

Effects of a Mitochondrial Genetic Variant on Sevoflurane Hypersensitivity

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Several recent communications from different anesthesiology societies reported on cases involving pediatric patients of Venezuelan origin who underwent surgery under general anesthesia and subsequently developed serious neurologic impairment or died
- The underlying causes of these neurologic morbidities or mortality remain undetermined, although genetic susceptibility to anesthesia exposure was hypothesized

What This Article Tells Us That Is New

- This report describes a cohort of seven individuals with acute perioperative events in temporal association with anesthetic exposure; no adverse events were observed in a subset of the same individuals when only intravenous anesthesia was used

ABSTRACT

Background: Recently, various anesthesiology societies have reported cases involving pediatric patients of Venezuelan origin who underwent surgery under general anesthesia and subsequently developed serious neurologic impairment or died. However, the cause of this condition was unknown.

Methods: Clinical and genetic studies were conducted on seven patients who experienced severe acute neurologic deterioration after the administration of general anesthetics during predominantly minor surgical procedures. Genetic–molecular, biochemical, and cellular studies were also performed on fibroblasts and cybrids treated with general anesthetics.

Results: In our cohort, acute perioperative events were observed in temporal association with anesthetic exposure. No adverse events were observed in a subset of the same individuals when only intravenous anesthesia was used. All patients shared a mitochondrial DNA haplotype that includes the m.11232T>C genetic variant, which results in the substitution of proline for leucine at position 158 (L158P) of the ND4 subunit of respiratory complex I in the electron transport chain. *In vitro* studies showed that sevoflurane exposure in cells carrying this genetic variant induces a pronounced suppression of mitochondrial oxygen consumption, with prominent effects on complex I–dependent respiratory pathways. In contrast, propofol did not elicit a differential effect in cells harboring this genetic variant.

Conclusions: These findings suggest that individuals carrying the m.11232T>C mitochondrial DNA variant may be at increased risk of severe neurologic deterioration after exposure to anesthetic agents. Further studies are needed to define the underlying mechanisms and to determine anesthetic-specific risks. These results have important implications for improving the safety of surgical interventions and open new avenues in the field of mitochondrial pharmacogenetics of general anesthesia.

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- All patients shared a mitochondrial DNA haplotype that includes the m.11232T>C genetic variant, which results in the substitution of proline for leucine at position 158 (L158P) of the ND4 subunit of respiratory complex I in the electron transport chain
- *In vitro* studies showed that sevoflurane but not propofol exposure in cells carrying this genetic variant induced a pronounced suppression of mitochondrial oxygen consumption, with prominent effects on complex I–dependent respiratory pathways

This article is featured in "This Month in ANESTHESIOLOGY," page A1. This article is accompanied by an editorial on p. 1269. This article has a related Infographic on p. A16. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). This article has an audio podcast. Part of the work presented in this article has been presented as oral communications at several conferences: I MitoSpain Congress, Alcalá de Henares and Madrid in Madrid, Spain, September 18 to 20, 2025; XXV Venezuelan Congress of Anesthesiology in Caracas, Venezuela, November 6 to 8, 2025; XV National Congress of Pediatric Anesthesiology, Resuscitation, and Pain Management in Madrid, Spain, November 13 to 15, 2025; and 15th Mitochondrial Disease Conference in Pisa, Italy, January 22 to 25, 2026. J.S.-P. and C.A.-H. contributed equally to this work. Y.H.-C. and P.B.-B. contributed equally as co-senior authors to this work.

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Abbreviations: DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; ETC, electron transport chain; LHON, Leber hereditary optic neuropathy; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PCR, polymerase chain reaction; STR, short tandem repeat

In July 2025, several anesthesiology societies from the Americas reported or echoed reports of a serious medical situation. According to these reports, several pediatric patients underwent surgery under general anesthesia and subsequently developed severe neurologic impairment or died. No errors were identified in the anesthetic procedures, and all affected patients were of Venezuelan origin. These anesthesiology societies referenced a study conducted in Spain describing similar clinical cases (<https://ronlitman.substack.com/p/joint-communication-from-the-spa>, accessed September 15, 2025).

The lack of scientific evidence on the causes of this condition is delaying decision-making, the development of detailed protocols, and the performance of genetic studies on patients before surgery. In addition, the recent public dissemination of cases has generated fear among both the parents of children who need surgery and the anesthesiologists responsible for treating them. On the other hand, the knowledge gap about the subject may lead to an increase in the reporting of new cases and a growing number of legal proceedings.

It is well established that, across diverse biologic systems, several general anesthetics can impair oxidative phosphorylation (OXPHOS) function. Tissues such as *Drosophila melanogaster* thoraxes or fronto-parietal cortex of aging mice; cell lines such as mouse BV-2 microglial, human H4 neuroglioma, or ovarian cancer cells; and isolated mitochondria from organs like mouse brain, rat liver, or pig or guinea pig heart have been used in various studies.^{1–9} In humans, exposure to volatile anesthetics has been associated with an increased risk of neurologic deterioration, with genetic background modulating individual sensitivity.^{10,11} In this study, we present a cohort of patients who developed severe acute neurologic deterioration after sevoflurane exposure,

along with studies that shed light on the underlying etiological factors.

Material and Methods

Patients and Controls

This project has been approved by two ethics committees: the Ethics Committee for Research of the Autonomous Community of Aragon (CEICA, C.I. PI23/264) in Spain and the Independent Bioethics Committee for Research of the National Bioethics Center (CIBI-CENABI, No. 16/2023) in Venezuela. All subjects, controls and patients (or their legal guardians), provided written informed consent to participate in the study and publish the results.

Genetic Analysis

Sample collection and DNA extraction: DNA from blood samples (patients), buccal swabs (control subjects), or cell pellets (cybrids and fibroblasts) was extracted using the NZY tissue gDNA isolation kit (NZYtech, Portugal), according to the manufacturer's protocol. The entire mitochondrial DNA (mtDNA) was sequenced as previously described.¹² Mitochondrial haplogroups were assigned according to the information collected in PhyloTree (<https://www.phyloree.org/>, accessed August 8, 2025).

To sequence specific mtDNA variants, shorter mtDNA regions were amplified by polymerase chain reaction (PCR), with PCR mix containing 200 μ M dNTPs, 0.05 unit of *Taq* polymerase (Bioron, USA), 0.2 μ M primer forward, 0.2 μ M primer reverse, 30 ng of DNA, and UltraPure dH₂O (Gibco, USA) to 50 μ L. For the detection of the m.15164T>C variant in *MT-CYB*, primer forward (hmtL14838:

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5'-TCCAACATCTCCGCATGATG-3') and primer reverse (hmtH15912: 5'-TCTCCGGTTTACAAGACTGG-3') were used. Detection of the Leber hereditary optic neuropathy (LHON) pathogenic mutation m.11778G>A in *MT-ND4* was performed as previously described.¹² For the detection of the m.11232T>C variant in *MT-ND4*, primer forward (hmtL11107): 5'-TTCACAGCCACAGAACTAATC-3' (5' m.11087T-m.11107C 3') and primer reverse (hmtH11381): 5'-AAGTGGAGTCCGTAAAGAGG-3' (3' m.11400T-m.11381C 5') were used. The DNA amplicons were analyzed by Sanger sequencing.

Alternatively, the m.11232T>C variant in *MT-ND4* was detected by PCR-restriction fragment length polymorphism. A 314-nt mtDNA fragment was PCR amplified from total DNA (100 ng) with primers hmtL11107 and hmtH11381 (see above). PCR conditions were 95°C for 5 min (95°C for 45 s, 60°C for 30 s, and 72°C for 90 s) × 35 cycles of 72°C for 5 min. The amplicon was digested with MnlI enzyme (Thermo Fisher) at 37°C overnight. MnlI digestion yields three fragments of 225, 76, and 13 base pairs for the control (m.11232T variant) and four fragments of 150, 76, 75, and 13 base pairs for the mutant (m.11232C variant). The bands were separated by electrophoresis in 2% agarose gel and stained with GreenSafe Premium (NZYtech). NZYDNA Ladder VI (NZYtech) and low DNA mass ladder (Invitrogen, USA) were used to estimate the size of the bands. The exomes were obtained following standard procedures.

The AmpFISTR Identifier PCR amplification kit from ThermoFisher Scientific (USA) was used for short tandem repeat (STR) profiling. It is a combined DNA index system–approved STR–typing kit that amplifies a set of 16 STR markers including the sex-determining marker amelogenin in a multiplex PCR.

Computational Methods

To determine the conservation index, defined as the relative frequency of the amino acid present in the human RefSeq polypeptide, mtDNA-encoded ND4 polypeptide sequences from organisms available in the GenBank RefSeq database (<https://www.ncbi.nlm.nih.gov/genbank/>, accessed July 26, 2025) were retrieved. All sequences above or less than 1.5 times the interquartile range of sequence lengths for the same protein were excluded before alignment. Multiple alignment of orthologous sequences was performed with MAFFT version 7.520. The fasta file obtained in the alignment was used as input argument for a Python script to calculate conservation. The values are represented as percentages.

The cryo-electron microscopy structure of human respiratory complex I (Protein Data Bank identifier 6zkm) was used to model the Leu158Pro variant in ND4. The structures were rendered with the PyMOL molecular visualization system.

Cells and Cell Culture

Primary human fibroblasts were obtained using standard methods. Fibroblasts with the specific Venezuelan haplotype were obtained from the mother of patient 2 (P2) and designated as F-Vh (haplogroup D1b and 7 private variants including m.11232T>C in *MT-ND4* and m.15164T>C in *MT-CYB*). Control fibroblasts were obtained from a 15-yr-old male control and designated as F-C (haplogroup H).

Cybrid cell lines were prepared following standard procedures.¹³ Briefly, platelets were isolated from blood samples by differential centrifugation and platelet mitochondria were transferred to p⁰ 143B osteosarcoma cells (completely devoid of mtDNA) by inducing plasma membrane fusion with 42% polyethylene glycol 1500 solution. Cybrid cells were selected by culturing the cell fusion mixture in uridine-free medium for at least 3 weeks, and it was verified that normal mtDNA levels were recovered. The cybrid cell line with specific Venezuelan haplotype was generated with platelets of the mother of P2 and designated as C-Vh. Cybrids used as control were designated as: C-C1 (haplogroup H); C-C2 (haplogroup H); C-C3 (haplogroup D); C-Cyb (haplogroup H with genetic variant m.15164T>C in *MT-CYB*); and C-LHON (haplogroup J with pathologic mutation m.11778G>A in *MT-ND4*).

The cells were propagated in high-glucose Dulbecco's modified Eagle's medium (DMEM; 25 mM glucose, 1 mM pyruvate, 2 mM L-glutamine; Gibco) supplemented with 50 µg/ml uridine and 10% fetal bovine serum (Gibco), in a CO₂ incubator at 37°C with a 5% (v/v) CO₂ atmosphere.

High-resolution Respirometry

The respiratory rate of intact or permeabilized cells was measured using the Oroboros O2k high-resolution respirometer (Oroboros Instruments, Austria) following standard protocols.¹⁴ Independent samples were analyzed simultaneously in the two chambers of the instrument. Chamber assignments were alternated in successive measurements of biologic replicates.

To assess the inhibitory effect of anesthetics on oxygen consumption, exponentially growing cells (at 80 to 90% confluence) were harvested by trypsinization. Cybrid cells were resuspended at 2.5 × 10⁶ cells/ml and fibroblasts were resuspended at 1.5 × 10⁶ cells/ml in high-glucose DMEM with 10% fetal bovine serum. A 2-ml aliquot of each cell suspension was transferred into the oxygraph chamber for measurement. The oxygen consumption rate of intact cells was continuously monitored as increasing doses of anesthetic were stepwise administered into the sealed oxygraph chamber *via* the capillary in the stopper.

For sevoflurane exposure, a 1.5 M stock solution of sevoflurane (catalog no. PHR3348; Sigma/Merck, Germany). A was prepared in dimethyl sulfoxide (DMSO) and stored at 4°C. From this stock, working solutions of 200 and

500 mM were prepared in DMSO. Sevoflurane was applied to the oxygraph chamber to achieve final concentrations of 0.05 and 0.1 mM (from the 200 mM working solution); final concentrations of 0.25, 0.50, and 0.75 mM (from the 500 mM working solution); and a final concentration of 1.5 mM (from the 1.5 M stock solution of sevoflurane in DMSO). Under clinical conditions, sevoflurane concentrations in blood are less than 0.5 mM.^{1,15}

For propofol exposure, a 30 mg/ml stock solution of propofol (catalog no. 1572503; Sigma/Merck) in DMSO was prepared and stored at 4°C. Propofol was added to the cells to achieve final concentrations of 2.5, 5, 10, 15, 20, 30, and 40 µg/ml (corresponding to 0.014, 0.028, 0.056, 0.084, 0.112, 0.168, and 0.224 mM) using a 4 mg/ml working solution in DMEM. The experiment used the active compound rather than the formulation used in clinical practice, which also contains salts and lipids. Under clinical conditions, propofol concentrations in blood are less than 0.06 mM.^{16,17}

To determine which respiratory complexes were targeted by sevoflurane, cybrid cells were resuspended at 1.25×10^6 cells/ml in Mir05 medium.¹⁴ A total of 2 ml of the cell suspension (2.5×10^6 cells) was transferred into the oxygraph chamber. After sealing the chamber, 7.5 µg/ml digitonin (Sigma/Merck) was added *via* the capillary to selectively permeabilize the plasma membrane. A substrate-inhibitor titration protocol was then applied to sequentially evaluate the activity of individual mitochondrial respiratory complexes.¹⁴ Substrates and inhibitors (from Sigma/Merck) were prepared according to the Oroboros O2k-Protocols (MiPNet03.02_Chemicals-Media.pdf) and were added sequentially: 2.5 mM ADP, 10 mM glutamate, 2 mM malate, 0.5 µM rotenone, 10 mM succinate, 10 mM glycerolphosphate, 2.5 µM antimycin A, 2 mM ascorbate, 0.5 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride, and 100 mM sodium azide. To correct for non-mitochondrial respiration, the oxygen consumption rate measured after addition of rotenone (respiratory complex I inhibition), antimycin A (respiratory complex III inhibition), or sodium azide (complex IV inhibition) was subtracted from the rates recorded before their addition.

Statistical Analysis

The experiments were performed in quadruplicate ($N = 4$, biologic replicates), unless otherwise indicated. The results are expressed as means $\pm$ SD. Given the descriptive and hypothesis-generating nature of this study, no formal statistical comparisons were performed.

Results

Subjects

We present a cohort of seven individuals of Venezuelan origin who underwent different surgical procedures under

general anesthesia. None of them showed evidence of an overt mitochondrial disorder. However, exposure to general anesthetics was followed by severe neurologic deterioration, including basal ganglia degeneration and severe motility problems, resulting in a clinical phenotype resembling mitochondrial diseases (table 1; supplemental fig. S1, <https://links.lww.com/ALN/E447>). The surgical procedures were performed in different countries (Venezuela, the United States, and Germany) and over several years, from 2003 to 2022. This seems to rule out the possibility that the underlying cause is a defective batch of any of the anesthetic agents used. Although only male patients are represented in our cohort, additional confirmed cases of hypersensitivity involving females reinforce the need for clinical awareness across both sexes (Candelo E, Arango A, Franco D, et al. Fatal outcome in a patient with an unknown mitochondrial disease after anesthetic exposure: A clinical literature review from the anesthetic perspective. Preprint. Posted online March 3, 2023. Research Square. doi:10.21203/rs.3.rs-2539495/v1). In five cases, the maternal lineage can be traced back to the state of Carabobo in Venezuela. A summary of the interventions, anesthetic protocols, and outcomes of the procedures is presented (table 2). In this series, acute perioperative events were observed in temporal association with anesthetic agents that consistently included sevoflurane.

Genetic and Functional Studies

In clinical practice, the end-tidal sevoflurane concentration differs among patients at the same depth of anesthesia, indicating that sensitivity to sevoflurane varies among patients.¹⁸ Since several nuclear genes have been associated with sevoflurane sensitivity,¹⁹ whole-exome sequencing of patients 1, 2, and 3 was performed (supplemental digital content, <https://links.lww.com/ALN/E447>). The reports were considered negative, and no shared genetic variants were observed in the cases studied. However, it is still possible that nuclear genetic variants behave as modifiers of the phenotype.

Analysis of the maternally inherited mtDNA sequences from five patients and the mothers of the remaining two (P4 and P5) revealed that all seven individuals shared an identical haplotype comprising 41 genetic variants. Of these, 34 are ancient variants that define haplogroup D1b (<https://www.phylotree.org/>, accessed August 8, 2025), while the remaining 7 are private variants: 3 (m.16176C>T, m.16261C>T, m.16327C>T) in the noncoding control region (*MT-CR*); 1 (m.4435A>G) in the gene for tRNA^{Met} (*MT-TM*); 1 synonymous (m.5273A>G) that does not cause amino acid substitution, in *MT-ND2*; and 2 non-synonymous (m.11232T>C and m.15164T>C) that cause amino acid substitution, in *MT-ND4* and *MT-CYB*, respectively (fig. 1A). The same haplotype was confirmed in the healthy mother of P2.

Given that genetic variants with deleterious effects are generally subject to purifying selection, which tends

to reduce their abundance, we analyzed the population frequency of the most significant private variants of this Venezuelan haplotype. The m.4435A>G transition has been identified in 212 of 314,945 sequences listed in Mitomap (www.mitomap.org, accessed August 8, 2025). However, according to the pathogenicity predictor APOGEE2, this variant is classified as probably benign. The m.15164T>C transition has been observed in 53 out of 314,969 Mitomap sequences. While APOGEE2 classifies this variant as likely benign, AlphaMissense (<https://alphamissense.hegelab.org/>, accessed August 8, 2025) predicts it to be likely pathogenic. The m.11232T>C transition has not been reported in any of the 285,539 sequences analyzed in Mitomap. Both the APOGEE2 and AlphaMissense predictors classify this variant as likely pathogenic. Before the clinical context described here, this variant had not been clearly associated with a severe neurologic phenotype. However, this latter mutation was previously described in a 48-yr-old man with chronic progressive external ophthalmoplegia,²⁰ and also in a 12-yr-old girl with severe sensorineural hearing loss who underwent a brain magnetic resonance imaging scan under general anesthesia that included propofol, midazolam, lidocaine, sevoflurane, and flumazenil (Candelo E, Arango A, Franco D, et al. Fatal outcome in a patient with an unknown mitochondrial disease after anesthetic exposure: A clinical literatura review from the anesthetic perspective. Preprint. Posted online March 3, 2023. Research Square. doi:10.21203/rs.3.rs-2539495/v1).

This particular mitochondrial Venezuelan haplotype has not been reported in 414 Venezuelan individuals from the literature (Supplementary Digital File – Control and Patient Information, <https://links.lww.com/ALN/E447>) nor in 71 Venezuelan population controls we have analyzed. Considering these values, the probability of analyzing 7 Venezuelan individuals and finding that they all belong to the same haplotype would be one in 6.3×10^{18} times, $[1/(414 + 71)]^7$ which strongly suggests that mtDNA haplotype and susceptibility to general anesthesia are not independent variables but are instead related.

Amino acid positions with functional importance are highly conserved across species. This is the case for phenylalanine at position 140 (m.15164T>C), conserved in 97.1% of 13,597 reference sequences of cytochrome *b* (CYB) subunit of respiratory complex III in the electron transport chain (ETC), encompassing organisms from protists to mammals (<https://www.ncbi.nlm.nih.gov/genbank/>, accessed July 26, 2025). Similarly, leucine at position 158 (m.11232T>C) of the NADH-ubiquinone oxidoreductase chain 4 subunit (ND4) of respiratory complex I is conserved in 91.0% of 14,894 ND4 reference sequences and is located very close to a functionally important region (fig. 1, B and C).^{21–24} It has been proposed that any molecule binding this ubiquinone/rotenone binding site could be inhibitory.²³

Regarding the functional relevance of the amino acid substitution, the m.15164T>C genetic variant provokes

a conservative substitution of leucine for phenylalanine (F140L) in CYB, while the m.11232T>C genetic variant causes a substitution of proline for leucine (L158P) in transmembrane helix 6 of ND4, which is likely to be important. It is well established that proline residues destabilize α -helices, altering the protein structure. Since the function of a protein depends on its structure and since the equivalent L158P substitution affects respiratory complex I activity when modeled in *Escherichia coli*,²⁵ we conducted functional studies on the ETC.

The oxygen consumption of fibroblasts obtained from the healthy mother of patient 2, who shares the mitochondrial haplotype with her son, was similar to that of the control fibroblasts (fig. 2A). However, ETC, involved in cellular oxygen consumption, is a well known target of general anesthetics.¹ Consistent with previous findings,^{7,8,10} we observed that sevoflurane reduces cellular oxygen consumption in a dose-dependent manner (fig. 2B; supplemental fig. S2, <https://links.lww.com/ALN/E447>). In control fibroblasts, at clinically relevant concentrations of this anesthetic, oxygen consumption remains above 75% of that observed in cells not exposed to sevoflurane (fig. 2B). Remarkably, in fibroblasts carrying the Venezuelan haplotype, at the same sevoflurane concentrations, oxygen consumption can reach 15% of that observed in unexposed cells (fig. 2B).

To investigate whether the susceptibility to general anesthetics is linked to the mtDNA haplotype, we generated transmitochondrial cell lines (cytoplasmic hybrids or cybrids) and confirmed that each carried the intended nuclear DNA and mtDNA. All cybrids generated shared culture conditions and an identical nuclear DNA background, which differed from that of the original mutant fibroblasts but have different mtDNA haplotypes (supplemental fig. S3 and supplemental tables S1 and S2, <https://links.lww.com/ALN/E447>). The oxygen consumption of cybrids with the Venezuelan haplotype was similar to that of the control cybrids (fig. 2C). When exposed to sevoflurane, cellular oxygen consumption was reduced dose-dependently in all cases (fig. 2D; supplemental fig. S4, <https://links.lww.com/ALN/E447>). In control cybrids, at clinically relevant concentrations of this anesthetic, oxygen consumption remains above 80% of that observed in cells not exposed to sevoflurane (fig. 2D). Cybrids harboring only the m.15164T>C genetic variant or a LHON pathologic mutation (m.11778G>A in *MT-ND4*) responded similarly, indicating that m.15164T>C variant of the Venezuelan haplotype is unlikely to be responsible for the observed phenotype and that not any pathologic ND4 variant induces sensitivity to sevoflurane (fig. 2D). However, in cybrids harboring the Venezuelan haplotype with the m.11232T>C genetic variant, at the same sevoflurane concentrations, oxygen consumption can reach 10% of that observed in unexposed cells (fig. 2D). Furthermore, when the sealed oxygraph chamber was opened to allow the release of volatile compounds, oxygen consumption in cybrids carrying

Table 1. Patient Information

Cases	1	2	3	4	5	6	7
Age/sex	17/Male	34/Male	18/Male	14/Male	34/Male	10/Male	14/Male
Country/year (first surgery with APE)	Venezuela/2016	Germany/2005	USA/2020	Venezuela/2022	Venezuela/2003	Venezuela/2018	Venezuela/2019
Venezuelan state of maternal ancestors*	Anzoátegui	Carabobo	Carabobo	Caracas	Carabobo	Carabobo	Carabobo
Family history	None	Maternal aunts: LA (coma 3 days); case 3	Maternal aunts: LA (coma 3 days); case 2	None	None	None	Maternal cousin: APE/death
APE	Immediate postoperative; vomiting and pallor	Immediate postoperative; active; hypotension 15 min; LA	Intraoperative; hypotension (80/40 to 180/100); LA	Immediate postoperative; active; LA, stupor	Immediate postoperative; active; LA, bradykinesia, stupor	Immediate postoperative; active; LA, stupor	LA
Initial neurologic signs	Vomiting and pallor, somnolence/psychomotor agitation, gait disturbance with ataxia, hypotonia, and dysarthria	Stupor, vomiting, dysarthria, bradykinesia	Vomiting, stupor, dysarthria, bradykinesia	Bradykinesia, dysarthria, gait disturbance	Consciousness deterioration	Bradykinesia, dysarthria	Gait disturbance; bradykinesia; stupor with vomiting (3 days); persistent hiccups until day 5; partial recovery on day 6 (alertness, trunk stability, sialorrhea, walking with support)
Evolution	Irritability, absence of response to verbal commands, dysphagia, tetraparesis	Generalized and buccolingual dystonia, ataxia, mutism	Painful dystonia, mutism, tetraparesis, scoliosis	Progressive generalized dystonia, rigor, ataxia	Generalized dystonia, ataxia, dysphagia, mutism	Progressive painful dystonia, mutism, inability to walk	Progressive generalized dystonia, rigor, ataxia over weeks to months
Current status	Wheelchair-bound, dysarthria, dystonia	Tetraparesis, PEG feeding	Wheelchair-bound, severe rigidity	Walks with dystonia, few words, rigidity	Walks few steps with help, rigidity, mutism	Bedridden, tetraparesis, mutism, dysphagia, scoliosis	Wheelchair-bound, nonambulatory, nonverbal, anti-equinus splints
Metabolic work-up	Normal (transient elevations up lactate 5.5 mM)	Abnormal (lactate 5.8 mM)	Normal	—	—	—	Normal
Brain imaging findings	Bilateral striatal necrosis and left parietal corticostriatal necrosis with ischemic features in the posterior watershed territory	48 h: GP + cerebellum lesions; later: frontal + GP necrosis ("eye of tiger"); atrophy	48 h: GP + cerebellum + brainstem; later: new bilateral neostriatal lesions	48 h: restricted diffusion GP + cerebellum; FLAIR hyperintensities	48 h: GP + cerebellum + brainstem lesions; later: basal ganglia necrosis	72 h: GP + cerebellum lesions; FLAIR hyperintensities	Cranial CT (day 5): ischemic lesions in basal ganglia

*Assuming that Venezuela has a population of 30,000,000 and the state of Carabobo has a population of 3,000,000, our data show that the haplotype of interest, defined by the m.11232T>C genetic variant and shared by all patients, is 15 times more common in Carabobo than in the rest of the country.

APE, acute perioperative event; CT, computed tomography; FLAIR, fluid-attenuated inversion recovery; GP, globus pallidus; LA, late awakening; PEG, percutaneous endoscopic gastrostomy.

Table 2. Results of Exposure to General Anesthesia

Patients	Interventions (age [yr])	Anesthetics	Result
1	Adenoid–tonsillectomy, partial turbinectomy (9)	F, M, P, Ro, S	APE
	Reimplantation of intrathecal baclofen pump (15)	P, R, Ro	NE
	Repairing the catheter rupture (15)	F, P, R, S	APE
	Brain MRI (15)	P	NE
	Brain MRI (16)	S	APE
2	Adenoidectomy (2)	U	NE
	Fracture repair (14)	F, P, S	APE
	Surgical hardware removal (15)	F, P	NE
	Fracture repair (16)	F, P, S	APE
	Surgical hardware removal (21)	F, P	NE
3	Fracture repair (28)	P, R	NE
	Herniotomy inguinal (2)	U	NE
	Knee arthroplasty (13)	F, P, S	APE
4	Baclofen pump implantation (16)	D, F, H, K, M, R	NE
	Fracture repair (11)	S + U	APE
5	Hydrocelectomy (4)	U	NE
	Varicocele and umbilical hernia repair (12)	S + U	APE
	Repairing foot deformities (17)	U	NE
6	Cleft lip and cleft palate repair (3)	P, R, S	APE
7	Cochlear implant (8)	S + U	APE
	Surgery for hip dislocation (14)	TIVA	NE

The first operations of patients 2 and 5 were before the approval of the use of sevoflurane in surgery.

APE, acute perioperative events; D, dexmedetomidine; E, etomidate; F, fentanyl; H, hydromorphone; K, ketamine; M, midazolam; MRI, magnetic resonance image; NE, no events; P, propofol; R, remifentanyl; Ro, rocuronium; S, sevoflurane; TIVA, total intravenous anesthesia; U, unknown.

the Venezuelan haplotype was restored. In contrast, opening the chamber after inhibition with rotenone, a nonvolatile compound, did not lead to recovery of respiration. These results suggest that sevoflurane-induced inhibition was neither covalent nor irreversible and, although the extent of sevoflurane elimination is unknown, that the observed effect was attributable to the volatile anesthetic (supplemental fig. S5, <https://links.lww.com/ALN/E447>).

Next, we performed oxygen consumption studies in permeabilized cells to identify which respiratory complex is the target of sevoflurane. In this context, permeabilized cybrids, whose ETC had been inhibited by sevoflurane, regained a normal oxygen consumption rate when specific substrates of respiratory complex II and III were administered (fig. 2, E and F; supplemental fig. S6, <https://links.lww.com/ALN/E447>). These assays pointed to respiratory complex I as the target of sevoflurane, as previously reported,^{1–5} which could explain previously published observations that patients with OXPHOS diseases associated with respiratory complex I deficits are more susceptible to the effects of sevoflurane.¹¹

Applying the criteria of the American College of Medical Genetics (Bethesda, Maryland) and the Association for Molecular Pathology (Bethesda, Maryland) to mtDNA genetic variants with incomplete penetrance is not straightforward,^{26,27} as is the case with the m.11232T>C variant. However, this variant meets several of these criteria that would support its possible pathogenicity (PS3, PS4, PM8, PP1, PP3, PP6, and PP7), although it also presents one (BS4) that could call it into question.

In anesthetic protocols, different compounds are used for both induction and maintenance of anesthesia. Several of these drugs, such as propofol, also adversely affect ETC.^{1,28,29} Propofol is one of the most widely used anesthetics and, together with sevoflurane, was administered to several patients included in this study. We observed that, in fibroblasts and cybrids, propofol reduced cellular oxygen consumption in a dose-related manner (fig. 3). However, in both control and mutant cells, at clinically relevant concentrations of this anesthetic, oxygen consumption is comparable and remains above 80% in fibroblasts and 90% in cybrids relative to that observed in cells not exposed to sevoflurane (fig. 3; supplemental figs. S7 and S8, <https://links.lww.com/ALN/E447>). This observation, along with the fact that several patients exhibited neurologic damage during procedures involving sevoflurane but not during those where propofol and other general anesthetics (no sevoflurane) were used instead (table 2), suggests that this haplotype may not cause hypersensitivity to propofol.

Discussion

Reduced OXPHOS function can increase susceptibility to volatile anesthetics. In the nematode *Caenorhabditis elegans*, a mutation in the *gas-1* gene that encodes a subunit homologous to the human NDUFS2 of respiratory complex I caused immobilization at lower concentrations of multiple volatile anesthetics.^{30–33} Similarly, *D. melanogaster* ND-23⁶⁰¹¹⁴ and ND-SGDH^{G6447}, carrying mutations in the ND-23

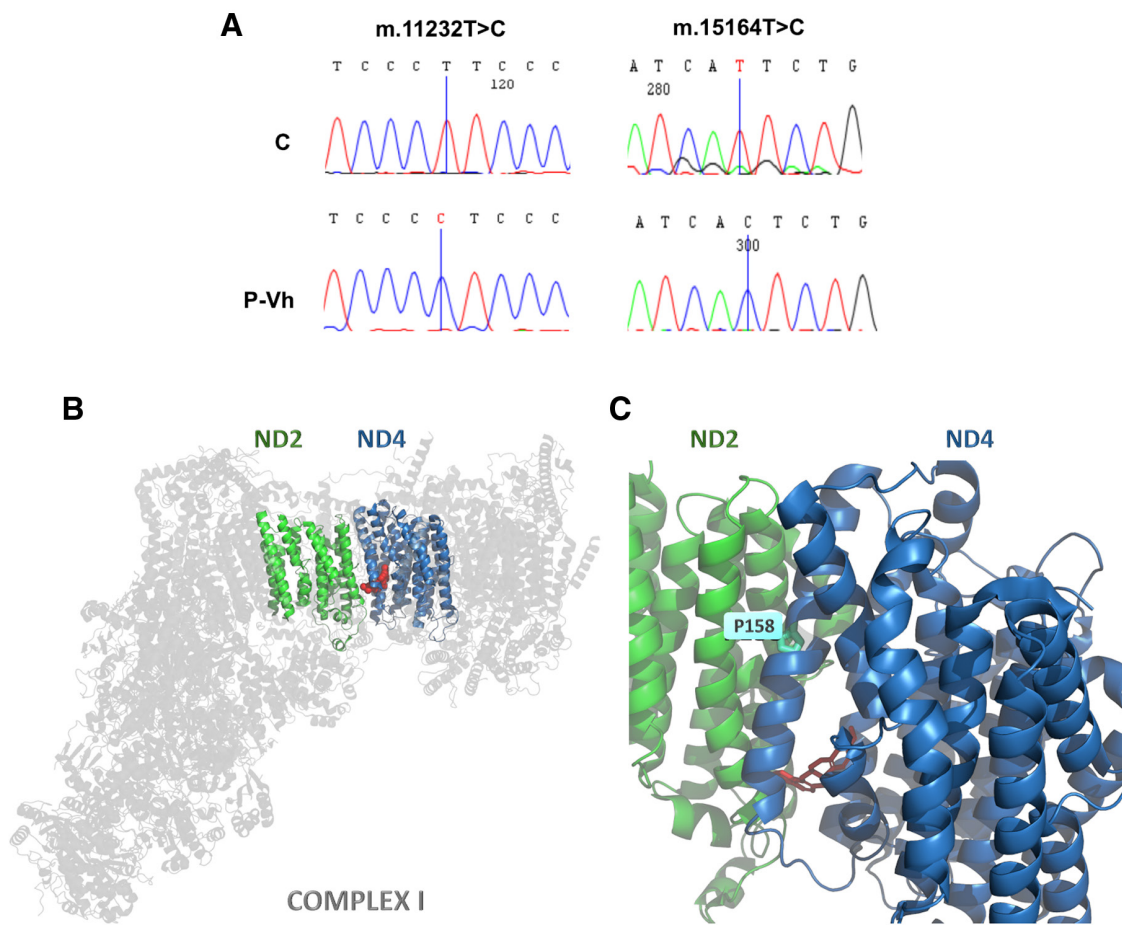


Fig. 1. Mitochondrial genetic variants of the Venezuelan haplotype and location of proline 158 in the ND4 subunit of respiratory complex I. (A) Electropherograms showing the m.11232T>C (*MT-ND4*) and the m.15164T>C (*MT-CYB*) genetic variants. Row C, control subject; row P-Vh, patient with the Venezuelan haplotype. (B) Ribbon structure of respiratory complex I (respiratory complex I of the mitochondrial electron transport chain [ETC]). (C) Interface between ND2 and ND4 subunits. The substitution of proline for leucine at position 158 (L158P) of ND4 is indicated in light blue. The ND2 subunit is in green; the ND4 subunit is in blue; and the rotenone molecule inside one of the ubiquinone/rotenone binding sites is in red.

and ND-SGDH subunits (homologous to NDUFS8 and NDUFB5 of human respiratory complex I), developed a neurotoxic phenotype after isoflurane exposure.^{34–36} Ndufs4-knockout mice, lacking the NDUFS4 subunit of respiratory complex I, exhibited marked hypersensitivity to volatile anesthetics.^{37–40} Collectively, these animal models demonstrate that mutations in respiratory complex I subunits increase OXPHOS sensitivity to volatile anesthetics. Consistently, patients with respiratory complex I deficits are more susceptible to the effects of sevoflurane.¹¹

Most of the patients included in this study did not present with any significant underlying health conditions. Moreover, the administered anesthetics are widely used for their established safety profile. Therefore, it is reasonable to assume that a combination of genetic and environmental factors underlies the appearance of this pathology.

In this study, we used sevoflurane and propofol to investigate mitochondrial sensitivity; we did not conduct

comparative assessments of clinical safety. Our results suggest that this Venezuelan mtDNA haplotype, which includes the m.11232T>C and m.15164T>C genetic variants, increases susceptibility to sevoflurane and leads to severe OXPHOS dysfunction. Additionally, we demonstrate that this mtDNA haplotype is associated with anesthesia-induced basal ganglia degeneration. Notably, dysfunction of the OXPHOS system is a well established cause of basal ganglia degeneration, as seen in Leigh syndrome.^{41,42}

Based on the available evidence, the absence of the homoplasmic m.11232T>C genetic variant in non-Venezuelan populations, together with its relatively high frequency among patients from Carabobo State, supports the hypothesis that this variant originated in a woman who lived in that region. In the absence of sevoflurane exposure, no deleterious phenotypic effects have been observed, either in maternally related relatives of affected individuals or in experimental assays using fibroblasts and cybrids.

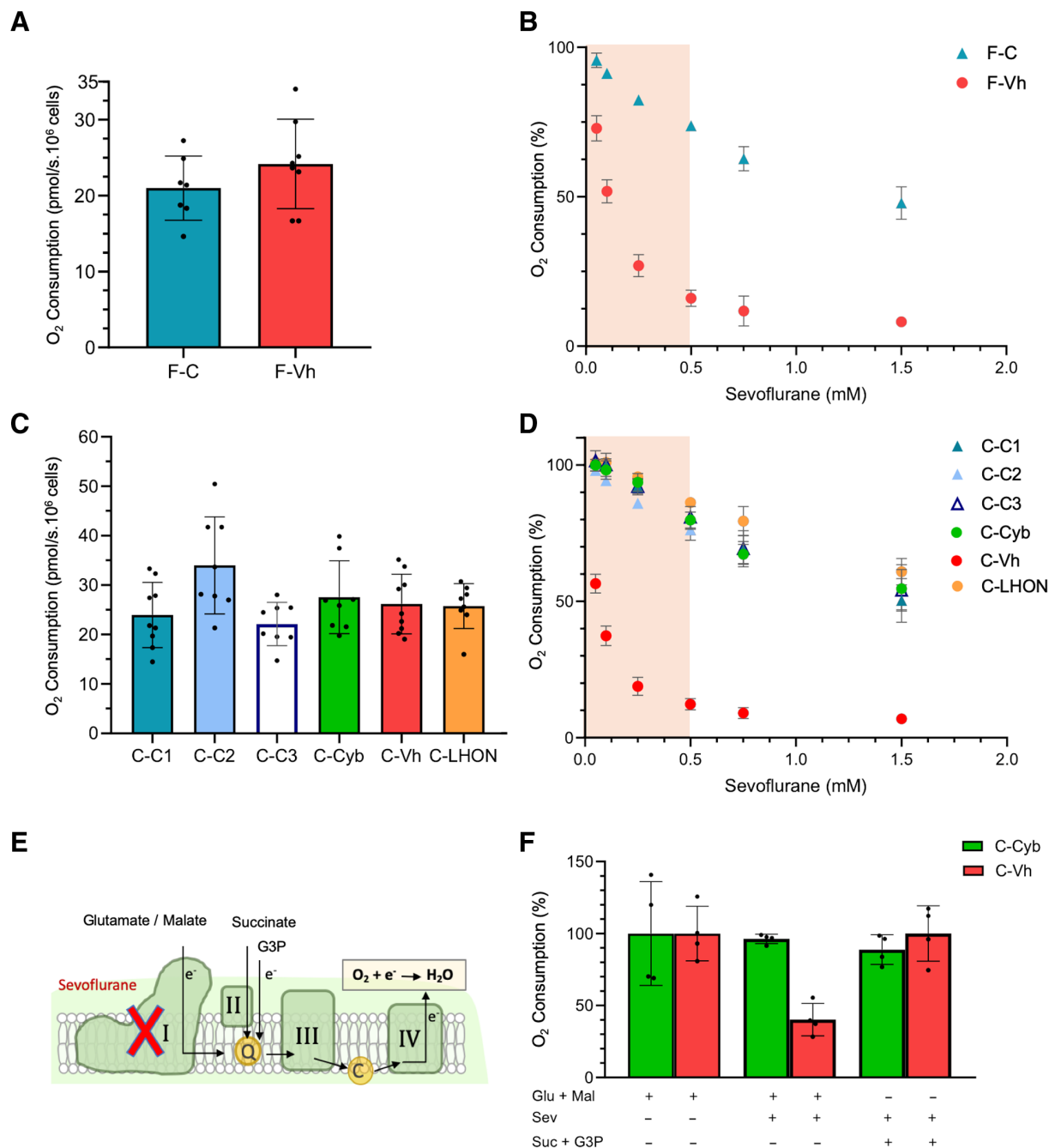
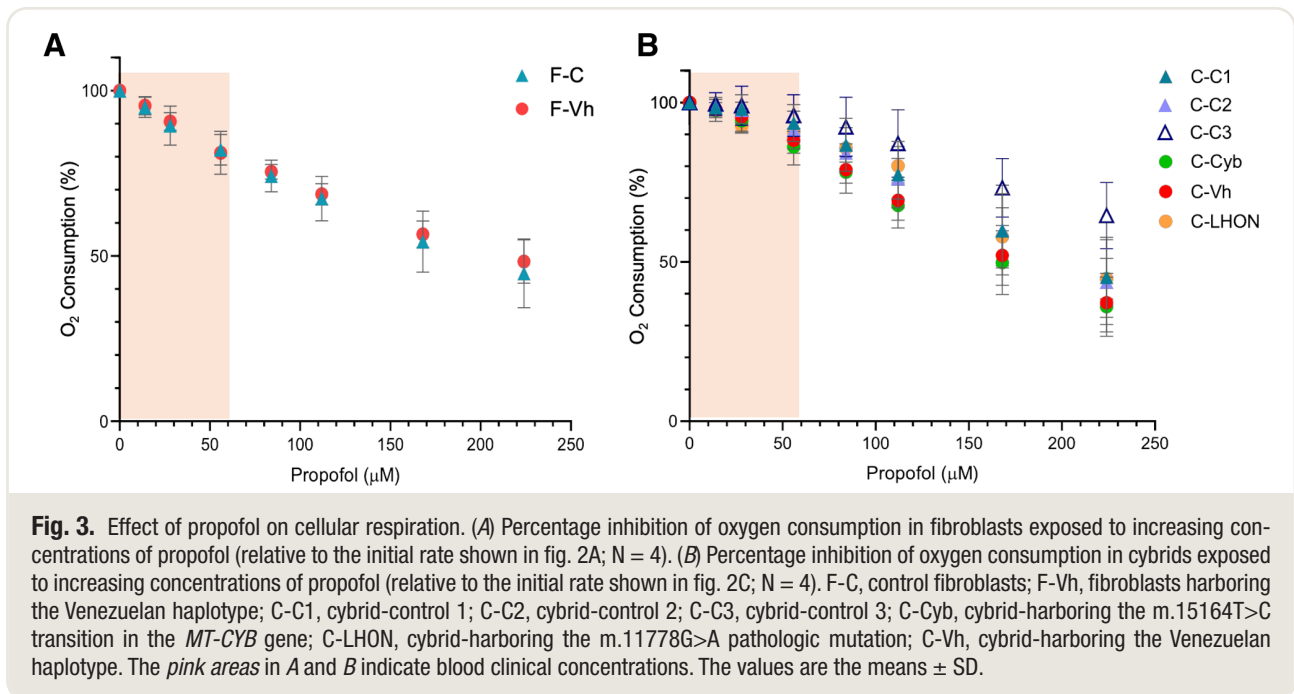


Fig. 2. Effect of sevoflurane on cellular respiration. (A) Oxygen consumption in fibroblasts (measured in intact cells suspended in high glucose medium; N = 7 to 8). (B) Percentage inhibition of oxygen consumption in fibroblasts exposed to increasing concentrations of sevoflurane (relative to the initial rate shown in A; N = 4). (C) Oxygen consumption in cybrids (measured in intact cells suspended in high glucose medium; N = 8 to 9). (D) Percentage inhibition of oxygen consumption in cybrids exposed to increasing concentrations of sevoflurane (relative to the initial rate shown in C; N = 4). (E) Schematic representation of the mitochondrial electron transport chain indicating the suspected site of sevoflurane action (red cross) and the complex-specific substrates used to determine the mitochondrial respiratory complex targeted by sevoflurane. (F) Inhibition of substrate-dependent oxygen consumption by sevoflurane (measured in permeabilized cells supplied with complex-specific substrates, as indicated in E, sevoflurane was added at 0.05 mM; N = 4). C-C1, cybrid-control 1; C-C2, cybrid-control 2; C-C3, cybrid-control 3; C-Cyb, cybrid-harboring the m.15164T>C transition in the *MT-CYB* gene; C-LHON, cybrid-harboring the m.11778G>A pathologic mutation; C-Vh, cybrid-harboring the Venezuelan haplotype; F-C, control fibroblasts; F-Vh, fibroblasts harboring the Venezuelan haplotype. The pink areas in B and D indicate blood clinical concentrations. I, II, III, IV, Q, and c code for respiratory complex I, II, III, IV, ubiquinone, and cytochrome c, respectively. The respiratory complex I substrates are glutamate (Glu) and malate (Mal). The respiratory complex II substrate is succinate (Suc). The respiratory complex III substrate via ubiquinone (Q) is glycerol-3-phosphate (G3P). The values are the means $\pm$ SD.



This suggests that the variant was not subjected to negative selective pressure and therefore became locally established. It was only after the introduction of sevoflurane into clinical practice⁴³ that this previously phenotypically neutral mtDNA genotype appears to have acquired pathogenic relevance. While the advent of sevoflurane has undoubtedly made surgical procedures safer and more efficient, our findings suggest that may also have unintended consequences, potentially rendering certain mtDNA genetic variants deleterious. This may represent yet another example of how pharmacologic advances can transform an ancient mtDNA genetic variant into a pathogenic one.⁴⁴

Similar cases to those described here are being reported in Chile,⁴⁵ Colombia, and the United States.⁴⁶ According to the United Nations High Commissioner for Refugees, “Nearly 7.9 million people have left Venezuela [...] The majority, more than 6.7 million people, are hosted in Latin American and Caribbean countries.”⁴⁷ Even though the m.11232T>C genetic variant is not common, the consequences of exposure to sevoflurane in individuals carrying this mtDNA haplotype could be devastating. If a woman carrying this mtDNA genotype migrates to another region of the world and has offspring with individuals from the local population, within a few generations, the nuclear genetic background and external morphological traits of her descendants will reflect those of the host population. However, the mtDNA genotype may have been introduced and become established in the new population (supplemental fig. S9, <https://links.lww.com/ALN/E447>). It is therefore especially important to study the effects of other inhalational or intravenous anesthetics on individuals harboring this mtDNA haplotype.

This study has some limitations. The apparent imbalance between male and female patients probably reflects the small number of identified cases with complete perioperative and follow-up data rather than a sex-specific effect. Although additional female individuals, carrying the same mitochondrial haplotype, with sevoflurane hypersensitivity have been reported, detailed information regarding phenotype severity and anesthetic exposure is not consistently available. Considering the absence of relevant information, it is conceivable that some patients had undergone previous surgical procedures involving exposure to sevoflurane, of which no records are available, without exhibiting adverse perioperative effects. Similarly, in the surgical interventions known to have been performed on mothers, for which the anesthetic protocol remains unspecified, halogenated inhalational agents may have been administered without the occurrence of perioperative complications. Assuming such exposures did occur, it is plausible that lower effective concentrations were employed.⁴⁸ Nevertheless, the absence of overt neurologic sequelae in mothers exposed to volatile anesthesia indicates that this Venezuelan mtDNA haplotype alone is insufficient to produce the observed phenotype. Taken together, all these observations further support a context-dependent vulnerability consistent with a rare pharmacogenetic interaction in which this mitochondrial genotype represents a necessary but not sufficient condition for severe acute neurologic deterioration. Additional modifying factors, including nuclear genetic background, brain development, and perioperative clinical conditions, are likely to influence phenotypic expression and clinical variability. Information regarding anesthetic exposure and neurologic outcomes in other maternally or paternally related

relatives will help further assessment of modifying genetic factors. We acknowledge the importance of these data and hope they can be recovered in the future, as its inclusion will be essential for addressing this issue in a more rational and evidence-based manner. We also consider important to supplement oxygen consumption studies with additional parameters related to the OXPHOS function.

In light of the complexity and incomplete nature of the clinical data, the current case series should be interpreted as descriptive and hypothesis-generating. Importantly, these findings should not be interpreted as a general contraindication to the use of sevoflurane in patients with mitochondrial disorders but rather as evidence of increased vulnerability in a highly specific genetic context. Moreover, it should be emphasized that sevoflurane is widely used and, in the vast majority of individuals, does not result in neurologic sequelae.

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Competing Interests

The authors declare no competing interests.

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Supplemental Digital Content

Supplemental Digital Content file, <https://links.lww.com/ALN/E447>

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