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GAMETE BIOLOGY

Isolation and expression of the human gametocyte-specific factor 1 gene (*GTSF1*) in fetal ovary, oocytes, and preimplantation embryos

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Abstract

Purpose Gametocyte-specific factor 1 has been shown in other species to be required for the silencing of retrotransposons via the Piwi-interacting RNA (piRNA) pathway. In this study, we aimed to isolate and assess expression of transcripts of the gametocyte-specific factor 1 (*GTSF1*) gene in the human female germline and in preimplantation embryos.
Methods Complementary DNA (cDNA) libraries from human fetal ovaries and testes, human oocytes and preimplantation embryos and ovarian follicles isolated from an adult ovarian cortex biopsy were used to as templates for PCR, cloning and sequencing, and real time PCR experiments of GTSF1 expression.
Results GTSF1 cDNA clones that covered the entire coding region were isolated from human oocytes and preimplantation embryos. GTSF1 mRNA expression was detected in archived cDNAs from staged human ovarian follicles, germinal vesicle (GV) stage oocytes, metaphase II oocytes, and morula and blastocyst stage preimplantation embryos. Within the adult female germline, expression was highest in GV oocytes. *GTSF1* mRNA expression was also assessed in human fetal ovary and was observed to increase during gestation, from 8 to 21 weeks, during which time oogonia enter meiosis and primordial follicle formation first occurs. In human fetal testis, *GTSF1* expression also increased from 8 to 19 weeks.
Conclusions To our knowledge, this report is the first to describe the expression of the human *GTSF1* gene in human gametes and preimplantation embryos.

Capsule The expression of transcripts of the human Gametocyte-Specific Factor 1 (*GTSF1*) gene were assessed in fetal gonads, the female germline and in preimplantation embryos.

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Introduction

The analysis of genes that are expressed in murine germ cells or whole ovaries has led to the identification of a novel gene, gametocyte-specific factor 1 (*Gtsf1*), also known as the computationally obtained undifferentiated and/or embryonic stem cell-specific 110 gene (Cue110) [1–3]. *Gtsf1* encodes a 167-amino acid protein that is a member of the Uncharacterized Protein Family 0224 (UPF0224).

Three recent reports have revealed that in *Drosophila*, gametocyte-specific factor 1 (DmGTSF1), is essential for P-element-induced wimpy testis (PIWI)-interacting RNA (piRNA)-mediated transcriptional repression and histone H3K9me3-mediated repression of transposons and their neighboring genes in the ovary [4–6]. DmGTSF1 interacts with Piwi via its C-terminal tail [4] and the two CHHC zinc-

finger motifs within the DmGTSF1 protein are required for its activity [5]. In the mouse, the *Gtsf1* gene is essential for spermatogenesis and has been revealed to function in transposon suppression in the mouse testes since increased expression of the long interspersed nucleotide element (line 1) and the intracisternal A-particle (IAP) retrotransposons were observed to occur in *Gtsf1*-null male mice [7]. The same study showed that *Gtsf1*-null male mice are sterile due to massive apoptosis of their germ cells and the cessation of meiotic progression before the zygotene stage. In humans, these observations are reflected by the observation that the testes of high infertility risk (HIR) cryptorchidism patients have reduced *GTSF1* expression and corresponding over-expression of LINE1 (L1) retrotransposons [8]. *GTSF1* has also been identified as a bovine spermatozoal transcript [9]. Elsewhere, *GTSF1* transcript expression has been observed to be up-regulated in certain leukemias and in lymphomas [10–14].

The precise role of the *Gtsf1* gene in the mammalian female germline is less clear since *Gtsf1*-null female mice were observed to be fertile [7]. The expression of *Gtsf1* transcripts has been detected in adult mouse primordial and primary follicles, whilst Gtsf1 protein has been detected in germ cell clusters and primordial follicles in newborn ovaries and in the oocytes of all follicle stages [3]. Significantly, *Gtsf1* was identified among the transcripts that were down-regulated in ovaries from mice deficient for the *Nobox* gene [2, 3]. Whilst not essential for fertility, a role for *Gtsf1* is implied during oogenesis and/or early embryonic development since other genes, with diverse functions during oogenesis, are also down-regulated in *Nobox*-deficient mice [15].

To our knowledge, this is the first report to describe cloning and expression analysis of *GTSF1* transcripts in human fetal ovaries and testis, adult human ovarian follicles, GV and metaphase II oocytes, and in preimplantation embryos.

Materials and methods

Samples

Human fetal ovaries and testes were obtained with informed consent, according to published methods [16]. Ethical approval was from the Lothian Research Ethics Committee (study code LREC 08/S1101/1). Briefly, human fetal gonads (gestations between 8 and 21 weeks) were obtained following medical termination of pregnancy and were subsequently snap-frozen and stored at −80 °C. RNA was extracted using the Qiagen RNeasy Mini Kit (14 weeks gestation onwards) or Qiagen RNeasy Micro Kit (8–12 weeks gestation) (Qiagen, Crawley, UK) with 500 ng RNA used for first-strand cDNA synthesis using the Superscript Vilo Reverse Transcriptase Master Mix (Life Technologies, Paisley, UK).

Methods for sample preparation of the cDNA libraries from human ovarian follicles and oocytes used in the present study were performed according to published methods [17, 18]. An ovarian cortex biopsy was donated for research by a 22-year-old woman attending Leeds General Infirmary hospital for gynaecological surgery under an ethically approved protocol as described [17]. Following harvest, the tissue was cryopreserved. Briefly, human ovarian follicles were isolated from the frozen-thawed ovarian cortex biopsy by dissection and staged according to size and morphology. Samples were collected from primordial follicles ($n = 28$), primordial/early primary follicles ($n = 45$), and primary follicles ($n = 7$), through to secondary stage follicles ($n = 7$). Diameters used for follicle staging were primordial follicles 34–38 mm, early primary follicles 34–53 mm, primary follicles 52–62 mm, and secondary follicles 62–86 mm. Mature metaphase II (MII) oocytes and preimplantation embryos that were surplus to requirement for clinical treatment were donated for research by patients attending the assisted conception unit at Leeds General Infirmary (Leeds, United Kingdom). Additionally, germinal vesicle (GV)-stage oocytes and cumulus and mural granulosa cells were harvested from nonluteinised antral follicles of 5 mm diameter that were aspirated from two patients during immature oocyte recovery as part of an in vitro maturation program as detailed in the protocol of Wynn et al. 1998 [19]. These oocytes and their surrounding granulosa cells had not been exposed to hCG prior to recovery. Cleavage stage embryos that were surplus to the patients' treatment needs were donated for research following embryo transfer on day 2, post insemination. Blastocysts were generated in vitro after a further 3–5 days of culture according to the methods detailed by Houghton et al. 2002 and, Ghassemifar et al. 2003 [20, 21]. Additionally, Cryopreserved embryos which were surplus to the patients' treatment requirements were donated for research under informed consent by couples attending Bourn Hall Clinic (Cambridge, United Kingdom). Cryopreserved embryos were thawed and cultured to the blastocyst stage in the HFEA licensed research laboratories in Leeds according to the methods of Houghton et al. (2002) and, Ghassemifar et al. (2003) [20, 21]. In summary, both fresh and frozen-thawed, cleavage staged preimplantation embryos were individually cultured in 4 μl droplets of Earle's Balanced Salt Solution, supplemented with 1 mM glucose, 5 mM lactate, 0.47 mM sodium pyruvate, 0.5 % (v/v) human serum albumin (Zenalb 20; Bio Products Laboratory), and amino acids at close-to-physiological concentrations, as defined by Tay et al. 1997 [22], under embryo tested mineral oil at 37 °C under 5 % CO_2 in air. At the end of culture, embryos were washed in Ca^{2+} and Mg^{2+} free phosphate buffered saline at 4 °C (Invitrogen, UK), before being snap frozen in liquid nitrogen in 20 μl

Dynal lysis buffer (Life Technologies, Ltd). All samples were obtained after informed consent under ethically approved protocols at the LGI, which were licensed in the UK by the Human Fertilisation and Embryology Authority.

Reverse transcription and cDNA amplification

Ovarian follicle samples, single embryos, and oocytes were collected and lysed at 80 °C in Dynal lysis buffer (Life Technologies, Ltd) and cDNA libraries were generated as previously described [23]. For the ovarian follicle samples as used in Fig. 1a, amplified cDNAs were generated from (i) pooled primordial follicles ($n = 28$), (ii) primordial/early primary follicles ($n = 45$), (iii) primary follicles ($n = 7$), and (iv), secondary stage follicles (n = 7).

Messenger RNA was extracted from preimplantation embryos with oligo-dT Dynabeads, and cDNA was generated using an adaptation of existing cDNA amplification protocols, using 1 μg each of primer (primer 1: 5′-aaacgacggccagtgaattgtaatacgactcactatagggcgct$_{24}$-3′ and primer 2: 5′-aagcagtggtatcaacgcagagtacgcggg-3′) and Superscript II RNAseH- Reverse Transcriptase (Life Technologies, Ltd) and associated reagents with incubation for 2 h at 42 °C. The cDNA was amplified by PCR using an additional 1 μg of each primer, 2 μl 50× Advantage 2 Polymerase (BD Clontech), in a thermal cycler for 32 cycles of 95 °C for 45 s, 65 °C for 6 min 45 s in a total volume of 50 μl.

Isolation of GTSF1 transcripts and expression analysis

Expression analysis for *GTSF1* was performed using 0.3 μl of amplified cDNA in a 12.5 μl reaction volume using BIOTAQ (BIOLINE). *GTSF1* primers (Table 1) were designed using the Primer3 programme (http://primer3.ut.ee/) and derived from human sequences found in GenBank (*GTSF1* AK098819; *GAPDH* AF261085, NM_144594.2). PCR was performed for 32 cycles for 45 s at each step of 94, 60, and 72 °C. The housekeeping gene (glyceraldehyde-3-phosphate
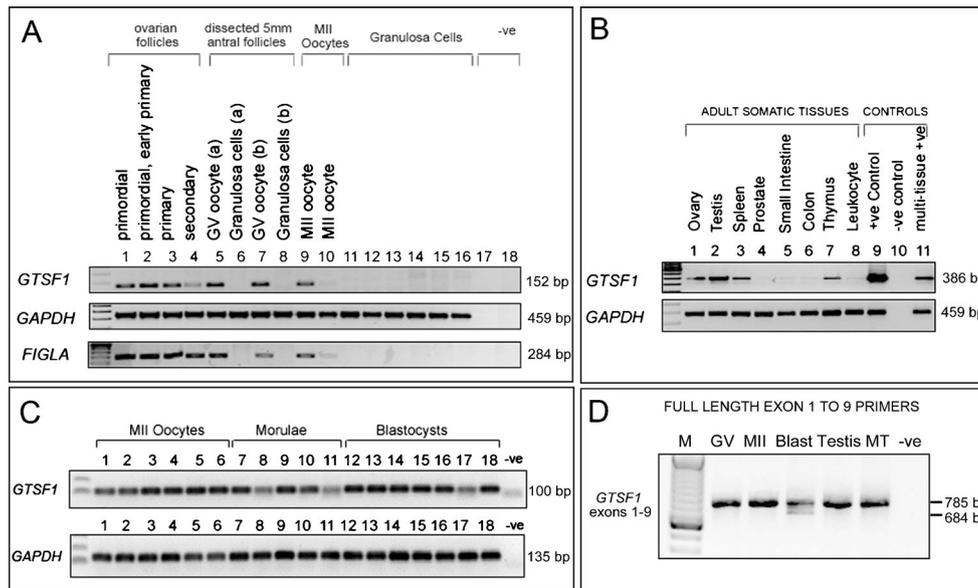
Fig. 1 Expression of *GTSF1* transcripts in the human female germline, preimplantation embryos, and adult tissues. **a** Expression of *GTSF1* (primers 7 F and 9R2: Size = 152 base pairs), *GAPDH*, and *FIGLA* gene transcripts by reverse transcription RT-PCR in amplified cDNA samples derived from human ovarian follicles, oocytes, and granulosa cells. The *FIGLA* PCR demonstrates the presence of oocyte cDNA in the ovarian follicle samples. Lane (1) Primordial follicles ($n = 28$ pooled follicles), (2) pooled primordial/early primary follicles ($n = 45$), (3) pooled primary follicles ($n = 7$), and (4) pooled secondary follicles ($n = 7$). Lanes (5) to (8) are cDNAs generated from two dissected 5 mm antral follicles (follicles a and b). Lane (5) Denuded single human GV stage oocytes isolated from the compact cumulus-enclosed oocyte complexes of a 5 mm non-luteinised antral follicle (follicle a), lane (6) granulosa cells isolated from follicle a. Lane (7) Denuded single human GV stage oocytes isolated from the compact cumulus-enclosed oocyte complexes of a 5 mm nonluteinised antral follicle (follicle b), lane 8 granulosa cells isolated from follicle b. Lanes (9) and (10) single MII oocytes, lanes 11 to 16 cDNA from cumulus granulosa cells isolated from 5–10 mm non-luteinised antral follicles containing failed fertilization metaphase II oocytes. Lanes (17) and (18) are negative PCR controls (no template, −ve). *Only weak PCR products for *FIGLA* and *GTSF1* were observed in one of the MII oocytes tested in this particular assay (lane 10). **b** Expression of *GTSF1* (primers 3 F and 7R) and *GAPDH* in cDNAs from adult human tissue (Clontech Multiple Tissue cDNA MTC panels) and controls. Lanes (1) ovary, (2) testis, (3) spleen, (4) prostate, (5) small intestine, (6) colon, (7) thymus, (8) leukocyte, (9) pooled GV oocyte (positive control, +ve), (10) negative control (no template, −ve), and (11) mixed adult human multiple tissue sample. **c** *GTSF1* RT-PCR (primers *GTSF1* real-time F and *GTSF1* real-time R), in individual human oocytes and preimplantation embryos. cDNAs were derived from pooled metaphase II oocytes ($n = 6$, lanes 1 to 6), morulae ($n = 5$, lanes 7–11), blastocysts ($n = 7$, lanes 12–18), and a negative control (−ve, no template). **d** *GTSF1* RT-PCR covering the entire coding region, from exons 1–9, in cDNAs derived from pooled germinal vesicle (GV) oocytes ($n = 6$), metaphase II oocytes ($n = 7$), blastocysts ($n = 7$), testis and mixed adult human multiple tissue samples (MT)

Table 1 PCR primers for conventional and real-time PCR

Gene	Primer name	Sequence 5′ to 3′
GTSF1	Exon1F	GGAGGAAGGTGACTGTGAGG
	Exon2F	CACTTGGATTCAGCTTCTTC
	Exon3F	GACCCTGAGAAGCTATTGCA
	Exon7F	CCCTGCGAGCAACATAGTTA
	Exon7R	TAACTATGTTGCTCGCAGGG
	Exon9R	CTGTATCAAAGGTTTATTTGGAAGC
	GTSF1 real-time F	ATTCAGCTTCTTCATTTCCAACA
	GTSF1 real-time R	CCTGATTTGATGGTTTTTGTCAT
FIGLA	*FIGLA* F1*	GATAAAAAATCTCAACCGTGG
	FIGLA R1*	CCCTCCTCTTCTTTCTTC
GAPDH	*GAPDH* F§	ACGGGAAGCTCACTGGCATGGC
	GAPDH R§	TCTTACTCCTTGGAGGCCATGTAGG
	GAPDH real-time F	TTGTCAAGCTCATTTCCTGGTAT
	GAPDH real-time R	TCTCTCTTCCTCTTGTGCTCTTG

FIGLA primers are from Huntriss et al. [17]

§ *GAPDH* primers are from Weisenberger et al. [24]

dehydrogenase) *GAPDH* was used as a positive control to demonstrate that the cDNA libraries that were generated from each sample were successful; *GAPDH* primers used in Fig. 1 a, b were taken from Weisenberger et al. 2002 [24]. The factor in the germline alpha (*FIGLA*) primers was from Huntriss et al. 2002 [17]. Products were run on 1.2 % agarose gels and visualised using ethidium bromide with reference to 100 base pair DNA size markers (Life Technologies, Ltd). Expression of the human *GTSF1* gene was also analysed using a range of normalized cDNA samples derived from various human tissues (Clontech MTC panels, Clontech).

Sequencing

GTSF1 PCR products that were obtained using various primer combinations were cloned into TOPO TA (Life Technologies). Primer sequences are given in Table 1. The M13 primer-amplified PCR products were sequenced at the Biomolecular Analysis Facility, University of Leeds. Sequences of PCR products were obtained in both directions and were identified by the Basic Local Alignment Search Tool (BLAST) http://www.ncbi.nlm.nih.gov/BLAST.

Real-time PCR

The real-time PCR reaction data was collected using an ABI PRISM 7900HT Real-Time PCR system using the SYBR Green method (Applied Biosystems). PCR primer sequences are described in Table 1. The 25 μl reaction mix contained 2 ng cDNA, 10 pmol of each primer. The PCR protocol was denaturation (95 °C for 10 min), amplification (94 °C 10 s,

60 °C 30 s, and 72 °C 30 s for 45 cycles). For quantitative assessment of *GTSF1* expression in the female germline, real-time PCR was performed on cDNA derived from primordial follicles, primordial/early primary follicles, primary follicles, secondary follicles, pooled cDNA from germinal vesicle (GV) stage oocytes ($n = 8$), pooled metaphase II oocytes ($n = 8$), and commercially available ovary and testis RNA (FirstChoice RNA, Ambion). Each sample was assessed in triplicate. Data are expressed as a percentage of the housekeeping gene *GAPDH* using the formula $2^{-\Delta CT} \times 100$, in which CT is the threshold cycle number. For real-time PCR analysis of *GTSF1* expression in human fetal ovary and testis experiments were performed as described previously [25], with the equivalent of approximately 2.5 ng of cDNA used per reaction in 10 μl reaction volumes. Significant changes in expression across gestation were determined by one-way ANOVA with Tukey's Multiple Comparison Test (GraphPadPrism 5.0 software). Due to the limiting amounts of cDNA that were available for ovarian follicle samples and human fetal gonads, we were unfortunately unable to assess further housekeeping genes across this series of samples as controls.

Results

Isolation and expression of GTSF1

A partial sequence of the *GTSF1* gene was originally isolated during differential display analysis from cDNAs derived from staged human ovarian follicles (J Huntriss, D Miller, HM Picton unpublished data). BLAST searches with this sequence, that were performed prior to the release of the *GSTF1* gene

sequence (for any species) in any gene database, initially identified a 100 % match over 138 nucleotides of readable sequence to the human sequences AK057504 and AK098819, both testis-derived transcripts. On translation, the partial sequence exhibited similarity to the Xenopus D7 oocyte protein that is represented by P13007 and NP_001081517 protein sequences. The human sequence was chosen for further study by virtue of the fact that it likely represented a novel human oocyte transcript with sequence similarity to the Xenopus D7 protein gene. Analysis by reverse transcription (RT)-PCR with gene-specific primers encompassing the partial sequence from AK057504 in samples derived from the adult human female germline indicated a pattern of expression consistent with being derived from oocytes (Fig. 1a). Figure 1a shows that amplicons were generated in ovarian follicle cDNAs (primordial through to secondary stages), germinal vesicle-stage (GV) oocytes, and metaphase II (MII) oocytes, and therefore in the same samples that we detected transcripts of *FIGLA*, another transcript observed in oocytes [17].

Subsequent BLAST searches identified the partial sequence as a fragment of novel gene, *FAM112B* (family with sequence similarity 112 member B) mapping to chromosome12q13.2. The *FAM112B* gene encodes a member of an uncharacterized protein family containing a conserved protein domain UPF0224. The gene has been more recently assigned the approved name Gametocyte-specific factor 1 and symbol (*GTSF1*), (GeneID: 121355, Ensembl gene ENSG00000170627). We performed further characterisation of the expression of *GTSF1* using primers designed from exons predicted in current *GTSF1* mRNA sequence NM_144594.2 and the related testis-derived mRNA sequences (AK057504 and AK098819) that pre-dated the NM_144594.2 sequence submission. Expression of *GTSF1* was examined across a range of normalized human cDNA samples utilizing primers from exons 3 and 7 of NM_144594.2 (Fig. 1b) and revealed expression in the ovary, testis, spleen thymus, and in the positive controls (cDNA from pooled GV oocytes (lane 9) and mRNA from multiple-tissues (lane 11). Expression of *GTSF1* transcripts was also assessed in a series of individual MII oocytes and in preimplantation embryos (Fig. 1c). *GTSF1* expression was observed in all tested samples: six additional single human MII oocytes, five morulae, and seven blastocysts. A PCR assay covering the entire coding region was designed upon the release of the NM_144594.2 sequence, utilizing primers designed from *GTSF1* exons 1 and 9. This assay amplified the appropriate-sized PCR product in samples of pooled GV oocytes, pooled MII oocytes, pooled blastocysts, testis, and mixed human adult tissues (Fig. 1d). In addition, a second smaller transcript was observed in blastocysts.

GTSF1 gene and protein sequence

Human *GTSF1* gene cDNA clones that were obtained from human oocytes and blastocysts were sequenced and data is shown in Supplemental Figure 1. This experimentally-defined transcript sequence spans the entire coding region sequence from exon 1 to 9 and the isolated sequence, and its translation, are in full agreement with the sequences NM_144594.2 and NP_653195 and the gene structure as represented by Ensembl GTSF1-001 splice variant that contains 9 exons (Transcript ID: ENST00000305879). The two CHHC zinc-finger domains (TRM13/UPF0224_CHHC_Znf_dom) are identified in Supplemental Figure 1. In blastocysts, the smaller transcript identified in Fig. 1d, that lacks exon 3, most likely represents the GTSF1-004 transcript (Transcript ID: OTTHUMT00000406189), a processed transcript that has no protein product and matches to ESTs (602638807 F1, 602552556 F1) that are derived from mucoepidermoid carcinoma and embryonal carcinoma, respectively. The *GTSF1* cDNA sequences that were isolated from the PCR products shown in Fig. 1d, were subsequently aligned on the browser (Supplemental Figure 2) using the BLAT tool on the UCSC browser (https://genome.ucsc.edu/FAQ/FAQblat.html). Shown are the blastocyst-specific *GTSF1* transcript variant lacking exon 3 (Track 1), the *GTSF1* transcript sequence as isolated from oocytes, blastocysts and all other stages of oogenesis and pre-implantation development (Track 2) and the UCSC gene (compiled from RefSeq, GenBank and other sources-Track 3).

Real-time PCR analysis of *GTSF1* expression in human fetal gonads and the adult female germline

Quantitative measurement of *GTSF1* expression was determined by real-time PCR relative to the housekeeping control gene *GAPDH*. *GTSF1* expression was assessed in the human fetal ovary and in the human fetal testis at gestations of 8–11 weeks, 14–16 weeks, and 17–21 weeks, using 4–5 samples per group (Fig. 2a). Over these periods, *GTSF1* expression was observed to increase in both the ovary and testis. Significant changes in expression across gestation were determined by one-way ANOVA with Tukey's Multiple Comparison Test (GraphPadPrism 5.0 software). Thus, ovary fold changes were 8–11 weeks vs. 14–16 weeks: 4-fold increase ($P < 0.01$) and 8–11 week vs. 17–21 weeks, 6.4-fold increase ($P < 0.001$) and 14–16 weeks vs. 17–21 weeks, and 1.6-fold increase ($P < 0.01$). Testis fold changes were 8–9 weeks vs. 14–16 weeks, 5.8-fold increase (not significant), 8–9 weeks vs. 17–19 weeks, 17.4-fold increase ($P < 0.01$) and 14–16 weeks vs. 17–19 weeks, and 3-fold increase ($P < 0.05$). Next, the expression of *GTSF1* during the ovarian follicle growth phase and final stages of oocyte maturation in the adult female ovary was approximated with a series of archived cDNAs [17] from the female germline that included staged ovarian follicles (primordial, primordial/early primary, primary follicles, and secondary follicles) and also included pooled cDNAs derived from GV-staged oocytes and MII oocytes
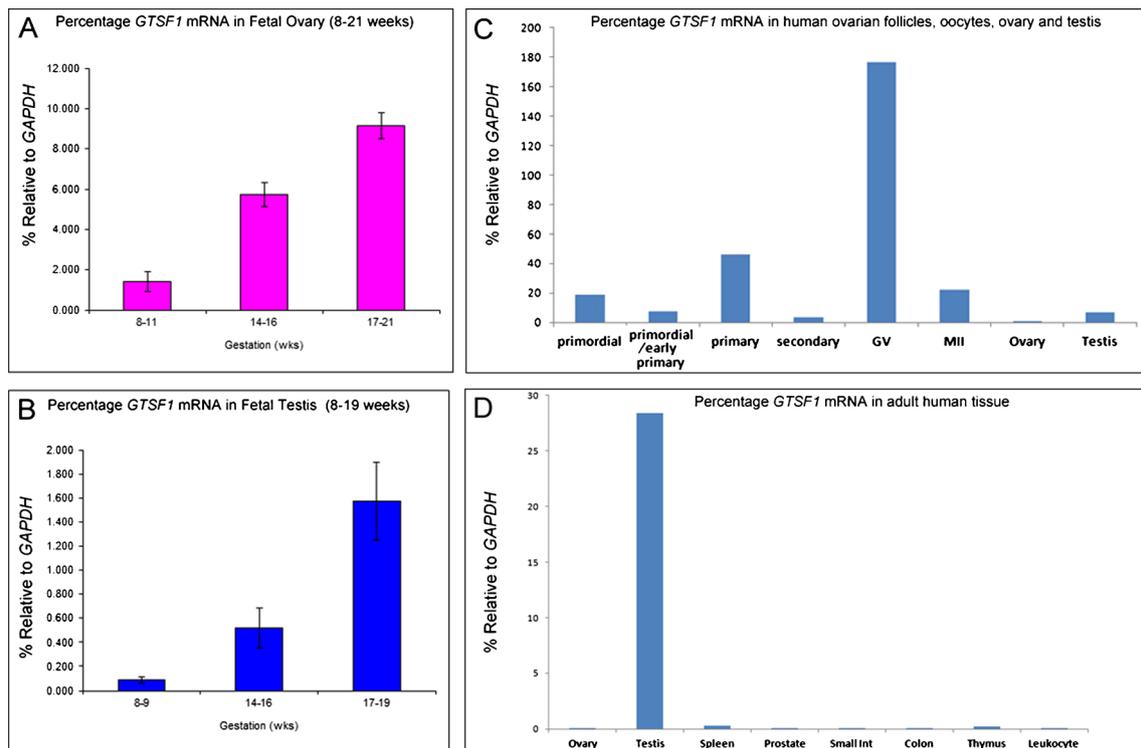
Fig. 2 Real-time PCR assessment of *GTSF1* expression. Real-time PCR assessment of *GTSF1* expression is expressed as a percentage of the housekeeping gene *GAPDH* and using primers *GTSF1* real-time F and R and *GAPDH* real-time F and R. **a** *GTSF1* expression in human fetal ovaries at different stages of gestation (total tested range was between 8 and 21 weeks) that were grouped into 8–11 weeks, 14–16 weeks, and 17–21 weeks of gestation, n = 4–5 samples per group. *Bars* indicate mean ± sem. **b** *GTSF1* expression in human fetal testis at different stages of gestation (total tested range was between 8 and 19 weeks) that were grouped into 8–9 weeks, 14–16 weeks, and 17–19 weeks of gestation,

n = 4 samples per group. **c** *GTSF1* expression by real-time PCR in cDNAs from human ovarian follicles (follicle number per sample as described in Fig. 1a) and pooled germinal vesicle (GV) oocytes (n = 8), pooled metaphase II oocytes (n = 8), and adult gonads. Samples left to right: primordial follicles, primordial/early primary follicles, primary follicles, secondary follicles, pooled cDNA from germinal vesicle (GV) stage oocytes (n = 8), pooled metaphase II oocytes (n = 8), human ovary (Ambion), and human testis (Ambion). **d** *GTSF1* expression by real-time PCR in cDNAs from human adult human tissue (Clontech Multiple Tissue cDNA panels, tissues as shown in Fig. 1b) and controls

(Fig. 2b). For comparison, cDNAs derived from adult human ovary and testis were included. *GTSF1* expression was detected in all ovarian follicles, and germinal vesicle-stage oocytes and metaphase II oocytes with the highest expression relative to *GAPDH* observed in germinal vesicle-stage oocytes. Finally, real-time PCR was performed using the same range of cDNA samples from adult human tissue as shown in Fig. 1b to reveal that expression of *GTSF1* was highest in the testis (Fig. 2c).

Conclusions

In this report, we have described the expression of the human *GTSF1* gene from cDNAs derived from fetal gonads, adult human ovarian follicles, GV oocytes, metaphase II oocytes, and preimplantation embryos. The main *GTSF1* transcript isolated from oocytes and preimplantation embryos corresponds to the GTSF1-001 transcript variant of *GTSF1* that encodes a 167 amino acid protein. *GTSF1* is a highly conserved gene that maps to 12q13.2 and has 91 % identity between the

mouse and human proteins in agreement with earlier studies [1, 3, 26], with the high conservation indicative of a common function across species.

In cDNAs derived from ovarian follicles that were isolated from the adult ovary, transcripts of *GTSF1* are detected from the primordial follicle stage and are expressed through to the metaphase II oocyte stage. Real-time PCR revealed that highest expression was observed in GV oocytes, with a subsequent decrease in MII oocytes, in agreement with data for mouse *Gtsf1* gene transcripts (http://www.ncbi.nlm.nih.gov/geoprofiles/29792642). Our PCR expression data reveals that *GTSF1* is also expressed during human preimplantation development, being detected in late preimplantation embryos. These observations indicate that expression is activated after zygotic gene activation, and this was supported by our observation of a blastocyst-specific transcript variant of *GTSF1* lacking exon 3. Our expression data for mature oocytes and preimplantation embryos are consistent with that described in the Human Embryo Resource HumER [27]. Our expression data for somatic tissues is consistent with microarray and RNAseq data from other sources

that indicate highest expression of the *GTSF1* transcripts within adult tissues occurs in the testis (http://biogps.org/ and http://www.ebi.ac.uk/). Our expression data for oocytes and preimplantation embryos is consistent with microarray data from http://www.ncbi.nlm.nih.gov/geoprofiles (geoprofile IDs 74253766 and 52847566). We were unable to perform analysis of the expression GTSF1 protein in our laboratory; however, GTSF1 protein expression has been described by the Human Protein Atlas ([28]: http://www.proteinatlas.org/ENSG00000170627-GTSF1/tissue). In agreement with our data for the *GTSF1* transcript, this resource reveals that GTSF1 protein (and RNA) expression is largely exclusive to the male and female reproductive tissues. Protein expression is described as being medium in ovarian follicles (but undetectable in ovarian stroma) enriched in the testis, but weakly expressed or undetectable in any other adult human tissues.

Here, we have reported for the first time the elevation of expression of *GTSF1* gene transcripts at the time of entry into meiosis and subsequent primordial follicle formation in the human fetal ovary. This may suggest a particular requirement for *GTSF1* during ovarian programming and early oogenesis and also in GV oocytes in particular. In the male germ line, there is now clear evidence that Piwi-mediated retrotransposon suppression is essential for fertility [29–33], and this includes a requirement for *Gtsf1* in this process [7]. Failure to suppress retrotranposons may affect genomic stability and lead to disease [34–36], and retrotransposons are abundant in germ cells and preimplantation embryos [37–42]. In contrast to the infertility observed in *Gtsf1*-null male mice, *Gtsf1*-null female mice were observed to be fertile with no histological abnormalities in the ovaries [7]. Retrotransposons are known to be silenced in murine primordial ovarian follicles via a piRNA-dependent mechanism, and this is believed to occur in nuage-like structures in the oocytes of primordial follicles [43]. However, mice with nuage-mutant primordial follicles defective for *Mvh*, *Mili*, or *Gasz* remain fertile, despite elevated transposon expression [43]. These studies, which reflect the observations of male-specific sterility vs. female fertility in *Gtsf1*-null mice [7], suggest that the PIWI-pathway in isolation may not be critical for female fertility. Retrotransposon suppression, at least that which is critical for female fertility, may be regulated in the mammalian female germ line by Meiosis arrest female 1 (MARF1) and accordingly, *Marf1* mutant females are infertile [44, 45].

We acknowledge that there are a number of shortcomings in our study. Firstly, for some of the samples, particularly the human ovarian follicles and fetal gonads, we were unable to obtain real-time PCR data for additional housekeeping genes across all stages because of the extremely limiting amounts of cDNA that were available to us. Accordingly, the real-time expression data is only normalised to one housekeeping gene, *GAPDH*. Secondly, we were only able to obtain ovarian follicles from a single patient for this study and therefore, we are not able to confirm whether the *GTSF1* transcript expression patterns

observed here are representative of the patterns that would be observed in a wider range of samples from different patients.

Currently, the role of *GTSF1* during mammalian oogenesis remains unclear. The function of *GTSF1*/*Gtsf1* has also been investigated by bioinformatic analysis which has revealed that the two U11-48 K-like CHHC-type zinc finger domains (Pfam: PF05253) within the human GTSF1 protein are found in spliceosomal proteins and tRNA modifying enzymes are predicted to have an RNA binding function [26, 46]. Indeed, the CHHC zinc-finger domain has been observed to specifically bind the 5′ splice site of U12-type introns in spliceosomal U11-48 K proteins [46]. We hypothesize however that although *Gtsf1* appears dispensable for female fertility in mice [7], the retention of the expression of *GTSF1*/*Gtsf1* in the female germline across several species including mouse [1, 3], human (this report), and bovine and ovine oocytes (H.M.Picton, J.Huntriss, J.Lu, G.Liperis- unpublished data, [47, 48]) is indicative of a retained function in the mammalian oocyte and/or early embryo. Of particular interest, the abundance of the GTSF1 protein in murine MII oocytes was observed to be significantly reduced upon postovulatory-ageing [49]; however, further experiments are required to understand the role of this gene in the mammalian oocyte.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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