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Serum Prokineticin-2 in Prepubertal and Adult Klinefelter Individuals

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The study was approved by the Sapienza University Hospital ethical committee (Ref. 5825). All the study procedures were under the Helsinki Declaration of 1975, as revised in 1983, for human experimentation.

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Data availability statement

Data are available on request

Abstract

The prokineticin-2 (PROK2) is a small peptide belonging to the prokineticin family. In humans and rodents this chemokine is primarily involved in the control of central and peripheral reproductive processes. Klinefelter's syndrome (KS) is the first cause of male genetic infertility, due to an extra X chromosome, which may occur with a classical karyotype (47, XXY) or mosaic forms (46, XY/47, XXY). In affected subjects, pubertal maturation usually begins at an adequate chronological age, but when development is almost complete, they display a primary gonadal failure, with early spermatogenesis damage, and later onset of testosterone insufficiency. Thus, the main aim of the present study was to investigate the serum levels of PROK2 in prepubertal and adult KS patients, comparing them with healthy subjects. We showed for the first time the presence of PROK2 in the children serum but with significant changes in KS individuals. Indeed, compared to healthy subjects characterized by PROK2 serum elevation during the growth, KS individuals showed constant serum levels during the sexual maturation phase (higher during the prepubertal phase but lower during the adult age). In conclusion, these data indicate that in KS individuals PROK2 may be considered a biomarker for investigating the SK infertility process.

Keywords: Klinefelter syndrome, prepubertal, adults, prokineticin-2, infertility

Introduction

The chemokine prokineticin-2 (PROK2) is a small peptide belonging to the prokineticin family. The prokineticins (PROK1 and PROK2) and their receptors (PKR1 and PKR2) are widely distributed in the entire organism and have been found to regulate a stunning array of biological functions. Indeed, they have been associated thereafter with multiple biological functions such as angiogenesis (LeCouter et al., 2003; Urayama et al., 2007), neuronal survival (Ngan & Tam, 2008), circadian rhythm (Cheng et al., 2012; J.-D. Li et al., 2006), nociception (Ngan & Tam, 2008), pain (Giannini et al., 2009), immune response (LeCouter, Zlot, Tejada, Peale, & Ferrara, 2004), hematopoiesis (LeCouter et al., 2004) and spermatogenesis (Maldonado-Pérez, Evans, Denison, Millar, & Jabbour, 2007). In both humans and rodents PROK2 is primarily involved in the control of central and peripheral reproductive processes (Masuda et al., 2002; Negri & Ferrara, 2018; Negri & Lattanzi, 2012; Negri, Lattanzi, Giannini, & Melchiorri, 2007).

Klinefelter syndrome (KS) is a male sex chromosome disorder that results from the presence of an extra X chromosome in cells. KS individuals have the usual X and Y chromosomes, plus one extra X chromosome, for a total of 47 chromosomes (47, XXY) (Lanfranco, Kamischke, Zitzmann, & Nieschlag, 2004). Some people with features of KS have the extra X chromosome in only some of their cells; this condition is described as a mosaic KS (46, XY/47, XXY). Individuals with mosaic KS may have milder signs and symptoms than those with the extra X chromosome in all of their cells, depending on what proportion of cells have the additional chromosome (Bonomi et al., 2017). In addition, KS must be distinguished from higher-grade aneuploidies, characterized by more complex karyotypes, with 48 or 49 chromosomes (Spaziani et al., 2021; Spaziani et al., 2018).

Adult KS men are characterized by infertility, small testes, azoospermia, gynecomastia, elevated gonadotropin levels, and serum testosterone concentrations usually below or in the low adult male range. Testicular biopsies from most adult patients demonstrate a complete absence of germ cells, seminiferous tubule hyalinization, and fibrosis, whereas Leydig cells show hyperplasia. In contrast, testicular histology obtained from infant boys with KS appears to be nearly normal, with primary

germ cells present (Wikström et al., 2004). Although an early alteration of the hypothalamus-pituitary-gonadal axis has been demonstrated, infant KS goes through a normal mini-puberty phase (Spaziani et al., 2021).

It is well known that infants and prepubertal boys with KS display higher adiposity and a significantly higher prevalence of metabolic syndrome features, compared to their healthy peers, despite comparable physiologically low serum testosterone levels (Bardsley et al., 2011; Davis et al., 2016; Davis et al., 2019, 2020). PROK2 seems to be highly expressed in the white adipose tissue of people with obesity, suggesting that PROK2 may be furtherly considered as a novel adipokine (Chaldakov et al., 2010; Davis et al., 2020; Tore et al., 2007). Indeed, in a recent study comparing 70 normal-weight children and 91 children with obesity, serum PROK2 levels were significantly higher in the obese group and correlating positively with several metabolic parameters (Wang et al., 2021).

Thus, the aims and novelties of the present study were to investigate serum levels of PROK2 in prepubertal and in adult individuals affected by KS (hypogonadal, eugonadal and hypogonadal under androgen replacement therapy), comparing them with healthy subjects, and making a correlation with metabolic parameters. In this light, to avoid confounding findings we aimed to recruit a very strict cohort of patients, choosing children with metabolic parameters in the range of normality.

Materials and methods

Subjects' Recruitment

We enrolled 31 KS subjects with classic 47, non-mosaic, XXY karyotype and 22 healthy age-matched controls, attending the Umberto I Policlinico, Sapienza University, Rome recruiting them by clinical symptoms and laboratory parameters measurements for the diagnosis of KS. The non-mosaic 47,XXY karyotype in the patients was proven by an analysis of 15 to 30 metaphases with G-banding and/or an analysis of 50 ± 100 interphases with FISH analysis in peripheral blood preparations and/or fibroblasts (Sarrate, Vidal, & Blanco, 2010).

In particular, prepubertal KS children (n=10), between 5 and 9.5 years, were selected at the Department of Pediatrics of the Sapienza University Hospital "Policlinico Umberto I" of Rome, Italy. Prepubertal age-matched healthy controls (n=8) were recruited in the Sapienza University hospital pediatric clinic. We selected these children because they attended the hospital for the investigation of the presumed pathologies that resulted not being present at all, defining thus the children as "healthy" (Tarani et al., 2020).

Adult KS patients (n=21), aged between 18 and 45 years, were enrolled by the Experimental Medicine Department, Section of Medical Pathophysiology, Food Science and Endocrinology. Adult voluntary healthy controls (n=12) were enrolled from the endocrine clinic in age comparable to that of the patients analyzed. We divided adult KS into three groups, depending on the serum testosterone levels, classifying them as follows: eugonadal (normal range of testosterone, named KS-EU, n=7), hypogonadal (low testosterone levels, named KS-HYP, n=7), and hypogonadal under testosterone replacement therapy (named KS-T, n=7). The KS-T group underwent a treatment with exogenous testosterone (via intramuscular or topical application, adapted to the patient's condition, to reach a blood testosterone concentration comprised between 15 and 30 nmol/L, the normal level of testosterone for that age). The karyotype analysis involved different steps: first the culture of lymphocytes, then the chromosome banding and, finally, a microscopic analysis (Bates, 2011). For all patients, a peripheral venous blood sample was collected and conserved in a 3 mL heparin tube

from which the lymphocytes were isolated and cultured for 72 hours at 37°C (Huang & Chen, 2017; Thalhammer, Koehler, Stark, & Heckl, 2001). The chromosomal banding was then carried out using the G-banding (Giemsa banding using Trypsin) kit by Thermo Fisher (Waltham, MA, USA) performed according to the manufacturer's instructions (Huang & Chen, 2017). For each patient blood sample, 100 metaphase cells with an average resolution of 400 bands were recorded.

The exclusion criteria (Ceccanti et al., 2015) for all the individuals enrolled were: 1) the presence of genetic mosaicism (for KS subjects), 2) the use of drugs that could influence the serum levels of PROK2 (antidepressant, immunosuppressant or immunomodulatory drugs), 3) inflammatory, endocrine, cardiovascular and autoimmune diseases not strictly related to the syndrome that could influence the serum levels of PROK2, 4) other systemic disorders that could influence the serum levels of PROK2.

The study was approved by the Sapienza University Hospital ethical committee (Ref. 5825); an informed consent was signed and all the study procedures were under the Helsinki Declaration of 1975, as revised in 1983, for human experimentation.

Clinical and metabolic assays

All participants were subjected to anthropometric and body composition examinations. Age, height, and weight were recorded by trained staff. Peripheral blood samples were taken from each participant, collected in heparinized tubes, and centrifuged at 3000 rpm for 15 min to separate serum from plasma. The serum was stored at -20°C till the day of the analysis.

Testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) serum levels were evaluated in KS (both prepubertal and adult patients), in the laboratory of the Experimental Medicine Department, Section of Medical Pathophysiology, Food Science and Endocrinology. These hormones were measured in duplicate with chemiluminescent microparticle immunoassay (CMIA, Architect System; Abbott Laboratories, IL, USA) with limits of detection (LOD) of 0.05 mIU/mL, 0.07 mIU/mL and 0.28 nmol/L respectively. Intra- and inter-assay coefficients of variation for our

laboratory were: 3.6% and 5.4% at 3.2 mIU/mL (FSH); 3.8 and 5.5% at 4.1 mIU/mL (LH); 2.1% and 3.6% at 10.08 nmol/L (T). Insulin (reference range: 2.6-24.9 μ IU/mL), was measured on a Cobas E 601 analyzer, using a sandwich immunological method that uses two monoclonal antibodies, together specific for human insulin; reagents were supplied by Roche Diagnostics GmbH (Mannheim, Germany). The interassay coefficient of variation (CV) for the insulin assay was 3.7% at serum insulin of 16.7 μ IU/mL.

Serum glucose (reference range: 70.3-100.9 mg/dL), triglycerides (reference range: male 45.1-235.4 mg/dL; female 45.1-163.7 mg/dL), total cholesterol (reference range: 81.1-235.5 mg/dL) and high-density lipoprotein (HDL)-cholesterol (reference range: male 34.7-56.0 mg/dL; female 44.4-64.9 mg/dL) were measured using standard colorimetric and enzymatic methods on a Cobas C 501 analyzer with reagents supplied by Roche Diagnostics GmbH (Mannheim, Germany). Low-density lipoprotein (LDL)-cholesterol levels (reference range: male 54.8-120.1 mg/dL; female 69.9-120.1 mg/dL) were calculated using Friedwald equation (Clin Chem. 1972 Jun;18(6):499-502. PMID: 4337382). Interassay CV was respectively 1.1% at a serum glucose of 95.5 mg/dL, 1.9% at a serum triglycerides concentration of 104 mg/dL, 1.6% at a serum cholesterol of 104 mg/dL, 0.8% at a serum HDL-cholesterol of 40.6 mg/dL. The HOMA index (Homeostasis Model Assessment - HOMAi) was also analyzed (Ostrowski & Dobrowolski, 2014).

PROK2 serum analysis

Serum PROK2 levels were assayed using a competitive commercial ELISA kit (Life Span BioSciences, Inc., Seattle, WA, USA) according to the manufacturer's instructions. The ELISA test was performed on 96-well microplates by experimenters who were blind to the experimental design. Each sample was run in triplicate with <5% intra-assay and <9% inter-assay coefficients of variation, expressing results as pg/mL.

Statistical analysis

Statistical significance was determined using the analysis of variance (ANOVA) test followed by a Tukey HSD test to assess the statistical significance of the results. P values less than 0.05 were considered as significant individuals according to methods previously described (Ceccanti et al., 2016; Manni, Aloe, & Fiore, 2009). The n values refer to the number of healthy or KS subjects. Post hoc comparisons were performed by using the Tukey's HSD test. The Spearman Correlation test was performed to investigate the association between serum PROK2 levels and the clinical and metabolic parameters. Data are presented as mean $\pm$ standard error mean (SEM).

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Results

Clinical and metabolic assays

There were no significant age differences between healthy and KS subjects, both prepubertal and adults. The clinical and laboratory characteristics are reported in Table 1. Prepubertal KS and healthy prepubertal individuals had comparable body mass index (BMI), hormonal profile (testosterone, FSH, LH) as well as metabolic parameters (cholesterol, HDL, LDL, triglycerides, glycemia, insulinemia, HOMAi).

In adult KS increased BMI, triglycerides and glycemia were found in KS-HYP individuals compared to healthy adult individuals [see F, dF and p in Table 1]. As expected, testosterone in KS-HYP adult individuals was lower compared to healthy adult individuals [see F, dF and p in Table 1]. Table 1 shows also elevated values of FSH, LH, cholesterol and LDL in KS-HYP, KS-EU or KS-T adult individuals compared to healthy adult individuals [see F, dF and p in Table 1].

PROK2 serum level

Figure 1, panel A shows that KS prepubertal children had a significant potentiation of PROK2 protein, compared to healthy prepubertal children [$F(1,16)=4.78$, $p<0.05$, see post-hocs in the figure].

PROK2 data in adult individuals and adult KS are shown in Figure 1, panel B. ANOVA investigation revealed significant low PROK2 values in KS-HYP, KS-EU and KS-T individuals when compared to healthy adult controls values [$F(3,29)=11.39$, $p<0.001$, see post-hocs in the figure].

As for the age differences in PROK2 values (Figure 1, panel C), one-way ANOVA, analyzing all recruited groups, shows that only in prepubertal healthy individuals PROK2 was significantly lower than those of healthy adults [$F(5,45)=15.82$, $p<0.0001$; 642.35 ± 51.06 vs 2527.41 ± 71.02 in pg/ml] while in KS adults and children serum PROK2 remained constant over time.

Spearman correlation PROK2 vs Clinical/Metabolic Parameters

Tables 2 and 3 show the Spearman correlation tests between serum PROK2 and the different investigated parameters, in prepubertal and adult individuals respectively. In prepubertal KS patients (Table 2), our data showed a correlation between PROK2 and testosterone serum levels, as well as for LH and FSH ($p < 0.05$). In particular, for high values of PROK2, low values of FSH, LH and testosterone were found (Table 2). On the contrary, no correlations were disclosed in adult KS between PROK2 and the analyzed parameters (Table 3). In healthy prepubertal individuals, we did find significant correlations between PROK2 and LDL and cholesterol (for high values of PROK2, low values of LDL and cholesterol, $p < 0.05$, Table 2). In adult healthy individuals (Table 3) we found a correlation between PROK2 and LH and FSH serum level (for high values of PROK2, low values of LH and FSH, $p < 0.05$) and for BMI, insulinemia and HOMA_i (for high values of PROK2, high values of BMI, insulinemia and HOMA_i, $p < 0.05$).

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Discussion

This is the first study demonstrating that the chemokine PROK2 is detectable in the serum of KS individuals. In KS children, the PROK2 serum level was higher than in healthy children; on the contrary, KS adults showed a decreased level compared to healthy individuals, regardless of the testosterone levels. Interestingly, PROK2 levels in adult KS were similar to those of prepubertal KS individuals, whilst in healthy children, PROK2 was lower when compared to adults.

In recent years, a few studies showed the presence of PROK2 in the serum of adult humans. Indeed, healthy controls in both middle and elderly age groups showed comparable levels of this protein (Lattanzi et al., 2019; Schirinzi et al., 2021). Our data on the adult control sample are in line with findings previously published. In the present study, we further displayed original data concerning PROK2 serum level in prepubertal children that is about four times lower than in healthy adult subjects. The reason underlying this difference is not clear but this could depend on the state of the endocrine system development.

Recent investigations recognized the key roles of PROKs in the control of human central and peripheral reproductive processes (Martin et al., 2011; Pasquali et al., 2006). Moreover, studies conducted in preclinical models support these pieces of evidence (Bassi et al., 2020; Kisliouk et al., 2005; Y. Li et al., 2020). PROK1 and PROK2 together with their receptors are expressed in tissues associated with reproductive functions (Martin et al., 2011). PROKs are pivotal regulators of the physiological functions of the ovary, uterus, placenta, and testis, with marked dysfunctions in various pathological conditions (pregnancy loss, preeclampsia) (Traboulsi et al., 2015). As for the male reproductive system in humans, a striking difference between PROK1 and PROK2 lies in the different local expressions in the human testis. PROK1 expression is restricted to the testosterone-producing Leydig cells (Martin et al., 2011), whilst PROK2 is present within seminiferous tubules in primary spermatocytes (LeCouter et al., 2003). The two receptors PKR1 and PKR2 are both expressed on the vascular endothelial cells of the interstitial tissue (LeCouter et al., 2003; Lin et al., 2002; Samson et al., 2004; Wechselberger et al., 1999). The presence of PROK2 in primary spermatocytes, along with

its known angiogenic role in the testis, suggests that PROK2 might play an important role in the spermatogenesis process. Once again, in a mouse model, Bv8 (the analogous of human PROK2) expression is predominantly confined in the testis and particularly in primary spermatocytes (LeCouter et al., 2003), strongly confirming the overlap of the model with humans. Bv8 contributes to the integrity of blood vessels in the testis and also induces proliferation, survival and migration of adrenal cortical capillary endothelial cells. Interestingly, as well as for endocrine-gland-derived vascular endothelial growth factor (EG-VEGF), a tissue-specific angiogenic factor, Bv8 gene expression is induced by hypoxic stress, an environmental condition existing within the seminiferous tubules (LeCouter et al., 2001). Intriguingly, the overexpression of VEGF in the mouse testis arrested spermatogenesis (Korpelainen et al., 1998).

The main clinical manifestations of KS are hypogonadism and the impaired maturation process of germinal cells, which determine persistent infertility. To compensate for testosterone deficiency (if present), androgen pharmacological supplementation could be necessary from pubertal age, allowing the normal appearance of secondary sexual characteristics and favoring the development of male-type body proportions. Of course, androgen replacement therapy is not sufficient to reverse infertility. This supports the hypothesis that other factors may play subtle roles in determining the irreversible impairment of gonadal development in men affected by KS.

In KS children we found an increased level of serum PROK2 in comparison with healthy children. Furthermore, their testosterone level seems to be normal compared to reference values in healthy children, whilst LH and FSH values remain comparable between the groups. In addition, the Spearman correlation analyses between PROK2 and testosterone levels in KS children revealed an inverse correlation between the two factors, showing that for high PROK2 values, low testosterone was present, and *vice versa*.

It has been shown that mutations in PROK2 and PKR2 genes are associated with the Kallmann syndrome and/or idiopathic hypogonadotropic hypogonadism, disorders characterized by delayed puberty and infertility, secondary to Gonadotropin-releasing hormone (GnRH) deficiency (Abreu et

al., 2008; Dodé et al., 2006). The Kallmann animal model confirmed the essential role of PROK2 system signaling for reproductive function (Cole et al., 2008). Pharmacological treatments of patients affected by Kallmann syndrome with FSH and LH can counteract GnRH deficiency (Fraietta, Zylberstejn, & Esteves, 2013), but in those patients presenting PROK2 mutation, primary gonadal defects persist (oligo/azoospermia) also afterward FSH and LH supplementation (Sinisi et al., 2008). Further investigations on PROK2 expression in KS gonadal tissues could be of interest. Indeed, more in-depth analyses are required at the cellular levels in the future to determine the specific function and signaling pathways of PROK2 in the male reproductive system. It would be quite interesting and important to include teenagers (12-17 years old) in the study to determine the PROK2 profile in that population that goes through puberty and hormonal changes to get more comprehensive information on the serum levels of PROK2 in all stages of human growth.

In conclusion, the data of the current investigation demonstrate that the endogenous PROK2 synthesis and release system are altered in KS individuals as demonstrated by disrupted serum levels during the development, differently from what happens in healthy individuals. PROK2 in males increases depending on age, according to sexual maturation. The fact that in KS individuals PROK2 serum levels remain unaltered strongly confirms the PROK2 role as an early biomarker of disrupted reproductive/endocrine systems. We do believe that the strength of this study depends on the quite restricted exclusion criteria for individuals' recruitment, leading, however, to a relatively low number of individuals enrolled in the experimentation. The present research may provide further information in the effort to unravel other early biomolecular markers aimed at disclosing prompt interventions in the KS endocrine system functionality and development. These findings could be of interest also for investigations in the fields of human genetic disorders.

References:

- Abreu, A. P., Trarbach, E. B., de Castro, M., Frade Costa, E. M., Versiani, B., Matias Baptista, M. T., ... Latronico, A. C. (2008). Loss-of-function mutations in the genes encoding prokineticin-2 or prokineticin receptor-2 cause autosomal recessive Kallmann syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 93(10), 4113–4118. <https://doi.org/10.1210/jc.2008-0958>
- Bardsley, M. Z., Falkner, B., Kowal, K., & Ross, J. L. (2011). Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome. *Acta Paediatrica (Oslo, Norway : 1992)*, 100(6), 866–870. <https://doi.org/10.1111/j.1651-2227.2011.02161.x>
- Bassi, I., Luzzani, F., Marelli, F., Vezzoli, V., Cotellessa, L., Prober, D. A., ... Bonomi, M. (2020). Knocking-down of the Prokineticin receptor 2 affects reveals its complex role in the regulation of the hypothalamus-pituitary-gonadal axis in the zebrafish model. *Scientific Reports*, 10(1), 7632. <https://doi.org/10.1038/s41598-020-64077-2>
- Bates, S. E. (2011). Classical cytogenetics: karyotyping techniques. In *Human Pluripotent Stem Cells* (pp. 177–190). Springer.
- Bonomi, M., Rochira, V., Pasquali, D., Balercia, G., Jannini, E. A., & Ferlin, A. (2017). Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. *Journal of Endocrinological Investigation*, 40(2), 123–134. <https://doi.org/10.1007/s40618-016-0541-6>
- Ceccanti, M., Coccorello, R., Carito, V., Ciafrè, S., Ferraguti, G., Giacobazzo, G., ... Fiore, M. (2016). Paternal alcohol exposure in mice alters brain NGF and BDNF and increases ethanol-elicited preference in male offspring. *Addiction Biology*, 21(4), 776–787. <https://doi.org/10.1111/adb.12255>
- Ceccanti, M., Hamilton, D., Coriale, G., Carito, V., Aloe, L., Chaldakov, G., ... Fiore, M. (2015). Spatial learning in men undergoing alcohol detoxification. *Physiology and Behavior*, 149. <https://doi.org/10.1016/j.physbeh.2015.06.034>
- Chaldakov, G. N., Fiore, M., Tonchev, A. B., & Aloe, L. (2010). Neuroadipology: A novel component of neuroendocrinology. *Cell Biology International*, 34(10), 1051–1053. <https://doi.org/10.1042/CBI20100509>
- Cheng, M. Y., Lee, A. G., Culbertson, C., Sun, G., Talati, R. K., Manley, N. C., ... Sapolsky, R. M. (2012). Prokineticin 2 is an endangering mediator of cerebral ischemic injury. *Proceedings of the National*

Academy of Sciences of the United States of America, 109(14), 5475–5480.

<https://doi.org/10.1073/pnas.1113363109>

Cole, L. W., Sidis, Y., Zhang, C., Quinton, R., Plummer, L., Pignatelli, D., ... Pitteloud, N. (2008).

Mutations in prokineticin 2 and prokineticin receptor 2 genes in human gonadotrophin-releasing hormone deficiency: molecular genetics and clinical spectrum. *The Journal of Clinical Endocrinology and Metabolism*, 93(9), 3551–3559. <https://doi.org/10.1210/jc.2007-2654>

Davis, S., Lahlou, N., Bardsley, M., Temple, M.-C., Kowal, K., Pyle, L., ... Ross, J. (2016). Gonadal function is associated with cardiometabolic health in pre-pubertal boys with Klinefelter syndrome. *Andrology*, 4(6), 1169–1177. <https://doi.org/https://doi.org/10.1111/andr.12275>

Davis, S. M., DeKlotz, S., Nadeau, K. J., Kelsey, M. M., Zeitler, P. S., & Tartaglia, N. R. (2020). High prevalence of cardiometabolic risk features in adolescents with 47,XXY/Klinefelter syndrome. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, 184(2), 327–333. <https://doi.org/10.1002/ajmg.c.31784>

Davis, S. M., Reynolds, R. M., Dabelea, D. M., Zeitler, P. S., & Tartaglia, N. R. (2019). Testosterone Treatment in Infants With 47,XXY: Effects on Body Composition. *Journal of the Endocrine Society*, 3(12), 2276–2285. <https://doi.org/10.1210/js.2019-00274>

Dodé, C., Teixeira, L., Levilliers, J., Fouveau, C., Bouchard, P., Kottler, M.-L., ... Hardelin, J.-P. (2006). Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genetics*, 2(10), e175. <https://doi.org/10.1371/journal.pgen.0020175>

Fraietta, R., Zylberstejn, D. S., & Esteves, S. C. (2013). Hypogonadotropic hypogonadism revisited. *Clinics (Sao Paulo, Brazil)*, 68 Suppl 1(Suppl 1), 81–88. [https://doi.org/10.6061/clinics/2013\(sup01\)09](https://doi.org/10.6061/clinics/2013(sup01)09)

Giannini, E., Lattanzi, R., Nicotra, A., Campese, A. F., Grazioli, P., Screpanti, I., ... Negri, L. (2009). The chemokine Bv8/prokineticin 2 is up-regulated in inflammatory granulocytes and modulates inflammatory pain. *Proceedings of the National Academy of Sciences of the United States of America*, 106(34), 14646–14651. <https://doi.org/10.1073/pnas.0903720106>

Huang, H., & Chen, J. (2017). Chromosome bandings. In *Cancer Cytogenetics* (pp. 59–66). Springer.

Kisliouk, T., Podlovni, H., Spanel-Borowski, K., Ovadia, O., Zhou, Q.-Y., & Meidan, R. (2005).

Prokineticins (endocrine gland-derived vascular endothelial growth factor and BV8) in the bovine

ovary: expression and role as mitogens and survival factors for corpus luteum-derived endothelial cells.

Endocrinology, 146(9), 3950–3958. <https://doi.org/10.1210/en.2005-0297>

Korpelainen, E. I., Karkkainen, M. J., Tenhunen, A., Lakso, M., Rauvala, H., Vierula, M., ... Alitalo, K.

(1998). Overexpression of VEGF in testis and epididymis causes infertility in transgenic mice:

evidence for nonendothelial targets for VEGF. *The Journal of Cell Biology*, 143(6), 1705–1712.

<https://doi.org/10.1083/jcb.143.6.1705>

Lanfranco, F., Kamischke, A., Zitzmann, M., & Nieschlag, E. (2004). Klinefelter's syndrome. *Lancet*

(London, England), 364(9430), 273–283. [https://doi.org/10.1016/S0140-6736\(04\)16678-6](https://doi.org/10.1016/S0140-6736(04)16678-6)

Lattanzi, R., Maftai, D., Petrella, C., Pieri, M., Sancesario, G., Schirinzi, T., ... Severini, C. (2019).

Involvement of the Chemokine Prokineticin-2 (PROK2) in Alzheimer's Disease: From Animal Models

to the Human Pathology. *Cells*, 8(11). <https://doi.org/10.3390/cells8111430>

LeCouter, J., Kowalski, J., Foster, J., Hass, P., Zhang, Z., Dillard-Telm, L., ... Ferrara, N. (2001).

Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature*, 412(6850),

877–884. <https://doi.org/10.1038/35091000>

LeCouter, J., Lin, R., Tejada, M., Frantz, G., Peale, F., Hillan, K., & Ferrara, N. (2003). The endocrine-

gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: Localization of Bv8

receptors to endothelial cells. *Proceedings of the National Academy of Sciences of the United States of*

America, 100(5), 2685–2690. <https://doi.org/10.1073/pnas.0337667100>

LeCouter, J., Zlot, C., Tejada, M., Peale, F., & Ferrara, N. (2004). Bv8 and endocrine gland-derived vascular

endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization. *Proceedings of*

the National Academy of Sciences of the United States of America, 101(48), 16813–16818.

<https://doi.org/10.1073/pnas.0407697101>

Li, J.-D., Hu, W.-P., Boehmer, L., Cheng, M. Y., Lee, A. G., Jilek, A., ... Zhou, Q.-Y. (2006). Attenuated

circadian rhythms in mice lacking the prokineticin 2 gene. *The Journal of Neuroscience : The Official*

Journal of the Society for Neuroscience, 26(45), 11615–11623.

<https://doi.org/10.1523/JNEUROSCI.3679-06.2006>

Li, Y., Zhou, T., Su, Y.-F., Hu, Z.-Y., Wei, J.-J., Wang, W., ... Zhang, H.-P. (2020). Prokineticin 2

overexpression induces spermatocyte apoptosis in varicocele in rats. *Asian Journal of Andrology*,

22(5), 500–506. https://doi.org/10.4103/aja.aja_109_19

- Lin, D. C.-H., Bullock, C. M., Ehler, F. J., Chen, J.-L., Tian, H., & Zhou, Q.-Y. (2002). Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. *The Journal of Biological Chemistry*, 277(22), 19276–19280. <https://doi.org/10.1074/jbc.M202139200>
- Maldonado-Pérez, D., Evans, J., Denison, F., Millar, R. P., & Jabbour, H. N. (2007). Potential roles of the prokineticins in reproduction. *Trends in Endocrinology and Metabolism: TEM*, 18(2), 66–72. <https://doi.org/10.1016/j.tem.2006.12.002>
- Manni, L., Aloe, L., & Fiore, M. (2009). Changes in cognition induced by social isolation in the mouse are restored by electro-acupuncture. *Physiology and Behavior*, 98(5), 537–542. <https://doi.org/10.1016/j.physbeh.2009.08.011>
- Martin, C., Balasubramanian, R., Dwyer, A. A., Au, M. G., Sidis, Y., Kaiser, U. B., ... Crowley, W. F. J. (2011). The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. *Endocrine Reviews*, 32(2), 225–246. <https://doi.org/10.1210/er.2010-0007>
- Masuda, Y., Takatsu, Y., Terao, Y., Kumano, S., Ishibashi, Y., Suenaga, M., ... Fujino, M. (2002). Isolation and identification of EG-VEGF/prokineticins as cognate ligands for two orphan G-protein-coupled receptors. *Biochemical and Biophysical Research Communications*, 293(1), 396–402. [https://doi.org/10.1016/S0006-291X\(02\)00239-5](https://doi.org/10.1016/S0006-291X(02)00239-5)
- Negri, L., & Ferrara, N. (2018). The Prokineticins: Neuromodulators and Mediators of Inflammation and Myeloid Cell-Dependent Angiogenesis. *Physiological Reviews*, 98(2), 1055–1082. <https://doi.org/10.1152/physrev.00012.2017>
- Negri, L., & Lattanzi, R. (2012). Bv8/PK2 and prokineticin receptors: a druggable pronociceptive system. *Current Opinion in Pharmacology*, 12(1), 62–66. <https://doi.org/10.1016/j.coph.2011.10.023>
- Negri, L., Lattanzi, R., Giannini, E., & Melchiorri, P. (2007). Bv8/Prokineticin proteins and their receptors. *Life Sciences*, 81(14), 1103–1116. <https://doi.org/10.1016/j.lfs.2007.08.011>
- Ngan, E. S. W., & Tam, P. K. H. (2008). Prokineticin-signaling pathway. *The International Journal of Biochemistry & Cell Biology*, 40(9), 1679–1684. <https://doi.org/10.1016/j.biocel.2008.03.010>

- Ostrowski, S., & Dobrowolski, J. C. (2014). What does the HOMA index really measure? *RSC Advances*, 4(83), 44158–44161. <https://doi.org/10.1039/c4ra06652a>
- Pasquali, D., Rossi, V., Staibano, S., De Rosa, G., Chieffi, P., Prezioso, D., ... Sinisi, A. A. (2006). The endocrine-gland-derived vascular endothelial growth factor (EG-VEGF)/prokineticin 1 and 2 and receptor expression in human prostate: Up-regulation of EG-VEGF/prokineticin 1 with malignancy. *Endocrinology*, 147(9), 4245–4251. <https://doi.org/10.1210/en.2006-0614>
- Samson, M., Peale, F. V. J., Frantz, G., Rioux-Leclercq, N., Rajpert-De Meyts, E., & Ferrara, N. (2004). Human endocrine gland-derived vascular endothelial growth factor: expression early in development and in Leydig cell tumors suggests roles in normal and pathological testis angiogenesis. *The Journal of Clinical Endocrinology and Metabolism*, 89(8), 4078–4088. <https://doi.org/10.1210/jc.2003-032024>
- Sarrate, Z., Vidal, F., & Blanco, J. (2010). Role of sperm fluorescent in situ hybridization studies in infertile patients: indications, study approach, and clinical relevance. *Fertility and Sterility*, 93(6), 1892–1902. <https://doi.org/10.1016/j.fertnstert.2008.12.139>
- Schirinzi, T., Maftai, D., Pieri, M., Bernardini, S., Mercuri, N. B., Lattanzi, R., & Severini, C. (2021, January). Increase of Prokineticin-2 in Serum of Patients with Parkinson's Disease. *Movement Disorders : Official Journal of the Movement Disorder Society*. <https://doi.org/10.1002/mds.28458>
- Sinisi, A. A., Asci, R., Bellastella, G., Maione, L., Esposito, D., Elefante, A., ... Iolascon, A. (2008). Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as reversible Kallmann syndrome and persistent oligozoospermia: case report. *Human Reproduction (Oxford, England)*, 23(10), 2380–2384. <https://doi.org/10.1093/humrep/den247>
- Spaziani, M., Granato, S., Liberati, N., Rossi, F. M., Tahani, N., Pozza, C., ... Radicioni, A. F. (2021). From mini-puberty to pre-puberty: early impairment of the hypothalamus-pituitary-gonadal axis with normal testicular function in children with non-mosaic Klinefelter syndrome. *Journal of Endocrinological Investigation*, 44(1), 127–138. <https://doi.org/10.1007/s40618-020-01281-x>
- Spaziani, M., Mileno, B., Rossi, F., Granato, S., Tahani, N., Anzuini, A., ... Radicioni, A. F. (2018). Endocrine and metabolic evaluation of classic Klinefelter syndrome and high-grade aneuploidies of sexual chromosomes with male phenotype: are they different clinical conditions? *European Journal of Endocrinology*, 178(4), 343–352. <https://doi.org/10.1530/EJE-17-0902>

- Spaziani, M., Tarantino, C., Pozza, C., Anzuini, A., Panimolle, F., Papi, G., ... Radicioni, A. F. (2020). Adverse pathophysiological influence of early testosterone therapy on the testes of boys with higher grade sex chromosome aneuploidies (HGAs): a retrospective, cross-sectional study. *Journal of Endocrinological Investigation*. <https://doi.org/10.1007/s40618-020-01452-w>
- Tarani, L., Carito, V., Ferraguti, G., Petrella, C., Greco, A., Ralli, M., ... Fiore, M. (2020). Neuroinflammatory Markers in the Serum of Prepubertal Children with down Syndrome. *Journal of Immunology Research*, 2020, 6937154. <https://doi.org/10.1155/2020/6937154>
- Thalhammer, S., Koehler, U., Stark, R. W., & Heckl, W. M. (2001). GTG banding pattern on human metaphase chromosomes revealed by high resolution atomic-force microscopy. *Journal of Microscopy*, 202(3), 464–467.
- Tore, F., Tonchev, A., Fiore, M., Tuncel, N., Atanassova, P., Aloe, L., & Chaldakov, G. (2007). From Adipose Tissue Protein Secretion to Adipopharmacology of Disease. *Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry*, 7(2), 149–155. <https://doi.org/10.2174/187152207780363712>
- Traboulsi, W., Brouillet, S., Sergent, F., Boufettal, H., Samouh, N., Aboussaouira, T., ... Alfaidy, N. (2015). Prokineticins in central and peripheral control of human reproduction. *Hormone Molecular Biology and Clinical Investigation*, 24(2), 73–81. <https://doi.org/10.1515/hmbci-2015-0040>
- Urayama, K., Guilini, C., Messaddeq, N., Hu, K., Steenman, M., Kurose, H., ... Nebigil, C. G. (2007). The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, 21(11), 2980–2993. <https://doi.org/10.1096/fj.07-8116com>
- Wang, H., Jia, Y., Yu, X., Peng, L., Mou, C., Song, Z., ... Li, X. (2021). Circulating Prokineticin 2 Levels Are Increased in Children with Obesity and Correlated with Insulin Resistance. *International Journal of Endocrinology*, 2021, 6630102. <https://doi.org/10.1155/2021/6630102>
- Wechselberger, C., Puglisi, R., Engel, E., Lepperdinger, G., Boitani, C., & Kreil, G. (1999). The mammalian homologues of frog Bv8 are mainly expressed in spermatocytes. *FEBS Letters*, 462(1–2), 177–181. [https://doi.org/10.1016/s0014-5793\(99\)01473-8](https://doi.org/10.1016/s0014-5793(99)01473-8)
- Wikström, A. M., Raivio, T., Hadziselimovic, F., Wikström, S., Tuuri, T., & Dunkel, L. (2004). Klinefelter

syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. *The Journal of Clinical Endocrinology and Metabolism*, 89(5), 2263–2270. <https://doi.org/10.1210/jc.2003-031725>

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Figure 1: PROK2 level (pg/ml) in the serum of healthy (n=8) and KS children (n=10) (panel A), of healthy (n=12) and KS adults (n=7 each group) (panel B) (KS-HYP = hypogonadal individuals; KS-EU = eugonadal individuals, KS-T = hypogonadal with replacement of testosterone). Panel C shows all groups analyzed by one-way ANOVA to better discriminate differences in PROK2 levels due to age. Data are presented as mean $\pm$ SEM. * $p<0.05$; ** $p<0.01$

Table 1

Statistics of the clinical, biochemical and hormonal parameters in the subjects enrolled in the study. Data are expressed as mean $\pm$ SEM (* $p < 0.05$; ** $P < 0.001$ vs healthy individuals in post-hocs).

Subjects and Data	BMI	Testosterone (nmol/L)	FSH (mU/mL)	LH (mU/mL)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	Glycemia (mg/dL)	Insulinemia (μ U/dL)	HOMA _i
Prepubertal:											
<i>Healthy</i> (n=8)	17.6 $\pm$ 3.2	0.094 $\pm$ 0.035	1.1 $\pm$ 0.2	0.35 $\pm$ 0.1	156.6 $\pm$ 18.9	29.2 $\pm$ 3.9	109.1 $\pm$ 20.1	90.2 $\pm$ 26	81.9 $\pm$ 7.3	8.2 $\pm$ 2.2	1.7 $\pm$ 0.5
KS (n=10)	24.2 $\pm$ 6.6	0.132 $\pm$ 0.042	2.5 $\pm$ 1.6	0.94 $\pm$ 0.56	134.6 $\pm$ 9.5	33.2 $\pm$ 1.2	90.9 $\pm$ 9.1	52.6 $\pm$ 4.8	63.8 $\pm$ 5.6	5.1 $\pm$ 1.1	0.8 $\pm$ 0.2
ANOVA	F(1,16)=0.68 p=0.42	F(1,16)=1.45 p=0.24	F(1,16)=0.58 p=0.45	F(1,16)=0.80 p=0.38	F(1,16)=1.19 p=0.29	F(1,16)=1.11 p=0.30	F(1,16)=0.79 p=0.28	F(1,16)=2.52 p=0.13	F(1,16)=3.99 p=0.062	F(1,16)=1.72 p=0.20	F(1,16)=3.29 p=0.088
Adult:											
<i>Healthy</i> (n=12)	23.8 $\pm$ 0.6	21.9 $\pm$ 1.9	3.8 $\pm$ 0.5	3.8 $\pm$ 0.5	127.2 $\pm$ 4.9	68.3 $\pm$ 4.9	42.5 $\pm$ 3.0	80.3 $\pm$ 10.6	83.7 $\pm$ 1.9	8.6 $\pm$ 1.8	1.8 $\pm$ 0.4
KS-HYP (n=7)	31.9 $\pm$ 3.2*	6.3 $\pm$ 1.3 **	21.7 $\pm$ 4.6**	14.2 $\pm$ 2.2**	176.5 $\pm$ 12.3*	54.7 $\pm$ 5.1	94.2 $\pm$ 10.5*	124.8 $\pm$ 10.4*	95 $\pm$ 2.4*	13.4 $\pm$ 3.2	3.2 $\pm$ 0.7
KS-EU (n=7)	23.9 $\pm$ 1.1	19.4 $\pm$ 2.0	23.7 $\pm$ 3.8**	12.8 $\pm$ 1.4**	189.3 $\pm$ 12.7*	54 $\pm$ 2.7	117.2 $\pm$ 9.2*	92.2 $\pm$ 10.1	87.7 $\pm$ 2.4	6.8 $\pm$ 1.3	2.6 $\pm$ 1.2
KS-T (n=7)	25.18 $\pm$ 1.3	17.4 $\pm$ 1.3	16.6 $\pm$ 5.8	8.8 $\pm$ 3.1	172.5 $\pm$ 7.6*	48.2 $\pm$ 3.4*	102.1 $\pm$ 7.2*	111.5 $\pm$ 10.1	85.2 $\pm$ 4.4	9.5 $\pm$ 1.6	2.1 $\pm$ 0.4
ANOVA	F(3,29)=5.10 p<0.01	F(3,29)=13.69 p<0.01	F(3,29)=8.65 p<0.01	F(3,29)=9.57 p<0.01	F(3,29)=11.19 p<0.01	F(3,29)=3.99 p<0.05	F(3,29)=25.78 p<0.01	F(3,29)=3.55 p<0.05	F(3,29)=3.44 p<0.05	F(3,29)=1.49 p<0.23	F(3,29)=0.77 p<0.51

Table 2

Spearman correlation between serum PROK2 levels (pg/ml) and different parameters in prepubertal individuals.

Subjects		Variables	Correlation coefficient (rho)	P value
PREPUBERTAL	Healthy (n=8)	BMI	-0.09524	0.8401
		Testosterone (nmol/L)	-0.5647	0.1548
		FSH (mU/mL)	0.5476	0.1710
		LH (mU/mL)	0.253	0.5411
		HDL (mg/dL)	-0.1078	0.8081
		LDL (mg/dL)	-0.8095	< 0.05*
		Cholesterol (nmol/L)	-0.7381	< 0.05*
		Triglycerides (mg/dL)	0.3571	0.3894
		Glycemia (mg/dL)	-0.3095	0.4618
		Insulinemia (μU/mL)	-0.3114	0.4505
		HOMAi	-0.4762	0.2431
	KS (n=10)	BMI	0.2242	0.5367
		Testosterone (nmol/L)	-0.6565	< 0.05*
		FSH (mU/mL)	-0.7697	< 0.05*
		LH (mU/mL)	-0.7915	< 0.01**
		HDL (mg/dL)	0.1341	0.7119
		LDL (mg/dL)	0.4061	0.2475
		Triglycerides (mg/dL)	0.03058	0.9379
		Cholesterol (nmol/L)	0.4195	0.2273
		Glycemia (mg/dL)	0.6	0.0734
		Insulinemia (μU/mL)	-0.04242	0.9184
		HOMAi	0.2606	0.4697

Table 3 Spearman correlations between serum PROK2 levels (pg/ml) and different parameters in adults.

Subjects		Variables	Correlation coefficient (rho)	P value
ADULTS	Healthy (n=12)	BMI	0.5919	< 0.05*
		Testosterone (nmol/L)	0.5175	0.0888
		FSH (mU/mL)	-0.6364	< 0.05*
		LH (mU/mL)	-0.5874	< 0.05*
		HDL (mg/dL)	0.1863	0.5597
		LDL (mg/dL)	-0.3713	0.2333
		Cholesterol (nmol/L)	0.07719	0.8124
		Triglycerides (mg/dL)	0	>0.9999
		Glycemia (mg/dL)	0.4061	0.1930
		Insulinemia (μ U/mL)	0.7483	< 0.01**
		HOMA _i	0.7483	< 0.01**
	KS-HYP (n=7)	BMI	0.5357	0.2357
		Testosterone (nmol/L)	-0.6429	0.1389
		FSH (mU/mL)	-0.1441	0.7571
		LH (mU/mL)	0.1071	0.8397
		HDL (mg/dL)	-0.75	0.0663
		LDL (mg/dL)	0.03571	0.9635
		Cholesterol (nmol/L)	-0.1786	0.7131
		Triglycerides (mg/dL)	0	>0.9999
		Glycemia (mg/dL)	0.5045	0.2548
		Insulinemia (μ U/mL)	-0.1786	0.7131
		HOMA _i	-0.1081	0.8190
	KS-EU (n=7)	BMI	-0.3929	0.3956
		Testosterone (nmol/L)	0.6429	0.1389
		FSH (mU/mL)	0.6786	0.1095
		LH (mU/mL)	0.2857	0.5560
		HDL (mg/dL)	0.2857	0.5560
		LDL (mg/dL)	-0.2857	0.5560
		Cholesterol (nmol/L)	-0.3571	0.4444
		Triglycerides (mg/dL)	-0.5714	0.2000
		Glycemia (mg/dL)	-0.8214	0.06
		Insulinemia (μ U/mL)	0.1071	0.8397
		HOMA _i	0.2143	0.6615
	KS-T (n=7)	BMI	-0.2857	0.5560
		Testosterone (nmol/L)	-0.1071	0.8397
		FSH (mU/mL)	0.4286	0.3536
		LH (mU/mL)	0.3571	0.4444
		HDL (mg/dL)	0.05406	0.9190
		LDL (mg/dL)	-0.5357	0.2357
		Cholesterol (nmol/L)	-0.6126	0.1579
		Triglycerides (mg/dL)	0.2857	0.5560
		Glycemia (mg/dL)	-0.6307	0.1413
		Insulinemia (μ U/mL)	-0.25	0.5948
		HOMA _i	-0.3929	0.3956