Achalasia and acromegaly: co-incidence of these diseases or a new syndrome?


Background. Acromegaly is a disorder associated with hypersecretion of growth hormone, most usually caused by a pituitary adenoma. Dysmotility of the gastrointestinal tract has been reported in acromegalic patients. Achalasia is a disorder characterized by aperistalsis of the oesophagus with incomplete lower oesophageal sphincter relaxation and whose aetiology remains unknown. Mutations in some genes have previously been associated with the development of acromegaly or achalasia. The study aims were to analyse mutations in selected genes in a woman having both of these diseases, to identify their aetiopathological factors, and to suggest explanations for the co-incidence of acromegaly and achalasia.

Methods and Results. A female patient with acromegaly, achalasia, and a multinodular thyroid gland with hyperplastic colloid nodules underwent successful treatment of achalasia via laparoscopic Heller myotomy, a thyroidectomy was performed, and the pituitary macroadenoma was surgically excised via transnasal endoscopic extirpation. Germline DNA from the leukocytes was analysed by sequencing methods for a panel of genes. No pathogenic mutation in AAAS, AIP, MEN1, CDKN1B, PRKAR1A, SDHB, GPR101, and GNAS genes was found in germline DNA. The somatic mutation c.601C>T/p. R201C in the GNAS gene was identified in DNA extracted from a tissue sample of the pituitary macroadenoma.

Conclusions. We here describe the first case report to our knowledge of a patient with both acromegaly and achalasia. Association of acromegaly and soft muscle tissue hypertrophy may contribute to achalasia’s development. If one of these diagnoses is determined, the other also should be considered along with increased risk of oesophageal and colorectal malignancy.

Key words: acromegaly, pituitary tumour, achalasia, autoimmune syndrome, gene, mutation, AAAS, GPR101, GNAS

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aDepartment of Gastroenterology and Internal Medicine, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
bDepartment of Surgery, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
cCentral European Institute of Technology, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic
dDepartment of Pediatrics, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
eDepartment of Neurosurgery, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
fDepartment of Radiology and Nuclear Medicine, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
gDepartment of Pathology, University Hospital Brno, Faculty of Medicine, Masaryk University, Jihlavská 20, 62500, Brno, Czech Republic
hDepartment of Pathophysiology, Faculty of Medicine, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic
iDepartment of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic
jDepartment of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic
kClinic of Stomatology, Institution Shared with St. Anne’s Faculty Hospital, Faculty of Medicine, Masaryk University, Pekarska 53, 656 91, Brno, Czech Republic
lInstitute of Medical Genetics and Genomics, Faculty of Medicine, Masaryk University, Kamenice 5, 62500, Brno, Czech Republic
Corresponding author: Lumir Kunovsky, e-mail: lumir.kunovsky@gmail.com

BACKGROUND

Acromegaly is a rare disease with incidence of approximately 3–5 persons per 1,000,000 (ref.1). This disorder is characterized by hypersecretion of growth hormone (GH), which is usually caused by a pituitary adenoma2. This leads to overgrowth of almost all tissues in the body, most especially of acral and soft tissues, and to visceromegaly3. Multisystemic complications develop in acromegalic patients due to high GH and insulin-like growth factor-I (IGF-1) levels, and these include joint pain, diabetes mellitus, hypertension, heart and respiratory failure, as well as disorders of the gastrointestinal (GI) system2. GI manifestations associated with acromegaly include adenomatous polyps, colon carcinoma, dolichocolon, and dysmotility (prolonged bowel transit time and impaired relaxation of the lower oesophageal sphincter [LES]) (ref.4). Acromegaly is associated with decreased survival rate4. Acromegalic patients experience mortality due to cardiovascular disease in 60% of cases, respiratory disorders in
(ref.3,7). This survival rate can be improved by successful surgical and adjunctive therapy3.

Although acromegaly occurs sporadically in 95% of all cases, almost 50% of childhood-onset cases have an identifiable genetic background, most commonly in the form of mutations in the genes encoding aryl hydrocarbon receptor interacting protein (AIP) or G-protein coupled receptor 101 (GPR101) (ref.3). In addition, mutations in genes such as multiple endocrine neoplasia type 1 (MEN1); cyclin-dependent kinase inhibitor 1B (CDKN1B); protein kinase A regulatory subunit type I alpha (PRKAR1A); genes of the succinate dehydrogenase family A, B, C, and D (SDHx); and the adenylyl cyclase stimulatory protein alpha subunit (GNAS) also have been associated with acromegaly/gigantism8.

Achalasia is an oesophageal motility disease characterized by aperistalsis of the oesophagus and impaired LES relaxation9,10. This disorder is caused by a degeneration of the myenteric plexus, but the aetiology of this degeneration remains unclear11. It is assumed that the plexus degeneration arises from a genetic predisposition caused by a viral infection and/or autoimmune process9,11. The incidence of this disease varies worldwide from 0.27 to 1.63 per 100,000 (ref.12-17).

The diagnosis is based on clinical symptoms, endoscopy, high-resolution oesophageal manometry (HRM), and/or swallow radiography. Treatment can be conservative, endoscopic, or surgical11,10.

Achalasia can develop independently or as a part of Allgrove (Triple A) syndrome (i.e., adrenal insufficiency, achalasia, and alacrimia) (ref.19). In the latter case, association with mutations in the AAAS gene has been identified20.

The aims of this study were to analyse the genes associated with acromegaly and achalasia in a woman with both of these diseases, to find their aetiopathogenetic factors, and to suggest some explanations for the co-incidence of acromegaly and achalasia.

CASE PRESENTATION

We present a female whose history consists solely of recurrent superficial venous thrombosis of the lower extremities. No hypercoagulation syndrome was noted. In the family history there was no oesophageal disease or acromegaly. The patient has a monozygotic twin. At 30 years of age (in 2008), a multinodular thyroid gland with hyperplastic colloid nodules was identified, resulting 1 year later in a total thyroidectomy (preoperative values of thyroid gland hormones: thyroid stimulating hormone 1.07 mU/L, thyroxine 12.86 pmol/L).

In 2009 (at 31 years), an investigation for oesophageal dysmotility was performed due to dysphagia. Achalasia was identified by manometry with characteristic findings (Fig. 1). Treatment was started with balloon dilation of the oesophagus, but the effect was only temporary. The control swallow radiograph can be seen in Fig. 2. A laparoscopic Heller myotomy (LHM) with Nissen–Rossetti fundoplication was performed. After this procedure, the patient recovered without any signs of dysphagia.

In 2016 (at 38 years of age), acromegaly was suspected because the typical acromegalic changes of the face (acral growth) as well as of the lower and upper extremities were observed. The patient started to experience severe headaches. Her foot size had grown 5 cm in 7 years, she was not able to put a ring on her finger, and changes in...
the patient’s voice also occurred. Laboratory tests gave reasons for suspicion of acromegaly (GH 48 mU/L, reference range 0−24 mU/L; IGF-1 725 µg/L, reference range 109−284 µg/L). The diagnosis of acromegaly was confirmed by serum GH concentrations assessed during oral glucose tolerance test (GH 48 mU/L at beginning of test, 28 mU/L after 1 h of glucose application, 22 mU/L after 2 h. The test was positive due to inadequate suppression of GH after application of 75 g of glucose. Magnetic resonance imaging (MRI) revealed a macroadenoma of the non-enhanced pituitary gland 14 × 10 × 9 mm in size (Fig. 3a, 3b). The optic chiasm was not affected by the lesion. The patient was referred to neurosurgery and transnasal endoscopic extirpation of the adenoma was performed. The histopathology of the adenoma of the pituitary gland can be seen in Fig. 4a and 4b. The patient was discharged 8 days after surgery. The control MRI showed a small residual amount of adenoma tissue, which was successfully treated by gamma knife radiosurgery. Subsequently, the levels of GH and IGF-1 normalized (hormone levels after surgery GH 7 mU/L, IGF-1 345 µg/L; after radiotherapy GH 15 mU/L, IGF-1 189 µg/L; 1 year after radiosurgery GH 8 mU/L, IGF-1 178 µg/L). We also performed a colonoscopy, with normal findings. An echocardiography showed normal heart function without signs of cardiomyopathy or valve dysfunction.

Fig. 2. The patient underwent sequential balloon dilations without treatment response. Fluoroscopy with barium revealed mild dilatation of the oesophagus with distal smooth, short-segment tapering.

Fig. 3a, 3b. Contrast-enhanced MRI - T1WI. Sagittal section (a), coronal section (b) showing a non-enhanced pituitary mass 14 × 10 × 9 mm in size. The optic chiasm was not affected by the lesion.
Genetic Analysis

Germline DNA was isolated from leukocytes of peripheral blood by a standard protocol (phenol–chloroform method).

An original protocol was created for identifying mutations in the **AAAS** gene associated with achalasia. Five pairs of specific polymerase chain reaction (PCR) primers were designed to amplify all 16 exons of the **AAAS** gene (NM_015665) (Table 1). The PCRs were performed using RED Taq® DNA polymerase (VWR, Radnor, PA, USA) in a 20 µL reaction volume. The conditions for PCR were as follows: 95 °C for 2.5 min activation/denaturation step, followed by 40 cycles of 95 °C for 20 s, 60 °C for 20 s, 72 °C for 60 s, with a final extension for 5 min. Amplicons were purified by Exo I-FastAP (Thermo Fisher Scientific, Waltham, MA, USA). The mixtures were incubated at 37 °C for 15 min and at 85 °C for 15 min to inactivate the enzymes, followed by sequencing with a BigDye Terminator v.3.1 (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified by EDTA/ethanol precipitation, resuspended in 15 µL Hi-Di Formamide (Applied Biosystems), then sequenced on an automated ABI 3500 Genetic Analyzer (Applied Biosystems).

Finally, sequences were compared using BioEdit v.7.0.9.0 (ref.21) with a standard sequence NM_015665 of the **AAAS** gene obtained from the GenBank database.

There were two differences between the patient’s sequence and the standard sequence NM_015665 of the **AAAS** gene. First, the patient was heterozygous (AT) in position 195 (counting according to the translated sequence). Second, the T base of the C855T single nucleotide polymorphism (SNP), known as rs1546808, was recorded on both strands (Supplementary data 1).

For identification of mutations in genes associated with acromegaly/gigantism in the patient, an NGS-based panel targeting 345 genes was used. This panel is customized and primarily designed for detecting mutations in

<p>| Table 1. Sequences of the specific primers used for PCR amplification of all 16 exons of the <strong>AAAS</strong> gene (NM_015665). |</p>
<table>
<thead>
<tr>
<th>Location of exons (NM_015665)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1..286 287..414</td>
<td>5′-GTGACCCAGAAACCCTTT-3′</td>
<td>5′-AGAAGCTTTGGAGGTTCTGTC-3′</td>
</tr>
<tr>
<td>415..470 471..562</td>
<td>5′-AAGGATAGGAATGAGGCGAGA-3′</td>
<td>5′-AAGCTGACCCACATTTTCCTC-3′</td>
</tr>
<tr>
<td>563..609 610..708 709..852</td>
<td>5′-AACTCTAGGCAAGGTTAAG-3′</td>
<td>5′-CCAGTGTTAGCTCTGCTATC-3′</td>
</tr>
<tr>
<td>853..973 974..1098 1099..1159</td>
<td>5′-TGACCAACCACAAATTCTGAA-3′</td>
<td>5′-TCTGACTCCACATCTCCTGT-3′</td>
</tr>
<tr>
<td>1160..1250 1251..1345 1345..1412</td>
<td>5′-CAACCTCCTGGAAGAACAGA-3′</td>
<td>5′-CTTGCAAGAACCTCTCCTGT-3′</td>
</tr>
<tr>
<td>1413..1494 1495..1579 1580..1837</td>
<td>5′-AACTCTCAGGCCAAGGTAAG-3′</td>
<td>5′-CCAGTGTTAGCTCTGCTATC-3′</td>
</tr>
</tbody>
</table>

<p>| Table 2. Common polymorphisms in the genes associated with acromegaly found in the patient. |</p>
<table>
<thead>
<tr>
<th>Gene</th>
<th>Variant cDNA</th>
<th>Variant protein</th>
<th>RefSeq accession</th>
<th>dbSNP</th>
<th>Hetero/homozygous</th>
<th>MAF in EU population (%)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN1</td>
<td>c.1621A&gt;G</td>
<td>p.T541A</td>
<td>NM_000244</td>
<td>rs2959656</td>
<td>homozygous</td>
<td>99.0</td>
<td>NGS panel</td>
</tr>
<tr>
<td>MEN1</td>
<td>c.1314T&gt;C</td>
<td>p.H438H</td>
<td>NM_000244</td>
<td>rs540012</td>
<td>homozygous</td>
<td>100.0</td>
<td>NGS panel</td>
</tr>
<tr>
<td>AIP</td>
<td>c.682C&gt;A</td>
<td>p.Q228K</td>
<td>NM_003977</td>
<td>rs641081</td>
<td>homozygous</td>
<td>100.0</td>
<td>NGS panel</td>
</tr>
<tr>
<td>AIP</td>
<td>c.920A&gt;G</td>
<td>p.Q307R</td>
<td>NM_003977</td>
<td>rs493099</td>
<td>homozygous</td>
<td>100.0</td>
<td>NGS panel</td>
</tr>
<tr>
<td>CDKN1B</td>
<td>c.326T&gt;G</td>
<td>p.V109G</td>
<td>NM_004064</td>
<td>rs2066827</td>
<td>heterozygous</td>
<td>24.3</td>
<td>NGS panel</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>c.998G&gt;A</td>
<td>p.S333N</td>
<td>NM_002734</td>
<td>rs9789047</td>
<td>heterozygous</td>
<td>20.6</td>
<td>NGS panel</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>c.349-5dupT</td>
<td>-</td>
<td>NM_002734</td>
<td>rs3841514</td>
<td>heterozygous</td>
<td>24.7</td>
<td>NGS panel</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>c.770-24G&gt;A</td>
<td>-</td>
<td>NM_002734</td>
<td>rs2302230</td>
<td>homozygous</td>
<td>68.0</td>
<td>NGS panel</td>
</tr>
<tr>
<td>PRKAR1A</td>
<td>c.892-34G&gt;T</td>
<td>-</td>
<td>NM_002734</td>
<td>rs2302231</td>
<td>homozygous</td>
<td>67.8</td>
<td>NGS panel</td>
</tr>
<tr>
<td>SDHB</td>
<td>c.201-36G&gt;T</td>
<td>-</td>
<td>NM_003000</td>
<td>rs1022580</td>
<td>homozygous</td>
<td>97.2</td>
<td>NGS panel</td>
</tr>
<tr>
<td>SDHB</td>
<td>c.18C&gt;A</td>
<td>p.A6A</td>
<td>NM_003000</td>
<td>rs2746462</td>
<td>homozygous</td>
<td>97.2</td>
<td>NGS panel</td>
</tr>
<tr>
<td>GPR101</td>
<td>c.370G&gt;T</td>
<td>p.V124L</td>
<td>NM_054021</td>
<td>rs1190736</td>
<td>homozygous</td>
<td>38.0</td>
<td>Sanger sequencing</td>
</tr>
</tbody>
</table>

Minor allele frequency (MAF) refers to the frequency at which the second most common allele occurs in a given population.
genes associated with a predisposition towards various cancers, but it includes five genes that have been found to be mutated also in patients with acromegaly: \textit{AIP}, \textit{MEN1}, \textit{CDKN1B}, \textit{PRKAR1A}, and \textit{SDHB} (ref.\textsuperscript{8}). The patient’s DNA extracted from the leukocytes was used as input material for the assay, followed by the preparation of sequencing libraries using SureSelectXT HS Target Enrichment System (Agilent, Santa Clara, CA, USA) according to the manufacturer’s protocol and sequencing on a NextSeq 500 device (Illumina, San Diego, CA, USA). In the bioinformatic analysis, sequencing reads in fastq format were mapped to the human reference genome GRCh37 with the bwa mem algorithm. PCR duplicates were marked in the resulting alignments with the Fgbio toolkit, which can utilize unique molecular identifiers included in the library. The quality of the final alignments was checked in respect to region coverage and depth and was used to assess germline variants. To minimize false negative calls, three different tools were used for germline variant calling (i.e., GATK HaplotypeCaller, VarDict, and Strelka2) and resulting variants were merged. Merged variants were annotated using the VEP annotation tool, which includes PolyPhen and SIFT scores for variant structural impact and reports co-located variants from several databases (snpDB, COSMIC, HGMD, NHLBI_ESP, and ClinVar). Variants were filtered manually. Examination using this NGS-based panel revealed only common polymorphisms in the genes of interest in our patient (reported in Table 2).

Because no pathogenic mutation was found using the NGS panel, we decided to take a closer look at the remaining genes that have been related to acromegaly: \textit{GPR101} and \textit{GNAS}.

Amplification and sequencing primers were designed (primer sequences and PCR conditions are available in Supplementary data 2) for the whole coding region of the \textit{GPR101} gene and the most common mutations in codons R201 and Q227 of the \textit{GNAS} gene.

DNA extracted from the formalin-fixed paraffin-embedded (FFPE) tissue sample of the pituitary macroadenoma and of the thyroid gland was used for identifying somatic mutations in the \textit{GNAS} gene.

Sequencing was performed on an ABI 3500 Genetic Analyzer device (Applied Biosystems) and sequences...
were analysed using the Mutation surveyor V4.0.9 software (SoftGenetics, State College, PA, USA).

The common polymorphism c.370G>T/p.V124L (NM_054021, rs1190736; see Table 2) was found using Sanger sequencing, while sequencing of the GNAS gene revealed the somatic mutation c.601C>T/p.R201C (NM_000516, rs11554273; Fig. 5) in tissue of the pituitary macroadenoma only but not in that of the thyroid gland. Peripheral blood samples were obtained from the patient’s monozygotic twin, mother, and father. DNA from leukocytes was extracted and the GNAS gene was analysed. No pathogenic mutation was found in this gene within the germline DNA of the patient’s relatives.

**DISCUSSION AND CONCLUSION**

The diagnosis of achalasia is based on clinical symptoms (dysphagia, regurgitation, retrosternal pain, vomiting, and weight loss), endoscopy, HRM, and/or swallow radiography. Treatment can be conservative (botulinum toxin, pneumatic dilation, or medical therapy), endoscopic (peroral endoscopic myotomy [POEM]), or surgical (LHM) (ref.11,18). The best long-term results in therapy are achieved by pneumatic dilation or LHM (ref.18,22). Long-term follow-up studies to evaluate the effectiveness of POEM are needed23,24. The main limitation of POEM may be its non-association with any anti-reflux procedure, which is in contrast to LHM (ref.23). Our patient achieved excellent postoperative results with LHM and the dysphagia disappeared.

The clinical manifestations of acromegaly usually develop slowly and the diagnosis is often determined late (from 4 years to more than 10 years after onset) (ref.1). So it is possible also in our patient that the acromegaly could already have started even before the diagnosis of achalasia. The average onset of the disease is at 49 years, with no difference by gender25. Because of the increased morbidity and mortality in acromegalic patients, early diagnosis of the disease is critical6. Making a diagnosis generally starts with a clinical suspicion (most typically acral growth and changes, as well as increased shoe and/or ring size). A diagnosis of acromegaly is confirmed, however, by high levels of GH and IGF-1 (ref.2,3).

Due to the excess of GH and IGF-1, soft tissue hypertrophy involving the GI organs occurs in acromegalic patients1. This results in an enlargement of the GI organs. For example, dolichocolon is often observed in patients with acromegaly. George et al.7 reported the case of an acromegalic patient with megaduodenum, where the destruction of neurons within the plexuses was mentioned as the possible aetiological cause.

Higher prevalence of colorectal neoplasm, prolonged bowel transit time, and dysmotility of the entire GI tract (including the oesophagus) have been described. Baldy-Waligorska et al.26 stated that long-lasting uncontrolled acromegaly significantly increased the risk of malignant neoplasms. Higher prevalence of colorectal neoplasm in acromegalic patients has been reported in many studies1,3,27. Because of an increased risk of colorectal neoplasms, more frequent endoscopic screening is recommended27.

Prolonged exposure to high levels of GH and IGF-1 also affects the heart muscle. Dilation and hypertrophy of the heart (dilated cardiomyopathy) can develop28.

Oesophageal dysmotility and impaired LES relaxation in acromegalic patients have been described previously4,29. Some theories of motility disorders have been associated with low levels of nitric oxide (as the main neurotransmitter in the GI tract causing smooth muscle relaxation). Another theory of oesophageal motility dysfunction considers alterations in GI hormones (ghrelin and somatostatin) and GH levels4.

An association between dilation and hypertrophy of soft tissue and muscle tissue (oesophageal dysmotility, prolonged bowel transit time, dolichocolon, megaduodenum, and dilated cardiomyopathy) in achalasia aetiology should be considered. Hypertrophy of the soft tissue possibly causes the destruction of neuron plexuses, which can be one of the possible aetiologies of achalasia.

A co-occurrence of achalasia and oesophageal cancer has also been reported21. A relationship between food stasis, chronic inflammation, and a potentially increased risk of carcinoma development has also been described20,31.
Based on these reports, a thorough endoscopic surveillance was required for our patient having co-occurrence of acromegaly and achalasia.

Some authors have reported an increased incidence of achalasia with autoimmune thyropathy. This strengthens the theory of achalasia's having an autoimmune disease aetiology. In our case, the thyroid gland of the patient had also been treated. Nevertheless, no autoimmune thyroid disorder was revealed.

A possible association of achalasia and polyglandular autoimmune syndrome type 2 (autoimmune Addison's disease in combination with autoimmune thyroid disease and/or type 1 diabetes mellitus) is also described in the literature. Moreover, there are several reports associating megaesophagus with adrenocortical insufficiency and other autoimmune endocrine diseases in dogs. In 1996 even described a human with megaesophagus and autoimmune adrenal failure as a feature of autoimmune polyglandular syndrome type 2. In addition to these findings, dysphagia and vomiting are also characteristic in untreated Addison patients, but they usually resolve after hormone substitution. It nevertheless remains unclear whether low cortisol levels could possibly play a role in the development of lower oesophageal dysmotility or achalasia. Moreover, our patient had normal cortisol levels.

Because of the possibility that our patient with achalasia might be suffering from Allgrove (Triple A) syndrome, the AAAS gene was analysed. This gene encoding the ALADIN protein (WD-repeat protein) is located on chromosome 12 and is strongly associated with aetiology. In our case, the thyroid gland of the patient had been treated. Nevertheless, no autoimmune thyroid disease in combination with autoimmune disease was diagnosed. We report the first co-occurrence of acromegaly and achalasia, and we suggest the association of acromegaly and soft muscle tissue hypertrophy as one of the factors contributing to achalasia development. If one of these two diagnoses is determined, therefore, presence of the other should be considered. Moreover, an increased risk of malignancy (oesophageal and colorectal) should also be considered. More studies are needed to clarify the aetiology and association of these two diseases.

**ABBREVIATIONS**

GH, Growth hormone; GI, Gastrointestinal; HRM, High-resolution oesophageal manometry; IGF-1, Insulin-like growth factor-1; LES, Lower oesophageal sphincter; LHM, Laparoscopic Heller myotomy; MAS, McCune-Albright syndrome; POEM, Peroral endoscopic myotomy; SNP, Single nucleotide polymorphism.

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**Author contributions:** JD: writing, main author, conception and design of the work, literature search; JK: consultant, writing, text editor, conception and design of the work, literature search, figures; RR, KS, PJ, TN, KM, TA, FM, ZK, and JV: consultants, text editors, conception and design of the work, figures, literature search; DS: histopathological examination; MZ, JL, HPN, OS: text editors, genetic analyses; LIH: consultant and text editor; PBL:
design of genetic analysis, literature search, text editor. All authors made critical revisions and gave their final approval for publishing.

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