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Mitochondrial Heteroplasmy: Detection and Significance

Kirk J. Mantione

Mito Genetics, LLC, 3 Bio Science Park Drive, Farmingdale, NY, USA

Abstract The existence of sequence variants within the mitochondrial DNA of an individual cell known as heteroplasmy can be detected using numerous methods. The development of sequencing methods beyond the traditional Sanger method to massively parallel sequencing has made it possible to produce rapidly vast amounts of sequence information. The levels of heteroplasmy reported vary and may depend on a multitude of factors, including technical artifacts. Most of the variability is attributed to the established threshold for mtDNA mutation detection. Technical artifacts can arise from the sequencing chemistry used as well as the data handling approaches used. Only after a standard method for generating and reporting heteroplasmy data exists will it be possible to move forward towards determining the significance of mitochondrial DNA variations. The amount of heteroplasmy required to cause disease must be examined in greater detail before an exact conclusion is reached. The present report will note that the focus of many researchers has been to argue about techniques instead of understanding the extent and implications of mitochondrial heteroplasmy.

Keywords Mitochondrial DNA, Heteroplasmy, Massively parallel sequencing

1. Introduction

Mitochondrial DNA (mtDNA) sequence differences within an individual are referred to as heteroplasmy. It manifests itself as a mixture of mtDNA sequences. These sequence differences can be present between tissue types [1, 2] or within a single mitochondrion. The length of a particular sequence may vary [3-5] or differences could exist at a single point or as a mixture of point mutations within a sequence [6-8]. The nature of the mitochondria and its rapid genetic turnover yields a high rate of somatic mutations [9-11].

In the past, detection of mitochondrial heteroplasmy was achieved by denaturing gradient gel electrophoresis, restriction fragment length polymorphisms, Southern blotting, polymerase chain reactions, or other electrophoretic methods [12-20]. Advancements in sequencing methods beyond the traditional Sanger method have made it possible to gain rapidly vast amounts of data with varying amounts of reliability and accuracy. The reported degree of heteroplasmy is mostly due to overestimation of the phenomenon. Most of the over reporting is caused by differences in sensitivity of the Sanger method and the massively parallel sequencing methods [21]. Recently, Just, Irwin [21] provided an important framework for future studies and implored those investigators engaging in this type of research to validate their findings. The future studies they suggested should provide better estimations of position specific mutation rates and determine the probability of heteroplasmy related to mutation rates in the coding region. Technical and interpretative problems exist leading to difficulties distinguishing between heteroplasmy and sequencing errors [22]. However, even a low level of heteroplasmy appears to be important to human health. Besides determining which sequences might lead to pathologies, the number of copies of a mutant sequence leading to illness must be determined [22]. Determining which changes are pathogenic should be a major goal of those researchers in the molecular medicine field. Resources need to be focused on methods to correct or prevent deleterious changes to mtDNA.

* Corresponding author:
kirk.mantione@mitogenetics.com (Kirk J. Mantione)
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2. Methodology Issues

Levels of heteroplasmy reported in the literature vary greatly [23, 24]. Most of the variability is attributed to the established threshold for mtDNA mutation detection. Sequences are compared to a reference genome. The degree of difference between the reference sequence and the investigated sequence is measured and evaluated. The selected threshold is usually a chosen minor allele frequency (MAF) that must be exceeded to declare a sequence to be different. Despite some studies using the same MAF, there are still reported differences in levels of heteroplasmy. This discrepancy may be due to contamination [25, 21]. Discernment between homoplasmic mixtures of individual cells with multiple haplotypes and heteroplasmy within or between cells is extremely difficult [26]. Furthermore, contamination can further complicate the task. Additional

differences in levels could be due to data handling mistakes, such as improper sequence alignment [27] and poor data interpretation practices [28]. For example, reports showing high levels of heteroplasmy have been generated by improper use of databases as well as failure to completely search all possible previous reports of so-called novel sequences [28]. Additionally, careful exclusion of possible contamination of sequences with nuclear mitochondrial DNA (NUMT) [29] also may occur. NUMT can be accounted for by scrupulous bioinformatic practices or by proper purification of mitochondria before mtDNA sequencing.

Alignment issues are more likely to occur when length heteroplasmy is present. Chemistry based sources of error in heteroplasmy measurements will continually be minimized as the techniques are improved. Bioinformatic approaches must be carefully vetted before the data they generate are reported [30]. Few standardized methods exist for mtDNA sequence data analysis [31]. Currently, there are no uniform ways of interpreting or reporting length heteroplasmy data [32]. As a result, it is imperative that a specialist is consulted or employed when length heteroplasmy is observed. The suggestion posed by Just, Irwin [21] regarding the creation of a catalog of regions where length heteroplasmy should be followed.

3. Implications

Mutations are likely caused by chronic alcohol consumption [33, 34], cigarette smoking [33, 35], other external environmental insults to the mtDNA, and reactive oxygen species generated by mitochondria. Mutagens could be causing damage at a particular location within the mtDNA. Point mutations that repeatedly occur at the same location within the mtDNA sequence, hotspots, exist in the control region (CR) [6, 36, 37, 5]. Coding region hotspots are rare [38, 39]. There is a need for reliable data to support the existence of this type of hotspot [21]. Many CR hotspots occur in areas that initiate the replication of mitochondria [2, 40, 41], thereby leading to a selective propagation of these mutations. When somatic mutation lead to reduced bioenergetic capacity of the mitochondria the compensatory reaction of the cell is to produce more mitochondria, thus increasing the level of the mutated mtDNA sequences [42]. Consequently, age-related diseases of those organs are observed [43]. Impairment of insulin secretion and reduced insulin sensitivity have been demonstrated to be maternally inherited and associated with tRNA mutations [44, 45]. Atherosclerosis and cardiovascular disease are major complications of untreated type 2 diabetes [46]. Oxidative stress is a hallmark of type 2 diabetes and related metabolic diseases and represents a significant risk factor for atherosclerosis [47]. Recently, evidence for the development of atherosclerosis due to somatic mitochondrial mutations has been presented [48-50]. Psychiatric diseases may result from somatic mutations leading to heteroplasmy [51]. Cancer is the most studied of the diseases that are thought to be caused by mtDNA heteroplasmy [52, 53]. Carcinogenic processes might target normal mitochondrial functioning and cause a disruption of the Krebs cycle and electron transport enzymes [54, 11, 55]. Furthermore, normative mitochondrial function in non-proliferating cells affects relatively high cytosolic ATP/ADP ratios, resulting in functional inhibition of aerobic glycolysis [56]. In contrast, the bioenergetics of the "Warburg" effect that has is linked to the metabolic phenotype of numerous cancer cell types is characterized by enhanced aerobic glycolysis and suppression of mitochondrial aerobic metabolism [57, 58, 54, 59]. Furthermore, aerobic respiration in proliferating cells may lead to the deleterious production of free radicals that can mutate mtDNA. Accordingly, free radical damage could exacerbate compromised mitochondrial functioning and subsequently increase the copy number of mutant mitochondria in cancer cells.

4. Conclusions

The growing body of evidence supports mtDNA mutation involvement in organ-specific disorders and, therefore, they don't emerge randomly [1, 7, 42, 15, 60, 2]. There are also cases of different locations within an organ having varying degrees of heteroplasmy [61, 49]. The non-random nature of many mtDNA somatic mutations could be explained by looking at the causes of the mutations. In the case discussed, in response to a paper by Bandelt and Salas [25], Prior and colleagues defended their data that demonstrated that the carcinogens in cigarette smoke can mutate mtDNA at specific locations [35]. While some of the arguments and suggestions presented by Bandelt and Salas [25] are valid, an overwhelming amount of data supports that many detected mtDNA mutations are not random [60, 2, 40] and are not due to technical errors or contamination [7]. Once again the focus should be shifted away from arguing about techniques but rather towards understanding why mutations occur at hotspots. We also need to continue to learn which selective processes allow heteroplasmy to exist.

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REFERENCES

[1] Calloway, C.D., Reynolds, R.L., Herrin, G.L., Jr., and Anderson, W.W., 2000, The frequency of heteroplasmy in the HVII region of mtDNA differs across tissue types and increases with age, Am J Hum Genet, 66(4), 1384-97.

[2] Samuels, D.C., Li, C., Li, B., Song, Z., Torstenson, E., Boyd Clay, H., Rokas, A., Thornton-Wells, T.A., Moore, J.H.,

Hughes, T.M., Hoffman, R.D., Haines, J.L., Murdock, D.G., Mortlock, D.P., and Williams, S.M., 2013, Recurrent tissue-specific mtDNA mutations are common in humans, PLoS Genet, 9(11), e1003929.

[3] Chung, U., Lee, H.Y., Yoo, J.E., Park, M.J., and Shin, K.J., 2005, Mitochondrial DNA CA dinucleotide repeats in Koreans: the presence of length heteroplasmy, Int J Legal Med, 119(1), 50-3.

[4] Lee, H.Y., Chung, U., Yoo, J.E., Park, M.J., and Shin, K.J., 2004, Quantitative and qualitative profiling of mitochondrial DNA length heteroplasmy, Electrophoresis, 25(1), 28-34.

[5] Lutz-Bonengel, S., Sanger, T., Pollak, S., and Szibor, R., 2004, Different methods to determine length heteroplasmy within the mitochondrial control region, Int J Legal Med, 118(5), 274-81.

[6] Bendall, K.E., Macaulay, V.A., Baker, J.R., and Sykes, B.C., 1996, Heteroplasmic point mutations in the human mtDNA control region, Am J Hum Genet, 59(6), 1276-87.

[7] Cavadas, B., Soares, P., Camacho, R., Brandao, A., Costa, M.D., Fernandes, V., Pereira, J.B., Rito, T., Samuels, D.C., and Pereira, L., 2015, Fine Time Scaling of Purifying Selection on Human Nonsynonymous mtDNA Mutations Based on the Worldwide Population Tree and Mother-Child Pairs, Hum Mutat, 36(11), 1100-11.

[8] Goto, H., Dickins, B., Afgan, E., Paul, I.M., Taylor, J., Makova, K.D., and Nekrutenko, A., 2011, Dynamics of mitochondrial heteroplasmy in three families investigated via a repeatable re-sequencing study, Genome Biol, 12(6), R59.

[9] Brown, W.M., George, M., Jr., and Wilson, A.C., 1979, Rapid evolution of animal mitochondrial DNA, Proc Natl Acad Sci U S A, 76(4), 1967-71.

[10] Pilonis, N. and Stefano, G.B., 2015, CNS Cancers: Early Diagnosis by Psychiatric Assessment, 1, 32-35.

[11] Stefano, G.B. and Kream, R.M., 2015, Cancer: Mitochondrial Origins, Medical Science Monitor, 21, 3736-9.

[12] Boles, R.G., Chaudhari, D., Soderkvist, J., Podberezin, M., and Ito, M., 2003, Quantification of mitochondrial DNA heteroplasmy by temporal temperature gradient gel electrophoresis, Clin Chem, 49(1), 198-200.

[13] He, L., Chinnery, P.F., Durham, S.E., Blakely, E.L., Wardell, T.M., Borthwick, G.M., Taylor, R.W., and Turnbull, D.M., 2002, Detection and quantification of mitochondrial DNA deletions in individual cells by real-time PCR, Nucleic Acids Res, 30(14), e68.

[14] Jacobi, F.K., Meyer, J., Pusch, C.M., and Wissinger, B., 2001, Quantitation of heteroplasmy in mitochondrial DNA mutations by primer extension using Vent(R)(exo-) DNA polymerase and RFLP analysis, Mutat Res, 478(1-2), 141-51.

[15] Lombes, A., Diaz, C., Romero, N.B., Ziegler, F., and Fardeau, M., 1992, Analysis of the tissue distribution and inheritance of heteroplasmic mitochondrial DNA point mutation by denaturing gradient gel electrophoresis in MERRF syndrome, Neuromuscul Disord, 2(5-6), 323-30.

[16] Morten, K.J., 1998, Diagnosis of mitochondrial disorders using the PCR, Methods Mol Med, 16, 171-87.

[17] Seo, S.B., Jang, B.S., Zhang, A., Yi, J.A., Kim, H.Y., Yoo, S.H., Lee, Y.S., and Lee, S.D., 2010, Alterations of length heteroplasmy in mitochondrial DNA under various amplification conditions, J Forensic Sci, 55(3), 719-22.

[18] Tanaka-Yamamoto, T., Tanaka, M., Ohno, K., Sato, W., Horai, S., and Ozawa, T., 1989, Specific amplification of deleted mitochondrial DNA from a myopathic patient and analysis of deleted region with S1 nuclease, Biochim Biophys Acta, 1009(2), 151-5.

[19] Tanno, Y., Yoneda, M., Tanaka, K., Tanaka, H., Yamazaki, M., Nishizawa, M., Wakabayashi, K., Ohama, E., and Tsuji, S., 1995, Quantitation of heteroplasmy of mitochondrial tRNA (Leu(UUR)) gene using PCR-SSCP, Muscle Nerve, 18(12), 1390-7.

[20] Theves, C., Keyser-Tracqui, C., Crubezy, E., Salles, J.P., Ludes, B., and Telmon, N., 2006, Detection and quantification of the age-related point mutation A189G in the human mitochondrial DNA, J Forensic Sci, 51(4), 865-73.

[21] Just, R.S., Irwin, J.A., and Parson, W., 2015, Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing, Forensic Sci Int Genet, 18, 131-9.

[22] Sobenin, I.A., Mitrofanov, K.Y., Zhelankin, A.V., Sazonova, M.A., Postnov, A.Y., Revin, V.V., Bobryshev, Y.V., and Orekhov, A.N., 2014, Quantitative assessment of heteroplasmy of mitochondrial genome: perspectives in diagnostics and methodological pitfalls, Biomed Res Int, 2014, 292017.

[23] Ramos, A., Santos, C., Mateiu, L., Gonzalez Mdel, M., Alvarez, L., Azevedo, L., Amorim, A., and Aluja, M.P., 2013, Frequency and pattern of heteroplasmy in the complete human mitochondrial genome, PLoS One, 8(10), e74636.

[24] Sosa, M.X., Sivakumar, I.K., Maragh, S., Veeramachaneni, V., Hariharan, R., Parulekar, M., Fredrikson, K.M., Harkins, T.T., Lin, J., Feldman, A.B., Tata, P., Ehret, G.B., and Chakravarti, A., 2012, Next-generation sequencing of human mitochondrial reference genomes uncovers high heteroplasmy frequency, PLoS Comput Biol, 8(10), e1002737.

[25] Bandelt, H.J. and Salas, A., 2009, Contamination and sample mix-up can best explain some patterns of mtDNA instabilities in buccal cells and oral squamous cell carcinoma, BMC Cancer, 9, 113.

[26] Jayaprakash, A.D., Benson, E.K., Gone, S., Liang, R., Shim, J., Lambertini, L., Toloue, M.M., Wigler, M., Aaronson, S.A., and Sachidanandam, R., 2015, Stable heteroplasmy at the single-cell level is facilitated by intercellular exchange of mtDNA, Nucleic Acids Res, 43(4), 2177-87.

[27] Cerezo, M., Bandelt, H.J., Martin-Guerrero, I., Ardanaz, M., Vega, A., Carracedo, A., Garcia-Orad, A., and Salas, A., 2009, High mitochondrial DNA stability in B-cell chronic lymphocytic leukemia, PLoS One, 4(11), e7902.

[28] Bandelt, H.J., Salas, A., Taylor, R.W., and Yao, Y.G., 2009, Exaggerated status of "novel" and "pathogenic" mtDNA sequence variants due to inadequate database searches, Hum Mutat, 30(2), 191-6.

[29] Lopez, J.V., Yuhki, N., Masuda, R., Modi, W., and O'Brien, S.J., 1994, Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat, J Mol Evol, 39(2), 174-90.

[30] Mantione, K.J., Kream, R.M., Kuzelova, H., Ptacek, R., Raboch, J., Samuel, J.M., and Stefano, G.B., 2014, Comparing bioinformatic gene expression profiling methods: Microarray and RNA-Seq, Med Sci Monit Basic Res, 20, 138-141.

[31] Guo, Y., Li, J., Li, C.I., Shyr, Y., and Samuels, D.C., 2013, MitoSeek: extracting mitochondria information and performing high-throughput mitochondria sequencing analysis, Bioinformatics, 29(9), 1210-1.

[32] Berger, C., Hatzer-Grubwieser, P., Hohoff, C., and Parson, W., 2011, Evaluating sequence-derived mtDNA length heteroplasmy by amplicon size analysis, Forensic Sci Int Genet, 5(2), 142-5.

[33] Kloss-Brandstatter, A., Weissensteiner, H., Erhart, G., Schafer, G., Forer, L., Schonherr, S., Pacher, D., Seifarth, C., Stockl, A., Fendt, L., Sottsas, I., Klocker, H., Huck, C.W., Rasse, M., Kronenberg, F., and Kloss, F.R., 2015, Validation of Next-Generation Sequencing of Entire Mitochondrial Genomes and the Diversity of Mitochondrial DNA Mutations in Oral Squamous Cell Carcinoma, PLoS One, 10(8), e0135643.

[34] Tsuchishima, M., Tsutsumi, M., Shiroeda, H., Yano, H., Ueshima, Y., Shimanaka, K., and Takase, S., 2000, Study of mitochondrial DNA deletion in alcoholics, Alcohol Clin Exp Res, 24(4 Suppl), 12S-15S.

[35] Prior, S.L., Griffiths, A.P., Baxter, J.M., Baxter, P.W., Hodder, S.C., Silvester, K.C., and Lewis, P.D., 2006, Mitochondrial DNA mutations in oral squamous cell carcinoma, Carcinogenesis, 27(5), 945-50.

[36] Irwin, J.A., Saunier, J.L., Niederstatter, H., Strouss, K.M., Sturk, K.A., Diegoli, T.M., Brandstatter, A., Parson, W., and Parsons, T.J., 2009, Investigation of heteroplasmy in the human mitochondrial DNA control region: a synthesis of observations from more than 5000 global population samples, J Mol Evol, 68(5), 516-27.

[37] Lagerstrom-Fermer, M., Olsson, C., Forsgren, L., and Syvanen, A.C., 2001, Heteroplasmy of the human mtDNA control region remains constant during life, Am J Hum Genet, 68(5), 1299-301.

[38] Rebolledo-Jaramillo, B., Su, M.S., Stoler, N., McElhoe, J.A., Dickins, B., Blankenberg, D., Korneliussen, T.S., Chiaromonte, F., Nielsen, R., Holland, M.M., Paul, I.M., Nekrutenko, A., and Makova, K.D., 2014, Maternal age effect and severe germ-line bottleneck in the inheritance of human mitochondrial DNA, Proc Natl Acad Sci U S A, 111(43), 15474-9.

[39] Skonieczna, K., Malyarchuk, B., Jawien, A., Marszalek, A., Banaszkiewicz, Z., Jarmocik, P., Borcz, M., Bala, P., and Grzybowski, T., 2015, Heteroplasmic substitutions in the entire mitochondrial genomes of human colon cells detected by ultra-deep 454 sequencing, Forensic Sci Int Genet, 15, 16-20.

[40] Stoneking, M., Hedgecock, D., Higuchi, R.G., Vigilant, L., and Erlich, H.A., 1991, Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence-specific oligonucleotide probes, Am J Hum Genet, 48(2), 370-82.

[41] Vigilant, L., Pennington, R., Harpending, H., Kocher, T.D., and Wilson, A.C., 1989, Mitochondrial DNA sequences in single hairs from a southern African population, Proc Natl Acad Sci U S A, 86(23), 9350-4.

[42] Li, M., Schroder, R., Ni, S., Madea, B., and Stoneking, M., 2015, Extensive tissue-related and allele-related mtDNA heteroplasmy suggests positive selection for somatic mutations, Proc Natl Acad Sci U S A, 112(8), 2491-6.

[43] Greaves, L.C., Reeve, A.K., Taylor, R.W., and Turnbull, D.M., 2012, Mitochondrial DNA and disease, J Pathol, 226(2), 274-86.

[44] Guttman, A., Gao, H.G., and Haas, R., 2001, Rapid analysis of mitochondrial DNA heteroplasmy in diabetes by gel-microchip electrophoresis, Clin Chem, 47(8), 1469-72.

[45] Suzuki, Y., Suzuki, S., Hinokio, Y., Chiba, M., Atsumi, Y., Hosokawa, K., Shimada, A., Asahina, T., and Matsuoka, K., 1997, Diabetes associated with a novel 3264 mitochondrial tRNA(Leu)(UUR) mutation, Diabetes Care, 20(7), 1138-40.

[46] Frostegard, J., 2013, Immune mechanisms in atherosclerosis, especially in diabetes type 2, Front Endocrinol. (Lausanne), 4, 162.

[47] Wang, F., Guo, X., Shen, X., Kream, R.M., Mantione, K.J., and Stefano, G.B., 2014, Vascular Dysfunction Associated with Type 2 Diabetes and Alzheimer's Disease: A Potential Etiological Linkage, Med Sci Monit Basic Res, 20, 118-129.

[48] Sazonova, M.A., Chicheva, M.M., Zhelankin, A.V., Sobenin, I.A., Bobryshev, Y.V., and Orekhov, A.N., 2015, Association of mutations in the mitochondrial genome with the subclinical carotid atherosclerosis in women, Exp Mol Pathol, 99(1), 25-32.

[49] Sazonova, M.A., Sinyov, V.V., Barinova, V.A., Ryzhkova, A.I., Zhelankin, A.V., Postnov, A.Y., Sobenin, I.A., Bobryshev, Y.V., and Orekhov, A.N., 2015, Mosaicism of mitochondrial genetic variation in atherosclerotic lesions of the human aorta, Biomed Res Int, 2015, 825468.

[50] Sobenin, I.A., Sazonova, M.A., Postnov, A.Y., Bobryshev, Y.V., and Orekhov, A.N., 2013, Changes of mitochondria in atherosclerosis: possible determinant in the pathogenesis of the disease, Atherosclerosis, 227(2), 283-8.

[51] Sequeira, A., Rollins, B., Magnan, C., van Oven, M., Baldi, P., Myers, R.M., Barchas, J.D., Schatzberg, A.F., Watson, S.J., Akil, H., Bunney, W.E., and Vawter, M.P., 2015, Mitochondrial mutations in subjects with psychiatric disorders, PLoS One, 10(5), e0127280.

[52] He, Y., Wu, J., Dressman, D.C., Iacobuzio-Donahue, C., Markowitz, S.D., Velculescu, V.E., Diaz, L.A., Jr., Kinzler, K.W., Vogelstein, B., and Papadopoulos, N., 2010, Heteroplasmic mitochondrial DNA mutations in normal and tumour cells, Nature, 464(7288), 610-4.

[53] Kloss-Brandstatter, A., Schafer, G., Erhart, G., Huttenhofer, A., Coassin, S., Seifarth, C., Summerer, M., Bektic, J., Klocker, H., and Kronenberg, F., 2010, Somatic mutations throughout the entire mitochondrial genome are associated with elevated PSA levels in prostate cancer patients, Am J Hum Genet, 87(6), 802-12.

[54] Gonzalez, M.J., Miranda Massari, J.R., Duconge, J., Riordan, N.H., Ichim, T., Quintero-Del-Rio, A.I., and Ortiz, N., 2012, The bio-energetic theory of carcinogenesis, Med.Hypotheses, 79(4), 433-439.

[55] Stefano, G.B. and Kream, R.M., 2015, Hypoxia Defined as a Common Culprit/Initiation Factor in Mitochondrial-Mediated Proinflammatory Processes, Med Sci Monit, 21, 1478-1484.

[56] Maldonado, E.N. and Lemasters, J.J., 2014, ATP/ADP ratio, the missed connection between mitochondria and the Warburg effect, Mitochondrion.

[57] Amoedo, N.D., Valencia, J.P., Rodrigues, M.F., Galina, A., and Rumjanek, F.D., 2013, How does the metabolism of tumour cells differ from that of normal cells, Biosci Rep, 33(6).

[58] Chen, X., Qian, Y., and Wu, S., 2014, The Warburg Effect: Evolving Interpretations Of An Established Concept, Free Radic.Biol.Med.

[59] Witkiewicz, H., Oh, P., and Schnitzer, J.E., 2013, III. Cellular ultrastructures in situ as key to understanding tumor energy metabolism: biological significance of the Warburg effect, F1000Res., 2, 10.

[60] Naue, J., Horer, S., Sanger, T., Strobl, C., Hatzer-Grubwieser, P., Parson, W., and Lutz-Bonengel, S., 2015, Evidence for frequent and tissue-specific sequence heteroplasmy in human mitochondrial DNA, Mitochondrion, 20, 82-94.

[61] Keogh, M.J. and Chinnery, P.F., 2015, Mitochondrial DNA mutations in neurodegeneration, Biochim Biophys Acta, 1847(11), 1401-11.