

# Prevalence of mitochondrial DNA common deletion in patients with gliomas and meningiomas: A first report from a Malaysian study group

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## Abstract

**Background:** The 4977-bp common deletion (mtDNA<sup>4977</sup>) is a well-established mitochondrial genome alteration that has been described in various types of human cancers. However, to date, no studies on mtDNA<sup>4977</sup> in brain tumors have been reported. The present study aimed to determine mtDNA<sup>4977</sup> prevalence in common brain tumors, specifically, low- and high-grade gliomas (LGGs and HGGs), and meningiomas in Malaysian cases. Its correlation with clinicopathological parameters was also evaluated.

**Methods:** A total of 50 patients with pathologically confirmed brain tumors (13 LGGs, 20 HGGs, and 17 meningiomas) were enrolled in this study. mtDNA<sup>4977</sup> was detected by using polymerase chain reaction (PCR) technique and later confirmed via Sanger DNA sequencing.

**Results:** Overall, mtDNA<sup>4977</sup> was observed in 16 (32%) patients and it was significantly correlated with the type of tumor group and sex, being more common in the HGG group and in male patients.

**Conclusion:** The prevalence of mtDNA<sup>4977</sup> in Malaysian glioma and meningioma cases has been described for the first time and it was, indeed, comparable with previously published studies. This study provides initial insights into mtDNA<sup>4977</sup> in brain tumor and these findings can serve as new data for the global mitochondrial DNA mutations database.

**Keywords:** Glioma; Meningioma; Mitochondrial DNA deletion

## 1. INTRODUCTION

Primary tumors of the central nervous system (CNS) are among the top 10 leading causes of death worldwide. It is estimated that 18 020 deaths occurred from these tumors in 2020.<sup>1</sup> The common types of primary CNS tumors in adults are gliomas and meningiomas.<sup>2</sup>

Gliomas are the most common intra-axial brain tumors, originating from glial cells, and comprise of three main types: astrocytomas, oligodendrogliomas, and ependymomas. According to the World Health Organization (WHO), these gliomas are further classified into four classes, more specifically, into low-grade gliomas (grades WHO I and II) and high-grade gliomas (grades WHO III and IV), based on histopathological and molecular features.<sup>3,4</sup> Low-grade gliomas (LGGs) are relatively slow-growing and benign histopathological characteristics, while high-grade

gliomas (HGGs) are highly malignant and aggressive tumors including anaplastic astrocytoma (WHO III) and glioblastoma multiforme (GBM) (WHO IV).<sup>3,4</sup>

On the other hand, meningiomas are the most common extra-axial tumors of the meninges in the intracranial space, originating from arachnoid cap cells.<sup>5</sup> They are usually benign and slow growing. These tumors are present in approximately 37% of all primary CNS tumors, and according to the WHO, they are classified into three grades (grade WHO I or benign, grade WHO II or atypical, and grade WHO III or malignant).<sup>4</sup>

Gliomas and meningiomas exhibit multiple complex interactions, with a variety of alterations in the genetic and epigenetic composition of the CNS that influence the patient prognosis.<sup>6,7</sup> To date, research on the genesis and development of these tumors has intensively focused on the alteration of the gene in the nucleus. Because of the complexity of genomic changes within these tumor cells, some researchers have shifted their focus to another genome. In addition to the nuclear genome, it is worth considering that there is another genome that needs to be investigated.

Mitochondria, which are remarkable organelles, are the ultimate energy power plants for producing ATP via an oxidative phosphorylation (OXPHOS) system and for retaining various cellular functions in cells.<sup>8</sup> Mitochondria contain our second genome, called mitochondrial DNA (mtDNA), which has a circular shape with an approximate size of 16 569 base pairs, encoding 13 proteins that are part of respiratory complexes I, III, IV, and V, 22 transfer RNAs (tRNAs) and two ribosomal RNAs (rRNAs).<sup>9</sup>

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In 1956, Otto Warburg<sup>10</sup> was the pioneer biochemistry researcher that proposed the relevance of mitochondria with the origin of cancer cells. Warburg's view believed that mitochondrial alterations in function might enhance tumor growth or promote cancer progression. From then on, different types of mtDNA aberrations have been characterized in human cancers such as point mutations, deletions, rearrangements, and changes in mtDNA content.<sup>11</sup>

Mitochondria are also the major source of cellular reactive oxygen species (ROS) and the preferred target of oxidative stress.<sup>12,13</sup> Relatively, ROS are well known to be toxic by-products during mitochondrial electron transport.<sup>14</sup> Excessive ROS exposure can trigger mtDNA damage and initiate a vicious cycle of damage within the mitochondria as well as the injuries implicated in carcinogenesis.<sup>15</sup> Furthermore, oxidative damage to mtDNA can also result in mtDNA deletions by causing double-strand breaks in the DNA.<sup>15</sup>

Historically, mitochondrial molecular medicine research has risen dramatically since the 1980s, when the discoveries of point mutations<sup>16</sup> and deletions<sup>17</sup> in mtDNA were believed to be linked to the diseases. In fact, large-scale mtDNA deletions were among the first mtDNA alterations to be discovered to cause human diseases.<sup>17-19</sup> To date, >180 mtDNA deletions that have been reported to be associated with various diseases have been stored in the Mitomap (<http://www.mitomap.org>), a mitochondrial genome database. One of the most essential large-scale mtDNA deletions that leads to a huge loss of the mitochondrial genome fragment is the 4977-bp mtDNA deletion (mtDNA<sup>4977</sup>).

Known as the "common deletion", this alteration involves the elimination of nucleotides from the 8470 to the 13 447 position of the human mitochondrial genome. mtDNA<sup>4977</sup> eliminates all five tRNA genes (tRNA<sup>Gly</sup>, tRNA<sup>Arg</sup>, tRNA<sup>His</sup>, tRNA<sup>Ser</sup>, and tRNA<sup>Leu</sup>) and seven genes encoding four Complex I subunits (ND3, ND4, ND4L, partial ND5), one Complex IV subunit (COX III), and two Complex V subunits (ATP6 and partial ATP8), that are vital for sustaining a normal mitochondrial OXPHOS function.<sup>20</sup>

mtDNA<sup>4977</sup> has been studied in various other tumor types, but there is little information about in the primary CNS tumors.<sup>21,22</sup> The present study is the first prospective study to determine the prevalence of mtDNA<sup>4977</sup> and its accumulation in patients with common primary CNS tumors, namely, gliomas and meningiomas.

## 2. METHODS

### 2.1. Patients and samples collection

This study was conducted on a Malay population of patients who referred to Hospital Universiti Sains Malaysia (HUSM) and the Department of Neurosciences during 2015-2018, with a brain tumor diagnosis, either with a glioma or a meningioma. A total of 50 patients were enrolled in the study after providing their written informed consent. Patients who had a previous history of receiving radiotherapy procedures to the brain or chemotherapy for any reason were excluded from the study. All patients underwent the neurological surgery. The histopathological diagnosis of the tumors was performed by a consultant neuropathologist following WHO criteria. This study was approved by the Research Ethics Committee of Universiti Sains Malaysia (USM) adhered to the tenets of the Declaration of Helsinki. The control group used in this study consisted of 20 archival paraffin-embedded tissues obtained from the autopsies of traffic accident victims, which were confirmed to be normal human brain tissues.

### 2.2. DNA extraction

All total DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) guided by the manufacturer's protocol. The concentration and quality of extracted DNA were measured using the NanoDrop ND1000 spectrophotometer and 1% gel agarose electrophoresis. All qualified DNAs were stored at -80°C until analysis.

### 2.3. PCR amplification of mtDNA<sup>4977</sup>

The multiplex PCR assay was performed to detect mtDNA<sup>4977</sup> using two sets of primers (P1/P2 and P3/P4). The primer sequences used were as follows: P1, 5'-CTGAGCCTTTTACCACTCCAG-3'; P2, 5'-GGTGATTGATACTCCTGATGCGA-3'; P3, 5'-CCCACTGTAAAGCTAACTTAGCATTAACCT-3'; P4, 5'-GGTTTCGATGAT GTGGTCTTTGG-3'. Samples were denatured at 94°C for 30s, followed by 30 cycles of denaturation (94°C for 30s), annealing (59°C for 30s), extension (72°C for 1min), and ultimately, a final extension at 72°C for 10min. PCR products were visualized using SYBR Green staining, following electrophoresis on 1.5% agarose gels.

In the case of mtDNA<sup>4977</sup> detection, another single PCR assay was performed with the second pair of primers for further confirmation of mtDNA<sup>4977</sup>. Primer pairs used to analyze 4977-bp deletion were as follows: L8150, Forward 5'-CCGGGGGTATACTACGGTCA-3' and H13650, reverse 5'-GGGGAAGCGAGGTTGACCTG-3'. The PCR conditions were as follows: 35 cycles of 30s at 94°C for denaturation, 30s at 58°C for annealing, and 1min at 72°C for the extension. The final extension was performed at 72°C for 5min.

### 2.4. Sanger DNA sequencing

All mtDNA<sup>4977</sup> were independently reassessed using Sanger DNA sequencing. Purified PCR products were sequenced using the same primers as that described in the PCR amplification. Sequencing was performed using a Big Dye Terminator cycle sequencing kit (Applied Biosystems Inc., Foster City, CA, USA), according to the manufacturer's protocol, and sequence reactions were analyzed on an ABI Prism 3700 DNA Analyzer automated sequencer (Applied Biosystems Inc.).

### 2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism software version 8.4.1 (GraphPad Software, Inc., San Diego, CA, USA). Demographic parameters of patients were summarized by descriptive statistics, including mean, SD, and range for continuous variables. For categorical variables, frequencies are described as absolute numbers and percentages. The association between mtDNA<sup>4977</sup> status and clinicopathological parameters was analyzed using either the chi-square test or Fisher's exact test. A *p*-value of <0.05 was considered statistically significant.

## 3. RESULTS

### 3.1. Characteristic profile of the study subjects

The main clinicopathological features of the patients are summarized in Table 1. There were 21 males (42%) and 29 females (58%) among 50 CNS tumor patients included in this study, with their age at diagnosis ranging from 15 to 70 years (mean: 51.2 ± 1.90) years.

The tumor groups were divided into three groups: 13 patients were included in the LGG group (2 patients with grade I and 11 patients with grade II gliomas), 20 patients were included in the HGG group (4 patients with grade III and 16 patients with grade IV gliomas), and 17 patients were included in the meningioma group, and all these patients were diagnosed with

**Table 1**  
**Clinicopathological information of patients with glioma and meningioma**

Patients' parameters	n	%
Age, y		
<50	22	44
>50	28	56
Mean $\pm$ SD	51.2 $\pm$ 1.90	
Range	15-70 y	
Gender		
Male	21	42
Female	29	58
Tumour type (grade)		
LGG	13	26
Pilocytic astrocytoma I	2	4
Astrocytoma II	5	10
Ependymoma II	4	8
Oligodendroglioma II	2	4
HGG	20	40
Anaplastic astrocytoma III	4	8
Glioblastoma multiforme IV	16	32
Meningioma	17	34

HGG = high-grade glioma; LGG = low-grade glioma.

meningioma grade I. Overall, there were 26% of LGG, 40% of HGG, and 34% of meningioma cases.

### 3.2. Detection of mtDNA<sup>4977</sup> in glioma and meningioma patients

To detect mtDNA<sup>4977</sup> in glioma and meningioma tissues of patients, mtDNA fragments were amplified using a multiplex PCR assay consisting of two pairs of primers (P1/P2 and P3/P4) (Fig. 1A). One primer pair, P1/P2, was designed to give a 142-bp product at mtDNA nucleotides 9500 to 9641 that are localized inside the mtDNA<sup>4977</sup> region. This 142-bp amplicon, corresponding to wild-type mtDNA, was used as an amplification control. Meanwhile, the other pair, P3/P4, that flanked the detected deletion region, allowed only a 262-bp DNA product, in case mtDNA<sup>4977</sup> was present. The existence of both amplicons (142 and 262 bp) indicates the presence of heteroplasmy.

In the case of mtDNA<sup>4977</sup> detection, the second set of PCR primers was used in a single PCR assay, to verify mtDNA<sup>4977</sup>. In the presence of mtDNA<sup>4977</sup>, the PCR amplification product was 524 bp in size (Fig. 1B). In the absence of the deletion or for the wild-type mtDNA amplification, there was no product yield because the fragment was too large (>5 kb) to be generated under the conditions used for PCR. mtDNA<sup>4977</sup> was identified in the patient tumor tissue, whereas this deletion was not observed in the normal control tissues. The deletion was verified by repeated analyses, which were finally confirmed using Sanger DNA sequencing (Fig. 2).

### 3.3. Prevalence of mtDNA<sup>4977</sup> and its clinicopathologic correlation

We determined a total of 16 mtDNA<sup>4977</sup> present in 50 enrolled patients by performing PCR, followed by sequencing, for validation. mtDNA<sup>4977</sup> was discovered in 32% (16/50) of the cases. All paired peripheral blood samples and brain tissue controls were mtDNA<sup>4977</sup>-negative. The most common mtDNA<sup>4977</sup> detected in 55.0% (11/20) of HGG cases, followed by 17.6% (3/17) of meningioma cases, and 15.4% (2/13) of LGG cases.

The clinicopathological information of the patients with and without mtDNA<sup>4977</sup> is summarized in Table 2. We further assessed the association of mtDNA<sup>4977</sup> with the clinicopathological

parameters of CNS tumor patients. Regarding tumor grading, we divided the patients into three groups: LGG, HGG, and meningioma.

Interestingly, in this study, it was revealed that there was a significant association between mtDNA<sup>4977</sup> status and tumor groups ( $p = 0.0172$ ). mtDNA<sup>4977</sup> was significantly more frequently detected in the HGG group (55.0%) than in the LGG (15.4%) and meningioma (17.6%) groups. Our data also showed that, mtDNA<sup>4977</sup> frequency in male patients was 52.4% ( $n = 11/21$ ), which was significantly higher than that in female patients (17.2%;  $n = 5/29$ ) ( $p = 0.0137$ ). Moreover, mtDNA<sup>4977</sup> frequency in the >50 years age group was 35.7% ( $n = 10/28$ ), which was higher than that in the <50 years age group (27.3%;  $n = 6/22$ ), but with no statistical significance between them ( $p = 0.5589$ ). It was concluded that there were significant correlations between mtDNA<sup>4977</sup> status with gender and tumor group. However, no significant association was identified between mtDNA<sup>4977</sup> status and age.

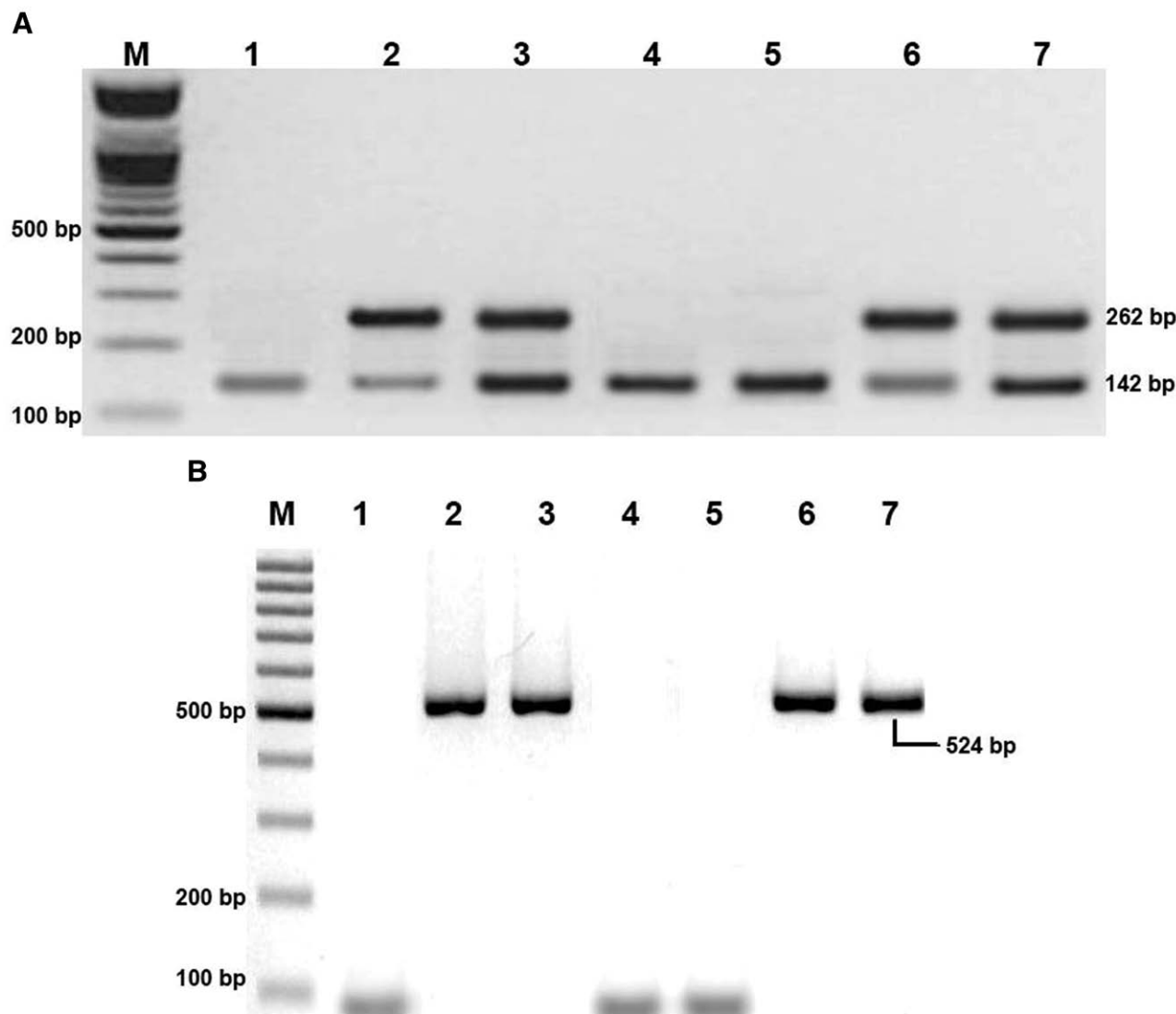
## 4. DISCUSSION

Gliomas and meningiomas are the most common types of adult brain tumors in Malaysia (accounting for approximately 28% and 36%, respectively, of brain tumors), with a poor prognosis.<sup>23</sup> The accumulation of genetic alterations is believed to be a direct reflection of the multiple steps involved in brain tumorigenesis. Some reports suggest that mtDNA may be altered in patients with brain tumors.<sup>21,22</sup> The deletion between nucleotides 8470 and 13 447 of the human mitochondrial genome (established known as mtDNA<sup>4977</sup>), which may lead to OXPHOS system failure, seems to be particularly crucial because of the elimination of some tRNAs and coding region genes, which are essential for the entire normal mitochondrial function and biogenesis process.<sup>24</sup>

Also considered as a pathogenic mutation, mtDNA<sup>4977</sup> is believed to play a physiological role in energy production cataprophes and aberrant ROS production of human diseases.<sup>25,26</sup> In humans, the brain is the most energy-demanding organ and tends to have a greater number of mitochondria. mtDNA deletions accumulate more rapidly in tissues with greater metabolic activity and a lower cell turnover, such as brain tissues, which leads to an enhanced oxidative stress that may contribute to dramatically accelerate deletions in brain tissues.

Mitochondrial OXPHOS is the predominant source of energy in cells, in which the energy transferred by electrons passing through the electron transport chain (ETC). The essential subunits of ETC complexes are encoded by mtDNA. As a consequence of alterations/deletions of mtDNA in cells, in particular mtDNA-encoded core subunits, cells exhibit increased oxidative stress and a defective OXPHOS function, which leads to protumorigenic effects.<sup>27</sup> Complex 1 represents about 40% of the proton-motive energy driving OXPHOS, making it, by far, the largest ETC complex. Complex 1 failure (due to heteroplasmic ND5 mutations) has been demonstrated to induce apoptosis resistance and activation of the PI3K/Akt pathway, which can potentially result in higher tumorigenicity.<sup>28</sup> In addition, the loss or disruption of some mitochondrial tRNA genes, particularly in mtDNA<sup>4977</sup>, is believed to trigger the impairment of the mtDNA-encoded proteins translation, consequently resulting in an enhanced ROS production and disease.<sup>24,29</sup>

mtDNA<sup>4977</sup> has been previously reported to accumulate in various disorders, including mitochondrial diseases and many types of cancer.<sup>21,30</sup> The initial finding of mtDNA<sup>4977</sup> was described in 1989 by Wallace's group in a patient with Kearns-Sayre/chronic external ophthalmoplegia plus syndrome,<sup>19</sup> and later it was often reported to accumulate in diverse human tissues with aging.<sup>31,32</sup> mtDNA<sup>4977</sup> has been reported in patients with cancer since the



**Fig. 1** Analysis of the mtDNA<sup>4977</sup> by agarose gel electrophoresis. (A) Multiplex PCR was used to amplify mtDNA<sup>4977</sup>. A 142-bp band corresponds to the wild-type mtDNA and a 262-bp band corresponds to the deleted 4977-bp region. The appearance of both bands indicates the presence of mtDNA<sup>4977</sup> (heteroplasmy). The appearance of the only 142-bp band indicates the absence of mtDNA<sup>4977</sup>. (B) Using the specific primers for validation, a 524-bp PCR product amplified from the mtDNA<sup>4977</sup> in tumor patients. The disappearance of any band indicates the absence of mtDNA<sup>4977</sup>. Lane M: 100-bp DNA marker, Lane 1: Normal sample—no deletion, Lanes 2 to 7: tumor samples.

earliest descriptions by Máximo et al,<sup>33,34</sup> who revealed a high frequency of mtDNA<sup>4977</sup> in primary sporadic gastric carcinomas. Furthermore, mtDNA<sup>4977</sup> was discovered to vary between 0% and 100% in a variety of human cancers.<sup>20</sup>

In this study, the prevalence of mtDNA<sup>4977</sup> in brain tumors, particularly in gliomas and meningiomas, and its association with clinicopathological parameters was investigated. mtDNA<sup>4977</sup> was found in 16 patients, resulting in a 32% prevalence among glioma and meningioma cases. To the best of our knowledge, to date, there have been no studies conducted on the mitochondrial genetic alterations involving mtDNA<sup>4977</sup> found in brain tumor cases. The present study is believed to be the first report of mtDNA<sup>4977</sup> detection in CNS/brain tumor in Malaysia patients. Therefore, there is little comparable data, especially in Asia, due to the limited number of mtDNA<sup>4977</sup> studies, mainly in gliomas and meningiomas.

By searching the literature published in PubMed, Scopus, Google Scholar, and other databases, mtDNA<sup>4977</sup> has been widely studied in breast cancer. A study in Vietnam reported that 68.8% of breast cancer cases had the mtDNA<sup>4977</sup>,<sup>35</sup> while another study in China reported a 48% mtDNA<sup>4977</sup> percentage in the blood of breast carcinoma patients.<sup>36</sup> A separate study conducted in China revealed the accumulation of mtDNA<sup>4977</sup> in all cases of primary breast cancer and benign breast disease.<sup>37</sup>

Other than breast cancer, mtDNA<sup>4977</sup> has also been reported in other cancer types. Perhaps the earliest study, by Lee et al<sup>38</sup> reported a 49% mtDNA<sup>4977</sup> percentage in oral cancer Taiwanese patients. A previous study involving Chinese patients performed by Dai et al<sup>39</sup> on lung cancer determined a 54.1% mtDNA<sup>4977</sup> frequency. In a study that involved gastric cancer also in China patients, Wang and Lu<sup>40</sup> reported a higher rate (79.6%) of mtDNA<sup>4977</sup> in gastric cancer tissues. In 2014, Dimberg et al<sup>41</sup>

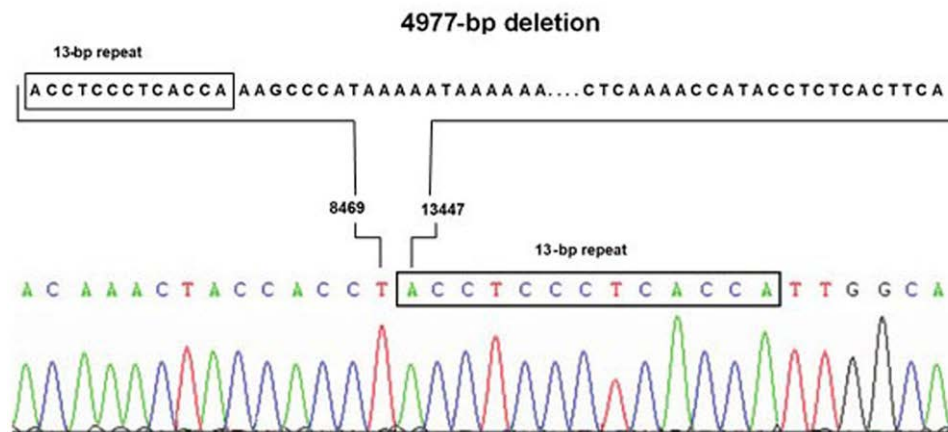


Fig. 2 Representative Sanger sequencing chromatogram demonstrating confirmation of mtDNA<sup>4977</sup> that contains only one of the 13-bp repeat.

revealed that 80.7% (71/88) of the colorectal cancer cases in Vietnamese patients were found to have mtDNA<sup>4977</sup>.

A lower percentage of the mtDNA<sup>4977</sup> in cancerous tissues has also been reported in other cancers. A study in Taiwan reported that 5% of breast cancer cases had the mtDNA<sup>4977</sup>,<sup>42</sup> whereas also in Taiwanese cases, Wu et al<sup>43</sup> detected only a 9.7% of mtDNA<sup>4977</sup> frequency in gastric carcinomas. In another study, Upadhyay et al<sup>44</sup> identified a low frequency of mtDNA<sup>4977</sup> (5.1%) in Indian patients with esophageal cancer. The lower incidence of the mtDNA<sup>4977</sup> has also been identified in hepatocellular cancer in Korean (11.1%) and Chinese (9.52%) patients, in two previous studies by Gwak et al<sup>45</sup> and Guo et al,<sup>46</sup> respectively. Compared with the previous studies for all different types of cancer in Asia, the prevalence of mtDNA<sup>4977</sup> in this study was comparable to that in other Asian countries.

Research on mtDNA<sup>4977</sup> from various cancer cases outside Asia reported higher prevalence results, compared with this

study. Studies from Caucasian populations, such as the United States, Brazil, and Poland, reported a prevalence of mtDNA<sup>4977</sup> in breast,<sup>47</sup> colorectal,<sup>48</sup> and endometrial cancer,<sup>49</sup> of 46.2%, 52.2%, and 81%, respectively. A study in Argentina by Pavicic and Richard<sup>50</sup> reported a prevalence of this deletion of 45.3% in breast cancer. A Swedish study by Dimberg et al<sup>41</sup> yielded a prevalence of 67.6% in colorectal cancer samples, which is twice the value obtained in this study.

Genetic variations among racial/ethnic groups, as well as the difference in cancer types, may influence the wide variation of mtDNA<sup>4977</sup> rates in Asian and Caucasian populations. Although numerous studies in different populations have been performed to determine associations between the mtDNA<sup>4977</sup> and various types of cancer, to date, no study has been conducted in brain tumor cases. Due to the limitations of existing data, the exact role of mtDNA<sup>4977</sup> in brain tumorigenesis is still unknown. However, it has been hypothesized that mtDNA deletions might appear either through spontaneous errors during mtDNA replication (replication slippage) or aberrancy of double-strand break repair (DBS).<sup>51-54</sup> Furthermore, it has been clarified that mtDNA<sup>4977</sup> is generated as a consequence of frequent fork stalling, a process that is mediated by the mitochondrial replisome, but independent of canonical DSB repair.<sup>55</sup> It is also believed that increased oxidative stress in cancer cells results in genomic instability in both mitochondria and the nucleus.<sup>56</sup> Therefore, it is suggested that under oxidative stress conditions, excessive ROS production can trigger the accumulation of deletions.<sup>57-59</sup>

The mitochondrial genome with the deleted 4977 bp has been noted to contribute to a deficient mitochondrial bioenergetic state.<sup>24,25</sup> Wei and his colleagues demonstrated that a self-accelerating vicious cycle of mitochondrial ROS is induced in cybrids harboring mtDNA<sup>4977</sup>, following a brief intense oxidative stress treatment.<sup>60</sup> These outcomes suggest that mtDNA<sup>4977</sup> plays a pivotal role in the pathophysiology process of the disease.<sup>60</sup> mtDNA deletion can result in elevated oxidative stress, which, in turn, creates more accumulation of the deleted mtDNA in cells. On the other hand, external environmental factors (including ionizing radiation, ultraviolet radiation, and exogenous chemicals) are thought to have an impact on mtDNA<sup>4977</sup> formation.<sup>61-65</sup> The combination of endogenous and exogenous factors may lead to deletion accumulation of mtDNA, which in turn may lead to the induction of human cancer.

mtDNA<sup>4977</sup> was detected in some tumor tissues, and this deletion was absent in all peripheral blood of patients and normal brain tissue subjects. It has been acknowledged that post-mitotic highly aerobic tissues (contain large numbers of mitochondria),

**Table 2**  
Association between clinicopathological parameters and mtDNA<sup>4977</sup> in patients with glioma and meningioma

Patients' parameters	mtDNA <sup>4977</sup> status		p
	mtDNA <sup>4977</sup> +	mtDNA <sup>4977</sup> -	
No. of patients (n)			
50	16 (32.0%)	34 (68.0%)	...
Gender			
Male	11 (52.4%)	10 (47.6%)	0.0137 <sup>a</sup>
Female	5 (17.2%)	24 (82.8%)	
Age, y			
<50	6 (27.3%)	16 (72.7%)	0.5589 <sup>a</sup>
>50	10 (35.7%)	18 (64.3%)	
Tumour type (grade)			
LGG			0.0172 <sup>b</sup>
Pilocytic astrocytoma I	0 (0%)	2 (100%)	
Astrocytoma II	1 (20.0%)	4 (80.0%)	
Ependymoma II	1 (25.0%)	3 (75.0%)	
Oligodendroglioma II	0 (0%)	2 (100%)	
HGG	11 (55.0%)	9 (45.0%)	
Anaplastic astrocytoma III	2 (50.0%)	2 (50.0%)	
Glioblastoma multiforme IV	9 (56.3%)	7 (43.7%)	
Meningioma	3 (17.6%)	14 (82.4%)	

HGG = high-grade glioma; LGG = low-grade glioma.

<sup>a</sup>p values correspond to two-sided Fisher's exact test.

<sup>b</sup>Chi-square test was used; p value <0.05 was considered significant (\*p < 0.05).

such as the brain, skeletal muscle, kidneys, and heart, tend to be the most affected, but theoretically, any organ can be involved.<sup>66-68</sup> Moreover, cancer cells require sufficient energy to support their uncontrolled rapid proliferation. Therefore, it is relevant that mtDNA alterations are often found at certain levels in post-mitotic tissues, as the clonal expansion of aberrations exists within these cells over time. Wallace<sup>69</sup> demonstrated high levels of clonally expanded mtDNA mutations in the brain and skeletal muscles. The rationale for the absence of deletions in other patients might be that cells undergo apoptosis when the mitochondrial mutation load increases to a certain threshold value.<sup>43</sup> Brain tumor cells, mostly high-grade tumors, tend to grow and spread more rapidly, and have the ability to invade and destroy the surrounding normal brain tissues. Besides, this tumor is difficult to remove without excessive damage to nearby normal brain tissues. Due to these reasons, non-tumor tissues adjacent to the tumor tissues were not used in this study. Alternatively, the patient's peripheral blood, as well as normal human brain tissues obtained from motor vehicle accidents, served as a control.

In the present study, we found a significant correlation between mtDNA<sup>4977</sup> and the tumor group (LGG, HGG, and meningioma), which means mtDNA<sup>4977</sup> was more common in HGG than in other groups ( $p = 0.0172$ ). It was also revealed that mtDNA<sup>4977</sup> status was significantly correlated with the gender group ( $p = 0.0137$ ). Notably, it was identified that the mtDNA<sup>4977</sup> frequency in the <50-year cases was only 27%, lower than that in the >50-year samples (35.7%), despite the lack of statistical significance between them ( $p = 0.5589$ ). In colorectal cancer research, Dimberg et al<sup>41</sup> observed that there was no statistically significant association between mtDNA<sup>4977</sup> and clinical parameters, such as gender, age, and tumor localization. Most of the findings from previous studies of mtDNA<sup>4977</sup> in human cancer reported no correlation with clinicopathological features.

Discrepancies in population characteristics and research methods may have also contributed to the prevalence of mtDNA<sup>4977</sup> in this study. The older age cases in this study may have influenced the deletion prevalence, as mtDNA<sup>4977</sup> has been found to be significantly higher in elderly individuals.<sup>15,70,71</sup> Corral-Debrinski et al<sup>70</sup> suggested that the accumulation of mtDNA deletions might contribute to the neurological impairment, often associated with human aging. More advanced or combination methods (such as real-time quantitative PCR, next-generation sequencing) could be used for further investigation to enhance the sensitivity of mtDNA<sup>4977</sup> detection.

In conclusion, we demonstrated that the overall prevalence of mtDNA<sup>4977</sup> in glioma and meningioma patients was 32%, which is in agreement with previously published results. There was a significant correlation between mtDNA<sup>4977</sup> and the tumor group, which shows that mtDNA<sup>4977</sup> was higher in the HGG group than in the LGG, and meningioma groups. Also, a statistically significant difference was found between mtDNA<sup>4977</sup> and gender, which indicates that mtDNA<sup>4977</sup> was prevalent in males than in females. This study provides initial insights into how mtDNA<sup>4977</sup> might contribute to brain tumor, and this finding can serve as new data for the global database of mtDNA mutations. Further studies with a larger sample sizes (with the extended follow-up and survival rate data) are required to investigate mtDNA<sup>4977</sup> and its associated clinicopathological features. A more detailed analysis is necessary to establish the exact role of this mitochondrial genetic alteration in brain tumor cases in the Malaysian population.

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