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Research paper

Deregulation of key signaling pathways involved in oocyte maturation in *FMR1* premutation carriers with Fragile X-associated primary ovarian insufficiency

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ABSTRACT

FMR1 premutation female carriers are at risk for Fragile X-associated primary ovarian insufficiency (FXPOI). Insights from knock-in mouse model have recently demonstrated that FXPOI is due to an increased rate of follicle depletion or an impaired development of the growing follicles. Molecular mechanisms responsible for this reduced viability are still unknown. In an attempt to provide new data on the mechanisms that lead to FXPOI, we report the first investigation involving transcription profiling of total blood from *FMR1* premutation female carriers with and without FXPOI. A total of 16 unrelated female individuals (6 *FMR1* premutated females with FXPOI; 6 *FMR1* premutated females without FXPOI; and 4 no-FXPOI females) were studied by whole human genome oligonucleotide microarray (Agilent Technologies). Fold change analysis did not show any genes with significant differential gene expression. However, functional profiling by gene set analysis showed large number of statistically significant deregulated GO annotations as well as numerous KEGG pathways in FXPOI females. These results suggest that the impairment of fertility in these females might be due to a generalized deregulation of key signaling pathways involved in oocyte maturation. In particular, the vasoendotelial growth factor signaling, the inositol phosphate metabolism, the cell cycle, and the MAPK signaling pathways were found to be down-regulated in FXPOI females. Furthermore, a high statistical enrichment of biological processes involved in cell death and survival were found deregulated among FXPOI females. Our results provide new strategic approaches to further investigate the molecular mechanisms and potential therapeutic targets for FXPOI not focused in a single gene but rather in the set of genes involved in these pathways.

1. Introduction

Primary ovarian insufficiency (POI), previously referred as premature ovarian failure (POF), is defined as the occurrence of amenorrhea for at least 4 months, before the age of 40 in women, accompanied with alteration of specific serum markers levels (follicle-stimulating hormone (FSH) >40 IU/L and estradiol <50 pg/mL) (Nelson, 2009).

Genetic factors affecting the ovary and the uterus are common causes of infertility. In fact it is reported that 7% of POI cases have a genetics etiology (Bachelot et al., 2009), involving genes with various

Abbreviations: POI, primary ovarian insufficiency; POF, premature ovarian failure; FSH, follicle-stimulating hormone; FXPOI, Fragile X-associated primary ovarian insufficiency; FMRP, Fragile X mental retardation protein; FXS, Fragile X syndrome; FXTAS, Fragile X-associated tremor/ataxia syndrome; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, False Discovery Rate; VEGF, vasoendotelial growth factor.

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biological functions such as regulation of the hypothalamic-pituitary ovarian axis, regulation of oogenesis, coordination of development of germ cell to primordial stage, regulation of development of further stages and participation in systemic endocrinal functions (Dixit et al., 2010).

The most common known genetic cause of 46, XX POI is the expansion of a CGG repeat located in the 5′ UTR region of the *FMR1* gene, referred as Fragile X-associated POI (FXPOI) (Sullivan et al., 2005) or POF1 (MIM # 311360). This CGG repeat element is polymorphic in the general population, ranging from 6 to 54 repeats. However, the CGG track can be unstable upon maternal transmission and the number of repeats can expand in the next generation. Full-mutated alleles (CGG >200 repeats) become transcriptionally silenced through a methylation mechanism, leading to an absence of *FMR1* protein (FMRP) (Oberlé et al., 1991; Verkerk et al., 1991; Yu et al., 1991), which is responsible for Fragile X syndrome (FXS) (OMIM # 300624). Individuals with alleles between 55 and 200 CGG repeats are called *FMR1* premutation carriers. In this premutation condition, the *FMR1* gene is hyperfunctional and the FMRP is at normal levels, and this is why carriers of *FMR1* premutation alleles were assumed to be clinically unaffected. However and contrary to expectation, a subgroup of male and female carriers does show some clinical affectation, which among others includes FXPOI and Fragile X-associated tremor/ataxia syndrome (FXTAS) (reviewed in Hagerman and Hagerman, 2004; Willemsen et al., 2011).

POI occurs in approximately 1% of the general female population (Coulam et al., 1986), whereas up to 20% of the *FMR1* premutation female carriers are affected of this condition (Sherman, 2000). Conversely, 2–14% of women with idiopathic sporadic POI are estimated to carry an *FMR1* premutation allele (Mallolas et al., 2001).

It was found that *FMR1* premutation alleles are transcriptionally up-regulated with some individuals presenting markedly increased (2–8-fold) production of the expanded CGG-repeat mRNA (Tassone et al., 2000). This fact together with the absence of FXTAS or FXPOI cases in the full-mutation range, promoted to propose an RNA "toxic gain-of-function" model for *FMR1* premutation associated pathologies (Hagerman and Hagerman, 2004).

Currently, the mechanism of the impaired ovarian function related to the *FMR1* premutation is unclear. In an attempt to provide new insights on the mechanisms that lead to FXPOI, we report here the first investigation involving transcription profiling of total blood from *FMR1* premutation females carriers with and without FXPOI.

2. Material and methods

2.1. Human samples

A total of 16 unrelated female individuals were selected and divided in three groups: 6 *FMR1* premutated females with FXPOI (Group 1); 6 *FMR1* premutated females without FXPOI (Group 2); and 4 no-POI females with normal *FMR1* alleles (Group 3). All *FMR1* premutation carriers were recruited from FXS families. Data from CGG repeat number and menopause onset of these individuals are summarized in Table 1. All subjects provided written informed consent for testing and for the research use of their phenotypic and genetic data. The study was approved by the Ethics Committee of the Hospital Clinic of Barcelona.

2.2. Total RNA isolation

For each individual, 2.5 mL of peripheral venous blood was collected in 5 mL PAXgene tubes (Qiagen, Valencia, CA, USA). Total RNA was isolated and purified with the PAXgene Blood RNA Kit according to manufacturer's instructions (Qiagen, Valencia, CA, USA). The RNA quality and quantity were evaluated on an Agilent 2100 Bioanalyzer with RNA6000 Nano Reagents and Supplies (Agilent Technologies, Santa Clara, CA, USA).

Table 1
Characteristics of *FMR1* premutated and non-premutated females.

	(CGG)n	Amenorrhea onset	Age
G1_1	33, 71	40	45
G1_2	30, 94	40	49
G1_3	30, 82	40	65
G1_4	29, 62	40	57
G1_5	30, 68	39	60
G1_6	30, 154	38	78
G2_1	20, 76	NA	48
G2_2	27, 60	47	67
G2_3	45, 80	52	56
G2_4	29, 70	50	50
G2_5	30, 80	NA	42
G2_6	30, 168	51	61
G3_1	29, 32	53	64
G3_2	28, 29	50	51
G3_3	29, 29	48	55
G3_4	27, 30	56	61

G1 (*FMR1* premutation female carriers with FXPOI).
G2 (*FMR1* premutation female carriers without FXPOI).
G3 (no-POI females with normal *FMR1* alleles).
NA (not applicable).

2.3. Gene expression microarray

Four hundred nanograms of the total RNA was labeled using Quick Amp Labeling kit following the manufacturer's instructions (One-Color Microarray-Based Gene Expression Analysis Quick Amp Labeling Protocol Version 5.7, Agilent Technologies, Santa Clara, CA, USA). Briefly, mRNA was reverse transcribed in the presence of T7-oligo-dT primer to produce cDNA. cDNA was then in vitro transcribed with T7 RNA polymerase in the presence of Cy3-CTP to produce labeled cRNA. The labeled cRNA was hybridized to an Agilent 4x44K Whole Human Genome 60-mer oligonucleotide microarray, according to the manufacturer's protocol (Agilent Technologies, Santa Clara, CA, USA). Afterwards the microarrays were washed and scanned on an Agilent G2565CA Microarray Scanner System (Agilent Technologies, Santa Clara, CA, USA). Data were extracted using Feature Extraction V10.7.3.1 (Agilent Technologies, Santa Clara, CA, USA).

2.4. Data processing and statistical analysis

Agilent Processed Signal (Agilent Feature Extraction Software) was standardized across arrays using *quantile* normalization (Bolstad et al., 2003). Differential gene expression was carried out using the limma package from Bioconductor (http://www.bioconductor.org/) (Smyth, 2004). Multiple testing adjustments of p-values were done according to Benjamini and Hochberg methodology (Benjamini and Hochberg, 1995).

Gene set analysis was carried out for the Gene Ontology (GO) terms and for the Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathways using FatiScan (Al-Shahrour et al., 2007) in Babelomics (http://babelomics.bioinfo.cipf.es/) (Medina et al., 2010). This method detects significantly up- or down-regulated blocks of functionally related genes in lists of genes ordered by differential expression. Given that many functional terms are simultaneously tested, the results of the test are corrected for multiple testing to obtain an adjusted p-value. Gene set analysis returns adjusted p-values based on False Discovery Rate method (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001). GO annotations for the genes in the microarray were taken from Ensembl 70 release (http://www.ensembl.org) and KEGG pathways from the KEGG web page (http://www.genome.jp/kegg/).

3. Results

Whole genome expression profile from three different unrelated female groups was obtained in an attempt to elucidate FXPOI molecular

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signature. Groups 1 and 2 consisted of *FMR1* premutation female carriers with and without FXPOI, respectively. Group 3 consisted of normal *FMR1* alleles female carriers with normal amenorrhea age of onset (Table 1). Fold change analysis did not show any genes with significant differential gene expression (adjusted p-value <0.05) when comparing all different groups (data not shown). However, functional profiling by gene set analysis showed large number of statistically significant deregulated GO annotations as well as numerous KEGG pathways in *FMR1* premutation female carriers with FXPOI (adjusted p-value <0.05) (Supplementary Fig. 1). We identified 22 statistically significant pathways that encompassed metabolism, regulatory and cell signaling (Fig. 1). Among them, several pathways that have been shown to play important roles in fertility such as the VEGF signaling (vasoendotelial growth factor) (hsa04370), the inositol phosphate metabolism (hsa00562), cell cycle (hsa04110), and the MAPK signaling (hsa04010) pathways were found to be down-regulated in *FMR1* premutation female carriers with FXPOI. When analyzing expression levels of genes related to these pathways, although none of them reached a significant p-value, the majority of them presented lower expression levels in *FMR1* premutation female carrier with FXPOI (Fig. 2).

To investigate the biological functions associated with FXPOI, gene set analysis was tested for function enrichment (Fig. 3). A high statistical enrichment of biological processes involved in cell cycle and cell death and survival was found. *FMR1* premutation female carriers with FXPOI showed down-regulation of processes related with apoptotic mechanisms and programmed cell death (Fig. 3).

4. Discussion

The ovary contains follicles at various developmental stages and its function includes the cyclic recruitment, development and regression of the follicles. The pool of primordial follicles is maintained in dormancy as a reserve to provide oocytes throughout the reproductive life. FXPOI involves infertility, irregular menses and an early menopause. Although in theory, FXPOI could arise from the presence of a smaller than normal primordial follicle pool at birth, insights from two different publications using a knock-in mouse model, demonstrated that FXPOI is due to an accelerated loss or an impaired development of the growing follicles (Hoffman et al., 2012; Lu et al., 2012). Regarding the *FMR1* CGG repeat number, it has also been described that normal alleles containing lower CGG repeat expansions (<26 CGGs) are associated with a polycystic ovarian-like phenotype that would lead to a prematurely diminished functional ovarian reserve (Gleicher et al., 2010, 2013).

Up to date, the only molecular signature associated to the *FMR1* premutation allele is the presence of significantly elevated levels of *FMR1* mRNA, which led to propose an RNA "toxic gain-of-function" model, in which the abnormal mRNA (expanded CGG repeat) itself is causative of the *FMR1* premutation-associated disorders. Although this mechanism is well supported for FXTAS, in FXPOI it is still under study (Willemsen et al., 2011).

Besides the RNA-gain-of-function toxicity hypothesis, other potential mechanisms have been explored (reviewed in Sullivan et al., 2011). Factors such as the repeat size tract, the sequence organization of the CGG repeat tract, the parental origin of the premutation, the X-chromosome inactivation pattern as well as familial aggregation and environmental factors have been examined (Murray et al., 2000; Sullivan et al., 2005; Hunter et al., 2008; Rodriguez-Revenga et al., 2009). In addition, FMRP might also contribute in the molecular mechanisms involved in the reduced fertility since it has been shown that controls germline proliferation during oogenesis (Epstein et al., 2009; Ferder et al., 2013). Although somehow all of them might have an impact on the development and severity of FXPOI, additional and as yet unidentified genetic factors might also be implicated.

In this study we present the first investigation involving transcription profiling of total blood from *FMR1* premutation female carriers with FXPOI. In particular, we have compared this group of individuals with two female groups (one of carriers of *FMR1* premutation alleles and the other with normal CGG repeat alleles) presenting amenorrhea at normal age of onset (Table 1). Differential expression analysis did not show any statistically significant difference between the three groups, although a sample size effect or tissue specificity cannot be fully discarded. However, gene set analysis, which search for groups of genes that are functionally related, identified several KEGG pathways (adj. p-value <0.05) that might be relevant in FXPOI pathology (Fig. 1). Remarkably, we found that signaling mechanisms necessary for the maintenance of the survival

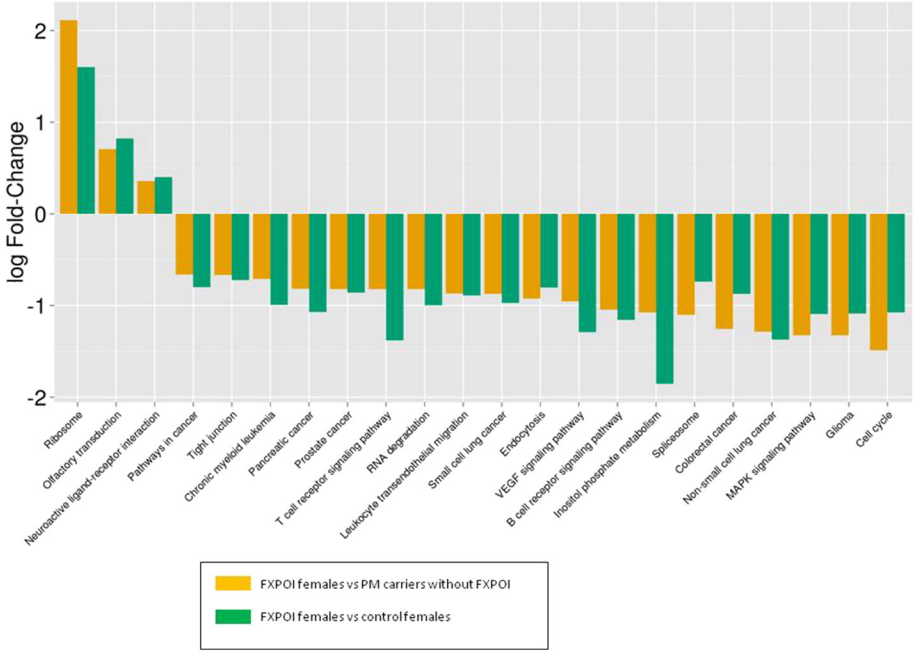

Fig. 1. KEGG pathways significantly deregulated in *FMR1* premutation female carriers with FXPOI. Yellow bars represent the fold change obtained when comparing Group 1 and Group 2. Green bars represent fold change obtained when comparing Group 1 and Group 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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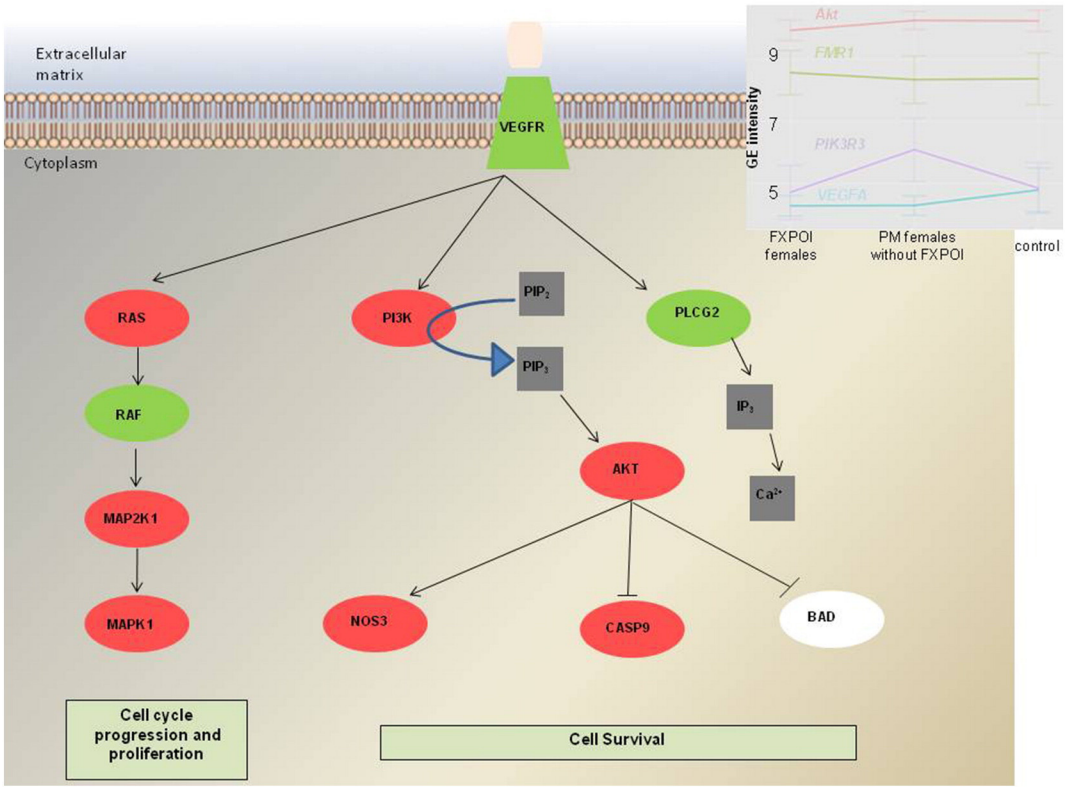

Fig. 2. VEGF, MAPK and PI3K/AKT signaling pathways deregulated in *FMR1* premutation female carriers with FXPOI. In red are represented genes with lower expression levels whereas those in green represent higher expression levels in this group rather than non-POI females. The graphic represents relative expression of *VEGF*, *PI3K3R*, *AKT2* and *FMR1* genes. The Y axis corresponds to gene expression microarray normalized results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and activation of the primordial follicle as well as oocyte maturation are down-regulated in FXPOI women. In particular, we have found that the VEGF signaling, the inositol phosphate metabolism the MAPK signaling and the cell cycle pathway are down-regulated in blood sample of FXPOI women (adj. p-value <0.05) (Fig. 1). VEGF is a key regulator of physiological angiogenesis during embryogenesis, skeletal growth and reproductive functions. Evidences demonstrate that VEGF and its receptors protect follicle and granulosa cells from apoptosis, suggesting that VEGF functions as a survival factor (Kosaka et al., 2007). In this study we identified under expression of members of VEGF pathway, including *PI3K* and

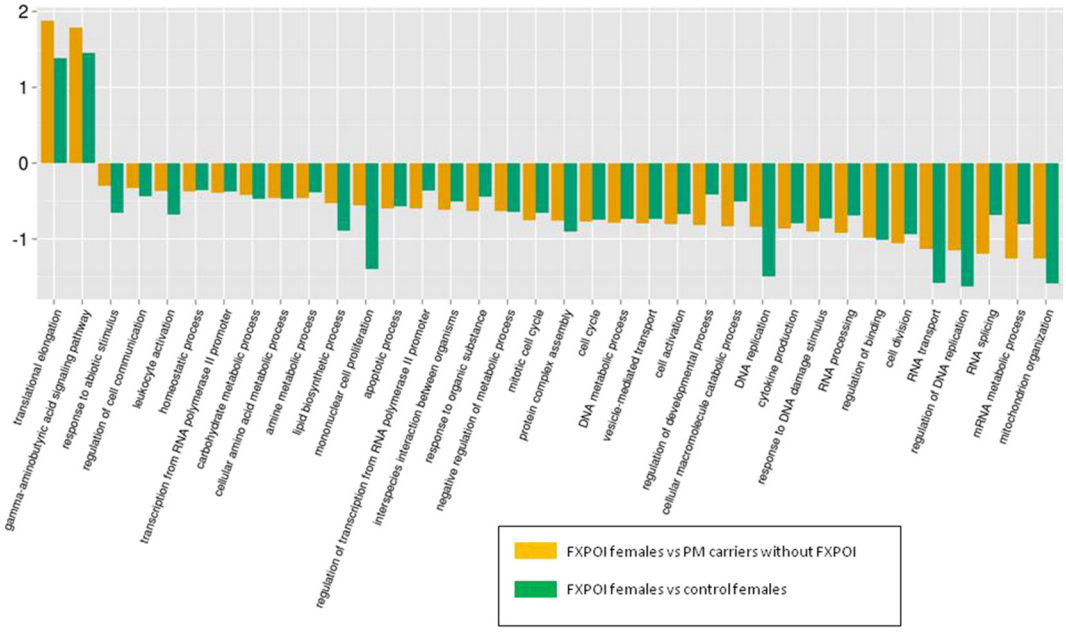

Fig. 3. Biological processes significantly deregulated in *FMR1* premutation female carriers with FXPOI. Yellow bars represent the fold change obtained when comparing Group 1 and Group 2. Green bars represent fold change obtained when comparing Group 1 and Group 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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AKT (Fig. 2). In consonance with this observation, the inositol phosphate metabolism pathway, which mediated the production of PI3K substrates, is also down-regulated in FXPOI females (adj. p-value <0.05) (Fig. 1). Several lines of evidence have indicated that the *PI3K/AKT* gene family is critical regulator of follicle growth, differentiation and survival (Cecconi et al., 2012). In fact, lack of basal level of PI3K activation leads to the premature depletion of the pool of primordial follicles and to the development of POI (Reddy et al., 2009). Furthermore, *Akt* knockout mice females display reduced fertility and abnormal estrous cyclicity. *Akt* knockout ovaries also have a reduced number of growing antral follicles, significantly larger primary and secondary oocytes and an increase in the number of degenerate oocytes (Brown et al., 2010). In addition, Lu et al. (2012) have recently demonstrated that *FMR1* expanded allele can lead to reduced phosphorylation levels of AKT protein in the ovary of FXPOI mice.

While the VEGF and downstream PI3K pathways are required to activate genes associated with cell growth and differentiation, it has been shown that additional pathways such as MAPK are also necessary (Hunzicker-Dunn and Maizels, 2006). Several members of the MAPK superfamily (ERK1/2, p38MAPK) have been demonstrated to play an important function in LH/FSH signaling pathways, resulting in maturation of ovarian follicles and ovulation (reviewed in Conti et al., 2012; Hunzicker-Dunn and Maizels, 2006).

Finally, when subjecting gene expression microarray results to gene set analysis and tested for function enrichment we found several significantly deregulated biological processes in FXPOI females, including cell-cycle progression/arrest, DNA repair and apoptosis (Fig. 3). These results are in consonance with the deregulation of the PI3K/AKT pathway found in FXPOI females, as it is well known that this pathway can regulate many aspects of cell function (Cecconi et al., 2012). In this scenario, Lu et al. (2012) found an increased apoptosis of follicle cells and an alteration of the AKT signaling cascade in ovaries of the *FMR1* knock-in model.

Blood expression profiling in *FMR1* premutation male carriers has recently identified an abundant gene expression deregulation among these individuals (Mateu-Huertas et al., 2014). Although these results are apparently not in concordance with the ones herein reported, it should be taken into consideration that female carriers have a second non-mutated *FMR1* gene on the other X-chromosome which could compensate the marked gene deregulation detected in *FMR1* premutation male carriers. Nevertheless, when performing gene enrichment analysis the molecular functions and biological pathways found to be deregulated, are consistent in both studies. In agreement with Mateu-Huertas et al. (2014), functions such as cell cycle, DNA repair and cell death and survival are statistically significant deregulated in *FMR1* premutation female carriers. We are aware that this study presents two limitations: tissue-specificity gene expression and the normal X-chromosome which might be masking deregulation promoted by the expanded allele in ovarian cells.

5. Conclusion

The gene expression profile of blood samples from FXPOI females suggested that the impairment of fertility might be due to a generalized deregulation of key signaling pathways involved in oocyte maturation. Our results provide new strategic approaches to further investigate the molecular mechanisms and potential therapeutic targets for FXPOI not focused in a single gene but rather in the set of genes involved in these pathways.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2015.06.039.

Conflict of interest

The authors declare no conflict of interest.

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References

Al-Shahrour, F., Arbiza, L., Dopazo, H., Huerta, J., Minguez, P., Montaner, D., Dopazo, J., 2007. From genes to functional classes in the study of biological systems. BMC Bioinforma. 8, 114.

Bachelot, A., Rouxel, A., Massin, N., Dulon, J., Courtillot, C., Matuchansky, C., Badachi, Y., Fortin, A., Paniel, B., Lecuru, F., et al., 2009. Phenotyping and genetic studies of 357 consecutive patients presenting with premature ovarian failure. Eur. J. Endocrinol. 161 (1), 179–187.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57 (1), 289–300.

Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. Ann. Stat. 29 (4), 919–1188.

Bolstad, B.M., Irizarry, R.A., Astrand, M., Speed, T.P., 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19 (2), 185–193.

Brown, C., LaRocca, J., Pietruska, J., Ota, M., Anderson, L., Smith, S.D., Weston, P., Rasoulpour, T., Hixon, M.L., 2010. Subfertility caused by altered follicular development and oocyte growth in female mice lacking PKB alpha/Akt1. Biol. Reprod. 82 (2), 246–256.

Cecconi, S., Mauro, A., Cellini, V., Patacchiola, F., 2012. The role of Akt signalling in the mammalian ovary. Int. J. Dev. Biol. 56 (10-12), 809–817.

Conti, M., Hsieh, M., Zamah, A.M., Oh, J.S., 2012. Novel signaling mechanisms in the ovary during oocyte maturation and ovulation. Mol. Cell. Endocrinol. 356 (1-2), 65–73 (Review).

Coulam, C.B., Adamson, S.C., Annegers, J.F., 1986. Incidence of premature ovarian failure. Obstet. Gynecol. 67 (4), 604–606.

Dixit, H., Rao, L., Padmalatha, V., Raseswari, T., Kapu, A.K., Panda, B., Murthy, K., Tosh, D., Nallari, P., Deenadayal, M., et al., 2010. Genes governing premature ovarian failure. Reprod. Biomed. Online 20, 724–740.

Epstein, A.M., Bauer, C.R., Ho, A., Bosco, G., Zarnescu, D.C., 2009. *Drosophila* Fragile X protein controls cellular proliferation by regulating cbl levels in the ovary. Dev. Biol. 330 (1), 83–92.

Ferder, I., Parborell, F., Sundblad, V., Chiauzzi, V., Gómez, K., Charreau, E.H., Tesone, M., Dain, L., 2013. Expression of fragile X mental retardation protein and FMR1 mRNA during folliculogenesis in the rat. Reproduction 145 (4), 335–343.

Gleicher, N., Weghofer, A., Lee, I.H., Barad, D.H., 2010. FMR1 genotype with autoimmunity-associated polycystic ovary-like phenotype and decreased pregnancy chance. PLoS One 5 (12), e15303.

Gleicher, N., Kim, A., Barad, D.H., Shohat-Tal, A., Lazzaroni, E., Michaeli, T., Lee, H.J., Kushnir, V.A., Weghofer, A., 2013. FMR1-dependent variability of ovarian aging patterns is already apparent in young oocyte donors. Reprod. Biol. Endocrinol. 11, 80.

Hagerman, P.J., Hagerman, R.J., 2004. The fragile-X premutation: a maturing perspective. Am. J. Hum. Genet. 74 (5), 805–816 (Review. Erratum in: Am J Hum Genet. 2004; 75(2):352).

Hoffman, G.E., Le, W.W., Entezam, A., Otsuka, N., Tong, Z.B., Nelson, L., Flaws, J.A., McDonald, J.H., Jafar, S., Usdin, K., 2012. Ovarian abnormalities in a mouse model of fragile X primary ovarian insufficiency. J. Histochem. Cytochem. 60 (6), 439–456.

Hunter, J.E., Epstein, M.P., Tinker, S.W., Charen, K.H., Sherman, S.L., 2008. Fragile X-associated primary ovarian insufficiency: evidence for additional genetic contributions to severity. Genet. Epidemiol. 32 (6), 553–559.

Hunzicker-Dunn, M., Maizels, E.T., 2006. FSH signaling pathways in immature granulosa cells that regulate target gene expression: branching out from protein kinase A. Cell. Signal. 18 (9), 1351–1959 (Review).

Kosaka, N., Sudo, N., Miyamoto, A., Shimizu, T., 2007. Vascular endothelial growth factor (VEGF) suppresses ovarian granulosa cell apoptosis in vitro. Biochem. Biophys. Res. Commun. 363 (3), 733–737.

Lu, C., Lin, L., Tan, H., Wu, H., Sherman, S.L., Gao, F., Jin, P., Chen, D., 2012. Fragile X premutation RNA is sufficient to cause primary ovarian insufficiency in mice. Hum. Mol. Genet. 21 (23), 5039–5047.

Mallolas, J., Duran, M., Sánchez, A., Jiménez, D., Castellví-Bel, S., Rifé, M., Milà, M., 2001. Implications of the *FMR1* gene in menopause: study of 147 Spanish women. Menopause 8 (2), 106–110.

Mateu-Huertas, E., Rodriguez-Revenga, L., Alvarez-Mora, M.I., Madrigal, I., Willemsen, R., Milà, M., Martí, E., Estivill, X., 2014. Blood expression profiles of fragile X premutation carriers identify candidate genes involved in neurodegenerative and infertility phenotypes. Neurobiol. Dis. 65, 43–54.

Medina, I., Carbonell, J., Pulido, L., Madeira, S.C., Goetz, S., Conesa, A., Tárraga, J., Pascual-Montano, A., Nogales-Cadenas, R., Santoyo, J., 2010. Babelomics: an integrative

platform for the analysis of transcriptomics, proteomics and genomic data with advanced functional profiling. Nucleic Acids Res. 38 (Web Server issue), W210–W213.
Murray, A., Ennis, S., MacSwiney, F., Webb, J., Morton, N.E., 2000. Reproductive and menstrual history of females with fragile X expansions. Eur. J. Hum. Genet. 8 (4), 247–252.
Nelson, L.M., 2009. Clinical practice. Primary ovarian insufficiency. N. Engl. J. Med. 360 (6), 606–614.
Oberlé, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boué, J., Bertheas, M.F., Mandel, J.L., 1991. Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 252, 1097–1102.
Reddy, P., Adhikari, D., Zheng, W., Liang, S., Hämäläinen, T., Tohonen, V., Ogawa, W., Noda, T., Volarevic, S., Huhtaniemi, I., et al., 2009. PDK1 signaling in oocytes controls reproductive aging and lifespan by manipulating the survival of primordial follicles. Hum. Mol. Genet. 18 (15), 2813–2824.
Rodriguez-Revenga, L., Madrigal, I., Badenas, C., Xunclà, M., Jiménez, L., Milà, M., 2009. Premature ovarian failure and fragile X female premutation carriers: no evidence for a skewed X-chromosome inactivation pattern. Menopause 16 (5), 944–949.
Sherman, S.L., 2000. Premature ovarian failure in the fragile X syndrome. Am. J. Med. Genet. 97, 189–194.
Smyth, G.K., 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3, 3.
Sullivan, A.K., Marcus, M., Epstein, M.P., Allen, E.G., Anido, A.E., Paquin, J.J., Yadav-Shah, M., Sherman, S.L., 2005. Association of *FMR1* repeat size with ovarian dysfunction. Hum. Reprod. 20 (2), 402–412.
Sullivan, S.D., Welt, C., Sherman, S., 2011. *FMR1* and the continuum of primary ovarian insufficiency. Semin. Reprod. Med. 29 (4), 299–307.
Tassone, F., Hagerman, R.J., Taylor, A.K., Gane, L.W., Godfrey, T.E., Hagerman, P.J., 2000. Elevated levels of *FMR1* mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. Am. J. Hum. Genet. 66 (1), 6–15.
Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, F.P., et al., 1991. Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65 (5), 905–914.
Willemsen, R., Levenga, J., Oostra, B.A., 2011. CGG repeat in the *FMR1* gene: size matters. Clin. Genet. 80 (3), 214–225.
Yu, S., Pritchard, M., Kremer, E., Lynch, M., Nancarrow, J., Baker, E., Holman, K., Mulley, J.C., Warren, S.T., Schlessinger, D., et al., 1991. Fragile X genotype characterized by an unstable region of DNA. Science 252, 1179–1181.

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