Frontiers in Health InformaticsISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2025; Vol 14: Issue 2
Open Access

Mitochondrial DNA Variations Increase the Risk of Glioblastoma Multiforme

Ali Azadi[1,3], Seyed Mohammad Musavi[1], Faridoddin Javaherian[1], Haniye Kazemi[2], Enayatolla Noori[1],Michael Van Haute[3]

[1]Qom University of Medical Sciences and Health Services, Qom, Iran
[2]Zanjan University of Medical Sciences, Zanjan, Iran
[3]De la salle Health Sciences Institute,College of Medicine,Cavite, Philippines

Cite this paper as: Ali Azadi Seyed Mohammad Musavi, Faridoddin Javaherian, Haniye Kazemi, Enayatolla Noori,Michael Van Haute (2025), Mitochondrial DNA Variations Increase the Risk of Glioblastoma Multiforme. *Frontiers in Health Informatics*, *14(2) 3043-3056*

ABSTRACT

Purpose: Gliomas account for 80% of all primary malignant brain tumors, and virtually 50% of gliomas give rise to glioblastoma multiforme (GBM).Given the possibility that GBM may significantly be influenced by genetic variations in complex I genes, we, for the first time, conducted a survey of ND genes in Iranian cases with GBM and attempted to seek for mutations.

Methods: A total of 25 patients and 79 non-cases were included in the present study. We amplified target genes of mtDNA using PCR, and sequenced them to evaluate mitochondrial complex I gene alterations. Based on the results, there were eight variants (ND1 T3394C, ND2 G5460A, ND3 A10398G, ND4 C11674T, ND5 A12662G, ND4L C10473G, ND5 C13061A, and ND6 C14426T)in the mitochondrial complex I gene.

Results: Our results also showed that the prevalence of ND4, ND4L, ND5, and ND6 gene variations was significantly higher in cases than in non-cases.

Conclusions: In conclusion, since the importance of mtDNA changes, as a potential molecular etiology for complex I deficiency more study is necessary to understand the role and effect of the mitochondrial variations in GBM pathogenesis.

Key words: Mitochondrial DNA, Glioblastoma multiforme, mitochondrial variations

1-INTRODUCTION

Brain tumors are recognized as the most frequent central nervous system tumors worldwide, and the second and fifth cause of cancer death among male and female adults, respectively [1,2]. These tumors are believed to be developed by various possible risk factors, particularly genetic conditions and environmental factors.

World Health Organization (WHO) has recorded 160 types of primary brain tumors, among which gliomas, medulloblastoma, and glioblastoma multiforme(GBM)are predominantly malignant. As per WHO severity scale, GBM has been graded as the most severe tumor (level 4) owingto its malignant nature [2]. Prognosis of this tumor is poor, reported as12–14 months after diagnosis. Indeed, only 3-5% of the patients survive more than three years [3,5]. The annual incidence of malignant GBM is estimated to be about five cases per 100,000 people worldwide [6]. For treating brain tumor,combined therapy by surgery, radiation, and chemotherapy is the most common treatment option [7].

Mitochondria,double-membranous organelles, are found a bundantlyin eukaryotic cells. The main role of these organelles is to synthesize ATP. Mitochondrial DNA (mtDNA) is a double-stranded molecule of 16.6

kb in length. This circular molecule carries 37 genes encoding 22 transfer RNAs, two ribosomal RNAs, and 13 polypeptides, which are vital for producing the ATP via the biochemical process of oxidative phosphorylation (OXPHOS). Complex I (NADH:ubiquinone oxidoreductase) is the largest complex of respiratory chain and comprises of at least 46 subunits, seven of which are encoded by the mtDNA, namely NADH dehydrogenase (ND)1, 2, 3, 4.4 L, 5, and 6.OXPHOS is of crucial importance for organs require high energy, such as the heart, muscles, and brain. Hence, it is not surprising that any failure in the OXPHOS would cause pathogenic disorders in these organs. It has been established that the mitochondria have a fundamental function in well-known diseases such as cancers and diabetes [8,13]. It has also been suggested that mutation in mtDNA is a factor affecting carcinogenesis processing and tumors [14].

Given the possibility that GBM may significantly be influenced by genetic variations in complex I genes, we, for the first time, conducted a survey of ND genes in Iranian cases with GBM and attempted to seek for mutations.

2-Material and Methods

A total of 25 cases of GBM and 79 non-cases were analyzed. Patients with brain cancer as well as the healthy individuals were selected from Cancer Institute of Tehran, Imam Khomeini Hospital and Shahid Beheshti Hospital Qom, Iran, respectively. None of the patients and non-cases had a family history of brain tumors or a prior history of significant ionizing radiation exposure. The Ethics Committee of the Qom University of Medical Sciences has approved the present research work, and all participants gave their written informed consents before enrolment.

2-1 DNA extraction, PCR amplification, and sequencing

Peripheral blood specimens were acquired from both patients and non-cases. DNA was extracted using a DNA extraction kit (QIAamp DNA Micro Kit; Qiagen, CA, USA) in accordance with the instruction provided by manufacturer. A list of the primers employed for the amplification of the mtDNA-encoded complex I subunit genes are illustrated in Table 1. PCR amplifications were accomplished in a final volume of 25 µl solution, comprising100 ng of genomic DNA, 10 pmol and 10 nmol of each primer and each deoxyribonucleotide triphosphates, respectively, 1.5 mmol of Mg2+, 1× PCR buffer, and 1 U of Taq polymerase. Separation of the amplified products were performed on2% agarose gel electrophoresis [15,16]. Direct sequencing method was utilized to analyze the amplified products by counting the mtDNA-complex I genes. To verify detected variations, we sequenced all the amplified fragments in both directions, forward and reverse. We also analyzed the chromatograms by the aid of the Chromas software. In the end, we compared the sequencing results with the MITOMAP database, the Human Mitochondrial Genome Databasehttp://www.genpat.uu.se/mtDB/and GenBank (http://www.ncbi.nlm.nih.gov/GenBank/index.html/NIH, Bethesda, MD).

2-2 Statistical analysis

All the data were statistically analyzed by using Stata 14.2 (StataCorp, College Station, TX, USA). The t-test for independent means was also exploited to compare the baseline characteristics of the cases and the non-cases, as well as the mean ages. However, for other variables, the t-test for independent means was applied. In general, the predictor variables of interest evaluated in the present study were as follows:(1) presence of mitochondrial gene variant(s) regardless of type; (2) presence of each particular mitochondrial gene variant type detected; (3) number of mitochondrial gene variants present. The relationship of each predictor variable with the occurrence of GBM was investigated by the chi-square (χ^2) and Fisher's exact tests. For each predictor variable, a logistic regression model was fitted. The possible confounders were age, sex, and smoking status, which were subsequently assessed for confounding effect by using the change-in-estimate criterion. The likelihood-ratio test statistic, G2, was then computed as measure of fit for the Firth's logistic

regression models to determine how well each model fits the data. Lastly, Tjur's coefficient of discrimination (Tjur's D) and the area under the ROC curve (AuROC) were calculated to evaluate the discriminatory ability of each model to predict GBM. For all statistical analyses, values of probability "p"were less than 0.05 and considered as statistically significant.

3-Results

In total,25 GBM cases and 79 non-cases were included in this study. The difference in the mean age between the two groups is near the significance threshold of 0.05 ($p = 0.0598$), with cases tending to be younger (45.76 years; SD: 5.07 years) than non-cases (48.09 years; SD: 5.41 years). Overall, eight variants were detected in the mitochondrial complex I (ND), including ND1 T3394C, ND2 G5460A, ND3 A10398G, ND4 C11674T, ND5 A12662G, ND4L C10473G, ND5 C13061A, and ND6 C14426T. The frequencies of the type and number of these gene variants, along with the other baseline characteristics of the patients included in the study, are summarized in Table 2.

Table 3 summarizes the associations between the different predictors of interest and GBM. Based on the Table, the presence of mitochondrial gene variants was significantly associated with GBM ($\chi 2df=1 = 15.22$, $p<0.001$); however, among the individuals with gene variants, non-cases had significantly a higher proportion of having only one variant compared to cases (83.37%, SD: 6.6% vs. 28.57%, SD: 9.9%, respectively; $p=0.0001$; Table 4). Besides, none of the patients in the non-cases group had more than two co-existing gene variants.

The crude and adjusted odds ratios (ORs) for GBM with each predictor variable of interest are summarized in Table 5. For each predictor variable, a regression model was fitted by applying Firth's logistic regression. Age, sex, and smoking status were assessed for possible confounding; only age had a change-in-estimate of >10% and was thus adjusted for each regression model. In all models, age had a significant effect on the outcome.

The number of coexisting gene variants, when treated as a continuous variable, was significantly associated with GBM (OR=5.63, 95% CI 2.69–11.79, after controlling for age; $p<0.001$). This variable can also be ordinal (LR $\chi 2df=3$–2.15 versus number as continuous, $p=1.0000$), thus further analyses were carried out for its treatment.

The measures of fit for each Firth's logistic regression model are represented in Table 6. Except for the model using predictor T3394C, which has a G2 slightly above the significance threshold of 0.05, all the other models had significant G2 statistics, indicating that the age-adjusted logistic model is well fitted with our data as compared to the intercept only model. However, only the models of two predictors had reasonable discriminatory power (i.e., Tjur's D between 0.3–0.4), that is the number of coexisting mitochondrial gene variants and the occurrence of C14426T variant). Similarly, the two models had corresponding AuROCs of >0.80 (Figure 1).

4-Discussion

Complex I (NADH–ubiquinone oxidoreductase) is known as the largest respiratory complex of the electron transport chain. In various pathological conditions, e.g.cancer, diabetes, and neurodegenerative diseases, mutations in mitochondrial and nuclear genes (encoding complex I subunits) are a contributing factor [17]. Mitochondria shape change as well as alterations in the number and function of mitochondria have been reported in different cancers. It has also been suggested that the conversion of ATP production from mitochondrial OXPHOS into glycolysis is a bioenergetics hallmark of cancer cells [18].

mtDNA is highly susceptible to oxidative damage, and its mutation rate is much greater than nDNA. The reason behind such high mutation is the lack of ineffectual DNA repair mechanism and protective histone protein. As mtDNA is near the respiratory chain, its vulnerability becomes higher when exposed to reactive oxygen species (ROS), a by-product of OXPHOS that causes damage to mtDNA. Based on emerging evidence, a ROS plays a key role in elevating tumor growth [19,20]. According to Mitomap (http://www.mitomap.org), more than 30 mutations and sequence variations have been identified in mtDNA, and all have a connection with brain tumors in mitochondrial genome database reported previously [7]. Moreover, some of the mutations in mtDNA inducing OXPHOS defects have pro-tumorigenic impacts. For instance, cancer cells mutated in ND4 and ND6, which gently reduces OXPHOS function, can enhance tumor development [18].Until date, there is very scant information on the prognosis of brain tumor with mtDNA changes. Additionally, investigation on this subject is limited, and their outcomes results are controversial [7].

Our results demonstrated eight variants in the mitochondrial complex I (ND) gene viz ND1 T3394C, ND2 G5460A, ND3 A10398G, ND4 C11674T, ND5 A12662G, ND4L C10473G, ND5 C13061A, and ND6 C14426T. Among the detected variants, seven (T3394C, G5460A, A10398G, C10473G, C13061A, and C14426T) were nonsynomous and one (C11674T)was synonymous (Table 7).Furthermore, ND4 C11674T, ND4L C10473G, ND5 A12662G, and ND6 C14426Tvariations were significantly higher in GBM patients than in non-cases. A number of the above-mentioned variations have previously been reported in several investigations. In a study conducted by Meixensberger et al. OXPHOS enzyme activities were down regulated in malignant gliomas, but not brain tissues. They explored that ND4 and ND6 at the mitochondrial genome assist cells to enhance GBM tumorigenesis [21,22]. They also observed that ND4 and ND6, which encode complex I subunits, are the most affected gene mutations, supporting the results of the present study and those of Yeung et al who verified the presence of the two aforesaid variants in high-grade gliomas [23].In a comparable study by DeHaan et al.,complex I ND subunits were found to be mutational hot spots in tumor mtDNA. They found that genetic mutations that change the function and structure of complex I could modify the capacity of a cell to respond to oxygen deficit and are likely help resistance to chemotherapeutic agents [24]. Zhang et al.affirmed our findings and demonstrated that patients with glioma and high mtDNA content had prolonged survival times [25]. In contrast, a number of researchers reported no effect on the prognosis of patients with brain tumor and mtDNA changes [7]. Montanini et al. indicated that mtDNA changes could not serve as an indication in the prognostic or diagnostic assessments of gliomas [26]. Vidone et al., the same as Montanini et al. [26], suggested that mtDNA genotyping could not be used as an effective molecular tool for the prediction of prognosis [27].

Considering the results of aforementioned research works, it is rationale to conclude that some of the mitochondrial complex I gene variations have a link with the pathogenesis of GBM. Such relationship between mitochondrial complex I gene variations and GBM, which was confirmed by our results, indicates that GBM susceptibility may be enhanced by these variations. However, the type and frequency of the mtDNA-encoded subunits of ND gene variations are differentin various studies. This discrepancy comes from the effect of racial dissimilarityof populations and/or differences in the number of studied subjects.

In conclusion, since the importance of mtDNA changes,as a potential molecular etiology for complex I deficiency, was not in hesitation, more study is necessary to understand the role and effect of the mitochondrial variations in GBM pathogenesis.

Limitations and recommendations

The main strength of this study is that we were able to quantify the association between a number of complex I mitochondrial gene variants and GBM. To address potential issues brought about by unconscious selection

bias, we assessed possible confounding and controlled them in each fitted model. Another strength point is our ability to demonstrate a significant association between increasing number of concurrent gene variants and GBM. This trend of increasing ORs was observed only in up to three coexisting gene variants, owing to the paucity of patients having four (or more) variants present. While our logistic regression models were wellfitted with our data, only two predictors,namely the number of concurrent gene variants and the occurrence of the C14426T variant, had promising discriminatory capability. Although this may well be due to a limited sample size, this bringsattention to increasing number of gene variants as having significant effect on the occurrence of GBM andnot just necessarily the variant type itself.The low Tjur's D values and unsatisfactory AuROCs of most ofthe fitted models suggest that other variables could be at play.It is important to stress that since our studyis cross-sectional in design, the period of time in which the gene variants have existed prior to the time oftheir ascertainment is invariably different for each patient, making this variable difficult to measure interms of duration. Apart from two strong risk factors in our samples'brain tumors, i.e. unknown history of exposure to ionizing radiation or family history of brain cancer, , other variables might be contributing to the risk of GBM. Allergies, certainviral infections, and head trauma have been proposed as possible causative factors. Much of the limitations of this study likewise hinges on the small sample size, as evidenced bythe wide confidence intervals of our estimates. For future studies, we recommend an increase in sample sizesuch as mtDNA copy number and grade of glioma, to allow for important variables to be included in the analysis and for interactions between the mentioned variables to be assessed. An increase in sample size could formally assess the number of coexisting gene variants for effective measure modification.

Acknowledgement

We are grateful toall the participants who have attendedin this study.

Conflict of interest

There are no potential conflicts of interest for each author concerning the submitted manuscript.

REFERENCES

1. M. Mehta, M.A. Vogelbaum, S. Chang, N. Patel, Neoplasms of the Central Nervous System: Epidemiology of Brain Tumors, DeVita, Vincent T.; Lawrence, Theodore S.; Rosenberg, Steven A. Cancer: Principles and Practice of Oncology. 2011; 1700–1749. http://www.brainlife.org/abstract/2011/Mehta_M110000.pdf.

2. World Health Organisation, GLOBOCAN Cancer Fact Sheets, WHO International Agency for Research on Cancer. 2012; doi:: http://globocan.iarc.fr(link is external).

3. J.A. Schwartzbaum, J.L. Fisher, K.D. Aldape, M. Wrensch, Epidemiology and molecular pathology of glioma. Nature Clinical Practice Neurology. 2006; 2(9):494-503.

4. B. Kunkle, C. Yoo, D. Roy, Discovering gene-environment interactions in Glioblastoma through a comprehensive data integration bioinformatics method. NeuroToxicology. 2013; 35:1-14

5. D. Krex, B. Klink, C. Hartmann, A. Von Deimling, T. Pietsch, M. Simon, M. Sabel, J.P. Steinbach, O. Heese, G. Reifenberger, M. Weller, G. Schackert, Long-term survival with glioblastoma multiforme. Brain. 2007; 130(Pt 10):2596-606

6. P.Y. Wen, S. Kesari, M. de Tayrac, E. Vauleon, R. Boniface, et al, Malignant Gliomas in Adults. New England Journal of Medicine. 2008; 359:492-507.

7. A. Mohamed Yusoff, Role of mitochondrial DNA mutations in brain tumors: A mini-review. Journal of Cancer Research and Therapeutics. 2015; 11(3):535-44.

8. S. DiMauro, E.A. Schon, Mitochondrial {DNA} mutations in human disease. American Journal of Medical Genetics. 2001; 106(1):18-26

9. A.K. Reeve, K.J. Krishnan, D. Turnbull, Mitochondrial DNA mutations in disease, aging, and neurodegeneration, in: Annals of the New York Academy of Sciences. 2008; 1147:21-9

10. J. Smeitink, L. Van Den Heuvel, S. DiMauro, The genetics and pathology of oxidative phosphorylation. Nature Reviews Genetics. 2001; 2(5):342-52

11. A.R. Rezaee, A. Azadi, M. Houshmand, F. Mahmoodi, Z. Purpak, S. Safaei, P. Karimi, M. Ghabaee, M.A. Sahraian, Mitochondrial and nuclear genes as the cause of complex I deficiency. Genetics and Molecular Research. 2013; 12(3):3551-4.

12. L. Iommarini, M.A. Calvaruso, I. Kurelac, G. Gasparre, A.M. Porcelli, Complex i impairment in mitochondrial diseases and cancer: Parallel roads leading to different outcomes. International Journal of Biochemistry and Cell Biology. 2013; 45(1):47-63.

13. S. Sethumadhavan, J. Vasquez-Vivar, R.Q. Migrino, L. Harmann, H.J. Jacob, J. Lazar, Mitochondrial DNA variant for complex I reveals a role in diabetic cardiac remodeling. Journal of Biological Chemistry. 2012; 287(26):22174-82.

14. O. Warburg, On Origin of Cancer Cells. Science. 1956; 123(3191):309-14.

15. A. Poursadegh Zonouzi, S. Ghorbian, M. Abkar, A.A. Poursadegh Zonouzi, A. Azadi, Mitochondrial complex i gene variations; As a potential genetic risk factor in pathogenesis of multiple sclerosis. Journal of the Neurological Sciences. 2014; 345(1-2):220-3

16. A. Azadi, D.J. Seo, H. Jafari Sasansara, M. Van Haute, Mitochondrial DNA variations are associated with recurrent pregnancy loss. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis. 2018; 29(5):674-678.

17. F.A. Urra, F. Muñoz, A. Lovy, C. Cárdenas, The Mitochondrial Complex(I)ty of Cancer. Frontiers in Oncology. 2017; 7: 118

18. J.M. Garcia-Heredia, A. Carnero, Decoding Warburg's hypothesis: tumor-related mutations in the mitochondrial respiratory chain. Oncotarget. 2015.

19. N.B. Larsen, M. Rasmussen, L.J. Rasmussen, Nuclear and mitochondrial DNA repair: Similar pathways?. Mitochondrion. 2005; 5(2):89-108.

20. M. Brandon, P. Baldi, D.C. Wallace, M. Brandon, P. Baldi, P. Baldi, D.C. Wallace, D.C. Wallace, Mitochondrial mutations in cancer. Oncogene. 2006;25(34):4647-62.

21. J. Meixensberger, B. Herting, W. Roggendorf, H. Reichmann, Metabolic patterns in malignant gliomas. Journal of Neuro-Oncology. 1995; 24(2):153-61

22. R. Günther Feichtinger, S. Weis, J. Adalbert Mayr, F. Zimmermann, R. Geilberger, W. Sperl, B. Kofler, Alterations of oxidative phosphorylation complexes in astrocytomas. GLIA. 2014; 62(4):514-25.

23. K.Y. Yeung, A. Dickinson, J.F. Donoghue, G. Polekhina, S.J. White, D.K. Grammatopoulos, M. McKenzie, T.G. Johns, J.C. St John, The identification of mitochondrial DNA variants in glioblastoma multiforme. Acta Neuropathologica Communications. 2014; 2:1.

24 C. DeHaan, B. Habibi-Nazhad, E. Yan, N. Salloum, M. Parliament, J. Allalunis-Turner, Mutation in mitochondrial complex I ND6 subunit is associated with defective response to hypoxia in human glioma cells. Molecular Cancer. 2004; 3: 19

25. Y. Zhang, Y. Qu, K. Gao, Q. Yang, B. Shi, P. Hou, M. Ji, High copy number of mitochondrial DNA

(mtDNA) predicts good prognosis in glioma patients. American Journal of Cancer Research. 2015; 5(3):1207-16

26. L. Montanini, C. Regna-Gladin, M. Eoli, R. Albarosa, F. Carrara, M. Zeviani, M.G. Bruzzone, G. Broggi, A. Boiardi, G. Finocchiaro, Instability of mitochondrial DNA and MRI and clinical correlations in malignant gliomas. Journal of Neuro-Oncology. 2005; 74(1):87-9.

27. M. Vidone, R. Clima, M. Santorsola, C. Calabrese, G. Girolimetti, I. Kurelac, L.B. Amato, L. Iommarini, E. Trevisan, M. Leone, R. Soffietti, I. Morra, G. Faccani, M. Attimonelli, A.M. Porcelli, G. Gasparre, A comprehensive characterization of mitochondrial DNA mutations in glioblastoma multiforme. International Journal of Biochemistry and Cell Biology. 2015; 63:46-54.

Table 1. Nucleotide sequence of primers used for amplification of mitochondrial complex I genes.

Gene	Sequence of primers	Annealing temperature (°C)	PCR product (bp)
ND1	F 5-CTCAACTTAGTATTATACCC-3 R 5-GAGCTTAGCGCTGTGATGAG-3	57	1353
ND2	F 5-GTCATCTACTCTACCTACTT-3 R 5-GGCGGGAGAAGTAGATTGAA-3	59	1249
ND3	F 5-CACTATCTGCTTCATCCGCC-3 R 5-GAGCGATATACTAGTATTCC-3	52	689
ND4	F 5-TCTCCAACACATATGGCCTA-3 R 5-ACTGTGAGTGCGTTCGTAGTTTGAG-3 F 5-GCGCAGTCATTCTCATAATC-3 R 5-TTTGTTAGGGTTAACGAGGG-3	54 54	1065 729
ND4L	F 5-TCTGGCCTATGAGTGACTAC-3 R 5-ACTGTGAGTGCGTTCGTAGTTTGAG-3	54	1415
ND5	F 5-TTTTGGTGCAACTCCAAA-3 R 5-GGTTGACCTGTTAGGGTGAG-3 F 5-GCAGTCTGCGCCCTACA-3 R 5-TCAGGGTTCATTCGGGAGGA-3	50 54	1369 1079
ND6	F 5-TTCATCATGCGGAGATGTTGGATGGGGTGG-3 R 5-CTCCAAAGACCACATCATCGAAAC-3	52	1334

Table 2. Baseline characteristics of the patients

Characteristic	Non-cases (n=79)	Cases (n=25)	p value
Age (yr)	48.09 ± 5.41	45.76 ± 5.07	0.0598

Female sex — no. (%)	32 (40.51)	9 (36)	0.6876
Medical History — no. (%)			
Hypertension	13 (16.46)	4 (16)	0.9568
Diabetes	9 (11.39)	4 (16)	0.5435
Ever smokers — no. (%)	30 (37.97)	10 (40%)	0.8557
Presence of mitochondrial genevariants — no. (%)	31 (39.24)	21 (84)	0.0003
Gene, variant type — no. (%)			
ND1, T3394C	11 (13.92)	2 (8)	0.4353
ND2, G5460A	4 (5.06)	2 (8)	0.5826
ND3, A10398G	4 (5.06)	3 (12)	0.2273
ND4, C11674T	6 (7.59)	11 (44)	<0.0001
ND5, A12662G	2 (2.53)	5 (20)	0.0024
ND4L, C10473G	0	3 (12)	0.0018
ND5, C13061A	6 (7.59)	5 (20)	0.0786
ND6, C14426T	3 (3.80)	14 (56)	<0.0001
Number of variants present — no. (%)			
0	48 (60.76)	4 (16)	0.0001
1	26 (32.91)	6 (24)	0.4002
2	5 (6.33)	7 (28)	0.0031
3	0	7 (28)	<0.0001
4	0	1 (4)	0.0741

Table 3. Association between the presence, type, and number of mitochondrial gene variants and occurrence of glioblastoma multiforme[a].

Presence, type, and number of variants	□2 statistics[b]	p value
Presence of mitochondrial gene variant/s regardless of type	15.22	<0.001
Variant type		
T3394C	0.61	0.729
G5460A	0.30	0.629
A10398G	1.46	0.355
C11674T	18.41	<0.001
A12662G	9.23	0.008
C10473G	9.76	0.013
C13061A	3.09	0.079
C14426T	37.85	<0.001
Number of variants present		
1	0.71	0.400
2	8.74	0.003
3	23.72	<0.001
4	3.19	0.240

[a]For cells with observations <1, Fisher's exact test was used.

[b]Statistically significant if □^{2}> critical value of 3.841 (df =1, □<0.05)

Table 4. Distribution of patients according to number of mitochondrial gene variants present.

Number of variants	Frequency		P value
	Non-cases (n =31)	Cases (n =21)	
Only one variant present			0.0001
T3394C	7	1	
G5460A	2	1	
A10398G	4	0	
C11674T	5	2	
A12662G	1	0	
C10473G	0	2	

C13061A	5	0	
C14426T	2	0	
Two variants present			0.1486
T3394C, C11674T	1	0	
T3394C, G5460A	1	0	
T3394C, C13061A	1	0	
T3394C, C14426T	1	1	
G5460A, A12662G	1	0	
A10398G, C14426T	0	1	
C11674T, C14426T	0	5	
Three variants present			0.00005
G5460A, A12662G, C14426T	0	1	
A10398G, C13061A, C14426T	0	1	
A10398G, A12662G, C14426T	0	1	
C11674T, C13061A, C14426T	0	2	
C11674T, A12662G, C14426T	0	1	
A12662G, C10473G, C13061A	0	1	
Four variants present			0.2200
C11674T, A12662G, C13061A, C14426T	0	1	

[a]*p*-values are based on results of *t*-test for independent proportions.

Table 5. Summary of crude and adjusted ORs for occurrence of GBM with each mitochondrial gene variant.

Predictor variable of interest	**Crude OR (95% CI)**	**Pvalue**	**Adjusted OR (95% CI)[a]**	**Pvalue**
Presence of mitochondrial gene variant/s regardless of type	7.36 (2.42–22.34)	<0.001	8.41 (2.62–26.95)	<0.001
Number of gene variants present				

0 1 2 3 4	1.00 (Reference) 2.64 (0.73–9.62) 14.70 (3.40–63.54) 161.67 (7.88–3316.92) 32.33 (1.14–915.31)	– 0.140 <0.001 0.001 0.042	1.00 (Reference) 3.00 (0.78–11.56) 21.15 (4.18–107.05) 182.93 (8.36–4003.77) 85.01 (2.51–2879.73)	– 0.110 <0.001 0.001 0.013
T3394C	0.63 (0.15–2.69)	0.536	0.78 (0.18–3.41)	0.741
G5460A	1.78 (0.36–8.96)	0.482	1.73 (0.34–8.91)	0.512
A10398G	2.61 (0.60–11.40)	0.202	3.00 (0.67–13.40)	0.150
C11674T	8.97 (2.94–27.34)	<0.001	7.95 (2.60–24.34)	<0.001
A12662G	8.32 (1.73–40.07)	0.008	8.10 (1.64–40.07)	0.010
C10473G	24.73 (1.23–496.74)	0.036	18.91 (0.92–386.69)	0.056
C13061A	3.03 (0.88–10.45)	0.079	3.62 (1.01–13.03)	0.049
C14426T	27.56 (7.35–103.33)	<0.001	40.12 (9.13–176.23)	<0.001

[a]Adjusted for age using Firth's logistic regression.

Table 6. Summary of G^2 statistics and Tjur's D for each logistic regression model.

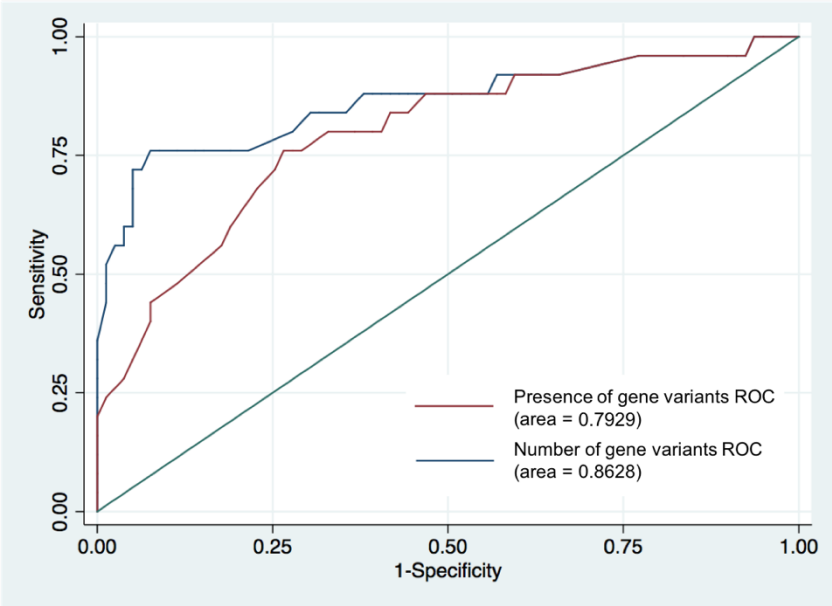
Predictor variable	G2statistic[a]	pvalue	Tjur's D[b]
Presence of mitochondrial gene variant/s	20.252	<0.001	0.202
Number of coexisting mitochondrial gene variants	41.068	<0.001	0.416

T3394C	3.506	0.061	0.035
G5460A	3.912	0.048	0.037
A10398G	5.386	0.021	0.048
C11674T	17.416	<0.001	0.188
A12662G	10.732	0.001	0.110
C10473G	9.616	0.002	0.098
C13061A	7.164	0.007	0.068
C14426T	38.48	<0.001	0.398

[a]TheG^2 statistic follows a □^{2} distribution; statistically significant if >3.841 (df =1, □<0.05).

Table 7. Detailed information of the polymorphism found in this study

Variation	Locus	Nucleotide changes	Amino acid changes
3394	ND1	T-C	Y-H
5460	ND2	G-A	A-T
10398	ND3	A-G	T-A
11674	ND4	C-T	T-T
10473	ND4L	C-G	P-A
12662	ND5	A-G	N-S
13061	ND5	C-A	P-Q
14426	ND6	C-T	G-E



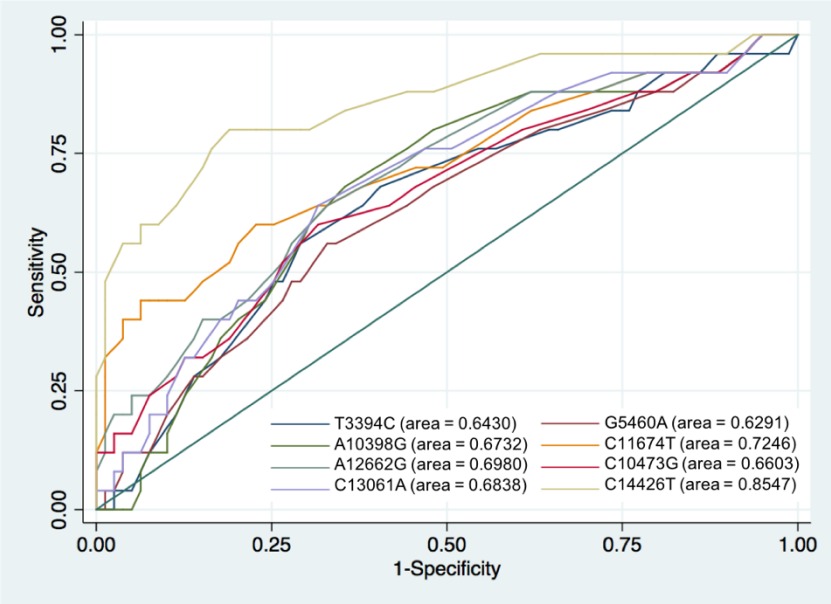


Figure 1. Receiver-operating characteristic (ROC) curves. (A)The presence and the number of mitochondrial gene variants and (B) different mitochondrial gene variants.