Testicular size development and reproductive hormones in boys and adult males with

Noonan syndrome: a longitudinal study

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Abstract

Objective: To characterize changes in testicular size and reproductive hormones, in order to investigate the aetiology of delayed puberty and impaired fertility in males with Noonan syndrome (NS).

Design: Twelve males with NS were followed longitudinally from pre/ early puberty until adulthood. Ten had no medical history other than NS and were divided into two groups; undescended testes (UT) and descended testes (DT) and compared with a reference population.

Methods: Hormone concentrations in serum were determined by immunoassays and testicular volume was measured using an orchidometer.

Results: Before puberty, reproductive hormone levels were within the expected range in almost all cases. In some cases, luteinizing hormone (LH), stimulating hormone (FSH), testosterone and estradiol concentrations started to increase during puberty and Inhibin B and anti-Müllerian hormone (AMH) declined to subnormal levels. Most of the boys studied had small testes, which, in the majority of cases, progressed to normal size in adulthood. No difference in reproductive hormones was observed between the groups UT and DT either during puberty or at adulthood. However, as adults, males with NS had higher LH (5.7 vs 4.0 U/L, p<0.01), FSH (7.1 vs 2.5 U/L, p<0.001), testosterone (18.7 vs 15.6 nmol/L, p<0.01), and estradiol (66 vs 46 pmol/L, p<0.001) levels, and lower AMH (33 vs 65 pmol/L, p<0.01) and inhibin B (median 108 vs 187 pg/mL, p<0.01) levels than the reference population.

Conclusions: In NS males, both Sertoli and Leydig cell dysfunction is common with reproductive hormone levels deteriorating progressively to adulthood.
Introduction

Noonan syndrome (NS) is an autosomal dominant disorder characterised by a phenotype including short stature, facial dysmorphology and congenital heart defects. In approximately 50% of cases, NS is due to mutations in the PTPN11 gene. In both sexes there is a delay in pubertal development. Fertility appears to be normal in females, but has been reported to be decreased in males, although male transmission of the disorder to the next generation is not uncommon. One possible cause of impaired male fertility is undescended testes (UT), reported in 60–77% of boys with NS. Undescended testes are associated with impaired fertility. A small numbers of males with NS have had assessment of gonadal function in prepubertal period as well as adulthood.

Theintz and Savage reported an elevated luteinizing hormone (LH) response in five prepubertal boys with NS who were all surgically treated for maldescended testes. Sinisi et al. and Elsawi et al. studied four and 11 males with NS, respectively. Half of the males in these studies had been surgically treated for bilateral UT during childhood. As adults, those with a history of bilateral UT had azoospermia, elevated follicle-stimulating hormone (FSH) levels and normal or elevated LH levels, whereas all of the men with descended testes (DT) had normal hormone levels. Unexpectedly, Marcus et al. studied nine males and found raised FSH and subnormal inhibin B concentrations in all three men with DT, indicating a primary gonadal dysfunction in males with NS.

The male hypothalamo–pituitary–gonadal axis is active from fetal life and regulates both the onset of puberty and spermatogenesis. The testosterone loop is also established in fetal life, and there is an important rise in gonadotropins and testosterone during early infancy. FSH is necessary for the initial establishment of mature germinal epithelium and also the initiation of
spermatogenesis early in puberty through Sertoli cell proliferation. Sertoli cell maturation is accompanied by increasing inhibin B and testosterone levels, but declining Anti-Müllerian hormone (AMH) levels. From fetal life until puberty testes produce high levels of AMH and secretion is induced by FSH but inhibited by testosterone. Testosterone also stimulates the early FSH-independent increase in inhibin B that precedes puberty. Moreover, inhibin B is germ-cell-independent before puberty and germ-cell-dependent after puberty. Therefore, inhibin B is commonly seen as a sensitive marker of germ cell damage in adults, whereas AMH reflects Sertoli cell function and testicular androgen action (reviewed in 14). In addition, AMH and inhibin B are clinically used to assess the presence and function of Sertoli cells during childhood.

The association between indices of fertility and concentrations of gonadotropins, inhibin B and sex steroids is well described in healthy men and males with bilateral UT. Together, these studies and the studies in males with NS, suggest that UT could be the principal but not the sole cause of impaired fertility. The question is to what extent abnormal gonadal function in males with NS is simply a consequence of UT or reflects a more fundamental hormonal dysfunction or testes dysgenesis. To investigate this, the present study followed testicular volume and hormonal status longitudinally from the start of puberty through to adulthood.

**Study subjects and methods**

**Males with NS**

The group studied comprised 12 males diagnosed with NS. All were investigated at the Queen Silvia Children’s Hospital in Göteborg, Sweden. Diagnoses were made by a single clinician (OW) based on the following major criteria: short stature, hypertelorism, low set ears, and low
set hairline, and at least one minor criterion: malformation of the thorax, ptosis, cubitus valgus, webbed neck, pulmonary stenosis and cryptorchidism. Subjects had normal thyroid function, normal karyotype, no major heart disease and were well nourished. Kidney and liver functions were normal. All subjects were treated with growth hormone (GH) due to short stature (growth data previously reported). None of the subjects were GH deficient.

The study was approved by the Ethics Committee of the Faculty of Medicine at the Sahlgrenska Academy, University of Gothenburg, and carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from the subjects and their parents.

**Pubertal classification**

Stage of puberty was evaluated every 6–12 months until adulthood. During each assessment, testicular volumes were determined using an orchidometer. Pubertal stage was classified according to the largest of the testes or testis *in situ* using the following stages: pre (testis 1–2 mL), early (testis 3–6 mL), mid (testis 8–12 mL) and late (15–25 mL) puberty as defined previously. According to this classification, ten boys were prepubertal and two (subjects 1 and 7) were in early puberty at the start of the investigations. Puberty was defined as delayed if a testicular volume of >3 mL was not reached by 14 years of age. Normal adult testicular volume was defined as ≥15 mL.

**Medical history**

Four of the 12 boys with NS were treated surgically for bilateral UT and one boy for unilateral UT (age range, 0.2–12.3 years; for individual ages see Table 1). One other boy was initially found to have DT and only later diagnosed with bilateral UT and therefore surgery was performed at 14.5 years of age. One boy was found to have central hypogonadism (not
common in subjects with NS) and he was the only subject who received hormone replacement therapy to induce puberty. Another male had juvenile arthritis and was treated during puberty with methotrexate (MTX), which has a toxic effect on the gonads (subject 6). Both are included in Table 1 but not in further analyses. No other subject had an unusual medical history. Data on individual ages at first and last visit, testicular volumes and surgery are presented in Table 1. The males were divided into two groups; UT and DT.

Methods
Blood samples were drawn between 8 am and 2 pm. After separation, sera were stored at −70 °C until assayed. All samples for hormone determination were assessed in duplicate at an accredited laboratory (SS-EN ISO 15189).
Serum estradiol concentrations were determined using an assay which involves a diethyl ether extraction step prior to quantification by a modified RIA (Spectria® Estradiol RIA, Orion Diagnostica; Espoo, Finland) as previously described \(^{21}\). The lower limit of detection for the extraction RIA was 4 pmol/L. The intra-assay coefficient of variation (CV) was 10–17%, while the interassay CV was 19% for 6 pmol/L and 8% for 70 pmol/L.
Serum testosterone concentrations were determined using a modified RIA (Spectria® Testosterone; Orion Diagnostica) as previously described \(^{22}\). The lower limit of detection was 0.03 nmol/L. The intra-assay and interassay CVs were 11% and 15%, respectively, for concentrations of 0.2 nmol/L and below, and 7% for concentrations above 0.9 nmol/L.
Serum anti-Müllerian hormone (AMH) concentrations were determined by AMH Gen II ELISA (Beckman coulter, Inc., Marseille). The lower limit of detection was 0.6 pmol/L. The intra-assay CV was less than 7% and the interassay CV was less than 10% within the range 25-550 pmol/L.
Serum inhibin B concentrations were determined by Inhibin B Gen II ELISA (Beckman Coulter, Inc., Webster). The lower limit of detection was 2.6 pg/mL. The intra-assay CV was less than 6% within the range 70–430 pg/mL. The interassay CV was 8% for 80 pg/mL and less than 6% for concentrations above 100 pg/mL.

Serum LH and FSH concentrations were determined using a time-resolved fluoroimmunoassay (AutoDELFIA; Wallac, Turku, Finland). The detection limits were 0.05 U/L for both. The intra-assay CV was less than 3%. The interassay CV was less than 7%.

Genetic screening of PTPN11 exons 2–4, 7–8 and 12–13 was performed in all subjects, as described by Tartaglia et al. 1. PCR fragments were either sequenced directly or screened for heterozygous positions using denaturing high-performance liquid chromatography, followed by sequencing of all fragments.

**Normal reference values**

Testicular size during puberty was compared to reference values according to Zachmann et al. 23. Gonadotropin and sex-steroid levels in males with NS prior to and during pubertal development were compared with previously published data on hormone levels in healthy subjects (age range, 5.0–18.6 years) 19,24,25. The adult control group consisted of 44 healthy males (age range, 20.1–24.8 years) and has previously been described 26. All controls’ hormonal values were determined by the same assays at GP-GRC laboratory as the NS group. Normal values for AMH and inhibin B levels during puberty were not available at the GP-GRC laboratory.

**Statistical analysis**

Individual data are presented in the figures, allowing the reader to track every individual subject. Tables 2 and 3 present median values and range for each group and pubertal stage.
Mixed between-within ANOVA (SPSS statistics version 17.0) was used for comparison of hormone concentrations between groups during pubertal development (repeated measures). Due to two missing values, prepubertal levels were excluded in that analysis. The Mann–Whitney test was used for comparison of hormone concentrations between groups at adulthood. A p-value of <0.05 was considered significant.

Results

Start of puberty and testicular size in NS

Progression of testicular size is presented in Fig. 1. According to the classical classification by Marshall and Tanner\(^{20}\), puberty was delayed in four of the 10 males with NS (subjects 3, 4, 7 and 8). All except two of the males with NS had testicular volumes below the median value for controls of the same age during pubertal development. Interestingly, testicular size had diminished after full pubertal maturation in three males (subjects 3, 10 and 11). Nevertheless, only two males with NS had subnormal testicular volumes as adults (Fig 1, Table 1).

Pituitary function prior to and during pubertal development

Individual serum concentrations of LH and FSH are shown in Fig. 2. LH and FSH levels did not differ from those of healthy boys prior to puberty. During late puberty, two males with UT had elevated LH levels; LH levels had already started to increase in early/mid puberty. All subjects in the DT group had normal LH levels. Three out of our five subjects in the UT group had increased FSH levels from late puberty. Elevated FSH levels were also found from mid-puberty in two subjects with DT both had diminished testicular size when assessed as adults.

There were no differences in serum LH or FSH levels during puberty, when males with NS who had UT were compared to those who had DT (Table 2).
Gonadal function prior to and during pubertal development

Individual serum testosterone, estradiol, AMH and inhibin B concentrations in males with NS in relation to pubertal development are shown in Fig. 3, 4 and 5. Before puberty, testosterone, estradiol, AMH and inhibin B levels were within the expected range in almost all cases. Testosterone concentrations were elevated in one subject during early to mid-puberty and in another one prior to and during early puberty. All boys had testosterone concentrations within the normal range during late puberty. Estradiol concentrations were elevated during early, mid- or late puberty for five of the 10 males with NS. In half of the cases (both UT and DT), AMH and inhibin B levels declined to subnormal levels in advanced pubertal stages. The lowest inhibin B levels were found in the four subjects treated for bilateral UT and in the two males with DT, but diminished testes (subject no 10 and 11).

There were no differences in serum testosterone, estradiol, AMH and inhibin B levels during puberty when males with NS with UT were compared to those who had DT (Table 2).

Hormone levels in NS adults

NS compared with controls

Both males with NS as a group and the subgroup with UT had higher serum levels of LH, FSH, testosterone, estradiol, and lower levels of AMH inhibin B than controls (Table 3). Adult males with DT also had higher serum levels of FSH and estradiol and lower levels of AMH and inhibin B than controls, but serum levels of LH and testosterone were normal (Table 3). Three NS males (1 UT and 2 DT) had all reproductive hormones within normal range.

UT group compared with DT group
In adulthood, there were no differences in serum LH, FSH, testosterone, estradiol, AMH and inhibin B levels between males with NS who had UT and those who had DT (Table 3).

**Genetic screening**

Five of the 12 boys were found to have a mutation in the *PTPN11* gene (Table 1). No other genes were tested. There was no association between reproductive hormones and PTPN11 gene mutation (data not shown).
Discussion

This longitudinal study is the first to describe the pituitary–gonadal endocrine changes during puberty in males with NS. Half of the group studied had UT and half had delayed puberty. Before puberty, reproductive hormone levels were within the expected range \(^{11, 12, 19, 24, 25}\).

During puberty, most of the boys studied had small testes, which, in the majority of cases, progressed to normal size at adulthood. No difference in hormone levels was observed between the UT and DT groups, either during puberty or in adulthood. However, as adults, males with NS had higher LH, FSH, testosterone, estradiol and lower AMH and inhibit B levels than controls. Overall, this study indicates Sertoli cell dysfunction in combination with impaired Leydig cell function in both NS groups, suggesting that UT is not the main contributing factor for impaired testicular function in males with NS.

The first overt sign of puberty in healthy boys is an increase in testicular volume, with Sertoli cells accounting for 93–95% of the seminiferous tubule cell mass, and germ cells accounting for 5–7\% \(^{27}\). During the months preceding this first pubertal sign, the gonadotropins have influenced the testes to develop and increase in size. This in turn results in increased testosterone production from the testes. The association between testicular volume and testosterone concentrations is strong during pubertal development \(^{19}\). At the end of puberty, germ cell mass accounts for more than 90% of seminiferous tubule volume and Sertoli cells account for less than 10%.

In this study, elevated testosterone and estradiol levels were found during pubertal development when testicular size was taken into account. Our findings indicate that these males do not have delayed gonadarche, but they do have reduced testicular volume. This is presumably due to impaired Sertoli cell function because their testicular volume is normal at adulthood. We believe that a deficit in Sertoli cell numbers; indicated in this study by elevated FSH and subnormal AMH and inhibit B levels; masks appropriate
pubertal development. Thus, based on our results, it is preferable to assess pubertal maturation in boys with NS by sex-steroid levels relative to appropriate paediatric reference intervals rather than by measurement of testicular size.

Several previous studies have indicated a strong association between subnormal inhibin B concentrations, elevated FSH concentrations and reduced fertility in otherwise healthy men\textsuperscript{13, 15}. It has also been reported that 40–50% of men who have formerly had bilateral UT were infertile, showing subnormal sperm counts and inhibin B levels, as well as elevated FSH levels but normal LH and testosterone levels\textsuperscript{7, 16}. Our results showed subnormal levels of inhibin B, elevated levels of LH and FSH but normal/high levels of testosterone and estradiol in adult men with a history of UT, despite being surgically treated and having at least one testis with a normal volume. According to their hormonal status they are anticipated to be infertile. The reason for this could be the surgery itself; however, UT is a frequent occurrence in NS (60–77%)\textsuperscript{3, 5} and we postulate that UT may be a secondary manifestation of primary gonadal dysfunction. Our speculation is strengthened by the findings of elevated LH and high-normal testosterone levels, not consistent with bilateral UT alone\textsuperscript{7, 16}, indicating a Leydig cell hyperplasia.

NS is not a homogenous entity concerning genotype nor phenotype, although all mutations are within the RAS/MAPK pathway. Thus, our study may demonstrate a mixture of results from different circumstances. In favour of dysgenetic testes hypothesis, elevated estradiol, LH and FSH levels and subnormal AMH and inhibin B levels have been found previously in Klinefelter syndrome\textsuperscript{28, 29}. Similar to Klinefelter syndrome, NS males may have late-onset testicular dysgenesis. Before puberty, hormone levels were normal, but the declining AMH and inhibin B concentrations to subnormal levels in adults indicate that Sertoli- and/ or germ-
cell function deteriorates progressively resulting in impaired spermatogenesis. Deterioration of Sertoli- and germ- cell function may be an explanation for the “shrinking” testes in three of our patients and an early sign of senescence. In order to validate our results, further studies involving testicular biopsies are needed.

None of the subjects in the present study were GH deficient. It has been speculated that GH-treatment may influence the initiation or tempo of pubertal development in non-GHD children. Our unpublished data in short otherwise healthy children reveal that GH treatment did not influence age of puberty onset and did not accelerate pubertal timing. However, in boys, GH treatment seemed to increase testicular volume. Thus, we presume that GH treatment does not change the conclusions of our current study.

Few data concerning correlations between gene mutation and gonadal function have been reported in subjects with NS. Some genotype–phenotype associations have been reported for other manifestations, including heart failure, cardiomyopathy, short stature, ectodermal features, and haematological abnormalities, but there are no known associations between genes and pubertal development, UT or fertility. In our study, about 50% of the subjects have the PTPN11 mutation, which is as expected in the NS population, but we did not find any correlations between this genotype and gonadal function. The lack of an association between gonadal function and the PTPN11 mutation was also shown in a previous study in nine males.

The main strength of the present study is the carefully longitudinally record, with few missing observations, made of clinical findings and associated laboratory results in males with NS from childhood through to adulthood. The weakness of the study is the few numbers of
observations and that may be the reason for lack of significances between the UT and DT groups. However, although hormonal abnormities are observed more frequent in those patients with UT, we still find some abnormal results in the DT group. This is in accordance with previous findings 10.

In conclusion, our data indicate that the declared delayed puberty, UT or infertility in males with NS is the result of a deficit in Sertoli and Leydig cell function. We suggest that the deficit in Sertoli cell numbers in NS males masks otherwise appropriate pubertal development. Moreover, NS males may have the same pattern of progressive deterioration in Sertoli cell function seen in other syndromes with late-onset testicular dysgenesis.

**Disclosure**

*Conflict of interest*

Novo Nordisk gave an unrestricted grant to perform the GH treatment study in the NS subjects as well as the analysis of genes. Jovanna Dahlgren received consulting fees for lectures and book chapters by Novo Nordisk, Pfizer, IPSEN and Merck Serono. Carina Ankarberg-Lindgren received consulting fees for lectures by Novo Nordisk.

*Funding*

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References


19. Ankarberg-Lindgren C & Norjavaara E. Changes of diurnal rhythm and levels of total and free testosterone secretion from pre to late puberty in boys: testis size of 3 ml is a transition stage to puberty. *Eur J Endocrinol* 2004 151 747-757.
Table 1. Clinical, genital and gene data in 12 males with Noonan Syndrome.

<table>
<thead>
<tr>
<th>Subject no</th>
<th>Age at first sampling (yrs)</th>
<th>Age at last sampling (yrs)</th>
<th>Largest testis at adult age (mL)</th>
<th>Smallest testis at adult age (mL)</th>
<th>Medical history</th>
<th>Age at surgery for UT (yrs)</th>
<th>Mutation PTPN11 gene</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>12,4</td>
<td>26,7</td>
<td>15</td>
<td>Missing</td>
<td>Surgically treated for bilateral UT</td>
<td>&lt;6</td>
<td>-</td>
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<tr>
<td>2</td>
<td>9,6</td>
<td>21,6</td>
<td>20</td>
<td>Missing</td>
<td>Surgically treated for bilateral UT</td>
<td>12,0</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>10,9</td>
<td>25,7</td>
<td>8</td>
<td>Not measurable due to hydrocele</td>
<td>Surgically treated for bilateral UT. Testis volume 20 mL at 17,0 yrs</td>
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<td>-</td>
</tr>
<tr>
<td>4</td>
<td>12,1</td>
<td>21,4</td>
<td>20</td>
<td>20</td>
<td>Initially, testes seemed normal. Surgically treated for bilateral UT</td>
<td>14,5</td>
<td>+</td>
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<tr>
<td>5</td>
<td>13,6</td>
<td>24,5</td>
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<td>1</td>
<td>Central</td>
<td>5,2</td>
<td>-</td>
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<td>hypogonadism, started with HRT at 16.6 yrs. Surgically treated for bilateral UT</td>
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<tr>
<td>6</td>
<td>13.0</td>
<td>23.6</td>
<td>15</td>
<td>12 Juvenile arthritis, treated with methotrexate</td>
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<td>7</td>
<td>14.2</td>
<td>28.9</td>
<td>20</td>
<td>15 Surgically treated for unilateral UT, soft testes</td>
<td>12.3 +</td>
<td></td>
<td></td>
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<td>8</td>
<td>13.8</td>
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<td>10.9</td>
<td>19.4</td>
<td>15</td>
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<tr>
<td>11</td>
<td>10.1</td>
<td>17.0</td>
<td>12</td>
<td>8 Testes volume 15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>mL at 13.1-14.3 yrs.</td>
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</tr>
<tr>
<td>12</td>
<td>10,1</td>
<td>18,2</td>
<td>20</td>
<td>20</td>
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<td>+</td>
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</tr>
</tbody>
</table>

UT= undescended testes
Table 2. Ages and reproductive hormone concentrations during pubertal development expressed as medians (ranges) in males with NS (5 with undescended testes (UT) and 5 with descended testes (DT)). Puberty was classified according to the largest of the testes or testis in situ.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre puberty</th>
<th>Early pub</th>
<th>Mid-puberty</th>
<th>Late puberty</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>UT</td>
<td>10.9 (9.6-12.1)</td>
<td>13.9 (11.6-14.2)</td>
<td>14.9 (13.6-16.2)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>10.7 (10.1-13.8)</td>
<td>12.8 (11.1-14.8)</td>
<td>14.8 (12.1-16.8)</td>
</tr>
<tr>
<td><strong>LH (U/L)</strong></td>
<td>UT</td>
<td>0.16 (0.10-0.3)</td>
<td>0.40 (&lt;0.05-3.7)</td>
<td>3.1 (0.93-5.0)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>0.10 (0.07-0.8)</td>
<td>1.2 (0.08-1.8)</td>
<td>1.7 (1.6-2.8)</td>
</tr>
<tr>
<td><strong>FSH (U/L)</strong></td>
<td>UT</td>
<td>1.9 (1.2-2.1)</td>
<td>2.2 (1.3-12.2)</td>
<td>5.6 (2.1-16.0)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>1.5 (0.8-1.8)</td>
<td>2.2 (1.2-4.7)</td>
<td>3.3 (1.9-9.0)</td>
</tr>
<tr>
<td><strong>Testosterone (nmol/L)</strong></td>
<td>UT</td>
<td>0.2 (0.2-0.3)</td>
<td>0.5 (0.2-12.4)</td>
<td>11.4 (4.3-17.2)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>0.3 (0.1-0.8)</td>
<td>1.4 (0.3-4.6)</td>
<td>7.1 (3.5-9.7)</td>
</tr>
<tr>
<td><strong>Estradiol (pmol/L)</strong></td>
<td>UT</td>
<td>6 (6-21)</td>
<td>9 (6-21)</td>
<td>21 (14-40)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>7 (6-12)</td>
<td>13 (6-16)</td>
<td>20 (13-33)</td>
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<tr>
<td><strong>AMH (pmol/L)</strong></td>
<td>UT</td>
<td>364 (161-793)</td>
<td>344 (33-864)</td>
<td>41 (26-118)</td>
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<tr>
<td></td>
<td>DT</td>
<td>548 (428-1035)</td>
<td>139 (90-940)</td>
<td>42 (20-74)</td>
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<tr>
<td><strong>Inhibin B (pg/mL)</strong></td>
<td>UT</td>
<td>54 (30-78)</td>
<td>116 (53-134)</td>
<td>93 (41-198)</td>
</tr>
<tr>
<td></td>
<td>DT</td>
<td>83 (49-164)</td>
<td>174 (33-186)</td>
<td>163 (54-188)</td>
</tr>
</tbody>
</table>
Table 3. Serum hormone concentrations subdivided by groups in 44 healthy controls and 10 adult males with Noonan syndrome.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Noonan males</th>
<th>Noonan Undescended testes group</th>
<th>Noonan Descended testes group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>4.0 (1.5-6.9)</td>
<td>5.7 (3.5-11.8)**</td>
<td>10.2 (4.0-11.8)**</td>
<td>5.2 (3.5-6.7)</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>2.5 (0.7-7.2)</td>
<td>7.1 (3.0-32.2)** ***</td>
<td>19.7 (3.0-32.2)** ***</td>
<td>5.9 (3.8-15.2)** **</td>
</tr>
<tr>
<td>Testosteron (nmol/L)</td>
<td>15.6 (6.5-27.3)</td>
<td>18.7 (13.2-25.3)**</td>
<td>18.7 (14.7-22.6)*</td>
<td>18.4 (13.2-25.3)</td>
</tr>
<tr>
<td>E2 (pmol/L)</td>
<td>46 (22-106)</td>
<td>66 (52-115)** ***</td>
<td>63 (52-115)** **</td>
<td>69 (54-95)*</td>
</tr>
<tr>
<td>AMH (pmol/L)</td>
<td>65 (26-206)</td>
<td>33 (15-63) **</td>
<td>37 (15-44) ** **</td>
<td>29 (25-63) *</td>
</tr>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>187 (67-295)</td>
<td>108 (28-251)**</td>
<td>68 (28-251)*</td>
<td>130 (101-203)*</td>
</tr>
</tbody>
</table>

Data are shown as median with range in parenthesis. *p<0.05, **p<0.01, ***p<0.001 compared to the control group.
Figure 4.