 30.79</td>
<td>26.08</td>
</tr>
<tr>
<td>Phenotype 1</td>
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<td>3.32</td>
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<td>Testosterone 0: 5.40</td>
<td>1.93</td>
</tr>
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<td>Testosterone 6: 10.79</td>
<td>4.01</td>
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<td>Estradiol -6: 4.60</td>
<td>2.78</td>
</tr>
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<td>Estradiol 0: 4.46</td>
<td>4.71</td>
</tr>
<tr>
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<td>N=37</td>
<td>Estradiol +6: 17.25</td>
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<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Testosterone -6: 3.79</td>
<td>1.60</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Testosterone 0: 4.55</td>
<td>1.55</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Testosterone 6: 5.72</td>
<td>1.52</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Estrone -6: 3.54</td>
<td>1.68</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Estrone 0: 4.10</td>
<td>1.72</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Estrone +6: 5.45</td>
<td>1.94</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>DHEA-S -6: 2.97</td>
<td>1.29</td>
</tr>
<tr>
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<td>DHEA-S 0: 3.39</td>
<td>1.72</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
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<td>DHEA-S +6: 3.44</td>
<td>1.26</td>
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<tr>
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<td>1.27</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
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<td>Estradiol 0: 3.43</td>
<td>1.98</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=74</td>
<td>Estradiol +6: 3.53</td>
<td>1.72</td>
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<tr>
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<td>N=96</td>
<td>Testosterone -6: 6.89</td>
<td>2.53</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Testosterone 0: 5.40</td>
<td>1.93</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
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<td>Testosterone 6: 10.79</td>
<td>4.01</td>
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<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Estrone -6: 5.24</td>
<td>2.11</td>
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<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Estrone 0: 5.17</td>
<td>2.31</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Estrone +6: 11.78</td>
<td>2.92</td>
</tr>
<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>DHEA-S -6: 32.93</td>
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<tr>
<td>Pheno1ype 3b</td>
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<td>DHEA-S 0: 24.91</td>
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<td>Pheno1ype 3b</td>
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<td>Estradiol -6: 4.60</td>
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<tr>
<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Estradiol 0: 4.46</td>
<td>4.71</td>
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<td>Pheno1ype 3b</td>
<td>N=96</td>
<td>Estradiol +6: 17.25</td>
<td>10.48</td>
</tr>
</tbody>
</table>

**Phenotype 1** – Higher DHEA-S (over 100% higher than the cohort mean at each time period)
- Testosterone and E1 values over 50% higher than the cohort mean

**Phenotype 2** – High E2 values across the time period from 30%-200% higher than the mean of the cohort
- E1 90% higher at +6
- Testosterone >50% higher at -6 and +6 vs cohort mean

**Phenotype 3a** – hormones lower than the cohort averages by about 10% except
- 20% lower for DHEA-S at -6
- >20% lower for all E2 time periods

**Phenotype 3b** – Over 30% lower levels for all hormones
- DHEA-S was 50% lower at all 3 time periods
- E1 was over 40% lower at 6 and
- E2 at -6 and 6 and E1 at 0 (over 20% lower)
Characteristics of Participants in each Phenotype (mean values unless noted)

Maturation and clinical characteristics of the girls according to the four phenotypes identified using principal component based cluster analysis

DHEA-S (ng/dL), estrone and estradiol (pg/mL), testosterone (ng/dL)

<table>
<thead>
<tr>
<th>Phenotype 1</th>
<th>Phenotype 2</th>
<th>Phenotype 3a</th>
<th>Phenotype 3b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cohort</strong></td>
<td><strong>High DHEA-S, T and E1</strong></td>
<td><strong>High E2, T and E1</strong></td>
<td><strong>No High Hormones</strong></td>
</tr>
<tr>
<td>N=269</td>
<td>N=42</td>
<td>N=37</td>
<td>N=74</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age of Thelarche (months)</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>113.24</td>
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<td>11.84</td>
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<td>13.06</td>
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<table>
<thead>
<tr>
<th>Tempo (time between)</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=269</td>
<td>39.01</td>
<td>13.06</td>
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<td>11.50</td>
<td>30.55</td>
<td>10.27</td>
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<td>10.87</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Baseline values for the cohort given as mean unless noted. P values represent tests for groupwise differences between the phenotypes; values for the phenotypes represent mean values within the phenotypes. Comparison between phenotypes using Kruskal-Wallis for continuous variables and \( \chi^2 \) for categorical.

- No differences between the phenotypes existed for BMI, ethnicity, family history of breast cancer, mother’s age of menarche or caregiver’s education.
- Pairwise comparisons proved the age of menarche to be different between Phenotypes 1 and 2.

Phenotype 1 – latest age of thelarche (5 months) but earlier pubarche by 2 months, latest to menarche, more girls entering pubarche prior to thelarche.

Phenotype 2 - later age of thelarche, earliest to achieve menarche, shortest tempo,

Phenotype 3a – Early to thelarche and earliest to pubarche, more girls entering pubarche prior to thelarche.

Phenotype 3b - Earliest to Thelarche, latest to pubarche, longest tempo.
Changes in hormone levels from one time window to the next (mean values unless noted)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>High DHEA-S, T and E1</th>
<th>High E2, T and E1</th>
<th>No High Hormones</th>
<th>All Low Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort</td>
<td>N=269</td>
<td>N=42</td>
<td>N=37</td>
<td>N=74</td>
</tr>
<tr>
<td><strong>Δ Testosterone from -6 to 0</strong></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>A</td>
<td>1.26</td>
<td>4.68</td>
<td>1.81</td>
<td>4.75</td>
</tr>
<tr>
<td>A Testosterone from 0 to 6</td>
<td>2.38</td>
<td>5.50</td>
<td>3.54</td>
<td>4.31</td>
</tr>
<tr>
<td>A Estrone from -6 to 0</td>
<td>1.32</td>
<td>3.74</td>
<td>1.24</td>
<td>4.71</td>
</tr>
<tr>
<td>A Estrone from 0 to 6</td>
<td>1.88</td>
<td>4.87</td>
<td>2.84</td>
<td>3.76</td>
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<tr>
<td>A DHEA-S from -6 to 0</td>
<td>9.68</td>
<td>24.85</td>
<td>17.15</td>
<td>21.26</td>
</tr>
<tr>
<td>A DHEA-S from 0 to 6</td>
<td>15.14</td>
<td>26.71</td>
<td>27.27</td>
<td>41.84</td>
</tr>
<tr>
<td>A E2 from -6 to 0</td>
<td>1.21</td>
<td>8.31</td>
<td>2.13</td>
<td>3.72</td>
</tr>
<tr>
<td>A E2 from 0 to 6</td>
<td>2.19</td>
<td>11.79</td>
<td>1.94</td>
<td>7.54</td>
</tr>
</tbody>
</table>

* Baseline values for the cohort given as mean unless noted. P values represent tests for groupwise differences between the phenotypes; values for the phenotypes represent mean values within the phenotypes. Comparison between phenotypes using Kruskal-Wallis for continuous variables and χ² for categorical.

Δ = change in hormone value

Increase between time periods

Decrease in values between -6 to 0 then an larger increase from 0 to 6

Phenotype 1 – large increase in DHEA-S from -6 to 0 and 0 to 6

Phenotype 2 – huge increase in E2 from 0 to 6, decrease in testosterone, estrone and DHEA-S from -6 to 0, larger increase all hormones from 0 to 6.

Phenotype 3a – larger increase in DHEA-S from -6 to 0 than the cohort

Phenotype 3b – very few changes in the hormones over the time periods
Survival Analysis

Survival analysis (Cox proportional-hazard models) were conducted in SAS using PHReg and controlling for covariates.

\[ h(t, x) = h_0(t) \exp(\beta x) \]

such that \( h_0(t) \) is the underlying hazard function which is independent of \( x \), \( x \) is the covariate and \( t \) stands for time. A unit increase in \( x \) multiplies the hazard by \( \exp(\beta) \) or \( e^\beta \) for all values of \( t \), \( \beta \) is assumed the same for all individuals.

Assumption of proportional hazards – additive changes in a variable cause corresponding multiplicative changes in the hazard function e.g. ratio of hazards for two individuals over time is constant

Girls who have not yet reached the pubertal milestone during the study or were lost to follow up prior to achieving it will be right censored.
Survival Analysis

Hazard Ratio ($\exp(\beta)$) is the measure of effect or risk of suffering the event/outcome.

- A ratio $<1$ indicates a reduced risk of reaching the pubertal outcome (e.g., delayed menarche) e.g. HR=.88 means a 22% decreased risk for reaching menarche for every unit increase in mother’s age of menarche.
- A ratio $>1$ indicates an increased risk of reaching menarche (e.g., early menarche) e.g. HR=1.44 means a 44% risk of reaching menarche every for unit of increase in DHEAS at -6 months.
- A ratio $=1$ indicates no relationship between the predictor and the age of pubertal outcome outcome.
Survival Analysis

Cox- Proportional model advantages

• does not require a certain probability function\textsuperscript{43,44}
• may include multiple covariates
• may include continuous and/or categorical covariates
• allows for the inclusion of girls who are right censored
• allows for interactions between covariates
• time varying covariates e.g. BMI\% that changes over time vs only including the BMI\% at the -12 month study visit
Survival Analysis

Cox-Proportional model assumption

Proportional hazards – additive changes in a variable cause corresponding multiplicative changes in the hazard function e.g. ratio of hazards for two individuals over time is constant

- Plot of the log-negative-log of the Kaplan Meier estimates of the survival function against the log of time should be parallel if the hazard is constant over time for a categorical predictor.\textsuperscript{44,45}

- If a variable fails the assumption, will include a multiplicative with time.
Survival Analysis

Non-parsimonious Model

Besides the phenotypes, the models will include the following even if they are insignificant because they are known risk markers for early pubertal timing

– Race
– BMIz - nearest but before age of the pubertal milestone
– Caregiver’s education
– Mother’s age of menarche

• Multiplicative interaction with time included if a variable failed the proportional hazard assumption
### Risk Estimates of Pubertal Milestones

Proportional hazard ratio analysis of risk factors for age at pubertal milestones

<table>
<thead>
<tr>
<th>Variable</th>
<th>Age of Pubarche (months)</th>
<th>Age of Thelarche (months)</th>
<th>Age of Menarche (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypes</td>
<td>Hazard Ratio 95% CI p-val</td>
<td>Hazard Ratio 95% CI p-val</td>
<td>Hazard Ratio 95% CI p-val</td>
</tr>
<tr>
<td>DHEA-S (1) vs high estradiol (2)</td>
<td>0.93 0.59 1.48 0.7600</td>
<td>0.91 0.57 1.46 0.7013</td>
<td>0.55 0.32 0.93 0.0264</td>
</tr>
<tr>
<td>DHEA-S (1) vs no high hormones (3a)</td>
<td>0.63 0.43 0.93 0.0196</td>
<td>0.77 0.52 1.15 0.2042</td>
<td>0.88 0.57 1.35 0.555</td>
</tr>
<tr>
<td>DHEA-S (1) vs all low hormones (3b)</td>
<td>0.45 0.31 0.66 &lt;0.0001</td>
<td>1.32 0.91 1.93 0.1493</td>
<td>0.87 0.57 1.33 0.514</td>
</tr>
<tr>
<td>high estradiol (2) vs no high hormones (3a)</td>
<td>0.68 0.45 1.03 0.0656</td>
<td>0.85 0.56 1.29 0.4433</td>
<td>1.61 1.02 2.54 0.0427</td>
</tr>
<tr>
<td>high estradiol (2) vs all low hormones (3b)</td>
<td>0.48 0.32 0.73 0.0006</td>
<td>1.45 0.96 2.18 0.0752</td>
<td>1.59 1.01 2.52 0.0466</td>
</tr>
<tr>
<td>no high hormones (3a) vs all low hormones</td>
<td>0.71 0.52 0.97 0.0337</td>
<td>1.71 1.24 2.35 0.0012</td>
<td>0.99 0.70 1.41 0.9647</td>
</tr>
<tr>
<td>Race (all other vs black)</td>
<td>0.67 0.51 0.88 0.0256</td>
<td>0.01 0.00 0.06 &lt;0.0001</td>
<td>0.01 0.00 0.30 0.0092</td>
</tr>
<tr>
<td>BMIZ closest to outcome</td>
<td>7.18 2.65 19.51 0.0001</td>
<td>1.16 1.05 1.30 0.0056</td>
<td>1.57 1.36 1.81 &lt;0.0001</td>
</tr>
<tr>
<td>Mother's age of menarche (years)</td>
<td>0.055 0.0837 0.1327</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Under 12 vs ages 12-14</td>
<td>1.34 0.96 1.87 0.0905</td>
<td>1.15 0.81 1.62 0.432</td>
<td>1.20 0.83 1.74 0.3418</td>
</tr>
<tr>
<td>Under 12 vs at least 14</td>
<td>1.68 1.10 2.57 0.0168</td>
<td>1.58 1.04 2.42 0.0336</td>
<td>1.61 1.01 2.59 0.0468</td>
</tr>
<tr>
<td>ages 12-14 vs at least 14</td>
<td>1.26 0.89 1.77 0.1931</td>
<td>1.38 0.98 1.95 0.0664</td>
<td>1.35 0.91 1.99 0.1334</td>
</tr>
<tr>
<td>Caregiver's education level</td>
<td>0.9763 0.6314 0.3051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or less vs at least an associate's or bachelor's degree</td>
<td>1.00 0.67 1.49 0.9967</td>
<td>0.88 0.58 1.33 0.5491</td>
<td>0.73 0.45 1.18 0.1946</td>
</tr>
<tr>
<td>High school or less vs master's degree or more</td>
<td>0.97 0.68 1.38 0.8618</td>
<td>0.84 0.59 1.20 0.3376</td>
<td>0.97 0.65 1.44 0.8747</td>
</tr>
<tr>
<td>At least an associate's or bachelor's degree or more</td>
<td>0.97 0.70 1.35 0.8568</td>
<td>0.95 0.68 1.33 0.7829</td>
<td>1.33 0.90 1.96 0.1478</td>
</tr>
<tr>
<td>race*age of milestone in months</td>
<td>0.96 0.94 0.98 0.0004</td>
<td>0.97 0.94 0.94 0.0166</td>
<td></td>
</tr>
<tr>
<td>bmiz closest to outcome* age of milestone in months</td>
<td>0.99 0.98 1.00 0.0036</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 1, 2 and 3a were less likely to enter early pubarche than 3b and 1 was also less likely than 3a.
- 3a was much more likely to enter thelarche earlier than 3b.
- 2 was 50% more likely to enter menarche earlier vs all 3 other phenotypes.
- White girls were half as likely to enter puberty early than black girls.
- Being heavier increased the likelihood of entering puberty early.
- Girls whose mother's who entered puberty prior to 12 years old had a much higher chance of entering early puberty than those whose mothers entered after 14 years old.
Characteristics of Participants in each Phenotype (mean values unless noted)

Maturation and clinical characteristics of the girls according to the four phenotypes identified using principal component based cluster analysis:

<table>
<thead>
<tr>
<th>Phenotype 1</th>
<th>Phenotype 2</th>
<th>Phenotype 3a</th>
<th>Phenotype 3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=269</td>
<td>N=42</td>
<td>N=37</td>
<td>N=74</td>
</tr>
<tr>
<td><strong>Age of Thelarche (months)</strong></td>
<td>108.26 ± 13.20</td>
<td>113.24 ± 11.29</td>
<td>112.49 ± 15.16</td>
</tr>
<tr>
<td><strong>Age of Pubarche (months)</strong></td>
<td>118.19 ± 16.24</td>
<td>116.83 ± 19.22</td>
<td>118.69 ± 11.39</td>
</tr>
<tr>
<td><strong>Age of Menarche (months)</strong></td>
<td>147.59 ± 13.75</td>
<td>150.70 ± 15.34</td>
<td>142.48 ± 11.84</td>
</tr>
<tr>
<td><strong>Tempo (time between thelarche and menarche in months)</strong></td>
<td>39.01 ± 13.06</td>
<td>36.39 ± 11.50</td>
<td>30.55 ± 10.27</td>
</tr>
<tr>
<td><strong>BMIZ</strong></td>
<td>0.33 ± 1.02</td>
<td>0.29 ± 0.96</td>
<td>0.14 ± 0.97</td>
</tr>
<tr>
<td><strong>BMI Percentile</strong></td>
<td>58.97 ± 29.56</td>
<td>57.67 ± 27.62</td>
<td>52.47 ± 28.56</td>
</tr>
</tbody>
</table>

**Mother’s Age of Menarche (%)**
- less than 12 years old: 20.07%
- at least 12 years but less than 14 years old: 59.85%
- at least 14 years or older: 20.07%

**Ethnicity (%)**
- Black: 31.60%
- Hispanic, White, Asian, All Other: 68.40%

**First or Second Degree Maternal Family Member Breast Cancer Diagnosis (%)**
- diagnosis of breast cancer: 12.64%
- no diagnosis of breast cancer: 80.30%
- missing: 7.06%

**Caregiver’s education (%)**
- high school degree or less: 29.00%
- associate’s or bachelor’s degree: 45.35%
- more than a bachelor’s degree: 25.62%

**Pubertal Pathway (%)**
- thelarche before pubarche: 69.14%
- pubarche before thelarche: 17.10%
- entered at the same time: 7.43%
- missing due to censorship: 6.32%

### Notes
- Δ = change in hormone value
- Baseline values for the cohort given as mean unless noted. P values represent tests for groupwise differences between the phenotypes; values for the phenotypes represent mean values within the phenotypes. Comparison between phenotypes using Kruskal-Wallis for continuous variables and χ² for categorical.

**Phenotype 1** – (High DHEA-S) latest age of thelarche (5 months) but earlier pubarche by 2 months, latest to menarche, more black

**Phenotype 2** – (High E2) later age of thelarche (4 months), very lean girls, less black vs all other

**Phenotype 3a** – (Not High) Early to thelarche and earliest to pubarche, more obese girls.

**Phenotype 3b** – (Low) Earliest to thelarche, latest to pubarche (longest time in between the two), more obese girls, more black
Assumptions of Tests

• Pearson correlation coefficient measures linear correlation between two continuous variables,
  – ranges between -1 and 1
  – based on covariance strength, normal and linear and not too many outliers
  – Can use Spearman’s Rank if not linear or normal

• Chi Square for categorical variables. Look at the proc freq race*education (use Fischer’s exact if expected value is <5 in proc freq of race*education) – independent categories, mutually exclusive, non-parametric (so no distribution assumption), groups are of equal sizes, not large amounts of categories (e.g.20), not paired e.g. mother and child,

• T Test assumptions – equal variance, random independent samples, normal if a small sample size
Study Measurements
Effect Modifier or Confounder?

• Modifier – magnitude of the effect on the outcome differs depending on a third variable; true relationship
  – e.g. a treatment may work for men but not women so sex is an effect modifier, UV induced cancer rate is higher for those with a rare hereditary defect
  – report individual stratified findings (they will be significantly different from each other)

• Confounder – variable has an association with both the exposure and the outcome but does not lie in the causal pathway; false relationship
  – e.g. birth order and mother’s age with outcome of down syndrome (if you stratify you see it is not birth order but age that effects the likelihood of down syndrome)
  – Stratify (these findings will be similar but different by at least 10% from the total finding) and use Mantel-Haenszel pooled odds ratio or risk ratio
  – Multivariable modeling

\[
\hat{RR}_{cmh} = \frac{\sum a_i(c_i + d_i)}{\sum c_i(a_i + b_i)} \quad \frac{n_i}{n_i} \quad \hat{OR}_{cmh} = \frac{\sum a_id_i}{\sum b_i c_i} \quad \frac{n_i}{n_i}
\]
Background – Hormones not included

- **SHBG** – declines during puberty
- **Insulin-like Growth Factor -1 (IGF-1)** – does not increase until later stages of thelarche and closer to menarche
- **DHEA** – 98% of circulating DHEA is in the form of DHEAS, DHEA and DHEAS levels run parallel and DHEAS is more stable to measure.
- **Androstenedione** – is either secreted or converted into testosterone, has few effects of its own.
- **Estrogen** – group of similar female hormones: estradiol, estrone, estiol (minor effect, best detected after a 24 urine collection)
References

References

References


34. Costofcancer.org


40. https://www.nidcd.nih.gov/about/mission
References

45. Kukhareva P. Cox Proportional Hazard model evaluation in one shot. Collaborative Studies Coordinating Center, UNC, Chapel Hill, NC. (n.d.)
An oral dissertation defense in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Division of Epidemiology, Department of Environmental Health, University of Cincinnati College of Medicine

DISSECTORATION COMMITTEE:
Susan Pinney, PhD (Chair, Advisor)
Frank Biro, MD
Iris Gutmark-Little, MD
Changchun Xie, PhD
Agenda

• Background
• Methods
• Objective, Hypothesis and Aims
• Aim 1
• Aim 2
• Aim 3
• Limitations
• Strengths
• Future Directions
Background
Puberty

- The beginning of puberty in girls is considered the beginning of breast development, thelarche.
- There are several pubertal milestones besides thelarche:
  - Pubarche – first appearance of pubic hair
  - Menarche – first menstrual bleeding, occurs about two years after thelarche
- Puberty can last 1-7 years.
- Puberty starts on average between the ages of 8 and 13.
- Puberty alters a girl’s body into one with full sexual capabilities.
- Girls experience changes in their physique, hormones levels, and brain development.
- Wide disparity in age of onset and tempo in girls as well as the pathway of puberty.
Age of Menarche

• Girls are experiencing menarche at an earlier age than in previous generations.¹
  – Girls born prior to 1920 had an average age of menarche of 13.3 years.¹
  – Girls born between 1980 and 1984 reported an average age of menarche of 12.4 years.¹
  – Girls born between 1998 and 1999 reported a median age of menarche of 12.25 years.²
• Risk factors linked to this earlier age of menarche include higher BMI (body mass index),¹ race/ethnicity (African American),³ and endocrine disrupters.⁴

Health Risks due to Early Menarche

• Early menarche has been linked with depression in the later teenage years, earlier sexual activity, eating disorders and substance abuse.

• Poor health outcomes later in life include increased risk of heart disease, higher BMI, increased fasting insulin and other risks of heart disease.

• Early menarche is the most widely known and established risk factor for breast cancer.
  - 9% decrease in risk for every year of delayed menarche in premenopausal women.
  - 4% decrease in risk for every year of delayed menarche in post menopausal women.
  - The cost of treating breast cancer was greater than $16.5 billion in 2010.

Hypothesis and Specific Aims
Previous Look at Individual Girl’s Hormones
Hypothesis

In young girls, relative levels or changes in DHEA-S, estrone, estradiol, and testosterone around the time of thelarche, when considered together as an individual hormone phenotype, are directly related to the age at pubertal milestones (thelarche, pubarche and menarche).
Specific Aims

• **Specific Aim 1** – Describe the serum DHEA-S, estradiol, estrone and testosterone levels in girls measured in 6 month increments from 18 months prior to 6 months after the age of thelarche.

• **Specific Aim 2** – Classify phenotypes of the sex hormones using the hormone levels across the different time windows relative to thelarche.

• **Specific Aim 3** – Use multivariable survival analysis to determine which among the sex hormone profile phenotypes (consisting of DHEA-S, estradiol, estrone and testosterone) are predictive of ages of thelarche, pubarche, and menarche to allow researchers to further understand why some girls experience pubertal milestones at an earlier age than others.
Methods
Five time windows relative to thelarche visit were defined.

-18 months: From -21 to < -15
-12 months: -15 ≤ to < -9
-6 months: -9 ≤ to < -3
3 months: -3 ≤ to < 3
9 months: 3 ≤ to < 9
Included in this analysis (n=269 girls)

Eligibility of Girls
Cincinnati Cohort (n=379)

<table>
<thead>
<tr>
<th></th>
<th>Eligible n</th>
<th>Eligible Girls</th>
<th>Observations</th>
<th>Total Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone 2015 Dataset</td>
<td>253</td>
<td>975</td>
<td>3480</td>
<td></td>
</tr>
<tr>
<td>Hormone 2016 Dataset</td>
<td>205</td>
<td>425</td>
<td>1360</td>
<td></td>
</tr>
</tbody>
</table>

Deletion of Observations

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Remove duplicates for a girl at the same visit</td>
<td>303</td>
<td>1307</td>
<td>4840</td>
<td></td>
</tr>
<tr>
<td>Keep only Hormones in -18 to +6 time period</td>
<td>-4</td>
<td>299</td>
<td>1046</td>
<td>3902</td>
</tr>
<tr>
<td>Keep girls with at least two hormone measurements during -18 to +6</td>
<td>-30</td>
<td>269</td>
<td>1009</td>
<td>3764</td>
</tr>
<tr>
<td>Keep only 1 visit per bucket*</td>
<td>269</td>
<td>935</td>
<td>3493</td>
<td></td>
</tr>
</tbody>
</table>
Sex Hormone Measurements

Serum hormone levels (continuous)

– Samples were frozen at -80°C.
– Analysis performed by Esoterix Labs.
– Estradiol, Estrone and Testosterone were measured by High Performance Liquid Chromatography with tandem mass spectrometry (HPLC-MS) which is very sensitive compared to other methods.
– Radioimmuno assay (RIA) was used to measure DHEAS for one batch and HPLC-MS for a second batch.
– Missing data was due to missing a study visit, refusing to have blood drawn or insufficient amount of blood serum to measure.
– Serum concentrations of these hormones are known to change during puberty.
Sex Hormone Measurements

Serum hormone levels (continuous)

Lower Limit of Quantification (LLOQ) is the value at which the coefficient of variation (standard deviation / mean) <20% (<25% for DHEAS).

- Measure of precision at low analyte levels
- If the measurement was <LLOQ (but not canceled or insufficient), the LLOQ/\sqrt{2} was used.

<table>
<thead>
<tr>
<th>LLOQ</th>
<th>1st Batch n=252</th>
<th>2nd Batch n=51</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHEA-S μg/dL</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Estradiol pg/mL</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Estrone pg/mL</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Testosterone ng/dL</td>
<td>3</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Aim 1
Aim 1

Describe the serum DHEA-S, estradiol (E2), estrone (E1) and testosterone (T) levels in girls measured in 6 month increments from 18 months prior to 6 months after the age of thelarche.
Maturation Information

Average Age of Thelarche – 108.26 months (9.02 years)
Average Age of Pubarche – 118.19 months (9.85 years)
Average Age of Menarche – 147.52 months (12.29 years)
Average tempo – 39 months (3.25 years)
Aim 2
Aim 2

Classify phenotypes of the sex hormones using hormone levels across the different time points relative to thelarche.

Two or more hormone data points used together as a phenotype in this analysis may refer to:

• two or more measurements of a certain hormone in an individual girl at different time points (such as the change in E2 from time=-18 to -12 months relative to thelarche)
• measurements of at least two hormones taken at the same or different time points in time (E2 and DHEA-S at time=-6) relative to thelarche.
All significant correlations are positive.

Lack of correlation of E2 with itself or other hormones, suggesting different longitudinal phenotypes.

DHEA-S is highly correlated with itself across all time periods.

Estrone is highly correlated with itself across all time periods.

Testosterone is highly correlated with itself across all time periods.

In general, DHEA-S, estrone and testosterone are highly correlated with each other across the time periods.

No correlation between DHEA-S and E2.

General lack of correlation between E2 and testosterone.

Correlation between E2 and estrone at corresponding time periods (eg. -18 with -18).
In general, there is a lack of either positive or negative correlations, implying girls with big changes in a hormone between time periods did not experience a big change in another hormone, suggesting different longitudinal phenotypes.

For all correlations between -18 to -12 is highly inversely correlated with next time period of -12 to -6.

For each time period estrone is negatively correlated with the change in the next time period.

No correlation between DHEA-S and estrone or E2, suggesting different longitudinal phenotypes.
Variable Dimension Reduction
Principal Component Analysis (PCA) - Proc Factor

• Variable reduction method

• Shared variance between several variables is explained in fewer unobserved variables (factors)

• Look for the number of factors that explain approximately 70% or more of the variance

• Variables are loaded on the factors by eigenvalues (>0.35). A high absolute value of an eigenvalue indicates a higher correlations with the factor.
Variable Dimension Reduction

PCA

- PCA does not handle missing data

- Looked at only time periods -6, 0 and 6 due to the large amount of missing data at time periods -18 and -12

- 9 girls were lost as all of their hormone measurements were outside of times -6, 0 and +6.

- Need to use Proc MI to handle the missing data as some girls might no have had blood drawn at every visit or the assays were too low to quantify.

- Ran Proc Mi for 30 imputations to ensure quality of the estimated values

- Factor analysis for each of the 30 imputed dataset

- Averaged the factor loadings (eigenvalues) across the 30 factor analysis results
Variable Dimension Reduction

PCA

Sensitivity Analysis - Factor Analysis on a smaller more complete data set to compare the results

- N=260 – all the girls included in the hormone analysis with at least some data at -6, 0 and 6 (30 imputed data sets then averaged the 30 factor analysis results)
- N=67 - all the girls with values for all four hormones at three time periods (-6, thelarche, +6 therefore no missing data)
Variable Dimension Reduction

PCA - Hormone Values at time = -6, 0, 6 – Only 3 of 12 factors shown

Factor loading from principal component analysis of hormones (n = 260)

Factor loadings greater than 35 are flagged by an ‘*’. 
Average of loadings from 30 PCAs of 30 Imputations

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone -6</td>
<td>7°</td>
<td>34°</td>
<td>6°</td>
</tr>
<tr>
<td>Testosterone 0</td>
<td>73°</td>
<td>26°</td>
<td>31°</td>
</tr>
<tr>
<td>Testosterone +6</td>
<td>55°</td>
<td>63°</td>
<td>7°</td>
</tr>
<tr>
<td>DHEA-S -6</td>
<td>87°</td>
<td>9°</td>
<td>4°</td>
</tr>
<tr>
<td>DHEA-S 0</td>
<td>88°</td>
<td>2°</td>
<td>3°</td>
</tr>
<tr>
<td>DHEA-S +6</td>
<td>87°</td>
<td>16°</td>
<td>8°</td>
</tr>
<tr>
<td>Estrone -6</td>
<td>61°</td>
<td>46°</td>
<td>36°</td>
</tr>
<tr>
<td>Estrone 0</td>
<td>60°</td>
<td>35°</td>
<td>60°</td>
</tr>
<tr>
<td>Estrone +6</td>
<td>33°</td>
<td>80°</td>
<td>20°</td>
</tr>
<tr>
<td>Estradiol -6</td>
<td>0°</td>
<td>40°</td>
<td>67°</td>
</tr>
<tr>
<td>Estradiol 0</td>
<td>11°</td>
<td>32°</td>
<td>83°</td>
</tr>
<tr>
<td>Estradiol +6</td>
<td>5°</td>
<td>82°</td>
<td>26°</td>
</tr>
</tbody>
</table>

*Hormones log transformed

Variance Explained by Factor

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.501302</td>
<td>0.1541</td>
<td>0.086107</td>
</tr>
</tbody>
</table>

Cumulative Variance

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.501302</td>
<td>0.655402</td>
<td>0.741509</td>
</tr>
</tbody>
</table>

Sensitivity Analysis

Factor loadings greater than 35 are flagged by an ‘*’. 
n = 67 (Girls who have all hormone values at times = -6, 0, 6)

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone -6</td>
<td>81*</td>
<td>5°</td>
<td>8°</td>
</tr>
<tr>
<td>Testosterone 0</td>
<td>80*</td>
<td>14°</td>
<td>10°</td>
</tr>
<tr>
<td>Testosterone +6</td>
<td>78*</td>
<td>4°</td>
<td>35°</td>
</tr>
<tr>
<td>DHEA-S -6</td>
<td>86*</td>
<td>8°</td>
<td>4°</td>
</tr>
<tr>
<td>DHEA-S 0</td>
<td>87*</td>
<td>12°</td>
<td>7°</td>
</tr>
<tr>
<td>DHEA-S +6</td>
<td>91*</td>
<td>4°</td>
<td>14°</td>
</tr>
<tr>
<td>Estrone -6</td>
<td>79*</td>
<td>33°</td>
<td>12°</td>
</tr>
<tr>
<td>Estrone 0</td>
<td>59°</td>
<td>54°</td>
<td>26°</td>
</tr>
<tr>
<td>Estrone +6</td>
<td>60*</td>
<td>22°</td>
<td>63°</td>
</tr>
<tr>
<td>Estradiol -6</td>
<td>20°</td>
<td>81°</td>
<td>4°</td>
</tr>
<tr>
<td>Estradiol 0</td>
<td>-9°</td>
<td>82°</td>
<td>26°</td>
</tr>
<tr>
<td>Estradiol +6</td>
<td>8°</td>
<td>15°</td>
<td>94°</td>
</tr>
</tbody>
</table>

*Hormones log transformed

All factors had eigenvalues > 1

Variance Explained by Factor

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5314</td>
<td>0.1413</td>
<td>0.0944</td>
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</table>

Cumulative Variance

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5314</td>
<td>0.6727</td>
<td>0.7671</td>
</tr>
</tbody>
</table>
PCA to Cluster (CA)

- PCA did not result in variable reduction of the absolute hormone values.
  - Should use all four absolute hormones measurements at -6, 0 and 6 as objective predictive variables in the cluster analysis as all the variables loaded onto the first three components that explained >74% of the variance.

- Use the subset of girls (n=260) with hormone data between -6 and 6 as the imputed data resulted in factors very similar to the reduced data set in the sensitivity analysis.
  - Factor 1 – same except estrone at 6
  - Factor 3 of N=260 the same as factor 2 of n=67

- Three factors imply 3 possible clusters
Participant Clustering
Proc Fastclus – Hormone Values at time =-6,0,6

• Assigns participants to a cluster where the other participants are more similar to them than participants in another cluster.

• Each participant belongs to only one cluster.

• Very sensitive to outliers and has a method to identify outliers in their own clusters (unlike other cluster procedures)
Cluster 1 (n=42)
Cluster 3 (n=172)

N=260  (results for n=67 produced similar cluster plot)

3 distinct clusters were produced

Canonical variable 1
discriminates between cluster
3 and cluster 2

Canonical variable 2
discriminates between cluster
1 and cluster 2 and 3

Cluster 2 (n=37)

Clusters -1 (n=2) and -2 (n=7)
represent outliers that do not fit
into any cluster

CA - Hormone Values at time =-6,0,6

All Girls included in Cluster Analysis - FASTCLUS

Participant Clustering
Mean hormone values for each cluster

Hormone phenotype objective predictors
Sex hormone serum concentrations of girls according to the three phenotypes identified using principal component analysis-based cluster analysis
DHEA-S (ug/dL), estrone and estradiol (pg/mL), testosterone (ng/dL)

Cluster 1
- Higher DHEA (over 100% higher than the cohort mean at each time period)
- T and E1 values (over 50% higher than the cohort mean)

Cluster 2
- High E2 values across each time period from 30% to 200% higher than the mean of the cohort
- E1 90% higher at +6
- T >50% higher at -6 and +6 vs the cohort mean

Cluster 3
- lower hormone values (approximately >20% lower) across the time period vs the mean of the entire cohort.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>No High Hormone Values</th>
<th>Significance (P value)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cohort</td>
<td>High DHEA, T and E1</td>
<td>High E2, T and E1</td>
<td>n=37</td>
</tr>
<tr>
<td>Testosterone -6 †</td>
<td>N=269</td>
<td>4.48</td>
<td>6.89</td>
<td>7.00</td>
</tr>
<tr>
<td>Testosterone 0 †</td>
<td>N=42</td>
<td>4.96</td>
<td>8.23</td>
<td>5.40</td>
</tr>
<tr>
<td>Testosterone 6 †</td>
<td>N=37</td>
<td>6.16</td>
<td>9.23</td>
<td>10.79</td>
</tr>
<tr>
<td>Estrone -6 †</td>
<td>N=269</td>
<td>4.00</td>
<td>6.18</td>
<td>5.24</td>
</tr>
<tr>
<td>Estrone 0 †</td>
<td>N=42</td>
<td>4.47</td>
<td>6.81</td>
<td>5.17</td>
</tr>
<tr>
<td>Estrone +6 †</td>
<td>N=37</td>
<td>6.09</td>
<td>8.10</td>
<td>11.78</td>
</tr>
<tr>
<td>DHEA -6 †</td>
<td>N=269</td>
<td>28.23</td>
<td>62.97</td>
<td>32.93</td>
</tr>
<tr>
<td>DHEA 0 †</td>
<td>N=42</td>
<td>30.79</td>
<td>68.13</td>
<td>24.91</td>
</tr>
<tr>
<td>DHEA +6 †</td>
<td>N=37</td>
<td>38.32</td>
<td>79.00</td>
<td>39.64</td>
</tr>
<tr>
<td>Estradiol -6</td>
<td>N=269</td>
<td>2.83</td>
<td>2.46</td>
<td>4.60</td>
</tr>
<tr>
<td>Estradiol 0 †</td>
<td>N=42</td>
<td>3.37</td>
<td>3.68</td>
<td>4.46</td>
</tr>
<tr>
<td>Estradiol +6 †</td>
<td>N=37</td>
<td>5.38</td>
<td>5.40</td>
<td>17.25</td>
</tr>
</tbody>
</table>

* Baseline values for the cohort given as mean unless noted. P values represent tests for groupwise differences between the phenotypes; Comparison between phenotypes using Kruskal-Wallis for continuous variables and χ² for categorical.

Considerably higher values than cohort and other phenotypes
3 clusters or not…. 

• Two clusters are very well defined by hormone values.

• One cluster (cluster 3) is a large group that is appears tightly clustered but not well defined except the hormone values are lower than the other clusters and the cohort as a whole. Could it possibly be broken into 2 or more clusters?
PCA on Cluster 3 (n=172) vs the cohort

PCA on the cluster still shows all variables loading and suggests using all 12 as predictive variables in CA

Factor loadings greater than 35 are flagged by an ‘*’. Average of loadings from 30 PCAs of 30 Imputations

Testosterone -6 59* 28 5
Testosterone 0 63* 37* 22
Testosterone +6 48* 64* -3
DHEA-S -6 81* -1 5
DHEA-S 0 83* 7 5
DHEA-S +6 78* 27 5
Estrone -6 44* 40* 43*
Estrone 0 47* 32 57*
Estrone +6 21 80* 21
Estradiol -6 -9 4 65*
Estradiol 0 5 19 70*
Estradiol +6 -9 72* 28

*Hormones log transformed
All factors had eigenvalues >1

Factor loading from principal component analysis restricted to girls in cluster 3 (n=172)

Factor loadings greater than 35 are flagged by an ‘*’. Average of loadings from 30 PCAs of 30 Imputations

Testosterone -6 70* 34 6
Testosterone 0 73* 26 31
Testosterone +6 55* 63* 7
DHEA-S -6 87* 9 4
DHEA-S 0 88* 2 3
DHEA-S +6 87* 16 8
Estrone -6 61* 46* 36
Estrone 0 60* 35 60*
Estrone +6 33 80* 20
Estradiol -6 0 40* 67*
Estradiol 0 11 32 85*
Estradiol +6 5 82* 26

*Hormones log transformed
All factors had eigenvalues >1
CA on Cluster 3 (n=172)

Cluster A n=74
Cluster B n=96
Cluster C n=2

Clusters A and B seem to be a large cluster cut in half.

Canonical Variable 1 discriminates between Cluster A and B

Cluster C is just too small and are the outliers.

Canonical Variable 2 does not discriminate between clusters
Clustering to Phenotype

- Phenotype 1 = Cluster 1, \( n=42 \) (from CA \( n=260 \))
- Phenotype 2 = Cluster 2, \( n=37 \) (from CA \( n=260 \))
- Phenotype 3a = Cluster 3A, \( n=74 \) (from CA on \( n=172 \))
- Phenotype 3b = Cluster 3B, \( n=96 \) (from CA on \( n=172 \))

- Outliers are not included in any clusters or phenotypes.
Further Characteristics of the Phenotypes after being agnostically defined by the hormones

• Statistical difference exists between the age of thelarche and the age of pubarche among the four phenotypes.
• Girls in phenotypes 1 and 3b had an average age of menarche statistically later than girls in phenotype 2.
• The tempo of girls in phenotype 2 was statistically shorter than that for girls in phenotype 3b.
• Girls in phenotype 3b were more likely to enter puberty via pubarche rather than thelarche which is different than the other phenotypes.
• No differences between the phenotypes existed for the following characteristics:
  • BMI
  • ethnicity
  • family history of 1st or 2nd degree breast cancer
  • mother’s age of menarche
  • caregiver’s education
Conclusions

• Classifying hormone heterogeneity prior to puberty is highly informative in unveiling different pathways through puberty.

• These analyses are the first to apply PCA-CA methods to longitudinal sex hormone data of girls going through puberty.

• PCA-CA did not result in variable reduction of the absolute hormone values but did identify four meaningful phenotypes of hormones levels in young girls in relationship to the timing of thelarche.

• The four distinct hormone phenotypes in girls indicate hormones levels relative to the age of thelarche are not the same in all girls and help to explain disparity in the age of onset.

• These findings underscore the need to better understand female sex hormones prior to puberty based on time related to puberty rather than chronological age or pubertal status.
Aim 3
Aim 3

Use multivariable survival analysis to determine which among the sex hormone profile phenotypes (consisting of DHEA-S, estradiol, estrone and testosterone) are predictive of age of thelarche, pubarche, and menarche to allow researchers to further understand why some girls experience pubertal milestones at an earlier age than others.
Survival Analysis

Cox proportional-hazard models (probability or likelihood of the event of interest) were conducted in SAS using PHReg and controlling for covariates.
- do not require a certain probability function
- may include multiple covariates
- may include continuous and/or categorical covariates
- allows for the inclusion of girls who are right censored
- allows for interactions between covariates

Assumption of proportional hazards – additive changes in a variable cause corresponding multiplicative changes in the hazard function e.g. ratio of hazards for two individuals over time is constant

Girls who have not yet reached the pubertal milestone during the study or were lost to follow up prior to achieving it will be right censored.
Survival Analysis (unadjusted)

Thelarche

Product-Limit Survival Estimates

Logrank p=0.0008

Cluster:
- all low hormones
- high DHEA-S plus E1 and T
- high E2 plus E1 and T
- no high hormones
Survival Analysis (unadjusted)

Pubarche

Menarche

Logrank p = 0.0333

Logrank p = 0.1975
Risk Estimates by Phenotypes

**Thelarche**
- no high hormones (3a) vs all low hormones (3b)
- high estradiol (2) vs all low hormones (3b)
- high estradiol (2) vs no high hormones (3a)
- DHEA-S (1) vs all low hormones (3b)
- DHEA-S (1) vs no high hormones (3a)
- DHEA-S (1) vs high estradiol (2)

**Pubarche**
- no high hormones (3a) vs all low hormones (3b)
- high estradiol (2) vs all low hormones (3b)
- high estradiol (2) vs no high hormones (3a)
- DHEA-S (1) vs all low hormones (3b)
- DHEA-S (1) vs no high hormones (3a)
- DHEA-S (1) vs high estradiol (2)

**Menarche**
- no high hormones (3a) vs all low hormones (3b)
- high estradiol (2) vs all low hormones (3b)
- high estradiol (2) vs no high hormones (3a)
- DHEA-S (1) vs all low hormones (3b)
- DHEA-S (1) vs no high hormones (3a)
- DHEA-S (1) vs high estradiol (2)

Conclusions

• Risk of earlier age of thelarche, pubarche and menarche differed by phenotypes, confirming heterogeneity of hormone phenotypes.

• Girls with mother’s ages of menarche younger than 12 years old had a 50% increased risk of reaching all three milestones earlier than girls with mother’s ages at least 14. This possibly indicates a genetic influence.
Summary
Participant Clustering
CA - Hormone Values at time = -6,0,6

CA on all girls (n=290)

Cluster 1 (n=42)
Cluster 2 (n=37)
Cluster 3 (n=172)

CA on Cluster 2 (n=172)

Large Cluster broken down - FASTCLUS

Cluster B n=96
Cluster A n=74

Cluster A  Cluster B
CA on Cluster 3 (n=172)

Cluster A
n=74

Cluster B
n=96
No statistically significant difference in race by phenotype.
No statistically significant difference in education by phenotype.

Phenotype 1
- High school degree or less: 24.43%
- Associate's or bachelor's degree: 40.48%

Phenotype 2
- High school degree or less: 29.73%
- Associate's or bachelor's degree: 16.22%

Phenotype 3a
- High school degree or less: 28.38%
- Associate's or bachelor's degree: 27.03%

Phenotype 3b
- High school degree or less: 27.08%
- Associate's or bachelor's degree: 29.17%
Phenotype 1

• Oldest age at thelarche
• Oldest age at menarche
• Less likely to enter thelarche early compared to 3a or 3b

![Graph showing hormone levels for Phenotype 1]
Phenotype 2

- Shortest tempo
- Youngest age at menarche
- 50% greater risk of earlier menarche than other phenotypes
- More likely to enter thelarche later than 3b

Hippocket??
Phenotype 3a

- Youngest age at pubarche
Phenotype 3b

- Youngest age at thelarche
- Second to oldest age at menarche
- Longest tempo
- Oldest age at pubarche