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Prostaglandins Leukot Essent Fatty Acids. Author manuscript; available in PMC 2022 July 04.

Published in final edited form as:
Prostaglandins Leukot Essent Fatty Acids. 2021 February ; 165: 102235. doi:10.1016/j.plefa.2020.102235.

Deletion of the Gene Encoding Prostamide/Prostaglandin F synthase Reveals an Important Role in Regulating Intraocular Pressure

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Abstract

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Declarations of interest
None, for all authors

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Prostamide/prostaglandin F synthase (PM/PGFS) is an enzyme with very narrow substrate specificity and is dedicated to the biosynthesis of prostamide $F_{2\alpha}$ and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The importance of this enzyme, relative to the aldo-keto reductase (AKR) series, in providing functional tissue prostamide $F_{2\alpha}$ levels was determined by creating a line of PM/PGFS gene deleted mice. Deletion of the gene encoding PM/PGFS (*Fam213b* / Prxl2b) was accomplished by a two exon disruption. Prostamide $F_{2\alpha}$ levels in wild type (WT) and PM/PGFS knock-out (KO) mice were determined by LC/MS/MS. Deletion of *Fam213b* (Prxl2b) had no observed effect on behavior, appetite, or fertility. In contrast, tonometrically measured intraocular pressure was significantly elevated by approximately 4 mmHg in PM/PGFS KO mice compared to littermate WT mice. Outflow facility was measured in enucleated mouse eyes using the *iPerfusion* system. No effect on pressure dependent outflow facility occurred, which is consistent with the effects of prostamide $F_{2\alpha}$ and $PGF_{2\alpha}$ increasing outflow through the unconventional pathway. The elevation of intraocular pressure caused by deletion of the gene encoding the PM/PGFS enzyme likely results from a diversion of the endoperoxide precursor pathway to provide increased levels of those prostanoids known to raise intraocular pressure, namely prostaglandin D_2 (PGD_2) and thromboxane A_2 (TxA_2). It follows that PM/PGFS may serve an important regulatory role in the eye by providing $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ to constrain the influence of those prostanoids that raise intraocular pressure.

Keywords

prostaglandins; prostamide/prostaglandin F synthase; *Fam213b* ; Prxl2b; prostamide $F_{2\alpha}$; prostaglandin $F_{2\alpha}$; intraocular pressure

1. INTRODUCTION

The prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogs are established as first line therapies for treating glaucoma. The selective FP receptor agonists were designed by virtue of the need to attenuate the ocular side effects produced by the naturally occurring prostanoid $PGF_{2\alpha}$ [1-3]. Similarly, bimatoprost is an analog of $PGF_{2\alpha}$ ethanolamide (prostamide $F_{2\alpha}$) [4, 5]. Both $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ are the products of identical biosynthetic pathways but differ with respect to their cyclo-oxygenase substrate. Thus, arachidonic acid is the primary precursor for $PGF_{2\alpha}$, whereas prostamide $F_{2\alpha}$ is biosynthesized from the naturally occurring mammalian endocannabinoid anandamide [6]. Following oxidation of arachidonic acid or anandamide, the respective endoperoxide intermediates PGG_2/PGH_2 and prostamides G_2/H_2, are converted to the active end-products by PGD, PGE, PGF, prostacyclin and thromboxane synthases [7, 8]. The first enzyme identified and named as a PGF synthase [PGFS] was an aldo-keto reductase (AKR)1C3 with broad substrate specificity [9-13]. More recently, a much more substrate-specific enzyme and a member of the thioredoxin-like superfamily was discovered and designated prostamide/prostaglandin F synthase [14,15], PM/PGFS, (*Fam213b* / Prxl2b). This enzyme facilitates a more restricted and targeted formation of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$, thereby conferring tighter control of regulatory function(s). Gene deletion is a classical approach for discovering biological functions and the first results of these studies are presented herein, together with the methodology for creating prostamide/prostaglandin F synthase (*Fam213b* / Prxl2b) knock-out mice.

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Given the importance of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ derived drugs in the treatment of glaucoma, a possible role for the parent molecules and their biosynthetic enzymes in the regulation of intraocular pressure seemed likely to exist. PGF synthase activity has been reported in rat ocular tissues [16] and more recently ocular prostamide F synthase activity was reported in mouse eyes [14]. The relative importance of the AKR series of PGFSs and PM/PGFS [15] was obscure. The role of PM/PGFS in regulating intraocular pressure was investigated by functional studies on intraocular pressure in mice where the gene encoding the enzyme was deleted.

2. METHODS

2.1 Creation of prostamide/prostaglandin F synthase [*Fam213b*/ Prxl2b] knock-out mice.

The prostamide/prostaglandin F synthase (PM/PGFS) knock-out mouse was generated using the RENKA embryonic stem (ES) cell line, derived from C57BL/6N mice [17]. To construct a PM/PGFS targeting vector, a 990 bp DNA fragment carrying exons 2 and 3, containing the enzyme active site of PM/PGFS, was amplified by PCR, and inserted into the targeting vector as described previously [18]. In this clone, a DNA fragment of a PGK promoter-driven neo-poly (A) cassette was flanked by two Frt sites. These were flanked by two LoxP sites located 139 bp upstream of exon 2 and 298 bp downstream of exon 3. The 5' side included 5.7 kb of the PM/PGFS gene and the 3' side 6.5 kb, followed by an MC1 promoter-driven diphtheria toxin (DTA) gene (Fig. 1A). The targeting vector was introduced into RENKA ES cells by electroporation and recombinant clones identified by Southern blot hybridization (Fig. 1B). To produce germline chimeras, recombinant ES clones were microinjected into eight cell stage embryos of CD1 mice. To generate PM/PGFS knock-out mice, chimeric mice were crossed with TLCN-Cre mice, by which recombination was introduced throughout the whole body [19].

All procedures on living mice were carried out in compliance with the Imperial College Statement for Use of Animals in Research, under UK home Office project license 70/9064 and in accordance with the ARRIVE guidelines and the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. Mice were housed in individually ventilated cages with access to food and water ad libitum, maintained at 21°C with a 12-hour light/dark cycle. Heterozygous mice were used for breeding and all pups genotyped prior to usage. Genotyping was carried out on DNA extracted from ear tissue following manufacturer's protocols (Express Extract, Kapa Biosystems, Cambridge, MA, USA). TaKaRa Ex Taq polymerase [RR001, Takara Biotech, Shiga, Japan] was used for PCR reactions, forward primer [CAACCAGTCTAACTAGGCT] and reverse primer [CATGTGACCTGAACCCCCT] were cycled 30 times with an annealing temperature of 65°C to yield predicted products of 650 bp for knock-outs and 1500 bp for wild-types. PCR products were resolved by gel electrophoresis (1% agarose) in the presence of a DNA gel stain (SYBR Safe, Invitrogen, Carlsbad, CA, USA). Bands were visualized on an imaging station (Biospectrum 500, UVP, Upland, CA).

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2.2 Quantitative real-time PCR

PM/PGFS knock-out, male mice (n=13) and littermate wild type mice of both sexes (n=15), 10 to 15 weeks old, were euthanized by cervical dislocation and harvested for eyes and approximately half were also harvested for inguinal, and gonadal white fat pads. Pairs of eyes were cleared of extraocular tissue, hemisected at the equator and lenses removed prior to homogenization in TRIzol Reagent (ThermoFisher Scientific, Waltham, Massachusetts, USA) with a rotor-stat homogenizer (VDI 12, VWR, Leicestershire, UK). Non-ocular fat pads were placed in TRIzol immediately after dissection, shortly followed by homogenization. Total RNA was extracted using PureLink RNA spin columns and DNase I treated following the manufacturer's protocol (ThermoFisher Scientific). RNA yield was determined using a NanoDrop ND-1000 spectrophotometer and Superscript VILO reverse transcriptase (ThermoFisher Scientific) was used to synthesize cDNA, following manufacturer's instructions. qPCR was carried out on 10 ng of starting RNA using SYBR select master mix (ThermoFisher Scientific]. PM/PGFS primers (Table 1) were designed with AlleleID software [Premier Biosoft]. cDNA was analyzed in triplicate on the QuantStudio 6 Flex (Applied Biosystems, ThermoFisher Scientific) Real-Time PCR system. GAPDH was used as the reference gene, RNA content was determined by the delta Ct method of comparative quantification (Ct (reference gene) - Ct [target gene]).

2.3 Measurement of tissue Fatty Acid Amide levels by LC/MS/MS

PM/PGFS knock-out mice (n=5) and littermate wild type mice (n=5), 10 to 15 weeks old and of both sexes, were humanely euthanized by cervical dislocation and harvested for eyes and inguinal and gonadal white fat pads. For quantification of prostamide $F_{2\alpha}$ and other fatty acid amides, the procedures were essentially as previously described [20, 21]. Briefly, the excised tissues were dounce-homogenized and extracted with acetone containing internal deuterated standards for anandamide (AEA), palmitoyl ethanolamide (PEA), oleoyl ethanolamdie (OEA) and prostaglandin $F_{2\alpha}$ ethanolamide (prostamide $F_{2\alpha}$), with quantification by isotope dilution ($[^2H]_8$AEA, $[^2H]_4$ PEA, $[^2H]_4$ OEA (Cayman Chemicals, MI, USA); $[^2H]_4$ prostamide $F_{2\alpha}$ (Target Molecules, Southampton, England). The lipid-containing organic phase was dried, weighed and pre-purified by open bed chromatography on silica gel. Fractions were obtained by eluting the column with 99:1, 90:10, 70:30 and 50:50 (v/v) chloroform/methanol. The 90:10 fraction was used for AEA, , PEA and OEA quantification by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS), as previously described [20] and using selected ion monitoring at M + 1 values for anandamide, entourage fatty acid amides and their deuterated homologues, as previously described in [21]. The 70:30 fraction was used for prostamide $F_{2\alpha}$, quantification by LC-MS-IT-TOF analysis. (Shimadzu Corporation, Kyoto, Japan). Prostamide $F_{2\alpha}$ was measured by LC-MS-MS, using an LC20AB coupled to a hybrid detector IT-TOF (Shimadzu Corporation, Kyoto, Japan) equipped with an ESI interface, using the multiple reaction monitoring mode. LC analysis was performed in the isocratic mode using a Discovery®C18 column (15cm x 2.1 mm, 5 μm) and methanol/water/acetic acid (53:47:0.05 by vol.) as mobile phase with a flow rate of 0.15 ml/min. Identification of prostamide $F_{2\alpha}$ was carried out using ESI ionization in the positive mode with nebulizing gas flow of 1.5 ml/min and curve desolvation line temperature of 250°C. The limit of detection (defined as the concentration at which the signal/noise ratio is greater

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than 3:1) of 25 fmol in the MS mode and 500 fmol in the MS/MS mode for all the compounds analysed was applied as previously [20].

2.4 Intraocular pressure measurement

PM/PGFS knock-out (n=18) and littermate wild type (n=27) mice, aged 10 to 15 weeks old and of both sexes, received bilateral intraocular pressure measurement between the hours of 10:00 AM and 12:00 noon using a commercial rebound tonometer (TonoLab; Icare, Helsinki, Finland). Intraocular pressure was calculated as the mean of three consecutive tonometer readings as previously described [22]. Statistical significance between groups was established using the unpaired Students T-test (mean ± 95% CI). Normal distribution was checked by the Kolgomorov-Smirnov test, equity of variances between groups was determined by Levene's test.

2.5 Measurement of outflow facility

PM/PGFS knock-out (n=18) and littermate wild type (n=22) mice, 10-15 weeks old and of either sex, were humanely culled by cervical dislocation and eyes enucleated immediately after death. Outflow facility was measured in individual eyes using the *iPerfusion* system, as previously described [23]. Briefly, eyes were glued to a support platform submerged in PBS regulated at 35°C. Anterior chambers were cannulated with 33-gauge beveled needles (NanoFil, NF33BV-2, World Precision Instruments) attached to micro-manipulators and equilibrated for 30 minutes at 9 mmHg. The perfusate comprised 0.2 μm filtered DBG (PBS including divalent cations and 5.5 mM glucose). Flow was measured at pressure steps from 6 to 15 mmHg in 6 increments. The steady state criterion per step was 1 minute of <0.1 nl/min/mmHg/min variation in ratio of flow rate to pressure. Pressure steps that failed to reach steady state were excluded from further analysis, paired eyes with 4 or more successful steps were analyzed.. Mean steady state flow Q and pressure P were calculated over a 4-minute window and fit by the relationship

$$Q = C_r\left(\frac{P}{P_r}\right)^{\beta}P$$

C_r represents outflow facility at a reference pressure P_r (8 mmHg) and β characterizes the non-linearity of the Q-P relationship. Note that there is no term for flow at zero pressure, as this has been demonstrated to be zero [24].

3. RESULTS

3.1 Transcription

Transcript for *Fam213b* (Prxl2b) was undetectable by qPCR in homogenized anterior eye segments and excised inguinal fat pads of homozygous PM/PGFS knock-out mice (Fig. 2). In littermate wild type mice, mRNA was detected in both fat pad and anterior eye segment homogenates.

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3.2 Tissue prostamide $F_{2\alpha}$ levels by LC/MS/MS detection

In whole eyes and gonadal white adipose tissue, endogenous prostamide $F_{2\alpha}$ was never detected in any of the PM/PGFS knock-out or littermate wild type mice (table 2). It should be noted that the diminutive mouse eye produces very low yield of ciliary body tissue that is diluted upon homogenization by the more abundant tissues within the anterior chamber of the eye. Although prostamide $F_{2\alpha}$ could not be detected in inguinal fat taken from PM/PGFS knock-out mice, inguinal fat taken from littermate wild type mice yielded consistently detectable amounts in all animals (table 2), thereby indicating that deletion of the gene encoding the PM/PGFS enzyme results in reduction of prostamide $F_{2\alpha}$ levels to undetectable. In addition to prostamide $F_{2\alpha,}$ the endogenous levels of other fatty acid amides and were measured (table 2). Deletion of the gene encoding PM/PGFS did not meaningfully affect anandamide, PEA, or OEA levels. In inguinal adipose tissue, where prostamide $F_{2\alpha}$ was detectable, its levels were lower than that of its precursor anandamide and that of the two anandamide congeners OEA and PEA.

3.3 Ocular effects

Intraocular pressure values obtained from PM/PGFS knock-out and littermate wild type mice are compared in figure 3. Intraocular pressure was significantly elevated by 3.6 (2.9, 4.3) mmHg [P<0.0001, N=45, mean (95% confidence interval)] in response to deletion of the PM/PGFS enzyme (Fig. 3A). Outflow facility, as measured in enucleated mouse eyes using the *iPerfusion* system, was 4.9 (4, 6) nl/min/mmHg (N=18) for PM/PGFS knock-out mice and 5.6 (5, 7) nl/min/mmHg (N=22) for littermate wild-type mice (Figure 3B). The facility at a physiological pressure drop, C_r, was compared between PM/PGFS knock-out and littermate wild type mouse eyes using an unpaired version of the weighted t-test of the log-transformed data as described previously [23]. Outflow facility was insignificantly different between the two groups [15% (−12, 51%) P=0.31, Fig. 3B].

4. DISCUSSION AND CONCLUSIONS

Two distinct families of enzymes are known to be involved in the biosynthesis of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$; the aldo-keto reductase family of reductases, notably AKR1C3 [8-13] and PM/PGFS, which is a member of the thioredoxin-like superfamily [14, 15]. To date the relative biosynthetic and regulatory importance of these two enzymes in living tissues has remained unclear. By studying prostamide $F_{2\alpha}$ tissue levels in inguinal fat, it became clear that deletion of the gene that encodes PM/PGFS prevents the biosynthesis of prostamide $F_{2\alpha}$. This correlated with the qPCR results, where no transcript was present in tissues excised from PM/PGFS gene deleted mice. Next, a functional study on intraocular pressure revealed a distinct increase in PM/PGFS knock-out mice, thereby revealing a regulatory function for PM/PGFS for the first time.

$PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ are known to produce profound and long-acting ocular hypotony [1, 25]. Despite these pronounced attributes, no evidence has emerged that $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ are involved in the physiological regulation of intraocular pressure. Deletion of the gene that encodes both the FP receptor and the heterodimeric prostamide $F_{2\alpha}$ receptor [26] essentially abolishes ocular hypotensive responses to prostanoid FP receptor agonist

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prodrugs and bimatoprost [27, 28]. Moreover, in FP receptor knock-out mice, there is no effect on baseline intraocular pressure or the diurnal increase in intraocular pressure that occurs in the early dark phase, 6:00pm – midnight [29]. In line with this result in FP knock-out mice is the common knowledge that clinical use of cyclo-oxygenase inhibitors does not result in any meaningful change in intraocular pressure. This is despite the fact that all the major prostanoids are found endogenously present in the eye [30-32]. It seems that, under most circumstances, prostanoids tending to either increase or decrease intraocular pressure are held in balance and that this balance is not altered, but rather nullified, by global inhibition of prostanoid biosynthesis. It was, therefore, initially unexpected that deletion of an enzyme involved in the biosynthesis of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ would significantly elevate resting intraocular pressure.

These findings are difficult to explain by the involvement of an aldo-keto reductase, despite these enzymes being eminently capable of substituting for absent PM/PGFS by producing $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$, which are both ocular hypotensive agents. Specifically, one member of the aldo-keto reductase family, AKR1C3, has been extensively characterized and is designated prostaglandin F Synthase [PGFS]. In terms of prostanoid formation, it is a dual function enzyme that converts the endoperoxide intermediate PGH_2 to $PGF_{2\alpha}$ and reduces PGD_2 to 11β-$PGF_{2\alpha}$ [9, 10], a stereoisomer almost as potent as $PGF_{2\alpha}$ [33]. Unlike $PGF_{2\alpha}$, the activity of prostamide $F_{2\alpha}$ is greatly reduced by re-arrangement of the 11-OH group to the β configuration [33]. PGFS [AKR1C3] does not appear to be expressed in the eye [15]. There are, however, alternative enzymes in the aldo-keto reductase superfamily [34], which could possess $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ synthase activities. AKR1C1, 2, 3, and 4 share more than 86% sequence identity [35]. Nevertheless, the marked increase in intraocular pressure in PM/PGFS knock-out mice, about 20%, suggests that the loss of PM/PGFS is not compensated for by functional expression of AKR1C1-4 in the eye. Although it was not possible to detect prostamide $F_{2\alpha}$ in ocular anterior segment tissue, it was reliably detected from inguinal fat from wild type mice, but not from PM/PGFS knockout mice. It appears that prostamide/PGF synthase is the primary source of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$, since (a) prostamide $F_{2\alpha}$ levels in inguinal fat were not detected in PM/PGFS knock-out mice and (b) a marked functional effect on intraocular pressure was observed in PM/PGFS knock-out mice.

The absence of $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ synthesizing enzymes from the ocular anterior segment and a resultant increase in intraocular pressure, would suggest that these prostanoids are involved in its negative regulation. Moreover, the increase in intraocular pressure recorded in PM/PGFS knock-out mice was substantial and similar to that associated with peak levels of diurnal intraocular pressure in mice [29]. This notion would, however, be flawed since disruption of the gene encoding FP and the prostamide $F_{2\alpha}$-sensitive receptors does not alter basal intraocular pressure [27, 28]. A more satisfactory explanation would be the diversion of the endoperoxide precursor PGH_2 to alternative PG synthases. This would likely be PGD and thromboxane [Tx] synthases, since their products PGD_2 and thromboxane A_2 [TxA_2] have been shown to markedly increase intraocular pressure [36, 37]. PGD_2 has been reported to produce a biphasic effect on intraocular pressure, with an initial ocular marked hypertensive phase [36]. TxA_2 is structurally highly unstable but its selective TP receptor analog U-46619 exclusively produces ocular hypertension that

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increases over time [37]. No effect on pressure-dependent outflow facility was observed in PM/PGFS knock-out mice, suggesting a possible effect on the uveoscleral pathway, aqueous humor secretion, or both in the ocular hypertension produced by deleting the PM/PGFS gene in mice.

In addition to producing decreased intraocular pressure, an important role for prostamide $F_{2\alpha}$ has been discovered in centrally mediated pain [20] and adipogenesis [38]. To date, however, the role of the enzyme PM/PGFS has not been directly studied in regulatory and disease processes. This is the first reported study on PM/PGFS using knock-out mice. The evidence that emerged suggests that this enzyme regulates intraocular pressure not by producing ocular hypotensive $PGF_{2\alpha}$ and prostamide $F_{2\alpha}$ but rather by diverting PGH_2 conversion to excessive production of PGD_2 and TxA_2, both of which cause ocular hypertension. In this series of experiments, the much more substrate specific enzyme PM/PGFS was the important enzyme for prostamide $F_{2\alpha}$ biosynthesis and not AKR1C3. Future studies should investigate whether deletion of the gene encoding PM/PGFS also results in altered pain thresholds and adipogenesis.

Acknowledgements

We gratefully thank Rie Natsume [Brain Research Institute, Niigata University] for her technical assistance. Yoko Inoue was a postdoctoral fellow at Niigata University, financially supported by Allergan Inc., who greatly contributed to the creation of a PM/PGFS knock-out mouse. She sadly passed away at a young age after completion of this research.

Funding:

These studies were financially supported by grants from JSPS KAKENHI Grant Number JP16H06276 (AdAMS) (K.S.), NIH Grant Number EY022359 (DRO) and Allergan Inc., USA.

Abbreviations

C	outflow facility
Fa	aqueous flow
Fu	uveoscleral outflow
IOP	intraocular pressure
PM/PGFS	prostamide/prostaglandin F synthase
PGFS	prostaglandin F synthase

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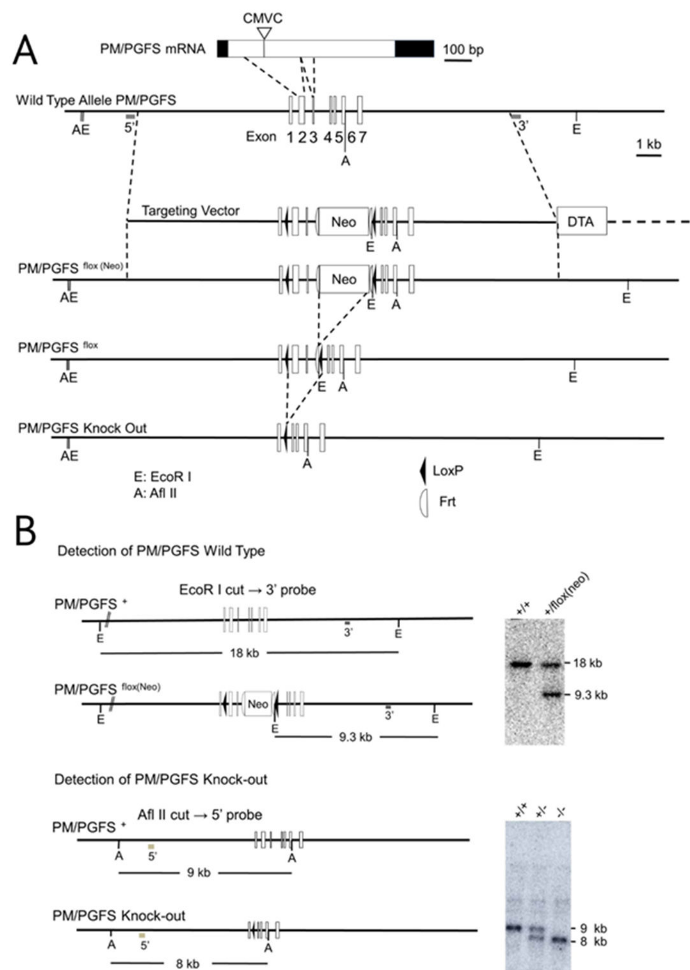

Figure 1.

(A) A schematic representation of PM/PGFS mRNA, genomic allele, targeting vector, targeted PM/PGFS floxed allele and knock-out allele. Numbered boxes of genomic allele represent the exon sequences of PM/PGFS. Frt sites are indicated by open half-circles. neo, PGK-promoter-neo-pA cassette; DTA, diphtheria toxin cassette; A, Afl II; E, EcoR I. Shadowed boxes indicate 5’- and 3’- Southern blot probes. **[B]** Southern blot analysis of EcoR I- or Afl II- digested genomic DNA prepared from the wild-type (WT), PM/PGFS-floxed (neo) and PM/PGFS knock-out mice.

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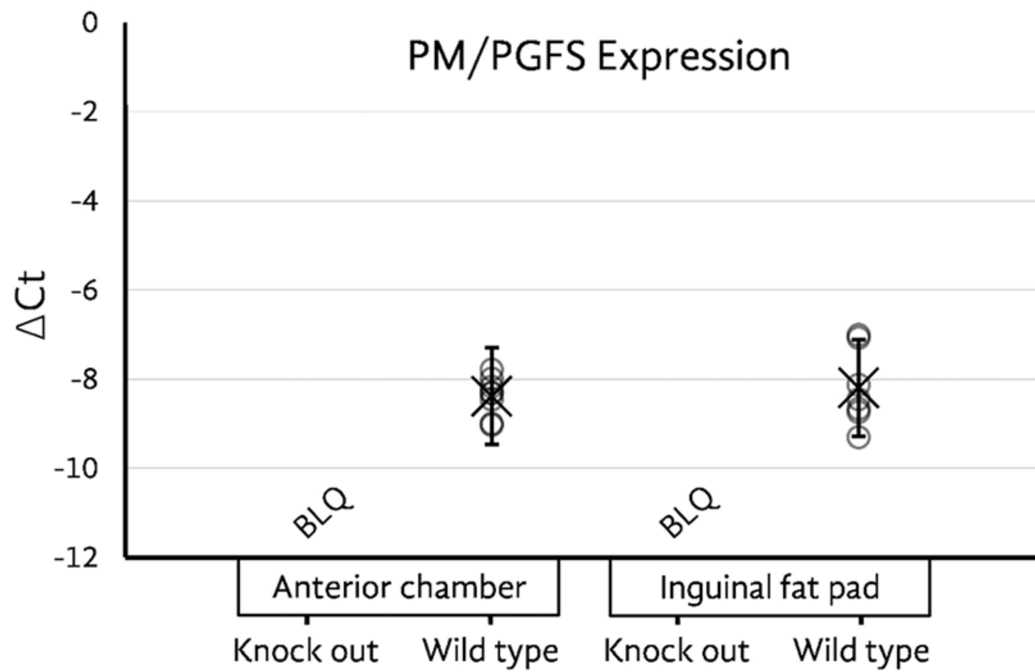

Figure 2. Gene expression analysis of the PM/PGFS by qPCR.

PM/PGFS mRNA was undetectable in homogenized anterior segments and inguinal fat pads of PM/PGFS knock-out mice. Similar levels of PM/PGFS mRNA were found in the homogenized anterior segments and inguinal fat pads of littermate wild type mice. Samples were run in triplicate, dCt = Ct (reference gene) - Ct (target gene), with GAPDH as the reference gene, Ct values greater than 35 cycles were excluded. Circles represent individual samples (○) and red crosses (X) show sample means with error bars as two standard deviations.

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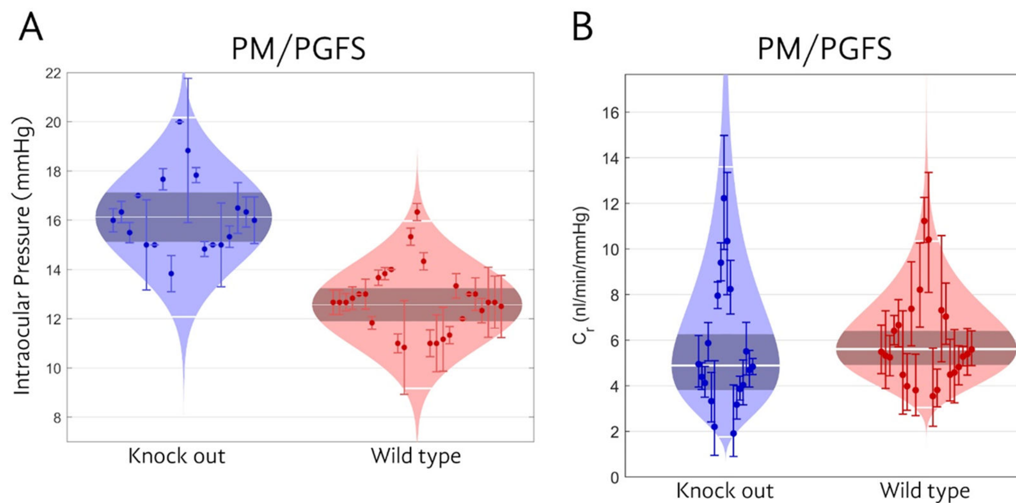

Figure 3.

(**A**) Intraocular pressure in PM/PGFS knock-out mice, 16 (15, 17) mmHg (N=18), was significantly greater than littermate wild type mice, 13 (12, 13) mmHg (N=27) (*P*<0.0001). However outflow facility (**B**) was not significantly different, 4.9 (4, 6) vs 5.6 (5, 7) nl/min/mmHg respectively [15% (−12, 51), mean, 95% CI; *P*=0.3, N=22]. IOP was measured in both eyes and averaged together, while Cr was measured in one eye only. Error bars represent the uncertainty (95% CI) on each measurement, accounting for the variability in the repeated measurements of IOP or the uncertainty on the fit to calculate Cr. The colored shading represents the best normal (A) or log-normal (B) distribution that best fits the data. The central line represents the mean (A) or geometric mean (B), while the outer white lines represent the range than encompasses 95% of the data. The central dark shaded region represents the 95% CI on the mean.

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Table 1.

Exon spanning gene expression primer sets for use with SYBR green qPCR. GAPDH was used as a housekeeping gene for relative mRNA abundance.

Gene	Forward Primer	Reverse Primer
PM/PGFS	TGATGAGAGCAAGCAAATCT	ACAGTACCTTGTCGCCAC
GAPDH	GCCTTCCGTGTTCCTACC	CCTGGTCCTCAGTGTAGC

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Table 2.

Prostamide $F_{2\alpha}$, anandamide, palmitoyl ethanolamide, and oleoyl ethanolamide levels [pmol/g tissue] in ocular anterior segment tissues, inguinal white adipose tissue, and gonadal white adipose tissue. Values are mean ± SD. When undetectable, a designation of below limit of quantification (BLQ) was applied N=5.

Tissue	Prostamide $F_{2\alpha}$ [pmol/g tissue]	Anandamide [pmol/g tissue]	Palmitoyl Ethanolamide [pmol/g tissue]	Oleoyl Ethanolamide [pmol/g tissue]
Ocular anterior segment +/+ mice	BLQ	41.1 ± 4.9	480 ± 75	860 ± 102
Ocular anterior segment −/− mice	BLQ	33.7 ± 8.4	460 ± 80	800 ± 276
Inguinal white adipose tissue +/+ mice	1.11 ± 0.56	16.9 ± 3.0	300 ± 253	940 ± 80
Inguinal white adipose tissue −/− mice	BLQ	16.6 ± 3.3	180 ± 40	800 ± 210
Gonadal white adipose tissue +/+ mice	BLQ	17.2 ± 1.8	180 ± 75	1200 ± 237
Gonadal white adipose tissue −/− mice	BLQ	17.7 ± 1.4	200 ± 10	960 ± 174