

SUPPLEMENTAL MATERIAL

Jiri Dolina, Lumir Kunovsky, Radek Kroupa, Karel Stary, Petr Jabandziev, Tereza Nesporova, Karel Maca, Tomas Andrasina, Filip Marek, Zdenek Kala, Jitka Vaculova, David Said, Martina Zapletalova, Jan Lochman, Hana Palova Noskova, Ondrej Slaby, Lydie Izakovicova Holla, Petra Borilova Linhartova. **Achalasia and acromegaly: co-incidence of these diseases or a new syndrome?** (doi: 10.5507/bp.2021.040)

	10	20	30	40	50	60	70	80	90	100	110	120
												
NM_015665	CCCGTTAGTCTTTTCTTCACTTCCGTTGAGTTCCGCCTCGCCGTTTGTCCCTTGCGGTACCCGTCGCGCATACGAATCTAGCCCGGGAACCGAGTTGCGGGAGTGCGGTCTGTGCCGTTCC											
Patient	-----TTCCGTTGAGTTCCGCCTCGCCGTTTGTCCCTTGCGGTACCCGTCGCGCATACGAATCTAGCCCGGGAACCGAGTTGCGGGAGTGCGGTCTGTGCCGTTCC											
	130	140	150	160	170	180	190	200	210	220	230	240
												
NM_015665	GGCCAGGAGTTTGCCGACTGCAGACGTCCTGCGAACCGGCAAGATGTGCTCTCTGGGGTTGTTCCCTCCTCCACCGCCTCGGGGTCAAGTCACCCTATATGAGCACAATAACGAGCTGGT											
Patient	GGCCAGGAGTTTGCCGACTGCAGACGTCCTGCGAACCGGCAAGATGTGCTCTCTGGGGTTGTTCCCTCCTCCACCGCCTCGGGGTCAAGTCACCCTATATGAGCACAATAACGAGCTGGT											
	250	260	270	280	290	300	310	320	330	340	350	360
												
NM_015665	GACGGGCAGTAGCTATGAGAGCCCGCCCCCGACTTCCGGGGCCAGTGGATCAATCTTCTGTCTCTACAACCTGACAAAGGATCCCTTAAAGACCCCTGGAAGGCTGGACCATGGCACAAG											
Patient	GACGGGCAGTAGCTATGAGAGCCCGCCCCCGACTTCCGGGGCCAGTGGATCAATCTTCTGTCTCTACAACCTGACAAAGGATCCCTTAAAGACCCCTGGAAGGCTGGACCATGGCACAAG											
	370	380	390	400	410	420	430	440	450	460	470	480
												
NM_015665	AACTGCCTTCATCCATCACC GGAGCAAGTGTGGAAGAGATGCATCAACATTGGCGTGATGTGGGCCTTTTGGGGTGCTAAATGAAATTGCAAACTCAGAAGAAGAGGTGTTTGTAGTG											
Patient	AACTGCCTTCATCCATCACC-----CGTGATGTGGGCCTTTTGGGGTGCTAAATGAAATTGCAAACTCAGAAGAAGAGGTGTTTGTAGTG											
	490	500	510	520	530	540	550	560	570	580	590	600
												
NM_015665	GGTGAAGACGGCATCCGGCTGGGCCCTGGCACTCTGTGATGGGCCTTCTCCCTCCATGGGTCCCTGTTCCCCCATCTGTCTCTCAGGAGCGAAGATCTGATCGCTGAATTTGCCCAAGT											
Patient	GGTGAAGACGGCATCCGGCTGGGCCCTGGCACTCTGTGATGGGCCTTCTCCCTCCATGGGTCCCTGTTCCCCCATCTGTCT-----ATCGCTGAATTTGCCCAAGT											
	610	620	630	640	650	660	670	680	690	700	710	720
												
NM_015665	CACAAATTGGTCCAGCTGCTGCTTGGCTGTCTTTGCATGGCACCACACCAACAAGTTTGAGTGGCCCTGCTAGATGACTCAGTCCGTGTGTATAATGCCAGCAGCACCATAGTCCC											
Patient	CACAAATTGGTCCAGCTGCTGCTTGGCTGTCTTTGCATGGCACCACACCAACAAGTTTGAGTGGCCCTGCTAGATGACTCAGTCCGTGTGTATAATGCCAGCAGCACCATAGTCCC											
	730	740	750	760	770	780	790	800	810	820	830	840
												
NM_015665	CTCCCTGAAGCACC GGCTGCAGCGAAATGTGGCGTCTCTGGCCTGGAAGCCCTTAGTGCTCTGTCTTGGCTGTGGCCTGCCAGAGCTGCATTCTTATCTGGACCCTGGACCCTACCTC											
Patient	CTCCCTGAAGCACC GGCTGCAGCGAAATGTGGCGTCTCTGGCCTGGAAGCCCTTAGTGCTCTGTCTTGGCTGTGGCCTGCCAGAGCTGCATTCTTATCTGGACCCTGGACCCTACCTC											
	850	860	870	880	890	900	910	920	930	940	950	960
												
NM_015665	CTTGCTTACCCGACCCTCTTCTGGCTGTGCCAAGTGTCTCTCACCTGGGCATACACCTGTTACCAGCTTGGCCTGGGGCCCCAGTGGGGGGCGGCTGCTCTCAGCTTCACCCGTGGA											
Patient	-----TTCTGGCTGTGCCAAGTGTCTCTCACCTGGGCATACACCTGTTACCAGCTTGGCCTGGGGCCCCAGTGGGGGGCGGCTGCTCTCAGCTTCACCCGTGGA											
	970	980	990	1000	1010	1020	1030	1040	1050	1060	1070	1080
												
NM_015665	TGCTGCTATCCGGGTATGGGATGTCTCAACAGAGACCTGTGTCCCCCTTCCCTGGTTCGAGGAGGTGGGGTGACCAACCTGCTCTGGTCCCCAGACGGCAGCAAAATCCTGGCTACCAC											
Patient	TGCTGCTATCCGGGTATGGGATGTCTCAACAGAGACCTGTGTCCCCCTTCCCTGGTTCGAGGAGGTGGGGTGACCAACCTGCTCTGGTCCCCAGACGGCAGCAAAATCCTGGCTACCAC											

rs1546808

	1090	1100	1110	1120	1130	1140	1150	1160	1170	1180	1190	1200
												
NM_015665	TCCTTCAGCTGTCTTTTCGAGTCTGGGAGGCCAGATGTGGACTTGTGAGAGGTGGCCTACTCTATCAGGGCGCTGTCTCAGACTGGCTGCTGGAGGCCAGATGGCAGCCGACTGCTGTTTCAC											
Patient	TCCTTCAGCTGTCTTTTCGAGTCTGGGAGGCCAGATGTGGACTTGTGAGAGGTGGCCTACTCTATCAGGGCGCTGTCTCAGACTGGCTGCTGGAGGCCAGATGGCAGCCGACTGCTGTTTCAC											
	1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320
												
NM_015665	TGTATTGGGAGAGCCACTGATTTTACTCCCTGTCTTTTCCAGAACGTTGTGGTGAGGGAAGGGGTGCGTTGGAGGTGCAAAAGTCAGCAACGATTGTGGCAGATCTGTCTGAGACAACAAT											
Patient	TGTATTGGGAGAGCCACTGATTTTACTCCCTGTCTTT-----GGCAGATCTGTCTGAGACAACAAT											
	1330	1340	1350	1360	1370	1380	1390	1400	1410	1420	1430	1440
												
NM_015665	ACAGACACCAGATGGTGAGGAGAGGCTTGGGGGAGAGGCTCACTCCATGGTCTGGGACCCAGTGGGGAACGCTGGCTGTGCTTATGAAAGGAAAGCCAAGGGTACAGGATGGTAAACC											
Patient	ACAGACACCAGATGGTGAGGAGAGGCTTGGGGGAGAGGCTCACTCCATGGTCTGGGACCCAGTGGGGAACGCTGGCTGTGCTTATGAAAGGAAAGCCAAGGGTACAGGATGGTAAACC											
	1450	1460	1470	1480	1490	1500	1510	1520	1530	1540	1550	1560
												
NM_015665	AGTCATCCTCCTTTTTTCGCACTCGAAACAGCCCTGTGTTTGAGCTCCTTCCCTGTGGCATTATCCAGGGGGAGCCAGGAGCCAGCCCGAGCTCATCACTTTCCATCCTTCCTTCAACAA											
Patient	AGTCATCCTCCTTTTTTCGCACTCGAAACAGCCCTGTGTTTGAGCTCCTTCCCTGTGGCATTATCCAGGGGGAGCCAGGAGCCAGCCCGAGCTCATCACTTTCCATCCTTCCTTCAACAA											
	1570	1580	1590	1600	1610	1620	1630	1640	1650	1660	1670	1680
												
NM_015665	AGGGGCCCTGCTCAGTGTGGGCTGGTCCACAGGCCGAATTGCCACATCCCGCTGTACTTTGTCAATGCCAGTTTCCACGTTTTAGCCAGTGCTTGGGCGGGGCCAGGAACCCCTGTC											
Patient	AGGGGCCCTGCTCAGTGTGGGCTGGTCCACAGGCCGAATTGCCACATCCCGCTGTACTTTGTCAATGCCAGTTTCCACGTTTTAGCCAGTGCTTGGGCGGGGCCAGGAACCCCTGTC											
	1690	1700	1710	1720	1730	1740	1750	1760	1770	1780	1790	1800
												
NM_015665	TGGGGGTGGAGGCTCTATTTCATGACCTGCCCTCTTTTACTGAGACATCCCCAACCTCTGCCCTTGGGACCTCTCCCAGGGCCACCACTGTTCTGCCCCACTCCCCACATTCCCACCT											
Patient	TGGGGGTGGAGGCTCTATTTCATGACCTGCCCTCTTTTACTGAGACATCCCCAACCTCTGCCCTTGGGACCTCTCCCAGGGCCACCACTGTTCTGCCCCACTCCCCACATTCCCACCT											
	1810	1820	1830	1840	1850							
												
NM_015665	CTAAGAATAAATAAGTTTTCCTTTTGTTCCTCACTCAAAAAAAAAAAAAAAAAA											
Patient	CTAAGAATAAATAAGTT-----											

Supplementary Data 1. Comparison of the obtained exonic sequence (patient) with the standard sequence (NM_015665) of the *AAAS* gene. Symbol “W” means mixed bases A and T. Symbol “—” indicates an unknown base in the sequence. The **red** colour marks differences between sequences. The **bold** font marks the translated region.

1) *GPR101* (whole gene in 2 PCR products)

Primer sequence 5'-3'

F1 - TCACCAGCATAGGCACTGAG

R1 - GGCGAAACTCACTCTCATCC

PCR product length: 889 bp

F2 - CGAGTCAAGGACTGTGTGGA

R2 - GCAACACCTTTGCCTGAAAT

PCR product length: 937 bp

2) *GNAS* (exons 8 and 9 for identification of the most common mutations c.601C>T/R201C, c.602G>A/R201H, and c.680A>G/Q227R (NM_000516.7) (<https://pubmed.ncbi.nlm.nih.gov/2549426/>))

Primer sequence 5'-3'

F - ACTATGTGCCGAGCGATCA

R - CCAAAGAGAGCAAAGCCAAG

PCR product length: 500 bp

PCR programme

95°C 3 min

95°C 45 sec

60°C 1 min

35×

72°C 1 min

72°C 10:00 min

6°C ∞

Supplementary Data 2.