Noonan syndrome males display Sertoli cell-specific primary testicular insufficiency

Sophie Moniez¹, Catherine Pienkowski¹, Benoit Lepage², Safouane Hamdi^{3,4}, Myriam Daudin⁴, Isabelle Oliver¹, Béatrice Jouret¹, Audrey Cartault¹, Gwenaëlle Diene¹, Alain Verloes⁵, Hélène Cavé⁵, Jean-Pierre Salles^{1,6}, Maïthé Tauber^{1,6}, Armelle Yart⁷ and Thomas Edouard^{1,6}

¹Endocrine, Bone Diseases, and Genetics Unit, Children's Hospital, ²Department of Epidemiology, ³Laboratory of Biochemistry and Hormonology, ⁴Fertility Centre, CECOS Midi-Pyrénées, EA 3694 Human Fertility Research Group, Toulouse University Hospital, Toulouse, France, ⁵Department of Genetics, Robert-Debré University Hospital, APHP, Paris, France, ⁶INSERM UMR 1043, Centre of Pathophysiology of Toulouse Purpan (CPTP), and ⁷INSERM UMR 1048, Institute of Cardiovascular and Metabolic Diseases (I2MC), University of Toulouse Paul Sabatier, Toulouse, France

Correspondence should be addressed to T Edouard
Email: edouard.t@chu-toulouse.fr

Abstract

Context: Abnormalities in the hypothalamo–pituitary–gonadal axis have long been reported in Noonan syndrome (NS) males with only few data available in prepubertal children.

Objective: The aim of this study was to describe the gonadal function of NS males from childhood to adulthood.

Design: It is a retrospective chart review.

Patients and methods: A total of 37 males with a genetically confirmed diagnosis of NS were included. Clinical and genetic features, as well as serum hormone levels (LH, FSH, testosterone, anti-Müllerian hormone (AMH), and inhibin B) were analysed.

Results: Of the 37 patients, 16 (43%) children had entered puberty at a median age of 13.5 years (range: 11.4–15.0 years); age at pubertal onset was negatively correlated with BMI SDS ($r = -0.541$; $P = 0.022$). In pubertal boys, testosterone levels were normal suggesting a normal Leydig cell function. In contrast, NS patients had significant lower levels of AMH (mean SDS: -0.6 ± 1.1 ; $P = 0.003$) and inhibin B (mean SDS: -1.1 ± 1.2 ; $P < 0.001$) compared with the general population, suggesting a Sertoli cell dysfunction. Lower AMH and inhibin B levels were found in *NS-PTPN11* patients, whereas these markers did not differ from healthy children in *SOS1* patients. No difference was found between cryptorchid and non-cryptorchid patients for AMH and inhibin B levels ($P = 0.43$ and 0.62 respectively). Four *NS-PTPN11* patients had a severe primary hypogonadism with azoospermia/cryptozoospermia.

Conclusions: NS males display Sertoli cell-specific primary testicular insufficiency, whereas Leydig cell function seems to be unaffected.

European Journal of
Endocrinology
(2018) 179, 409–418

Introduction

Noonan syndrome (NS; Mendelian inheritance in man (MIM) #163950) is a common autosomal dominant genetic disorder characterized by the association of craniofacial abnormalities, congenital heart defects (i.e. pulmonary valve stenosis and hypertrophic cardiomyopathy), short stature and skeletal abnormalities (i.e. pectus, scoliosis), variable developmental delay/learning disability and predisposition to myeloproliferative disorders (1, 2, 3).

NS is caused by germline mutations in genes encoding components or regulators of the RAS/extracellular signal-regulated kinases (ERK) signalling pathway. This pathway plays key roles in development and homeostasis by regulating various cellular processes including cell survival, proliferation, differentiation, migration and adhesion (3, 4). To date, nearly 20 genes have been found to be implicated in NS, but the classical form is mostly

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	410
----------------	---------------------	---	-------	-----

associated with heterozygous missense mutations in four genes: about 50% of NS patients have a mutation in the *protein tyrosine phosphatase non-receptor type 11* (*PTPN11*) gene, along with less common mutations notably in *SOS1*, *RAF1* and *KRAS* genes, accounting for 10, 10, and <2%, respectively (3). Genetic screening remains negative in about 30% of NS patients. A specific cluster of mutations in *PTPN11* gene are also found in about 90% of patients with NS with multiple lentigines (NSML; MIM # 151100). This syndrome previously referred to as LEOPARD syndrome (acronym for multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth, sensorineural Deafness) is closely related to NS and might be difficult to differentiate in early life. The *PTPN11* gene encodes the Src-homology 2 domain-containing tyrosine phosphatase 2 (SHP2), a widely expressed non-receptor protein tyrosine phosphatase that positively regulates the RAS/ERK signalling pathway. From a biochemical point of view, NS-causing mutants induce hyperactivation of the phosphatase (gain-of-function mutation), leading to an enhancement of RAS/ERK activation which is responsible for the different features of NS, notably craniofacial and heart defects, as well as growth retardation (review in (5, 6)).

Abnormalities in the hypothalamic–pituitary–gonadal axis have long been reported in NS patients. In both sexes, the onset of puberty is usually delayed and is associated with a decreased pubertal growth spurt (7, 8, 9). Although fertility does not seem to be affected in NS females, gonadal dysfunction with deficient spermatogenesis has been described in NS males (10, 11); this may explain the sex ratio distortion/predominant transmission by the mother in familial cases. It has been suggested that impaired fertility may be related to cryptorchidism, which is reported in up to 80% of NS males (8). However, Sertoli cell dysfunction has also been reported in NS male with normal testicular descent that could suggest an intrinsic defect of these cells (12, 13). This hypothesis is strengthened by the critical role of *PTPN11* in the regulation of spermatogenesis and subsequently male fertility (review in (14)). Thus, *PTPN11* is expressed in immature germ cells and Sertoli cells, through all developmental stages from birth to adulthood (15). Global conditional or germ cell-specific deletion of *Ptpn11* in mice cause infertility as a result of altered blood–testis barrier (BTB) or impaired maintenance of spermatogonial stem cells (SSC), which are the precursors for germ cells (16, 17). Interestingly, Sertoli cells either lacking SHP2 or expressing a constitutively active mutant

of SHP2 do not support SSCs renewal and differentiation, suggesting that fine-tuned levels of SHP2 activity in Sertoli cells are required for proper spermatogenesis (15, 17). Finally, SHP2 regulates steroidogenesis in Leydig cells. Indeed, treatment of primary Leydig cell cultures with SHP2 inhibitor or shRNA-mediated *Ptpn11* knockdown decreased testosterone production, whereas SHP2 overexpression increased steroid production (18).

Most studies in NS have focused on pubertal and adult gonadal function with only few data available in prepubertal children. To our knowledge, only one study has investigated markers of testicular function, such as anti-Müllerian hormone (AMH) and inhibin B, in NS prepubertal boys (12). Moreover, the better knowledge of the genetic bases of NS made possible to look for genotype–phenotype correlations, notably with cardiac, growth and haematological defects (1, 19), but these correlations have not been investigated for gonadal function. In the present study, we aimed to describe the gonadal function of boys with NS and NSML according to their phenotype, notably the presence or not of cryptorchidism and evaluate whether mutations in the different genes involved in NS and NSML may have differential impact. To achieve this goal, we performed a retrospective chart review in 37 boys with NS or NSML harbouring mutations in the *PTPN11*, *SOS1*, *RAF1* or *KRAS* genes.

Subjects and methods

Patient population

The study population included 37 male patients with a genetically confirmed diagnosis of NS who were followed at the Children's Hospital in Toulouse, France, between 2008 and 2018. The genetic diagnosis was defined as the presence of a germline mutation in one of the following genes: *PTPN11*, *SOS1*, *RAF1* or *KRAS*. Two types of *PTPN11* mutations were distinguished: NS-associated *PTPN11* mutations (*NS-PTPN11*) (gain-of-function mutation) and NSML-associated *PTPN11* mutations (*NSML-PTPN11*) (loss-of-function mutation). A total of 37 boys were identified with *NS-PTPN11* mutations in 26 (70.3%) patients, *NSML-PTPN11* mutations in 2 (5.4%), *SOS1* mutations in 6 (16.2%), *RAF1* mutations in 2 (5.4%), and *KRAS* mutations in 1 (2.7%).

Personal history (including cardiopathy, short stature and cryptorchidism) and clinical data (including height, weight and Tanner stages) were obtained by retrospective chart review. The median age at the last visit was 11.1 years

(range: 1–30 years) with a median duration of follow-up of 2.9 years (range: 0.1–16.7 years).

Due to the retrospective design of the study, the approval by an independent ethical committee was not required according to the French law (Jardé law dealing with the Research Implying Human Person (RIHP), November 16th, 2016). Patient information was pseudonymized and de-identified prior to analysis. Written informed consent of parents and assent of the children were obtained for the molecular diagnosis of NS in the framework of standard clinical management.

Clinical data

BMI was calculated as the ratio of weight in kg divided by the square of height in metres. Height, weight and BMI measurements were converted to age and sex-specific standard deviation score (SDS) on the basis of published reference data (20, 21). Pubertal development was evaluated according to Marshall and Tanner staging (22).

Semen collection and analyses

Four patients who had the association of high FSH levels and low inhibin B levels were referred to the fertility centre for evaluation and fertility preservation.

At the fertility centre, informed consent before the cryopreservation was obtained from all patients and from parents for minor subjects. Semen samples were collected by masturbation after a recommended 2–6 days of sexual abstinence. The semen analysis was performed according to the 2010 World Health Organization (WHO) guidelines (5th edition). The characteristics considered were sexual abstinence (days), ejaculate volume (mL) and pH, sperm concentration ($10^6/\text{mL}$), round cell concentration ($10^6/\text{mL}$), spermatozoa vitality (%) and progressive motility (PR, %), non-progressive motility (NP, %) and total sperm count ($10^6/\text{ejaculate}$). After semen analysis, if spermatozoa were found even in the pellet, each sample was diluted in cryoprotectant medium (SpermFreeze, FertiPro) and aspirated into high-security straws (CryoBioSystem). Straws were frozen and stored in liquid nitrogen.

Biochemical measurements

Blood samples were taken as part of the clinical follow-up of the patients. During the study period, different measurement kits were used. Testosterone was assessed using radio-immunoassay (RIA) kit (Cis-Bio International, Gif-sur-Yvette, France). Serum luteinizing hormone

(LH) and follicle-stimulating hormone (FSH) were measured using one of two automated immunoassays (ADVIA Centaur, Siemens Healthcare and Cobas, Roche Diagnostics). Serum inhibin B level was determined using one of three enzyme-linked immunoassay (ELISA) kits (OBI-DSL, Oxford, UK; Beckman-Coulter, Inc.; Anshlabs, Webster, TX, USA). Serum AMH was quantified using one of three enzyme-linked immunoassay (ELISA) kits (DSL-Beckman-Coulter; GenII Beckman-Coulter; Cobas, Roche Diagnostics). Dates, sensitivity, inter- and intra-assay coefficient of variations, low detection limit, as well as correction factors between different hormonal assays are indicated in Supplementary Table 1 (see section on [supplementary data](#) given at the end of this article). Serum AMH results were transformed to sex-, age- and Tanner stages-specific SDS from the average result in the reference population using the data published by Grinspon *et al.* (23). Serum inhibin B results were transformed to sex- and age-specific SDS using the reference published by Crofton *et al.* (24).

Molecular analyses

All molecular analyses were performed at the Department of Genetics of Robert-Debré Hospital (H Cavé, A Verloes), Paris, France. DNA samples were obtained from peripheral leukocytes. Mutation screening was performed by direct bidirectional sequencing of exons and their flanking intron–exon boundaries. The entire coding regions of *PTPN11*, *SOS1*, *RAF1* and *KRAS* genes were tested in all patients. Primers and PCR conditions are available on request.

The PCR products were sequenced (Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and reaction products run on an automated capillary sequencer (ABI 3100 Genetic Analyzer, Applied Biosystems). Sequences were aligned using Seqscape analysis software (Applied Biosystems) and compared with the reference sequences for genomic DNA and mRNA. GenBank accession numbers for genomic and mRNA reference sequences, respectively, are as follows: *PTPN11* NC_000012 and NM_002834, *SOS1* NC_000002 and NM_005633, *RAF1* NC_000007 and NM_004333 and *KRAS* NC_000012 and NM_033360 (isoform a) or NM_004985 (isoform b).

Statistical analysis

Raw results were transformed to age- and sex-specific SDS from the average result in the reference population using

the published reference data cited in the description of measurement techniques. Data were reported as mean $\pm$ S.D. or median (interquartile range, IQR) as appropriate.

First, mean SDS for the entire NS sample and the different subgroups (presence of cryptorchidism or not, genotypes) were compared with those of the general population using the one-sample Student test, assuming an average z-score of 0 in the general population. For repeated measures, a linear mixed model was applied with a robust variance estimator in order to estimate means, CIs and to compare subgroups according to the presence or not of cryptorchidism and genotype. Associations were given as Pearson correlation or Spearman rank correlation, as appropriate. All tests were two-tailed and throughout the study $P < 0.05$ was considered significant. Statistical analyses were performed using Stata Statistical Software, version 11 for Windows (Stata Corporation).

Results

Clinical and genetic features of NS patients

The main clinical and genetic features of the 37 NS patients are summarized in Supplementary Table 2.

Growth hormone (GH) therapy was started in 9 (24%) patients at a mean age of 8.2 ± 3.9 years; only auxological data from non-GH-treated NS patients were reported. As previously reported, NS patients were significantly shorter (height SDS: -1.4 ± 1.2 ; $P < 0.001$) and leaner (BMI SDS: -1.1 ± 0.9 ; $P < 0.001$) compared with the general population. Bone age was delayed of -1.4 ± 1.1 years. Serum leptin levels assessed in 27 patients were low (mean: 1.3 ± 0.9 pg/mL; normal values: 2.0–5.6) with 88% of the values below the lower range limit.

Cryptorchidism was found in 24 (65%) of NS boys, it was bilateral in 14 (58%) patients, and patients were operated on at a median age of 2.5 years (range: 1.4–10.4 years).

Onset of puberty and testicular volume

During the study period, 16 (43%) children had entered puberty (defined as testicular volume ≥ 4 mL or Tanner stage G2) at a median age of 13.5 years (range: 11.4–15.0 years). Puberty was delayed (defined as testicular volume < 4 mL or Tanner stage G1 after the age of 14 years) in 3 (19%) patients, but none of these patients required puberty induction treatment. Age at pubertal onset was negatively correlated with BMI SDS ($r = -0.54$; $P = 0.022$).

During pubertal development, all NS patients had a testicular size in the normal range of healthy boys (Fig. 1C).

Serum LH, FSH and testosterone levels

Serum LH, FSH and testosterone levels were low or undetectable during childhood and consequently uninformative, thereafter these hormones progressively increased from the onset of puberty (Fig. 1A, B and D). In pubertal boys, LH levels were positively correlated with testosterone levels ($r = 0.82$; $P < 0.001$), and FSH levels were

Figure 1

(A and B) Leydig cell markers (serum LH and testosterone levels) and (C, D, E, F, G and H) Sertoli cell markers (testes volume, serum FSH, AMH and inhibin B levels) according to age, normal testes/cryptorchidism and genotype. Filled symbols represent males with descended testes and open symbols represent males with cryptorchidism. The genotype is represented by circle (NS-PTPN11), star (NSML-PTPN11), square (SOS1), diamond (RAF1) and sun (KRAS) symbols. A line connects all measures from each individual child.

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	413
----------------	---------------------	---	-------	-----

negatively correlated with inhibin B ($r=-0.72$; $P=0.002$) and AMH ($r=-0.75$; $P=0.001$) levels.

On the 36 available serum testosterone assessments, 32 (95%) values were above the 3rd percentile and 18 (66%) above the 50th percentile of normal reference values suggesting a normal Leydig cell function. In contrast, serum FSH levels were dramatically increased in four patients suggesting a Sertoli cell dysfunction.

Serum inhibin B and AMH levels

In contrast to the gonadotropins, the serum concentration of AMH and inhibin B, which are markers of Sertoli cells function, were above the detection limit throughout life, notably the prepubertal period, with marked variations according to age and pubertal status. The patterns of AMH and inhibin B secretion in NS patients were comparable to those of healthy children (Fig. 1E and F). Thus, serum AMH levels remained at a relatively stable level through childhood until the onset of puberty, when they progressively declined until adulthood. In contrast, serum inhibin B levels were high in infants, decreased gradually to reach a plateau around 5 years, then increased in early adolescence to reach a new plateau in late puberty. Before puberty, AMH levels were positively correlated with inhibin B levels ($r=0.69$; $P<0.001$). However, despite the same patterns of secretion, NS patients had significant lower levels of AMH (mean SDS: -0.6 ; $P=0.003$) and

inhibin B (mean SDS: -0.9 ; $P<0.001$) compared with the general population (Fig. 1G, H and Tables 1, 2), suggesting a Sertoli cell dysfunction.

The levels of these hormones varied according to the genotype. Lower AMH levels were found in *NS-PTPN11* compared with healthy children (mean SDS: -0.8 ; $P<0.001$). In contrast, inhibin B levels in *SOS1* patients did not differ from healthy children (mean SDS: -0.2 ; $P=0.56$) and tended to be higher compared to *NS-PTPN11* patients (mean difference SDS: 0.8 ; $P=0.07$).

Despite the fact that cryptorchid patients had significant lower AMH levels compared with healthy children, no difference was found between cryptorchid and non-cryptorchid patients for AMH and inhibin B levels ($P=0.43$ and 0.62 respectively).

Surprisingly, GH-treated NS patients had significant lower serum AMH levels compared to non-treated NS patients (mean SDS: -1.1 vs -0.4 ; <0.001), whereas serum inhibin B levels were similar (mean SDS: -1.4 vs -0.9 ; $P=0.318$).

Patients with primary hypogonadism

As mentioned above, four patients had the association of high FSH levels and low inhibin B levels suggestive of primary hypogonadism and were referred to the fertility centre for evaluation and fertility preservation. Clinical and biological characteristics are shown in Table 3. All these

Table 1 Comparison of serum AMH levels SDS in NS boys with the normal reference values and according to phenotype and genotype.

	Patients, n	Measures, n	Mean (s.d.)	Median (IQR)	Estimated mean (95% CI)	P*
All patients	33	83	-0.6 (1.1)	-0.9 (-1.3; -0.1)	-0.6 (-0.9; -0.2)	0.003 [†]
Cryptorchidism or not						
No	11	25	-0.3 (1.2)	-0.6 (-1.0; 0.3)	-0.3 (-1.0; 0.4)	0.42 [†]
Yes	22	58	-0.7 (1.0)	-0.9 (-1.3; -0.4)	-0.7 (-1.1; -0.3)	0.001 [†]
Genotype						
<i>NS-PTPN11</i>	22	57	-0.8 (1.0)	-1.0 (-1.3; -0.6)	-0.8 (-1.2; -0.4)	<0.001 [†]
<i>KRAS</i>	1	1	0.4	-	0.4 (-)	-
<i>NSML-PTPN11</i>	2	6	-0.7 (1.1)	-1.1 (-1.3; 0.4)	-0.4 (-1.8; 1.0)	0.57 [†]
<i>RAF1</i>	2	6	-0.4 (0.6)	-0.4 (-1.0; 0.3)	-0.6 (-1.1; -0.1)	0.027 [†]
<i>SOS1</i>	6	13	0.2 (1.3)	-0.4 (-0.6; 0.7)	0.2 (-0.8; 1.2)	0.68 [†]
Comparisons						
Cryptorchidism vs without	-	-	-0.4	-	-0.4 (-1.2; 0.4)	0.32
<i>KRAS</i> vs <i>NS-PTPN11</i>	-	-	1.2	-	1.2 (-)	-
<i>NSML-PTPN11</i> vs <i>NS-PTPN11</i>	-	-	0.1	-	0.4 (-1.0; 1.9)	0.56
<i>RAF1</i> vs <i>NS-PTPN11</i>	-	-	0.4	-	0.2 (-0.4; 0.9)	0.49
<i>SOS1</i> vs <i>NS-PTPN11</i>	-	-	1.0	-	1.0 (0.0; 2.1)	0.06

*Estimated means, confidence intervals (CIs) and *P* values were calculated using a linear mixed model for repeated measures with a robust variance estimator; [†]means SDS were compared with those of the general population using the one-sample Student test, assuming an average SDS of 0 in the general population.

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	414
----------------	---------------------	---	-------	-----

Table 2 Comparison of serum inhibin B levels SDS in NS boys with the normal reference values and according to phenotype and genotype.

	Patients, n	Measures, n	Mean (s.d.)	Median (IQR)	Estimated mean (95% CI)	P*
All patients	32	82	−1.1 (1.2)	−1.2 (−2.0; −0.4)	−0.9 (−1.3; −0.5)	<0.001 [†]
Cryptorchidism						
No	11	26	−0.8 (0.8)	−1.1 (−1.3; −0.4)	−0.7 (−1.2; −0.2)	0.004 [†]
Yes	21	56	−1.2 (1.3)	−1.4 (−2.2; −0.1)	−1.0 (−1.5; −0.4)	<0.001 [†]
Genotype						
<i>NS-PTPN11</i>	21	56	−1.2 (1.2)	−1.3 (−2.1; −0.4)	−1.0 (−1.5; −0.5)	<0.001 [†]
<i>KRAS</i>	1	1	0.8 (−)	0.8 (0.8; 0.8)	0.8 (−)	–
<i>NSML-PTPN11</i>	2	6	−1.5 (0.9)	−1.6 (−2.0; −1.4)	−1.4 (−1.5; −1.4)	<0.001 [†]
<i>RAF1</i>	2	6	−1.4 (0.6)	−1.3 (−1.6; −1.2)	−1.7 (−2.6; −0.9)	<0.001 [†]
<i>SOS1</i>	6	13	0.0 (1.0)	0.0 (−0.8; 0.7)	−0.2 (−0.9; 0.5)	0.56 [†]
Comparisons						
Cryptorchidism vs without	–	–	−0.4 (−)	–	−0.3 (−1.0; 0.5)	0.46
<i>KRAS</i> vs <i>NS-PTPN11</i>	–	–	2.1 (−)	–	1.9 (−)	–
<i>NSML-PTPN11</i> vs <i>NS-PTPN11</i>	–	–	−0.2 (−)	–	−0.4 (−0.9; 0.1)	0.10
<i>RAF1</i> vs <i>NS-PTPN11</i>	–	–	−0.2 (−)	–	−0.7 (−1.7; 0.3)	0.16
<i>SOS1</i> vs <i>NS-PTPN11</i>	–	–	1.2 (−)	–	0.8 (−0.1; 1.7)	0.07

*Estimated means, confidence intervals (CIs) and *P* values were calculated using a linear mixed model for repeated measures with a robust variance estimator; [†]means SDS were compared with those of the general population using the one-sample Student test, assuming an average SDS of 0 in the general population.

four patients had a *NS-PTPN11* mutation and a history of bilateral cryptorchidism. They had entered puberty spontaneously with normal progression and satisfactory development of secondary sexual characteristics. Serum testosterone concentrations were normal in all cases. Semen analysis revealed azoospermia in one patient and severe cryptozoospermia in the three other patients. One patient had scrotal ultrasonography, which found testicular microlithiasis.

Discussion

In this study, we report the gonadal function, covering the pituitary gonadotropins and both tubular and interstitial functions of the testes, in a large series of boys with a genetically confirmed diagnosis of NS.

First, we found that the onset of puberty was delayed in NS boys. The mean age of pubertal onset in our patients (13.5 years) was delayed of about 2 years compared with the general population (11.7 years in healthy Danish boys) (25). This is consistent with previous data reporting a mean age of pubertal onset between 13.5 and 14.5 years in NS boys (7, 8, 9, 26). Interestingly, age at puberty onset was negatively correlated with body fat, boys with a lower BMI being more likely to have delayed puberty. It is extensively reported that nutrition and adiposity are major determinants of pubertal onset and timing. Despite extensive data demonstrating an inverse association between body fat and age of pubertal onset in girls (27, 28),

relationship between BMI and onset of puberty in boys is less consistent. It has been suggested that the association between body fat and age of pubertal onset in boys may be nonlinear with delayed puberty seen in very lean as well as in very obese boys (25). As previously reported in NS (19, 29, 30), our patients displayed a lean phenotype with a BMI in the lower normal range which may influence the timing of puberty. The relationship between fat mass and puberty may be mediated by several factors, including leptin, the levels of which are very low in our patients. Many animal experiments and human studies have shown that leptin is an important signal in the initiation of puberty (31). Thus, mice or humans who have mutation in leptin gene exhibit central hypogonadism, which is reversed by leptin treatment. Lower leptin levels have also been reported in adolescent boys with constitutional delay of growth and puberty (32). Interestingly, besides its role on the hypothalamus–pituitary–gonadal axis, leptin may also have local effects on the function of testis and spermatogenesis. Thus, leptin inhibit testosterone secretion by downregulating cAMP-dependent activation of steroidogenic genes expression (33).

Despite the fact that it was delayed, the puberty started spontaneously in all our patients suggesting a normal hypothalamic–pituitary input. In contrast, our data suggest a primary testicular insufficiency in NS patients. Normal testicular function results from the combination and interaction between seminiferous tubules and interstitial tissue functions. Tubular function can be assessed by the determination of serum AMH and

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	415
----------------	---------------------	---	-------	-----

Table 3 Clinical and biological characteristics of NS patients with severe primary hypogonadism. All these four patients had a NS-PTPN11 mutation and a history of bilateral cryptorchidism.

	Patient number			
	11	8	6	1
Age (years)	16.0	16.4	18.0	30.0
Testis volume (mL) (NR: 20–25)	15	9	20	ND
Puberty onset (years)	12.5	13	12.3	ND
LH (IU/L) (NR: 1.3–6.3)	9.7	8.9	8.6	22.1
FSH (IU/L) (NR: 1.1–7.0)	37.6	34.7	34.2	106.1
Testosterone (ng/dL) (NR: 114–682)	438	467	365	330
Inhibin B (pg/mL=ng/L) (NR: 74–470)	29	38	27	16
AMH (pmol/L) (NR: 25–137)	16	9	2	1
Semen characteristics ^a				
Samples obtained	2	11	3	1
Ejaculate volume (mL)	1.9	1.1	1.8	2.3
Sperm concentration (10 ⁶ /mL)	<0.05	0.01	0.08	0
Total sperm count (10 ⁶ /sample)	<0.1	0.01	0.14	0
Total motility (PR+NP, %)	0	30	5	ND
Total straws	6	34	10	0

^aIn case of multiple samples, only the best sample with the highest total progressive sperm count (i.e. the sample that would take priority for assisted reproduction treatment) is reported.

NP, non-progressive motility; PR, progressive motility; NR, normal range.

inhibin B levels which are markers of Sertoli cell function, and in adults, by sperm analysis which reflects germ cell development. Interstitial tissue function can be assessed by the determination of serum testosterone, which is secreted by Leydig cells. Primary hypogonadism may result either from the impaired function of all testicular cell populations or from the predominant dysfunction of one specific cell population. In our study, NS adolescents display normal to high testosterone levels suggesting a normal or compensated Leydig cell function, which is in accordance with the positive role of SHP2 on testosterone production (18). However, a future Leydig cell impairment in older NS adults cannot be excluded. In contrast, despite similar patterns of secretion, serum AMH and inhibin B levels of the entire NS cohort were in the low normal range throughout all development stages. Moreover, four patients among the 16 children (25%) who had entered puberty had azoospermia or severe oligospermia. All these data suggest a Sertoli cell-specific primary insufficiency in NS at least in adolescent and young adults. In order to validate our results, further studies involving semen analysis and testicular biopsies would be interesting. In the literature, histological analyses of testes performed in few NS patients, all of them with cryptorchidism, mostly found interstitial fibrosis associated with abnormalities of the seminiferous tubules (i.e. reduction of tubular diameter, Leydig cells per seminiferous tubules, and spermatogonia per tubule) (34, 35). To our knowledge, semen analysis was reported once with low sperm count in one NS male (8).

Whether the abnormalities observed in testicular function in cryptorchid NS patients are due to the ectopic gonadal position or to the primary genetic disorder is difficult to determine. Indeed, it has been suggested that cryptorchidism reported in up to 80% of NS males (8) may be responsible for impaired fertility. Indeed, young males (without NS) who had been operated on for cryptorchidism in childhood often display Sertoli cell dysfunction characterized by low testicular volume, high FSH levels and impaired sperm quality (36, 37, 38); in these cryptorchid patients, Leydig cell function seems to be compensated with normal testosterone levels associated with normal to increased LH levels. However, in our study, no difference in Sertoli cell markers was shown between cryptorchid and non-cryptorchid NS patients. This point is in accordance with previous reports showing Sertoli cell dysfunction in NS male with normal testicular descent (12, 13) that could suggest an intrinsic defect of these cells. SHP2 inhibition/gene invalidation has previously revealed a positive role for SHP2 male gonadal function. Its invalidation in Sertoli cell or in germ cells impair BTB and SSC maintenance, resulting in male infertility (16, 17). These effects are associated to ERK signalling downregulation and reduced expression of FSH and testosterone target genes. Consistent with this, disruption of MEK/ERK signalling in Sertoli or Leydig cells impair spermatogenesis and reduced fertility (39, 40). This is at odds with our finding that NS-causing mutations, which activate SHP2 and the RAS/ERK pathway, also result in Sertoli cell impairment and reduced fertility.

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	416
----------------	---------------------	---	-------	-----

These apparent discrepancies could be explained by the fact that fine-tuned regulation of SHP2 and RAS/ERK might be necessary to ensure proper Sertoli cell function. Supporting this view, expression of a hyperactivating mutant of SHP2 alter Sertoli cell function, and it has been shown that periodic activation of ERK 1/2 is necessary for Sertoli cell maturation (15). Such spatiotemporal regulation could also explain that both gain-of-function and loss-of-function mutations of PTPN11 could give rise to similar phenotype.

Genotype–phenotype correlations have been reported for certain features of NS, but few data are available concerning the relationship between genes and gonadal function. In our study, the frequencies of the genotypes were similar to those previously described in patients with a genetically confirmed diagnosis of NS, with a vast majority of *NS-PTPN11* patients (about 70%) (2). Interestingly, Sertoli cell markers levels significantly varied according to the genotype with lower levels in *NS-PTPN11* and *RAF1* patients than in *SOS1* patients. Of note, the same impact of genes was found for growth impairment, infants with *SOS1* mutations being less short than those with *NS-PTPN11* or *RAF1* mutations (19). These differences between genotypes may be explained by variations in the degree of activation of the RAS/ERK signalling pathway or by differential, genotype-dependent, tissue specificity. Indeed, it has been reported that a periodic activation of ERK1/2 is necessary for Sertoli cells maturation, so that both quantitative and qualitative changes in this signalling pathway can result in Sertoli cell dysfunction (39). However, no genotype/phenotype correlation studies have been reported to quantitatively assess the level of RAS/ERK hyperactivation or to document the tissue/organ pattern of RAS/ERK dysregulation. Alternatively, some specific mutants may induce dysregulation of other signalling pathways, which might counteract or synergize with RAS/ERK hyperactivation. These data may provide insight into the mechanisms underlying delayed puberty and infertility, which are still incompletely understood.

Surprisingly, serum AMH and inhibin B levels were significantly lower in GH-treated NS patients. This is discordant with other article reporting that GH therapy has no significant effect on testicular function (24). This observation may only reflect the severity of the disease, the shortest NS patient also displaying the more severe testicular impairment.

This study reports clinical and hormonal data on gonadal function, during childhood and puberty, in one of the largest series of NS male patients. Another strength of this study lies in the genetic diagnosis of NS in all patients

that allow us to look for genotype–phenotype correlations for the first time. However, only few patients were longitudinally followed up and except for the *NS-PTPN11* patients, this study was limited by the small quantity of data in the other subcohorts. Another limitation of our study is that, because of the retrospective design of the study, several kits were used for hormone assays.

Conclusion

This retrospective study suggests a normal albeit delayed onset at puberty. In contrast, our data reveal a Sertoli cell-specific primary insufficiency in NS at least in adolescent and young adults. Prospective long-term follow-up studies are required to further investigate gonadal function and fertility in NS adults.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-18-0582>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors thank the many individuals with NS and their families who participated in the research studies cited in this report. They thank Claire Bouvattier for fruitful scientific discussions.

References

- Romano AA, Allanson JE, Dahlgren J, Gelb BD, Hall B, Pierpont ME, Roberts AE, Robinson W, Takemoto CM & Noonan JA. Noonan syndrome: clinical features, diagnosis, and management guidelines. *Pediatrics* 2010 **126** 746–759. (<https://doi.org/10.1542/peds.2009-3207>)
- Roberts AE, Allanson JE, Tartaglia M & Gelb BD. Noonan syndrome. *Lancet* 2013 **381** 333–342. ([https://doi.org/10.1016/S0140-6736\(12\)61023-X](https://doi.org/10.1016/S0140-6736(12)61023-X))
- Tartaglia M, Gelb BD & Zenker M. Noonan syndrome and clinically related disorders. *Best Practice and Research: Clinical Endocrinology and Metabolism* 2011 **25** 161–179. (<https://doi.org/10.1016/j.beem.2010.09.002>)
- Tidyman WE & Rauen KA. Expansion of the RASopathies. *Current Genetic Medicine Reports* 2016 **4** 57–64. (<https://doi.org/10.1007/s40142-016-0100-7>)
- Tajan M, Paccoud R, Branka S, Edouard T & Yart A. The Rasopathy family: consequences of germline activation of the RAS/MAPK

- pathway. *Endocrine Reviews* 2018 **39** 676–700. (<https://doi.org/10.1210/er.2017-00232>)
- 6 Tajan M, Pernin-Grandjean J, Beton N, Gennero I, Capilla F, Neel BG, Araki T, Valet P, Tauber M, Salles JP *et al.* Noonan syndrome-causing SHP2 mutants impair ERK-dependent chondrocyte differentiation during endochondral bone growth. *Human Molecular Genetics* 2018 **27** 2276–2289. (<https://doi.org/10.1093/hmg/ddy133>)
 - 7 Ranke MB, Heidemann P, Knupfer C, Enders H, Schmaltz AA & Bierich JR. Noonan syndrome: growth and clinical manifestations in 144 cases. *European Journal of Pediatrics* 1988 **148** 220–227. (<https://doi.org/10.1007/BF00441408>)
 - 8 Sharland M, Burch M, McKenna WM & Paton MA. A clinical study of Noonan syndrome. *Archives of Disease in Childhood* 1992 **67** 178–183. (<https://doi.org/10.1136/adc.67.2.178>)
 - 9 Shaw AC, Kalidas K, Crosby AH, Jeffery S & Patton MA. The natural history of Noonan syndrome: a long-term follow-up study. *Archives of Disease in Childhood* 2007 **92** 128–132. (<https://doi.org/10.1136/adc.2006.104547>)
 - 10 Elswawi MM, Pryor JP, Klufio G, Barnes C & Patton MA. Genital tract function in men with Noonan syndrome. *Journal of Medical Genetics* 1994 **31** 468–470. (<https://doi.org/10.1136/jmg.31.6.468>)
 - 11 Sinisi AA, Criscuolo T, Maresca F, Quarto C, Di Finizio B, Bellastella A & Faggiano M. Endocrine profile in Noonan's syndrome. *Minerva Endocrinologica* 1987 **12** 13–17.
 - 12 Ankarberg-Lindgren C, Westphal O & Dahlgren J. Testicular size development and reproductive hormones in boys and adult males with Noonan syndrome: a longitudinal study. *European Journal of Endocrinology* 2011 **165** 137–144. (<https://doi.org/10.1530/EJE-11-0092>)
 - 13 Marcus KA, Sweep CG, van der Burgt I & Noordam C. Impaired Sertoli cell function in males diagnosed with Noonan syndrome. *Journal of Pediatric Endocrinology and Metabolism* 2008 **21** 1079–1084.
 - 14 Puri P & Walker WH. The regulation of male fertility by the PTPN11 tyrosine phosphatase. *Seminars in Cell and Developmental Biology* 2016 **59** 27–34. (<https://doi.org/10.1016/j.semcdb.2016.01.020>)
 - 15 Puri P, Phillips BT, Suzuki H, Orwig KE, Rajkovic A, Lapinski PE, King PD, Feng GS & Walker WH. The transition from stem cell to progenitor spermatogonia and male fertility requires the SHP2 protein tyrosine phosphatase. *Stem Cells* 2014 **32** 741–753. (<https://doi.org/10.1002/stem.1572>)
 - 16 Puri P & Walker WH. The tyrosine phosphatase SHP2 regulates Sertoli cell junction complexes. *Biology of Reproduction* 2013 **88** 59. (<https://doi.org/10.1095/biolreprod.112.104414>)
 - 17 Hu X, Tang Z, Li Y, Liu W, Zhang S, Wang B, Tian Y, Zhao Y, Ran H, Liu W *et al.* Deletion of the tyrosine phosphatase Shp2 in Sertoli cells causes infertility in mice. *Scientific Reports* 2015 **5** 12982. (<https://doi.org/10.1038/srep12982>)
 - 18 Cooke M, Orlando U, Maloberti P, Podesta EJ & Cornejo Maciel F. Tyrosine phosphatase SHP2 regulates the expression of acyl-CoA synthetase ACSL4. *Journal of Lipid Research* 2011 **52** 1936–1948. (<https://doi.org/10.1194/jlr.M015552>)
 - 19 Cessans C, Ehlinger V, Arnaud C, Yart A, Capri Y, Barat P, Cammas B, Lacombe D, Coutant R, David A *et al.* Growth patterns of patients with Noonan syndrome: correlation with age and genotype. *European Journal of Endocrinology* 2016 **174** 641–650. (<https://doi.org/10.1530/EJE-15-0922>)
 - 20 Masse N & Sempe M. Somatic growth and development of French children and adolescents. Summary of our knowledge and suggestions for surveys. *Pediatrerie* 1967 **22** 593–607.
 - 21 Rolland-Cachera ME, Cole TJ, Sempe M, Tichet J, Rossignol C & Charraud A. Body mass index variations: centiles from birth to 87 years. *European Journal of Clinical Nutrition* 1991 **45** 13–21.
 - 22 Marshall WA & Tanner JM. Variations in the pattern of pubertal changes in boys. *Archives of Disease in Childhood* 1970 **45** 13–23. (<https://doi.org/10.1136/adc.45.239.13>)
 - 23 Grinspon RP, Bedecarras P, Ballerini MG, Iniguez G, Rocha A, Mantovani Rodrigues Resende EA, Brito VN, Milani C, Figueroa Gacitua V, Chiesa A *et al.* Early onset of primary hypogonadism revealed by serum anti-Müllerian hormone determination during infancy and childhood in trisomy 21. *International Journal of Andrology* 2011 **34** e487–e498. (<https://doi.org/10.1111/j.1365-2605.2011.01210.x>)
 - 24 Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV & Kelnar CJ. Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clinical Endocrinology* 2002 **56** 215–221. (<https://doi.org/10.1046/j.0300-0664.2001.01448.x>)
 - 25 Tinggaard J, Mieritz MG, Sorensen K, Mouritsen A, Hagen CP, Aksglaede L, Wohlfahrt-Veje C & Juul A. The physiology and timing of male puberty. *Current Opinion in Endocrinology, Diabetes and Obesity* 2012 **19** 197–203. (<https://doi.org/10.1097/MED.0b013e3283535614>)
 - 26 Romano AA, Dana K, Bakker B, Davis DA, Hunold JJ, Jacobs J & Lippe B. Growth response, near-adult height, and patterns of growth and puberty in patients with Noonan syndrome treated with growth hormone. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 2338–2344. (<https://doi.org/10.1210/jc.2008-2094>)
 - 27 Lee JM, Appugliese D, Kaciroti N, Corwyn RF, Bradley RH & Lumeng JC. Weight status in young girls and the onset of puberty. *Pediatrics* 2007 **119** e624–e630. (<https://doi.org/10.1542/peds.2006-2188>)
 - 28 Kaplowitz PB. Link between body fat and the timing of puberty. *Pediatrics* 2008 **121** (Supplement 3) S208–S217. (<https://doi.org/10.1542/peds.2007-1813F>)
 - 29 da Silva FM, Jorge AA, Malaquias A, da Costa Pereira A, Yamamoto GL, Kim CA & Bertola D. Nutritional aspects of Noonan syndrome and Noonan-related disorders. *American Journal of Medical Genetics Part A* 2016 **170** 1525–1531. (<https://doi.org/10.1002/ajmg.a.37639>)
 - 30 Malaquias AC, Brasil AS, Pereira AC, Arnhold JJ, Mendonça BB, Bertola DR & Jorge AA. Growth standards of patients with Noonan and Noonan-like syndromes with mutations in the RAS/MAPK pathway. *American Journal of Medical Genetics Part A* 2012 **158A** 2700–2706. (<https://doi.org/10.1002/ajmg.a.35519>)
 - 31 Zhang J & Gong M. Review of the role of leptin in the regulation of male reproductive function. *Andrologia* 2018 Epub. (<https://doi.org/10.1111/and.12965>)
 - 32 El-Eshmary MM, Abdel Aal IA & El Hawary AK. Association of ghrelin and leptin with reproductive hormones in constitutional delay of growth and puberty. *Reproductive Biology and Endocrinology* 2010 **8** 153. (<https://doi.org/10.1186/1477-7827-8-153>)
 - 33 Lin Q, Poon SL, Chen J, Cheng L, HoYuen B & Leung PC. Leptin interferes with 3',5'-cyclic adenosine monophosphate (cAMP) signaling to inhibit steroidogenesis in human granulosa cells. *Reproductive Biology and Endocrinology* 2009 **7** 115. (<https://doi.org/10.1186/1477-7827-7-115>)
 - 34 Okuyama A, Nishimoto N, Yoshioka T, Namiki M, Itatani H, Takaha M, Mizutani S, Aono T, Matsumoto K & Sonoda T. Gonadal findings in cryptorchid boys with Noonan's phenotype. *European Urology* 1981 **7** 274–277. (<https://doi.org/10.1159/000473239>)
 - 35 Sasagawa I, Nakada T, Kubota Y, Sawamura T, Tateno T & Ishigooka M. Gonadal function and testicular histology in Noonan's syndrome with bilateral cryptorchidism. *Archives of Andrology* 1994 **32** 135–140. (<https://doi.org/10.3109/01485019408987778>)
 - 36 Cortes D, Thorup J, Lindenberg S & Visfeldt J. Infertility despite surgery for cryptorchidism in childhood can be classified by patients with normal or elevated follicle-stimulating hormone and identified at orchidopexy. *BJU International* 2003 **91** 670–674. (<https://doi.org/10.1046/j.1464-410X.2003.04177.x>)
 - 37 Gracia J, Sanchez Zalabardo J, Sanchez Garcia J, Garcia C & Ferrandez A. Clinical, physical, sperm and hormonal data in 251 adults operated on for cryptorchidism in childhood. *BJU International* 2000 **85** 1100–1103. (<https://doi.org/10.1046/j.1464-410X.2000.00662.x>)

Clinical Study	S Moniez and others	Gonadal function of Noonan syndrome males	179:6	418
----------------	---------------------	---	-------	-----

- 38 Trsinar B & Muravec UR. Fertility potential after unilateral and bilateral orchidopexy for cryptorchidism. *World Journal of Urology* 2009 **27** 513–519. (<https://doi.org/10.1007/s00345-009-0406-0>)
- 39 Hasegawa K, Namekawa SH & Saga Y. MEK/ERK signaling directly and indirectly contributes to the cyclical self-renewal of

- spermatogonial stem cells. *Stem Cells* 2013 **31** 2517–2527. (<https://doi.org/10.1002/stem.1486>)
- 40 Yamashita S, Tai P, Charron J, Ko C & Ascoli M. The Leydig cell MEK/ERK pathway is critical for maintaining a functional population of adult Leydig cells and for fertility. *Molecular Endocrinology* 2011 **25** 1211–1222. (<https://doi.org/10.1210/me.2011-0059>)

Received 12 July 2018

Revised version received 19 September 2018

Accepted 1 October 2018