The Influence of Mitophagy and Apoptosis on The Embryo Development in Kartagener and Globozoospermia Cases

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Case Report

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Abstract

In this case study, we aimed to detect the gene expression profiles in two rare male infertility syndromes. The first syndrome is Globozoospermia which occurs via detecting acrosomal-free spherical sperm. Another rare and genetic syndrome is known as primary ciliary dyskinesia, Kartagener Syndrome which is caused by mutations that affect the motility of cilia. We aimed to identify the role of apoptosis and mitophagy genetic markers in embryonic waste culture medium of Globozoospermia and Kartagener Syndrome cases. Waste embryo culture medium samples of Globoospermia and Kartagener patients were collected. Total RNAs were isolated and MFN1, MFN2, Cyt C and p53 gene expressions were determined by using RT-PCR. KS sample with developing embryos have the lowest apoptotic pathway activity compared to both arrest KS and NR samples ($p<0.001$).

Although there is a genetic component to about half of cases of infertility, many genetic reasons of infertility in humans are still unknown. Over the past two decades, the discovery of gene variants related with infertility in patients has been made easier by the development of high-throughput sequencing tools.

Introduction

Globozoospermia is normally diagnosed by the detection of round-headed sperm heads during routine light microscopic examination of a semen sample. The absence of an acrosome is another major feature, which is best visualized by transmission electron microscopy. Furthermore, sperm cells tend to have multiple defects involving the cytoskeleton such as a round nucleus, absence of the post-acrosomal sheath, separation of the nuclear membranes and frequently coiled tails. Also, maturation defects such as a persisting residual cytoplasmic body/droplet surrounding the nucleus or the midpiece have frequently been reported [1]. The mammalian acrosome is a secretory vesicle attached to the sperm nucleus whose fusion with the overlying plasma membrane is required to achieve fertilization. The most extreme case of acrosome biogenesis failure is globozoospermia syndrome, which is primarily characterized by the presence of round-headed spermatozoa without acrosomes with cytoskeleton defects around the nucleus and infertility [2].

Kartagener syndrome (KS), also known as primary ciliary dyskinesia (PCD), is a rare genetic disease with autosomal recessive inheritance that causes defects in the activity of cilia in the body. Mutations affecting cilia's motility are the main cause of this syndrome. In community, PCD is claimed to affect 1 in 10,000–20,000 live newborns. However, its prevalence may increase up to 5% in live births with respiratory tract infection. Male and female infertility or subfertility are other non-respiratory complications related to sperm dysmotility and ciliary dysfunction in the fallopian tubes, respectively [3]. Although ciliary activity is crucial for both male and female reproductive health, ciliary malfunction and abnormal sperm motility are seen in the fallopian tubes in cases of infertility. The cilia's mobility, which is directly tied to mitochondrial decrease or failure, results in improper cilia function [4].

Apoptosis, or "programmed cell death," and mitophagy, also known as "mitochondrial autophagy," are two of the key biological pathways involved in mitochondrial dysfunction. Mitofusin (MFN) genes, whose expression is increased during spermatogonial differentiation, are directly related to mitophagy. By inducing an apoptotic mechanism linked to DNA oxidation in the development of spermatogonia and spermatocytes, MFN deletion causes male infertility [5]. High Mitofusin (MFN) gene expressions were observed with increased mitochondrial and endoplasmic reticulum (ER) activities during spermatogonial differentiation. Deletion of MFN gene leads to DNA oxidation and apoptosis, especially in the differentiation of spermatogonia and spermatocytes, which in turn causes male sterility [5].

MFN2 controls spermatogenesis and affects these two processes, in contrast to MFN1. Germ cell development defects in MFN2 mutants are corrected by MFN2 but not by MFN1 in mitochondria and ER, so this study provides critical insights into mitochondrial determinants of male fertility by revealing a fundamental requirement for MFN-mediated mitochondrial and ER coordination in spermatogenesis [6]. Although the role of mitochondrial morphology in cellular homeostasis is well known, insufficient information is available on whether mitochondrial remodeling is required for postnatal germ cell development. The initiative signal of apoptosis is cytochrome C (cyt C), which is an essential component of the electron transport chain found in the inner membrane of the mitochondria, released from mitochondria [7].

Our goal was to identify potential genetic indicators of apoptosis and mitophagy in the patient's embryonic waste culture media for both Globozoospermia and Kartagener Syndrome Cases.

Materials and Methods

Sample Collection: Spare human embryos donated to research were obtained with informed consent from IVF patients at Bahçeci Health Group Umut IVF Laboratory, Istanbul (Ethic Number: 2022/20 – 05). Sperm samples with KS were obtained by TESA procedure. No movement was observed in sperms treated with pentoxifylline before the procedure. The ejaculate sperm of GS patient was used by for microinjection. No acrosome was observed in the sperm head structure. Following microinjection at both samples, the oocytes were treated with Ca-ionophore. Oocytes were cultured individually in a unique pre-equilibrated culture dish. Single continuous culture complete (CSCM-C) with human serum albumin (single-step media; Irvine Scientific, CA, USA) was used for the embryo cultures throughout the culture period. All embryos were cultured until day 5 or 6 of embryo development (MIRI, ESCO Medical, Singapore). For comprising with the control group, D6 stage waste embryonic fluid of normoresponder (NR) patient was used.

A three-point grading system was used to award a cleavage-stage morphologic score on the third day of embryo development. Features that were taken into consideration were cell number, fragmentation, symmetry, and form. The expansion stage, inner cell mass (ICM) quality, and TE quality were the three factors used to calculate the morphologic score at the blastocyst stage.

The waste embryo culture medium was collected after finishing the culture. Single culture droplets had been overlaid with oil; thus, pipetting the medium had to be made to avoid taking the oil. Single waste embryo culture medium samples were stored at 80°C.

Total RNA Isolation and Real Time Polymerase Chain Reaction (RT-PCR)

Nucleospin RNA Kit (Macherey-Nagel, Germany) was used to isolate total RNA in compliance with the manufacturer's instructions. Reverse transcription was used to transform the total RNAs into cDNA (Nucleogene, Turkey). BmLabosis (Ankara, Turkey) synthesized and supplied SYBR Green primer sets for the amplification of p53, Cytochrome C (Cyt C), Mitofusin 1 (MFN1), Mitofusin 2 (MFN2), and Glyseraldehide-3-phosphate dehydrogenase (GAPDH). RT-PCR was performed in PowerAmp96 (Kogene, Korea). Gene expressions were determined using the ΔCT method.

Statistical Evaluation

The IBM SPSS Statistics 26.0 program was used for all statistical analyses. The acquired information was presented as the mean ± standard deviation. The Kolmogorov-Smirnov test was used to determine if the continuous variables had a normal distribution. Sidak test was used to determine multiple comparisons.

Results

Gene expression levels in Kartagener syndrome

MFN1 gene expressions of both arrest (16.541 ± 0.011) and developing embryo (17.572 ± 0.013) samples were increased compared to the NR samples (8.668 ± 0.005; $p < 0.001$). Oppositely, arrest (3.020 ± 0.059) and developing embryo (2.520 ± 0.013) samples' MFN2 gene expressions were decreased compared to the NR samples (3.260 ± 0.001; $p < 0.001$). Both p53 and Cyt C expression were reduced in KS samples with arrest (0.670 ± 0.023; 2.222 ± 0.199) and developing (0.360 ± 0.019; 2.312 ± 0.014) embryos in comparison to the sample from the NR patient (1.140 ± 0.007; 5.600 ± 0.02; $p < 0.001$; Fig. 1).

Gene expression levels in Globozoospermia syndrome

MFN1 gene expressions of both arrested (2.668 ± 0.082) and developing embryo (7.551 ± 1.608) samples were decreased compared to NR samples (8.668 ± 0.005; $p < 0.001$). In addition, MFN2 gene expressions of arrested (-1.453 ± 0.326) and developing embryo (1.535 ± 0.536) samples were decreased compared to NR samples (3.260 ± 0.001; $p < 0.001$). Both p53 and Cyt C expression were decreased in globozoospermia samples with arrested (-2.144 ± 0.586; -4.750 ± 0.215) and developing (-1.864 ± 0.392; -2.965 ± 0.470) embryos compared to the sample from the NR patient (1.140 ± 0.007; 5,600 ± 0.02; $p < 0.001$; Fig. 2).

Discussion

Morphological defects in human sperm cells raise suspicion for further anomalies within the sperm cell and could have implications in clinical practice. Reproductive and respiratory issues can arise from primary ciliary dyskinesia (PCD), formerly known as immotile cilia syndrome. The patients experience issues with their upper respiratory tracts because of the compromised microtubule mobility and other problem with these patients have been infertility too. Several attempts have been proposed to bring back sperm motion and to improve intracytoplasmic sperm injection (ICSI) results.

Since causative mutations for PCD were identified in over 50 genes, the role of these genes in sperm development should be investigated to understand the effect of PCD mutations on male fertility. Variations in the impact of mutations on airways and fertility may be partially explained by the presence of distinct dynein arm heavy chains in sperm flagellum and respiratory cilia, as demonstrated by previous investigations [8]. Recent research has also indicated that the motile cilia of the male reproductive tract may be crucial for the maturation and movement of sperm. Reportedly decreased sperm counts in certain PCD patients could be the result of reproductive tract motile cilia failure rather than sperm production issues. Thus, more research is needed to determine the precise functions of PCD genes in male fertility as well as the best ways to treat them.

Primary ciliary dyskinesia (PCD) may present with structurally abnormal completely immotile sperm, with seemingly little prospect of fertility, and moderate respiratory dysfunction supporting the presence of an underlying ciliopathy. Despite testicular sperm also being immotile and showing profound structural defects that would seem to preclude fertilization, more morphologically normal sperm can establish a normal pregnancy [9].

There are also studies in the literature reporting live births in patients with intracytoplasmic sperm injection (ICSI) with testicular sperm in men with Kartagener syndrome [10, 11]. His diagnosis was verified with transmission electron microscopy and genetic mutation screening, revealing total absence of dynein arms in sperm tails and homozygous mutation in the ZMYND10, heterozygous mutations in the ARMC4 and DNAH5 genes. Laser assisted viability assay (LAVA) was performed by shooting the sperm tails during sperm retrieval for microinjection, following detection of pentoxifylline resistant immotile sperm. Live births of healthy triplets, one boy and two monozygotic girls, was achieved after double blastocyst transfer [12].

A successful delivery was documented following intracytoplasmic sperm injection (ICSI) using ejaculated sperm extracted from a patient diagnosed with Kartagener syndrome. Couples with Kartagener's syndrome may be able to conceive successfully using ejaculated sperm following ICSI. Kartagener's syndrome is a heterogeneous group of disorders with similar clinical presentations, and treatment should be individualized depending on sperm motility [13].

Both motile cilia and flagella are microtubule-based organelles, and they share similar 9 + 2 axonemal structures. PCD, a hallmark motile cilia-related disease, is characterized by deficient mucociliary

clearance and chronic airway disease coupled with laterality disturbances and subfertility [14]. Although motile cilia and sperm flagella share a similar axonemal structure, those organelles may display some differences in structural and protein content. This could be the reason why not all men with PCD exhibit infertility and axonemal defects are sometimes restricted to the sperm flagella [15].

Apoptosis in the KS sample was discovered to be lower in arrest and developing embryos compared to the NR sample. Mitophagy has taken place since MFN2 expression has increased in both KS sample whereas MFN1 gene expression has reduced. However, according to the embryonal development, arrest KS sample showed low expression compared to the developing embryo KS sample. When all characteristics were considered, it was shown that KS sample with developing embryos have the lowest apoptotic pathway activity compared to both arrest KS and NR samples. Mitophagy genes have demonstrated and verified why developing embryo KS sample might develop as opposed to the arrest KS sample. The fact that embryo waste culture is a helpful sample for gene expression research was also shown by this investigation.

The pathogenesis of globozoospermia most probably originates in spermiogenesis, more specifically in acrosome formation and sperm head elongation. In several knockout mouse models, a phenotype like that in humans was found. Together with the occurrence of affected siblings, these findings indicate a genetic origin, which makes globozoospermia a good candidate for genetic analysis. More research is needed to elucidate the pathogenesis of human globozoospermia to further understand globozoospermia as well as (abnormalities in) spermiogenesis and spermatogenesis in general [16].

Semen parameters were described in detail in 72 cases and varied widely. Besides the general observation of mild asthenozoospermia and typical morphological features, no abnormality in semen parameters can be characterized as a typical feature of globozoospermia. Closer investigation with a computerized digital image analysis system did not reveal any abnormalities in sperm motility properties in globozoospermia [17].

The introduction of ICSI [18] provided a solution in fertility treatment for patients suffering from globozoospermia [19]. Since then, numerous reports have described successful attempts to achieve either fertilization or pregnancy following ICSI with globozoospermic sperm cells [20–26].

In general, ICSI with globozoospermic cells is less successful compared with ICSI in general. Several authors reported a low to absent fertilization [21, 27–28]. Therefore, to speed up discovering pathogenic mutations responsible for globozoospermia, a bigger globozoospermic cohort needs to be investigated. However, aberrant sperm morphology may be linked to genetic changes in the sperm cells, which may have long-term effects on the progeny produced by artificial reproduction.

Apoptosis in GS sample was discovered to be lower in arrest and developing embryos compared to the NR sample. It was observed that both MFN1 and MFN-2 gene expressions, which are related to mitophagy, were decreased in GS sample (arrest and developing embryos) compared to the NR sample.

An investigation was carried out to characterize the reproductive consequences of a diverse range of male infertility disorders resulting in dramatic changes in sperm parameters (counts, motility, and morphology) due to chromosomal, genetic, or still unknown reasons. Source of sperm, fertilization, pregnancy, live birth, and miscarriage rates of patients with Klinefelter's syndrome, Kartagener's syndrome, round-head sperm, dysplasia of the fibrous sheath (DFS), and stump-tail sperm were reviewed. In Klinefelter's and Kartagener's syndrome, the fertilization potential and the live-birth rate are close to that obtained from nonspecific causes of sperm defects. Round-head sperm shows a lower fertility potential, but the addition of assisted oocyte activation and the use of intracytoplasmic morphologically selected sperm injection increased the rates of live birth. Conditions such as DFS and stump tail have a poor prognosis, but the number of cases described in the literature is too limited for drawing final conclusions [29].

It is now possible to base embryo selection criteria on quantifiable biological characteristics of the blastocyst rather than on subjective measures like morphology. This is the first report to directly compare a physiological mixture of levels of mitophagy and apoptosis markers by human preimplantation embryos of Kartagener Syndrome and globozoospermia syndrome blastocyst embryos and link aspects of profile with gene expressions levels assessments of embryo health.

These data suggest that the noninvasive method of mitophagy and apoptosis gene expression levels profiling will be of value as a tool for routine preimplantation embryo selection among specific or all patient groups, as this technology can provide insight into human pre-implantation embryo genetic health.

Considering the great importance of microtubule and acrosomal structures in the formation and maintenance of the physiological functions of sperm cells, this serves as a scientific trigger to encourage further research in this direction on male infertility. It is important to use our current understanding to identify avenues and priorities for future research in the field of genetic causes of infertility as well as to apply mutation knowledge to risk prediction, genetic diagnosis, and potential treatment for human infertility.

Declarations

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Declaration of interests: The authors have no competing interests to declare that are relevant to the content of this article.

Conflict of Interest Statement: None of the authors disclose any conflict of interest.

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References

1. Bhattarai, L., Gautam, B., Raut, B. B., & Chettri, S. (2023). Globozoospermia. *Kathmandu Univ Med J (KUMJ), 21*(81), 98-99.
2. Moreno, R. D. (2023). Human globozoospermia-related genes and their role in acrosome biogenesis. *WIREs Mech Dis, 15*(2), e1589. doi: 10.1002/wsbm.1589
3. Ferkol, T. W., & Leigh, M. W. (2012). Ciliopathies: the central role of cilia in a spectrum of pediatric disorders. *J Pediatr, 160*(3), 366-371. doi: 10.1016/j.jpeds.2011.11.024
4. Mitchell, D. R. (2007). The evolution of eukaryotic cilia and flagella as motile and sensory organelles. *Adv Exp Med Biol, 607*, 130-140. doi: 10.1007/978-0-387-74021-8_11
5. Chen, W., Sun, Y., Sun, Q., Zhang, J., Jiang, M., Chang, C., . . . Wang, Y. (2020). MFN2 Plays a Distinct Role from MFN1 in Regulating Spermatogonial Differentiation. *Stem Cell Reports, 14*(5), 803-817. doi: 10.1016/j.stemcr.2020.03.024
6. Cozzolino, M., Ergun, Y., & Seli, E. (2023). Targeted Deletion of Mitofusin 1 and Mitofusin 2 Causes Female Infertility and Loss of Follicular Reserve. *Reprod Sci, 30*(2), 560-568. doi: 10.1007/s43032-022-01014-w
7. Babbitt, S.E., Sutherland, M.C., San Francisco, B., Mendez, D.L., & Kranz, R.G. (2015). Mitochondrial cytochrome c biogenesis: no longer an enigma. Trends in biochemical sciences, 40 8, 446-55
8. Jayasena, C. N., & Sironen, A. (2021). Diagnostics and Management of Male Infertility in Primary Ciliary Dyskinesia. Diagnostics (Basel, Switzerland), 11(9), 1550. https://doi.org/10.3390/diagnostics11091550
9. McLachlan, R. I., Ishikawa, T., Osianlis, T., Robinson, P., Merriner, D. J., Healy, D., de Kretser, D., & O'Bryan, M. K. (2012). Normal live birth after testicular sperm extraction and intracytoplasmic sperm injection in variant primary ciliary dyskinesia with completely immotile sperm and structurally abnormal sperm tails. Fertility and sterility, 97(2), 313–318. https://doi.org/10.1016/j.fertnstert.2011.11.003
10. Kaushal, M., & Baxi, A. (2007). Birth after intracytoplasmic sperm injection with use of testicular sperm from men with Kartagener or immotile cilia syndrome. Fertility and sterility, 88(2), . https://doi.org/10.1016/j.fertnstert.2006.11.113
11. Cayan, S., Conaghan, J., Schriock, E. D., Ryan, I. P., Black, L. D., & Turek, P. J. (2001). Birth after intracytoplasmic sperm injection with use of testicular sperm from men with Kartagener/immotile cilia syndrome. Fertility and sterility, 76(3), 612–614. https://doi.org/10.1016/s0015-0282(01)01974-4
12. Ozkavukcu, S., Celik-Ozenci, C., Konuk, E., & Atabekoglu, C. (2018). Live birth after Laser Assisted Viability Assessment (LAVA) to detect pentoxifylline resistant ejaculated immotile spermatozoa during ICSI in a couple with male Kartagener's syndrome. Reproductive biology and endocrinology : RB&E, 16(1), 10. https://doi.org/10.1186/s12958-018-0321-6

13. Matsumoto, Y., Goto, S., Hashimoto, H., Kokeguchi, S., Shiotani, M., & Okada, H. (2010). A healthy birth after intracytoplasmic sperm injection using ejaculated spermatozoa from a patient with Kartagener's syndrome. Fertility and sterility, 93(6), 2074.e17–2074.e2.074E29. https://doi.org/10.1016/j.fertnstert.2009.11.042
14. Knowles, M. R., Daniels, L. A., Davis, S. D., Zariwala, M. A., & Leigh, M. W. (2013). Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. American journal of respiratory and critical care medicine, 188(8), 913–922. https://doi.org/10.1164/rccm.201301-0059CI
15. Jiao, S. Y., Yang, Y. H., & Chen, S. R. (2021). Molecular genetics of infertility: loss-of-function mutations in humans and corresponding knockout/mutated mice. Hum Reprod Update, 27(1), 154-189. doi: 10.1093/humupd/dmaa034
16. Dam, A. H., Feenstra, I., Westphal, J. R., Ramos, L., van Golde, R. J., & Kremer, J. A. (2007). Globozoospermia revisited. Human reproduction update, 13(1), 63–75. https://doi.org/10.1093/humupd/dml047
17. Aitken RJ, Kerr L, Bolton V and Hargreave T (1990) Analysis of sperm function in globozoospermia: implications for the mechanism of sperm–zona interaction. Fertil Steril 54,701–707.
18. Palermo G, Joris H, Devroey P and Van Steirteghem AC (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340,17–18.
19. Hamberger L, Lundin K, Sjogren A and Soderlund B (1998) Indications for intracytoplasmic sperm injection. Hum Reprod 13(Suppl 1),128–133., 128–133.
20. Bourne H, Liu DY, Clarke GN and Baker HW (1995) Normal fertilization and embryo development by intracytoplasmic sperm injection of round-headed acrosomeless sperm. Fertil Steril 63,1329–1332.
21. Liu J, Nagy Z, Joris H, Tournaye H, Devroey P and Van Steirteghem A (1995) Successful fertilization and establishment of pregnancies after intracytoplasmic sperm injection in patients with globozoospermia. Hum Reprod 10,626–629.
22. Trokoudes KM, Danos N, Kalogirou L, Vlachou R, Lysiotis T, Georghiades N, Lerios S and Kyriacou K (1995) Pregnancy with spermatozoa from a globozoospermic man after intracytoplasmic sperm injection treatment. Hum Reprod 10,880–882.
23. Nardo LG, Sinatra F, Bartoloni G, Zafarana S and Nardo F (2002) Ultrastructural features and ICSI treatment of severe teratozoospermia: report of two human cases of globozoospermia. Eur J Obstet Gynecol Reprod Biol 104,40–42.
24. Tesarik J, Rienzi L, Ubaldi F, Mendoza C and Greco E (2002) Use of a modified intracytoplasmic sperm injection technique to overcome sperm-borne and oocyte-borne oocyte activation failures. Fertil Steril 78,619–624.
25. Zeyneloglu HB, Baltaci V, Duran HE, Erdemli E and Batioglu S (2002) Achievement of pregnancy in globozoospermia with Y chromosome microdeletion after ICSI. Hum Reprod 17,1833–1836.
26. Heindryckx B, Van der Elst J, De Sutter P and Dhont M (2005) Treatment option for sperm- or oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. Hum

Reprod 20,2237–2241.

27. Stone S, O'Mahony F, Khalaf Y, Taylor A and Braude P (2000) A normal livebirth after intracytoplasmic sperm injection for globozoospermia without assisted oocyte activation: case report. Hum Reprod 15,139–141.
28. Kilani Z, Ismail R, Ghunaim S, Mohamed H, Hughes D, Brewis I and Barratt CL (2004) Evaluation and treatment of familial globozoospermia in five brothers. Fertil Steril 82,1436–1439.
29. Dávila Garza, S. A., & Patrizio, P. (2013). Reproductive outcomes in patients with male infertility because of Klinefelter's syndrome, Kartagener's syndrome, round-head sperm, dysplasia fibrous sheath, and 'stump' tail sperm: an updated literature review. Current opinion in obstetrics & gynecology, 25(3), 229–246. https://doi.org/10.1097/GCO.0b013e32835faae5

Figures

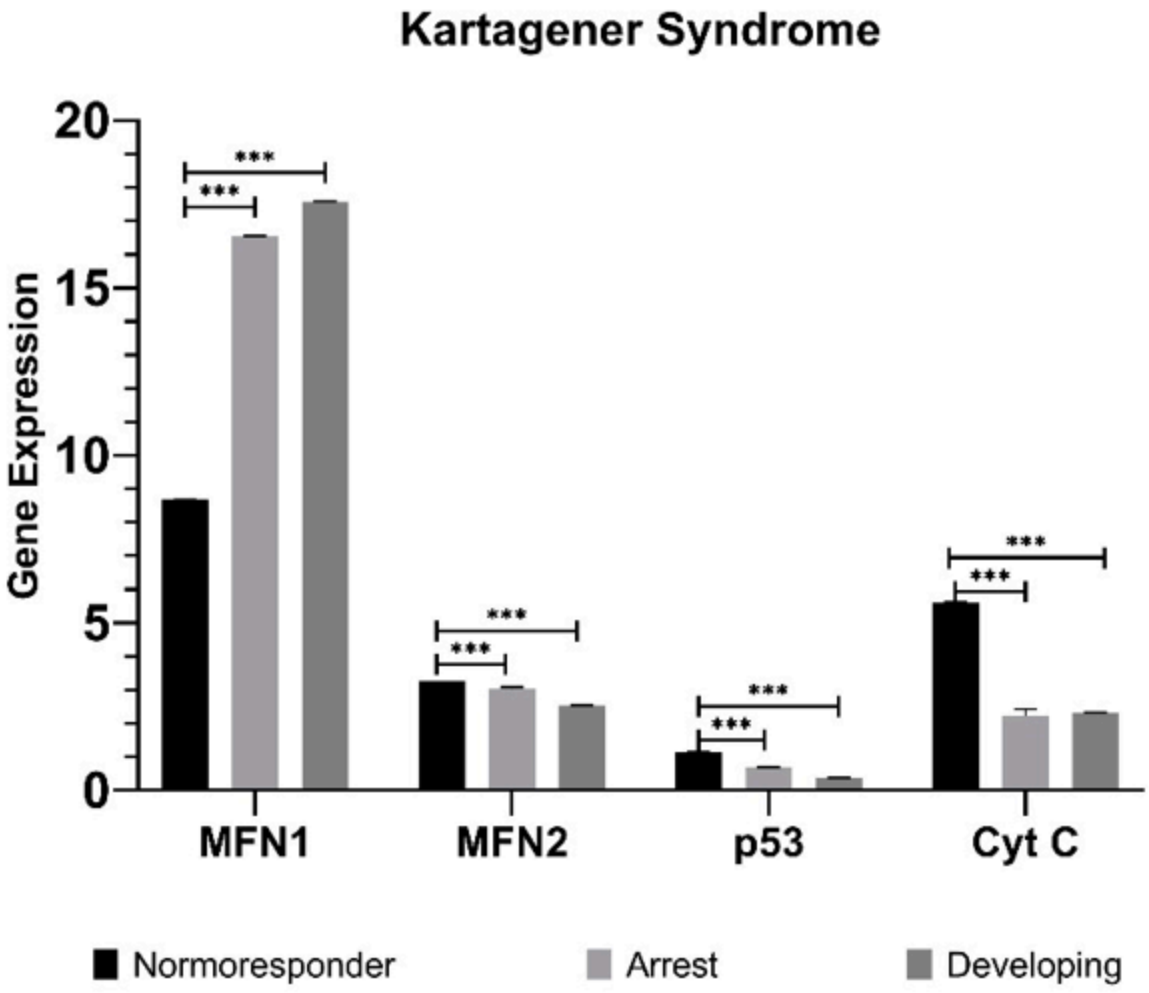

Figure 1

The p53, Cyt C, MFN1 and MFN2 Gene Expression Profiles in KS.

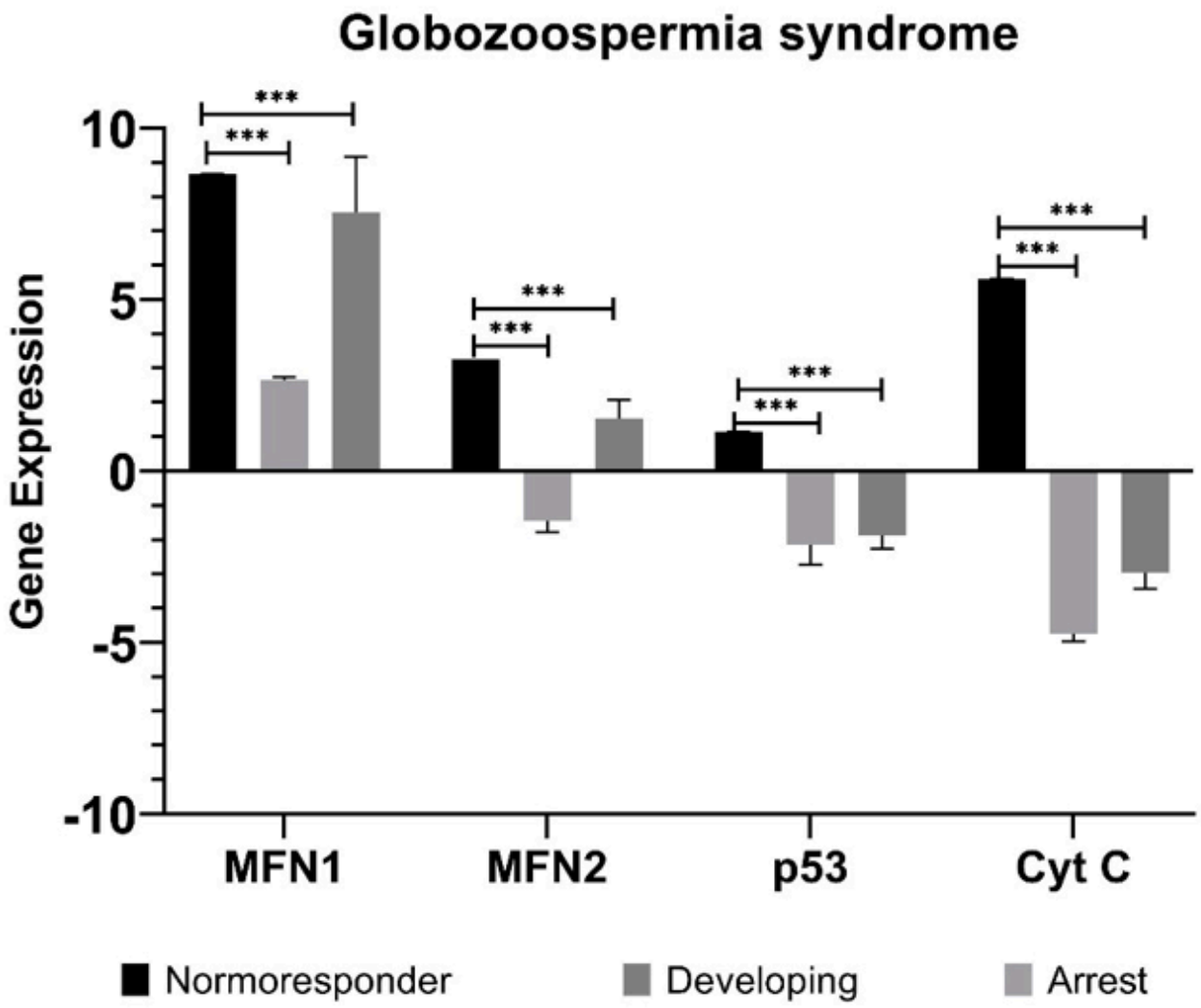

Figure 2

The p53, Cyt C, MFN1 and MFN2 Gene Expression Profiles in GS.