Expression of chemerin and its receptors in rat testes and its action on testosterone secretion

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Abstract

The novel adipokine chemerin plays a role in the regulation of lipid and carbohydrate metabolism, and recent reports of elevated chemerin levels in polycystic ovarian syndrome and preeclampsia have pointed to an emerging role of chemerin in reproduction. We hypothesised that chemerin, like other adipokines, may function to regulate male gonadal steroidogenesis. In this study, we show that chemerin and its three receptors chemokine-like receptor 1 (CMKLR1), G-protein-coupled receptor 1 (GPR1) and chemokine (C-C motif) receptor-like 2 were expressed in male reproductive tracts, liver and white adipose tissue. CMKLR1 and GPR1 proteins were localised specifically in the Leydig cells of human and rat testes by immunohistochemistry. The expression of *chemerin* and its receptors in rat testes was developmentally regulated and highly expressed in Leydig cells. *In vitro* treatment with chemerin suppressed the human chorionic gonadotropin (hCG)-induced testosterone production from primary Leydig cells, which was accompanied by the inhibition of 3 β -hydroxysteroid dehydrogenase gene and protein expression. The hCG-activated p44/42 MAPK (Erk1/2) pathway in Leydig cells was also inhibited by chemerin cotreatment. Together, these data suggest that chemerin is a novel regulator of male gonadal steroidogenesis.

Key Words

- ▶ chemerin
- ▶ steroidogenesis
- ▶ testosterone
- ▶ adipokine
- ▶ Leydig cell

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Introduction

Chemerin, also known as retinoic acid receptor responder protein 2 (RARRES2), was initially identified as a chemoattractant ligand for the G protein-coupled receptor (GPR), chemokine-like receptor 1 (CMKLR1, also known as ChemR23 or DEZ) (Meder *et al.* 2003, Wittamer *et al.* 2003, Zabel *et al.* 2005b). It was subsequently identified as a novel adipokine that regulates adipogenesis and adipocyte metabolism, with high expression levels in

white adipose tissue (Goralski *et al.* 2007). Chemerin is secreted as an 18-kDa inactive precursor protein and undergoes extracellular serine protease cleavage of the C-terminus to generate the 16-kDa active form (Wittamer *et al.* 2003, Zabel *et al.* 2005a). Chemerin is expressed at relatively high levels not only in liver, white adipose tissue and placenta, but is also present in skin, adrenal gland, all parts of the gut, pancreas, the airways and the kidney

(Wittamer *et al.* 2003, Goralski *et al.* 2007). Chemerin attracted great interest for its proposed roles in adaptive and innate immunity, inflammation, lipid and carbohydrate metabolism and its association with obesity and diabetes (Bondue *et al.* 2011). Elevated plasma chemerin levels are correlated with obesity and diabetes in both patients (Bozaoglu *et al.* 2007) and animal models (Ernst *et al.* 2010, Parlee *et al.* 2010).

To date, three receptors have been shown to bind chemerin: CMKLR1 (Meder *et al.* 2003, Wittamer *et al.* 2003, Zabel *et al.* 2005b), chemokine (C-C motif) receptor-like 2 (CCRL2; Zabel *et al.* 2008) and G protein-coupled receptor 1 (GPR1; Barnea *et al.* 2008); all of which are heptahelical receptors. Chemerin binding to CMKLR1 leads to internalisation of the chemerin–receptor complex and promotes leukocyte chemotaxis; chemerin binding to GPR1 stimulates receptor internalisation with no reported physiological roles (Barnea *et al.* 2008); chemerin binding to CCRL2 does not stimulate internalisation or chemotaxis, but might present chemerin to nearby cell displaying functional receptors (Monnier *et al.* 2012). The activation of chemerin/CMKLR1 resulted in intracellular calcium release, inhibition of cAMP accumulation and phosphorylation of MAPK ERK1/2 or the phosphatidylinositol 3-kinase/Akt pathway (Yoshimura & Oppenheim 2011).

The critical roles played by adipokines in fertility and reproduction have been recently reviewed (Hausman & Barb 2010, Poulos *et al.* 2010, Tersigni *et al.* 2011). For example, leptin antagonised the augmenting effect of several growth factors (insulin-like growth factor 1 (IGF1) and transforming growth factor) and hormones (insulin and glucocorticoids) on gonadotropin-stimulated steroidogenesis in both follicular and theca ovarian cells throughout the menstrual cycle (Spicer & Francisco 1998, Agarwal *et al.* 1999, Barkan *et al.* 1999, Brannian *et al.* 1999, Kitawaki *et al.* 1999, Zachow *et al.* 1999). Though most of the published research focused on the relevance of adipokines in female fertility and reproduction, several studies described the important roles of adipokines in testicular steroidogenesis as well. Leptin and adiponectin were both found to inhibit basal and human chorionic gonadotropin (hCG)-induced testosterone production in rat testis, while resistin-stimulated testosterone production in rat testis (Tena-Sempere *et al.* 1999, Nogueiras *et al.* 2004).

Recent reports point to a role of the novel adipokine chemerin in reproduction as well, focusing on female reproductive disorders. Polycystic ovary syndrome patients have elevated circulating and adipose tissue chemerin levels (Tan *et al.* 2009), preeclampsia patients

have elevated circulating chemerin levels (Duan *et al.* 2011, Stepan *et al.* 2011) and fetuses of obese mothers have increased chemerin concentrations, which correlates with maternal insulin sensitivity (Barker *et al.* 2012). Moreover, chemerin level has been quantified in rat placenta which has been found to be higher than in the liver (Garces *et al.* 2012). Expression of chemerin and its receptor CMKLR1 was reported in human granulosa cell, and chemerin treatment inhibited IGF1-induced progesterone and oestradiol (E₂) secretion (Reverchon *et al.* 2012). A similar suppressive effect of chemerin on FSH-stimulated progesterone and E₂ secretion was reported in rat granulosa cells (Wang *et al.* 2012). Although chemerin and its receptors are expressed in human and mouse testes (Wittamer *et al.* 2003, Takahashi *et al.* 2011), their biological actions in the male gonad remains unexplored. In this study, we characterised the cellular expression of chemerin and its receptors in rat testes and show that chemerin functions to suppress testicular steroidogenesis.

Materials and methods

Human sample and animals

Normal human testis paraffin sections were purchased from Pantomics, Inc. (San Francisco, CA, USA). Sprague–Dawley rats (postnatal 5-, 15-, 30-, 60- and 90-day-old) were obtained from the Laboratory Animal Center, Institutes of Biomedicine and Health, Chinese Academy of Sciences. All procedures related to animal usage were approved by the Committee on the Use of Live Animals for Teaching and Research, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences.

RNA analysis by QPCR

Total RNA from the tissues and cells was extracted using TRIzol Reagent (Invitrogen) and subjected to QPCR analysis. RNA samples (1 µg) were reverse transcribed into cDNA according to the manufacturer's instructions (Bio-Rad Laboratories). The PCR mixtures contained 10 µl SYBR Premix Ex Taq II (Takara, Shiga, Japan), 500 nM of each primer, 1 µl template cDNA and DNase-free water to a final volume of 20 µl. Cycle conditions were 95 °C for 10 s, followed by 45 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 30 s. The reaction was completed with a dissociation step for melting point analysis from 50 to 95 °C (in increments of 0.5 °C for 10 s each). The primers were designed on the basis of the published sequences of *chemerin* (forward, TGTGCAGTGGGCCTTCCA; reverse, CAAAGGTGCCAG

CTGAGAAGA), *Cmklr1* (forward, CAAGCAAACAGCCAC-TACCA; reverse, TAGATGCCGGAGTCGTTGTAA), *Gpr1* (forward, GGAGCTCAGC ATTCATCACA; reverse, GACAGGCTCTTGGTTTCAGC), *Ccr12* (forward, CTCTGCTTGTCCCTCGTGCTT; reverse, GCCCACTGTTGT-CCAGGTAG), and steroidogenic acute regulatory protein (*Star*; forward, CTGCTA GACCAGCCCATGGAC; reverse, TGATTTCCTTGACATTGCGGTCC), cytochrome P450 cholesterol side-chain cleavage (*Cyp11a1*; forward, CTATGCCATGGGTGCGAGAAT; reverse, CAGCACGTTGAT-GAGGAAGA), 3 β -hydroxysteroid dehydrogenase (*Hsd3b*; forward, AGCAAAA AGATGGCCGAGAA; reverse, GGCA-CAAGTATGCAATGTGCC) and *Hsd17b1* (forward, AATGTGCTTTC CATTTGCAAGGT; reverse, ATGC-CACTGGCAGAGGAGATG), β -actin (forward, GGAAATCG TGCCTGACATTA; reverse, AGGAAGGAAG GCTGGAA-GAG) and ribosomal protein L19 (*Rpl19*; forward, ATCGC-CAA TGCCAACTCC; reverse, TCATC CTTCT CATCCAGGTCA). The relative gene expression was normalised to *Rpl19* in the developmental study and to β -actin in the comparison between the whole testis and the Leydig cells of the 3-month-old rats. The RNA levels were calculated using the $\Delta\Delta CT$ method, where CT was the cycle threshold (Livak & Schmittgen 2001). Melt curve analysis for each primer set revealed only one peak for each product. The size of the PCR products was confirmed by comparing the size with a commercial ladder after agarose gel electrophoresis.

Immunohistochemistry

The testes were dissected from 3-month-old Sprague–Dawley rats after decapitation and then, fixed, processed for embedding in paraffin and sectioned. Normal human testis paraffin sections were purchased from Pantomics, Inc. Immunohistochemistry was carried out on 5 μ m sections of paraffin-embedded tissues with a Peroxidase-labelling Kit (Vector Laboratories, Burlingame, CA, USA). The antibodies used for the immunohistochemistry were mouse MAB to GPR1 (clone 043, gift from Drs B Zabel and E Butcher, Stanford University, USA), mouse polyclonal antibody to CCRL2 (ab88632, Abcam, Cambridge, MA, USA), goat polyclonal antibody to GPR1, goat polyclonal antibody to ChemR23 (CMKLR1), goat polyclonal antibody to chemerin (sc-48179, sc-32651, sc-47479 all from Santa Cruz). Staining was visualised using a DAB Substrate Kit for peroxidase (Vector Laboratories), counterstained with haematoxylin. The primary antibody was replaced with IgG from control sections to check for non-specific staining.

Primary Leydig cell culture

Leydig cells were isolated from the testes of 3-month-old sexually mature Sprague–Dawley male rats as described previously with some modifications (Li & Wong 2008). The testes from five rats were excised rapidly after decapitation and washed twice in 1 $\times$ PBS. The decapsulated testes were digested for 15 min by shaking at 80 cycles/min at 34 $^{\circ}$ C in DMEM/F12 Ham (1:1) (Gibco-BRL) containing 0.1% BSA and supplemented with 0.5 mg/ml collagenase type IA, 0.25 mg/ml soybean trypsin inhibitor (all from Sigma). To stop the digestion, ice-cold medium was added to the flask and the suspension was allowed to settle for 5 min. Then the supernatant containing Leydig cells was filtered through cell strainers (70 μ m nylon, Falcon BD Biosciences, Franklin Lakes, NJ, USA). The tubules were dispersed in another 50 ml medium, and the supernatant was pooled and centrifuged. Discontinuous Percoll (Amersham Biosciences) gradients (with six density fractions ranging from 1.030 to 1.096 g/ml) were used to separate the Leydig cells. The Leydig cells located at the boundary between fractions of 1.070 and 1.096 g/ml densities were collected and washed twice with ice-cold DMEM/F12–0.1% BSA medium. The collected Leydig cells were seeded in a NUNC 24-multiwell plate (Nunc, Roskilde, Denmark). The cells were pre-incubated in DMEM/F12–0.1% BSA at 34 $^{\circ}$ C in a humidified atmosphere of 5% CO₂/95% air. The Leydig cells were incubated for 24 h with one of the following treatments: 0.01 IU/ml of hCG (Sigma), 1, 10 and 100 nM recombinant chemerin (2324-CM-025, R&D Systems, Minneapolis, MN, USA), or the combination of hCG and chemerin or culture media only. The conditioned media were collected for testosterone measurement. The cells were lysed for QPCR analysis or western blot analysis. A batch of cells loaded in parallel were prepared and the cell purity was determined by 3 β -hydroxysteroid dehydrogenase (3 β -HSD) staining after 24 h pre-incubation.

Western blot

The cells were lysed by M-PER Mammalian Protein Extraction Reagent (Pierce, Thermo Scientific, Rockford, IL, USA). After 10 min incubation on ice, the cell lysates were centrifuged and the supernatants were assayed for protein concentration using a BCA Protein Assay Kit (Pierce, Thermo Scientific). The samples containing 30 μ g total protein were subjected to electrophoresis on NuPage 4–12% Bis–Tris gradient gels (Invitrogen). The gels were blotted onto the PVDF membranes (PerkinElmer Life Sciences, Boston, MA, USA). Western blot was conducted

using rabbit MAB to 3 β -HSD (1:5000, ab150384, Abcam), rabbit MAB to phospho-p44/42 MAPK (Erk1/2) (1:2000, #4370, Cell signaling, Boston, MA, USA), rabbit MAB to p44/42 MAPK (Erk1/2) (1:1000, #4695, Cell signaling), followed by an incubation with HRP-conjugated goat anti-rabbit IgG (1:8000, ab136817, Abcam). Specific bands were visualised with a chemiluminescent reagent (Western-lightening Plus, PerkinElmer Life Sciences). The blots were then washed in PBS and were re-probed with rabbit anti-actin serum (1:5000, Sigma). PageRuler Prestained Protein Ladder 10–170K (Pierce, Thermo Scientific) was loaded in a well next to the samples, fractionated on the same gel and transferred to the membrane. Sizes of the specific bands were determined with the prestained protein ladder band profile. Specific bands of a size consistent with that of the target proteins were detected by western blotting for 3 β -HSD (42 kDa), phospho-p44/42 MAPK (44, 42 kDa) and p44/42 MAPK (42, 44 kDa).

Testosterone measurements by RIA

Testosterone levels in the conditioned media were measured using commercial Iodine [¹²⁵I] RIA Kits (Lareneen, Guangzhou, China). The sensitivity of the testosterone RIA assay was 20 ng/ml. The intra-assay error and inter-assay error were <10 and 15% respectively.

Statistical analysis

All data are expressed as mean $\pm$ S.E.M., and statistical significance was assessed by either one-way ANOVA followed by Fisher's least significant difference test for *post-hoc* comparisons or Student's *t*-test (GraphPad Prism, San Diego, CA, USA). *P* < 0.05 was considered statistically significant.

Results

The expression and localisation of *chemerin* and its receptors *Cmklr1*, *Gpr1* and *Ccr12* in rat and human testes

Although *chemerin* RNA levels were relatively low in the adult testis (Fig. 1) compared with liver, epididymal fat, chemerin protein was detected in the cytoplasm of the interstitial Leydig cells and to a lesser extent in the spermatogonia and spermatocytes inside the seminiferous tubules (Fig. 2A). *Cmklr1* and *Gpr1* RNA were expressed at significantly higher levels in the testes compared with other tissues of the male reproductive system, as well as liver and epididymal fat (Fig. 1). Consistent with the high gene

Figure 1

Expression of *chemerin* and its receptors in male reproductive tissues. Analysis of relative gene expression of *chemerin*, *Cmklr1*, *Gpr1* and *Ccr12* by QPCR in adult rat male reproductive tissues including testis, epididymis, seminal vesicle, coagulating gland and prostate and using liver and epididymal adipose tissue as controls; β -actin served as the reference gene; *n* = 5–8; all data were expressed as mean $\pm$ S.E.M. **P* < 0.05 compared with epididymal fat, #*P* < 0.05 compared with testis for one-way ANOVA followed by Fisher's least significant difference test.

expression in the testis, antibodies against CMKLR1 and GPR1 stained interstitial Leydig cells in rat and human testes (Fig. 2). *Ccr12* RNA was expressed at relatively higher levels in the testes and seminal vesicles compared with other male reproductive tissues, or liver and epididymal fat (Fig. 1), although there was only weak to negligible CCRL2 immunostaining in human and rat testes (Fig. 2).

The expression of *chemerin* and its receptors *Cmklr1*, *Gpr1* and *Ccr12* in rat testes is developmentally regulated and highly expressed in Leydig cells

We next asked whether the expression of *chemerin* and its receptors in the testes changed over time during postnatal development. Testicular *chemerin* expression consistently decreased over time from the neonatal period to adulthood (Fig. 3). In contrast, expression of *Cmklr1*, *Gpr1* and *Ccr12* increased over this period – *Cmklr1* and *Gpr1* at the transitional period from pre-adolescence (day 15) to puberty (day 30) and *Ccr12* at day 60 (Fig. 3). The primary Leydig cells were isolated and the gene expression of *chemerin* and its receptors *Cmklr1*, *Gpr1* and *Ccr12* was compared with that of whole testis. Leydig cells in general showed a higher expression level of *chemerin* and its receptors *Cmklr1* and *Gpr1* (Fig. 4). Thus, in male

Figure 2

Immunolocalisation of chemerin and its receptors in rat and human testes sections. Chemerin and its receptors *CMKLR1* and *GRP1* were localised to interstitial Leydig cells and to a lesser extent in the spermatogonia and spermatocytes inside the seminiferous tubules. Parallel sections with

primary antibody replaced by non-immune serum serve as control (result not shown); scale bars, 0.05 mm; LC, interstitial Leydig cells; ST, seminiferous tubule; similar results were obtained with rats and human ($n=3$).

reproductive tissues, expression of *chemerin* and its receptors is developmentally regulated and is cell-type selective.

Chemerin suppresses testicular steroidogenesis accompanied by the inhibition of both 3β -HSD expression and p44/42 MAPK (Erk1/2) phosphorylation

The high expression of *chemerin* and its receptors in Leydig cells prompted us to explore the possible effects of chemerin on steroidogenesis by a direct *in vitro* study

of the Leydig cells. In cultured primary Leydig cells, 24 h incubation with hCG induced considerable secretion of testosterone (Fig. 5A), which was significantly suppressed by cotreatment with chemerin at doses of 1, 10 and 100 nM (Fig. 5A). HCG stimulation resulted in the upregulation of steroidogenic key factors including *Hsd3b* (Fig. 5B), *Star*, *Cyp11a1* and *Hsd17b1* (data not shown). The suppressive effect of chemerin on hCG-stimulated testosterone production was accompanied by the suppression of hCG-stimulated gene and

Figure 3

The relative gene expression of *chemerin* and its receptors during postnatal development in testes from 5-, 15-, 30-, 60- and 90-day old rats. $n=4-5$, L19 ribosomal protein mRNA served as the reference gene; all data were expressed as mean $\pm$ S.E.M.; * $P<0.05$ compared with day-5 and # $P<0.05$ compared with day-15 for one-way ANOVA followed by Fisher's least significant difference test.

protein expression of 3β -HSD (Fig. 5B and C), while leaving expression of *Star*, *Cyp11a1* and *Hsd17b1* unaffected (data not shown). Chemerin alone had no effect on basal testosterone secretion or 3β -HSD expression (Fig. 5A, B and C). Also cotreatment of chemerin inhibited the hCG-activated p44/42 MAPK (Erk1/2) pathway, while chemerin alone showed no effect on the phosphorylation of p44/42 MAPK (Fig. 6).

Discussion

At least two noteworthy observations have surfaced from our investigations on the role of chemerin and its receptors in reproduction. First, in male reproductive tissue, expression of *chemerin* and its receptors is highly regulated by developmental factors and is cell-type selective, with GPR1, CMKLR1 and chemerin protein highly expressed in the testes, specifically Leydig cells. Second, chemerin suppresses hCG-induced testosterone secretion by primary Leydig cells at 1, 10 and 100 nM and the suppression is accompanied by the inhibition of 3β -HSD expression and p44/42 MAPK (Erk1/2) pathway. The estimated plasma and serum concentrations of chemerin were 3.0 and 4.4 nM in human and 0.6 and 0.5 nM in mouse respectively (Zabel *et al.* 2006). The effective doses in our study were within the physiological levels. Our study is the first to report the expression of

the novel adipokine chemerin and its receptors CMKLR1 and GPR1 in human testis and to characterise its direct biological effects in mammalian male gonad.

Testicular expression of *chemerin* and its receptors appears to be under the regulation of developmental cues. The peri-puberty (30-day) period seems to be a switching point at which the *chemerin* level remains steady till adulthood while all the receptors increase after puberty. The importance of high *chemerin* level in neonatal rat remains to be explored while the increased receptors levels in post-puberty rat might suggest the growing importance of chemerin during adulthood. Actually our data indicated that chemerin suppressed the hCG-stimulated testosterone production, suggesting a regulatory role of chemerin in steroidogenesis of adult rats. Considering the expression of chemerin and its receptors in both the interstitium and seminiferous tubules, we cannot limit the biological functions of chemerin and its receptors to Leydig cells only. Its possible roles in spermatogenesis need more investigation. The decrease in *chemerin* gene expression level towards adulthood might be due to the presence of a feedback loop, i.e. the increase in the receptors (and therefore action) leading to a decrease in the ligand. The low protein level of CCRL2 in adulthood

Figure 4

The relative gene expression of *chemerin* and its receptors in rat Leydig cells and whole testicular tissue. β -actin served as the reference gene; $n=4-5$, all data were expressed as mean $\pm$ S.E.M.; * $P<0.05$ for Student's *t*-test.

Figure 5

In vitro suppressive effect of chemerin on steroidogenesis in rat Leydig cells. In primary Leydig cell culture, chemerin showed no effect on basal testosterone production, but suppressed hCG-induced testosterone production (A), gene expression of *Hsd3b* (B) and protein expression of 3β-HSD (C). For (B), β -actin served as the reference gene; each experiment was repeated at least three times; all data were expressed as mean $\pm$ S.E.M.; * $P < 0.05$ compared with control group with no chemerin and no hCG treatment; # $P < 0.05$ compared with hCG alone treatment group for one-way ANOVA followed by Fisher's least significant difference test.

needs to be confirmed and any discrepancy with the increase in *Ccr12* gene expression towards adulthood requires further investigation.

Among all the three receptors of chemerin, CMKLR1 and GPR1 are more likely to mediate the binding of chemerin in Leydig cells and direct its following signaling pathways. There was only weak to negligible CCRL2

immunostaining found in either human or rat testes compared with the strong staining of CMKLR1 and GPR1. This could be due to a lower avidity of the anti-serum used compared with the antisera for other receptors or a lower level of the receptor protein. There was also no significant difference in the gene expression levels of *Ccr12* between isolated Leydig cells and the whole testis. This could be the result of a decrease in *Ccr12* gene expression in the Leydig cells or an increase in other cell types in the testis. The chemotactic activity of chemerin was mediated by binding to CMKLR1 (Barnea *et al.* 2008) and no biological actions of chemerin/GPR1 or chemerin/CCRL2 was reported except that binding of chemerin to CCRL2 might present chemerin to nearby cell displaying functional receptors (Monnier *et al.* 2012). The activation of chemerin/CMKLR1 pathway has been reported to result in the inhibition of the phosphorylation of MAPK ERK1/2 (Yoshimura & Oppenheim 2011). In the primary Leydig cells of this study, the suppression of hCG-stimulated testosterone production by chemerin was accompanied by the inhibition of hCG-activated p44/42 MAPK (Erk1/2) pathway, suggesting that the activation of chemerin/CMKLR1 pathway is involved. The colocalisation of chemerin and its receptor CMKLR1 and GPR1 protein in Leydig cells of adult testes, the high expression level of chemerin in adjacent epididymis adipose tissue (Goralski *et al.* 2007) and the high circulating chemerin level reported (Zabel *et al.* 2006) suggest that the action of chemerin in testis could be autocrine, paracrine or endocrine mediating the binding of chemerin in Leydig cells and the subsequent signaling pathways. More investigations are needed to

Figure 6

Chemerin inhibited the hCG-activated p44/42 MAPK (Erk1/2) pathway in primary Leydig cells. In primary Leydig cells, chemerin alone showed no effect on the phosphorylation of p44/42 MAPK. HCG treatment significantly activated the p44/42 MAPK (Erk1/2) pathway which was inhibited by chemerin cotreatment.

identity which receptor(s) are responsible for the actions of chemerin in testis.

In summary, our data demonstrate the expression of chemerin in human and rat testes and provide the first evidence for the direct action of chemerin in testosterone secretion. This, together with the suppressive effects of chemerin on steroid hormone secretion from human (Reverchon *et al.* 2012) and rat (Wang *et al.* 2012) granulosa cells, suggesting that chemerin is a novel regulator of gonadal steroidogenesis.

Declaration of interest

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

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Author contribution statement

L L conceived and designed the experiments. L L, P M, C H, Y L, Y Z, C G and T X performed the experiments. L L analysed the data. J V Z, B A Z and P-G R contributed reagents/materials/analysis tools. L L and B A Z wrote the paper.

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