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CLINICAL STUDY

Identification of somatostatin receptor type 5 gene polymorphisms associated with acromegaly

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Abstract

Objective: The aim of this study was to characterize the genetic variance of somatostatin receptor 5 (SSTR5) and investigate the possible correlation of such variants with acromegaly risk and different disease characteristics.

Design and methods: The SSTR5 gene coding region and 2000 bp upstream region was sequenced in 48 patients with acromegaly and 96 control subjects. Further, three single nucleotide polymorphisms (SNPs) were analyzed in the same group of acromegaly patients and in an additional group of 475 age- and sex-matched controls.

Results: In total, 19 SNPs were identified in the SSTR5 gene locus by direct sequencing. Three SNPs (rs34037914, rs169068, and rs642249) were significantly associated with the presence of acromegaly using the initial controls. The allele frequencies were significantly ($P<0.01$) different between the acromegaly patients and the additional large control group. rs34037914 and rs642249 remained significantly associated with acromegaly after Bonferroni correction and permutation tests (odds ratio (OR)=3.38; 95% confidence interval (CI), 1.78–6.42; $P=0.00016$ and OR=2.41; 95% CI, 1.41–4.13; $P=0.0014$ respectively). Haplotype reconstruction revealed two possible risk haplotypes determined by rs34037914 (633T) and rs642249 (1044A) alleles. Both haplotypes were found in significantly higher frequency in acromegaly patients compared with controls ($P<0.001$). In addition, the 663T allele was significantly associated with a younger age of acromegaly diagnosis (unstandardized regression coefficient $\beta=-10.4$; $P=0.002$), increased body mass index ($\beta=4.1$; $P=0.004$), higher number of adenoma resection ($P<0.001$) and lack of observable tumor shrinkage after somatostatin analog treatment ($P=0.014$).

Conclusions: Our results demonstrate a previously undetected strong association of two SSTR5 SNPs with acromegaly. The data also suggest a possible involvement of SSTR5 variants in decreased suppression of GH production and increased tumor proliferation.

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Introduction

Acromegaly is a rare chronic disease mainly caused by pituitary GH overexpressing adenomas (1). The general characteristics of acromegaly are elevated levels of GH and insulin-like growth factor 1 (IGF1). Disease incites high morbidity and increased risk of mortality if it is not properly treated (2). One of the main physiological mediators of GH secretion and regulation of tumor proliferation is somatostatin acting through the somatostatin receptors (SSTRs), a family of G protein-coupled receptors (GPCRs) including five subtypes (SSTR1–5) (3, 4). Two of these receptor subtypes SSTR2 and SSTR5 are found in high levels in pituitary somatotropinomas (5), which is in contrast to other SSTR subtypes. Accordingly, somatostatin analogs (SA) such as octreotide and lanreotide, that have the highest affinity to SSTR2 and SSTR5, are used to repress GH secretion in acromegaly patients (6, 7). Although the introduction of these drugs has greatly improved therapy for the disease, a significant proportion of the patients do not adequately respond to treatment resulting in incomplete reduction in GH and IGF1 levels (8, 9). Moreover, 10% of the acromegaly patients show no changes in GH levels upon the SA treatment (10).

The genetic basis of acromegaly and drug resistance remains largely unclear (reviewed in (11)). It has been shown that somatic mutations in the Gsα gene (*GNAS*)

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are associated with constitutive activity of adenylyl cyclase and that these mutations are found in roughly 40% of somatotropinomas resulting in increased sensitivity to the inhibitory action of somatostatin (12). Very few germline mutations are known that are implicated in inherited pituitary tumor risk. Recently, it was shown that pituitary adenoma predisposition in familial cases are due to mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene (13) and may also play a role in aggressive disease development in sporadic cases of somatotropinomas (14, 15). As far as the acromegaly and SSTRs are concerned, only a few genetic association studies have been conducted. Filopanti *et al.* (16) identified that the rs3751830 and rs169068 alleles are weakly associated with altered GH and IGF1 levels in patients with acromegaly. To date, the only non-synonymous SSTR mutation that may be associated with drug resistance in acromegaly treatment is a germline R240W mutation in the SSTR5 that was found in one patient resistant to octreotide (17). Since almost no genetic mutations of SSTR2 and SSTR5 have been found in resistant patients (18, 19), the resistance has been attributed to impaired SSTR2 and SSTR5 expression in tumor tissues (10, 20).

In this study, we performed sequencing of the SSTR5 gene in 144 subjects and investigated potential correlations between SSTR5 gene variants and disease outcome as well as the clinical and hormonal characteristics in 48 acromegaly patients and 475 controls.

Materials and methods

Study group

We followed the STREGA guidelines (21) to describe the study group selection and association analysis. Case–control study groups were selected from the Latvian Genome Database (LGDB), a government funded biobank. All participants of LGDB were over 18-years of age, and information about their health status was affirmed by physicians using International Classification of Diseases (ICD)-10 codes. Anthropometric measurements (including weight and stature) were obtained by direct measurement; ethnic, social, environmental information, and familial health status were obtained using a questionnaire-based interview. Participants of the LGDB were recruited by medical personnel in hospitals or general practices. Recruitment was population based (a specific health condition was not the obligatory requirement for involvement). Signed consent forms were acquired from all participants. The Biobank protocol was approved by the Central Medical Ethics Committee of Latvia (protocols no. A-30, 2005 and A-7, 2007).

Forty-eight acromegaly patients recruited for this study were enrolled on to the LGDB between 2004 and 2008 from two main hospitals: Pauls Stradins Clinical University Hospital (41 patients) and Riga Eastern Clinical University Hospital (seven patients) representing ~80% of all the acromegaly patients registered in Latvia as recorded in October 2008 (diagnosed with acromegaly from 1985 to 2007). Additional data were collected based on hospital records and interviews for all the patients selected for the study (ICD-10 code E22.0). Forty-five patients received the SA octreotide (Sandostatin LAR) at dose 10–30 mg in every 28 days or lanreotide (Somatuline Autogel) at dose 60–90 mg in every 28 days. Tumor size was measured as the maximum diameter obtained from magnetic resonance imaging data and tumors were classified accordingly as microadenomas (<10 mm) or macroadenomas (≥ 10 mm). The effect of SA on tumor proliferation and IGF1 level normalization was estimated by comparing the tumor sizes and serum IGF1 (μg/ml) measurements during the course of therapy. To estimate the dynamics of adenoma size, the data from last follow-up was compared with the first available measurement with at least a 12-month period in between. We excluded all cases where the therapy was interrupted or adenoma resections were performed during this period. Two groups were defined: 'reduced' with observable tumor shrinkage ($n=11$) and 'unchanged' with no observable tumor shrinkage ($n=22$) together with the cases that showed prolonged expansion ($n=2$). Only a limited number of cases had IGF1 measurements available before the therapy. For the IGF1 response, only the data at least 6 months after the start of the SA therapy were considered for analysis and only if the therapy was not interrupted. In those cases where several IGF1 measurements were available, the mean IGF1 was calculated, excluding the outliers where possible. Non-responsiveness was defined as the mean IFG1 value above the upper limit of normal (ULN) value at the corresponding age. Owing to the lack of uniformity in the GH measurements, we did not include the GH levels in this analysis.

As controls (control I), 96 samples were randomly chosen for sequencing from LGDB participants excluding patients with metabolic and endocrine diseases. To minimize the risk of false positivity, we selected an additional control group (control II) who were sex- and age-matched consisting of 475 LGDB participants. As per the selection criteria, we used 63 participants from the control I who were also included in the control II group. Detailed sample selection procedure is described in Supplementary Material, see section on supplementary data given at the end of this article. Study protocol was approved by Central Medical Ethics Committee of Latvia (protocols no. A-33, 2005 and A-3, 2008).

DNA analysis

DNA samples were provided by LGDB and aliquoted into 96-well PCR plates or PCR tubes by Tecan Freedom Evo robotic pipette. The final DNA amount was 28 ng/well.

The SSTR5 gene containing genomic DNA region including 5′ and entire coding region (from −2239 to +1294 respective to start codon) were amplified in six PCR reactions and sequenced using BigDye chemistry and ABI Prism 3100 (AME Bioscience, Toroed, Norway) capillary electrophoresis sequencer. All chromatograms were manually inspected using Contig Express Software of Vector NTI Advance 9.0 package (Invitrogen). Presence of polymorphisms was confirmed by opposite strand analysis. Genotyping was carried out using minisequencing and subsequent MALDI-TOF mass spectrometry analysis. Primers were designed on Primer3 Software (source code available at http://fokker.wi.mit.edu/primer3/) and using the program CalcDalton (www.uni-leipzig.de/~ahnert/calcdalton.htm). Detailed experimental procedures can be found in Supplementary Material and primer sequences can be found in Supplementary Table 1, see section on supplementary data given at the end of this article.

Statistical analysis

Statistical analysis was performed by the PLINK 1.07 (22) and SPSS (standard version 13; SPSS, Chicago, IL, USA) software. Deviation from Hardy–Weinberg equilibrium was assessed by the exact test described by Wigginton *et al.* (23) which is considered more accurate for rare genotypes. The Cochran–Armitage trend test was used for association analysis in the case–control group and Bonferroni correction was applied. Haplotype association was performed as implemented in PLINK. For the quantitative analyses, the IGF1 data were transformed as a normalized percentage of ULN of appropriate age according to formula $(C_{IGF1}-ULN_{IGF1})/ULN_{IGF1}\times 100)$. Normalized IGF and all other continuous variables displayed normal distribution and were further used in linear regression analysis. Two-sided Fisher exact test was used to test the allelic distribution in the case of categorical clinical variables, except the analysis of number of adenoma resections where Pearson χ^2 was calculated from 3×3 table. Permutation tests with 100 000 permutations were performed for each analysis and we used corrected (EMP2) *P* values. These values are corrected based on calculation of the proportion of permutations in which any of the test statistics exceeds the particular observed statistic and are more stringent than uncorrected *P* values.

Results

DNA samples of all 48 available acromegaly patients and 96 control individuals were subjected to direct sequencing of the SSTR5 gene including both the coding and the flanking regions (−2239 to 1294 respective to start codon). Baseline demographic and clinical characteristics are given in Table 1. In total, 19 polymorphisms were identified (relative positions, sequencing success rate and Hardy–Weinberg test results are shown in Supplementary Table 2, see section on supplementary data given at the end of this article). All single nucleotide polymorphisms (SNPs) were in Hardy–Weinberg equilibrium. Fourteen SNPs passed the quality and minimal minor allele frequency (MAF) criteria and were included in the subsequent analysis. Three SNPs were excluded due to low sequencing success rate (<95%) and two SNPs were excluded due to low MAF (<0.01).

Table 1 Characteristics of the study population. Data are presented as mean (S.D.) or *n* (%).

Variables	Acromegaly patients (*n*=48)	Control I (*n*=96)	Control II (*n*=475)
Sex			
Female	32 (67%)	67 (70%)	324 (68%)
Male	16 (33%)	29 (30%)	151 (32%)
Age (years)	55.8 (12.8)	53.2 (17.4)	54.3 (13.4)
BMI (kg/m^2)	29.9 (5.5)	25.4 (4.4)	27.6 (5.2)
Waist (cm)	91.0 (13.7)	–	–
Age at diagnosis (years)	47.4 (13.1)	–	–
Tumour size			
Macroadenoma	31 (64%)	–	–
Microadenoma	17 (36%)		
Effect of SA on tumor size (*n*=35)			
Reduced	11 (31.5%)	–	–
Unchanged	24 (68.5%)		
Expanded			
Adenoma resections per patient			
1	23 (47.9%)	–	–
2	2 (4.1%)		
3	2 (4.1%)		
IGF1 norm			
% ULN before treatment (*n*=20)	178.8 (87.9)	–	–
% ULN after treatment (*n*=39)	50.8 (77.8)		
IGF1 responsiveness (*n*=39)			
<ULN	12 (30%)		
>ULN	27 (70%)		

BMI, body mass index; IGF1, insulin-like growth factor 1; ULN, upper limit of normal at corresponding age group; IGF1 norm, % ULN, normalized percentage of ULN $((C_{IGF1}-ULN_{IGF1})/ULN_{IGF1}\times 100)$.

The minor alleles of three SNPs were independently associated with the presence of acromegaly (Table 2) using Cochran–Armitage trend test. T and A alleles of rs34037914 and rs642249, respectively, remained associated with acromegaly after adjusting for multiple comparisons using both Bonferroni correction and adjusted permutation test. The first control group (control I) contained some individuals with malignant and benign neoplasms (23%). To avoid the influence of age, gender, or medical conditions on the association results, as well as to minimize type I error, we selected an additional age- and gender-matched control group representing 475 healthy individuals. Genotyping of the three SNPs (rs34037914, rs169068, and rs642249) associated with acromegaly was performed in this study group. Genotyping results for these individuals corresponded to the genotypes previously obtained from the

Table 2 Single nucleotide polymorphism (SNP) association analysis in acromegaly patients and controls.

SNP codes	Alleles/ position[a]	AA change	MAF case	MAF controls	Allelic OR (95% CI)	*P* trend[b]	*P* perm[c]
Acromegaly patients ($n=48$) versus control I sample ($n=96$)							
rs550713	T-2190G		0.078	0.036	2.23 (0.76–6.56)	0.16	0.84
NA	T-2138delT		0.427	0.479	0.81 (0.49–1.34)	0.43	1
rs535338	A-1670G		0.202	0.266	0.7 (0.38–1.27)	0.24	0.97
NA	C-805G		0.043	0.044	0.97 (0.28–3.3)	0.96	1
rs4988479	G27A		0.021	0.031	0.67 (0.13–3.4)	0.63	1
rs4988483	C142A	P335L	0.021	0.016	1.34 (0.22–8.16)	0.75	1
rs4988484	C155T	A52V	0.021	0.042	0.49 (0.1–2.35)	0.40	1
rs4988487	C325T	P109S	0.021	0.042	0.49 (0.1–2.35)	0.40	1
rs35072648	G516A		0.031	0.047	0.66 (0.17–2.48)	0.56	1
rs34947461	G573A		0.021	0.031	0.66 (0.13–3.33)	0.65	1
rs34037914	**C633T**		**0.146**	**0.036**	**4.51 (1.76–11.6)**	**0.0015***	**0.019**
NA	G693A		0.021	0.016	1.34 (0.22–8.16)	0.75	1
rs169068	**T1004C**	**P335L**	**0.594**	**0.429**	**1.94 (1.18–3.21)**	**0.013**	**0.11**
rs642249	**G1044A**		**0.208**	**0.073**	**3.35 (1.61–6.97)**	**0.0016***	**0.020**
Acromegaly patients ($n=48$) versus Control II sample ($n=475$)							
rs34037914	**C633T**		**0.146**	**0.048**	**3.38 (1.78–6.42)**	**0.00016***	**0.0012**
rs169068	**T1004C**	**P335L**	**0.594**	**0.434**	**1.91 (1.25–2.93)**	**0.0029**	**0.0089**
rs642249	**G1044A**		**0.208**	**0.098**	**2.41 (1.41–4.13)**	**0.0014***	**0.0064**

AA, amino acid; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; NA, not acquired; association results for SNPs with $P<0.05$ are marked with bold. *Significant after Bonferroni correction (significances level 0.0029).
[a]Nucleotide position relative to the SSTR5 start codons, major allele is shown before and minor allele after the number indicating the nucleotide position.
[b]*P* value from Cochran–Armitage trend test.
[c]*P* value obtained from 100 000 permutation and corrected for multiple testing (EMP2).

sequencing reactions. All three SNPs were significantly associated with the presence of acromegaly (Table 2).

Six haplotypes were derived from the three candidate SNPs in acromegaly patients and control II group (Table 3). Four haplotypes (frequency >1% in control group) were subjected to the haplotype-based association analysis. The overall joint test of the SSTR5 haplotypes on presence of acromegaly (comparing acromegaly patients and control II group) was highly significant ($P=3.4\times10^{-6}$). For haplotype-specific tests the most common haplotype (56%) that contains only the common alleles of the three included SNPs (C-T-G) was chosen as a reference haplotype (Hap1). Hap3 (C-C-A) and Hap4 (T-C-G) contained the independently associated alleles of rs642249 and rs34037914, respectively, and were found with significantly higher frequency in acromegaly patients than in controls. Both these haplotypes also contain the C allele of rs169068 that was independently associated with acromegaly. On the contrary, Hap2 (C-C-G), which in addition to common rs642249 and rs34037914 alleles contains the rs169068 C allele, was not associated with acromegaly indicating that the association of this allele with the acromegaly is due to linkage disequilibrium (LD) with the minor alleles of both other SNPs.

We also tested the association of rs642249 and rs34037914 with different disease characteristics and phenotypes affected by acromegaly (Tables 4 and 5). The following categorical variables in the group of acromegaly patients were tested: size of adenoma (microadenoma versus macroadenoma), change in the size of adenoma as a result of use of medication (reduced versus unchanged and increased); number of adenoma resections per patient (0 vs 1 vs 2–3); and IGF1 levels

Table 3 Haplotype based association in acromegaly patients and control II sample.

Names	Haplotypes[a]	Haplotype frequency (number)		OR (95% CI)	*P* values[b]
		Cases	Control II		
Hap1	C-T-G	0.385 (37)	0.562 (534)	Ref	Ref
Hap2	C-C-G	0.260 (25)	0.293 (279)	1.3 (0.76–2.2)	0.3358
Hap3	C-C-A	0.188 (18)	0.096 (91)	**2.85 (1.56–5.22)**	**0.0016**
Hap4	T-C-G	0.146 (14)	0.045 (43)	**4.69 (2.35–9.34)**	**0.000053**
Hap5	C-T-A	0.021 (2)	0.002 (2)	NC	NC
Hap6	T-T-G	0 (0)	0.001 (1)	NC	NC

OR, odds ratio; CI, confidence interval; Ref, reference group; NC, not calculated; association results for haplotypes with $P<0.05$ are marked with bold.
[a]SNP order=rs34037914 – rs169068 – rs642249.
[b]*P* value from Fisher exact test.

Table 4 Categorical analysis of SNP association with different phenotypes in acromegaly patients.

Phenotype	Genotype distribution	MAF	Allelic OR (0.95% CI)	*P* value[a]	*P* perm[b]
SNP: rs34037914					
TT/TC/CC					
Size of adenoma			1.44 (0.42–5.00)	0.56	0.62
Micro	0/4/13	0.11			
Macro	2/6/23	0.16			
Effect of SA on tumor size			NA	**0.014**	**0.02**
Reduced	0/0/11	0			
Enlarged	2/7/15	0.22			
IGF1 after treatment			3.27 (0.64–16.54)	0.13	0.17
Below ULN	0/2/11	0.07			
Above ULN	2/5/14	0.21			
Number of adenoma resections		NC	NA	**0.0001**[c]	NC
0	0/4/16				
1	0/5/18				
2–3	2/1/1				
SNP: rs642249					
AA/AG/GG					
Size of adenoma			1.03 (0.28–3.82)	0.63	0.68
Micro	2/4/11	0.19			
Macro	2/8/21	0.24			
Effect of SA on tumor size			0.78 (0.28–2.15)	0.96	0.96
Reduced	1/2/8	0.19			
Enlarged	2/5/17	0.18			
IGF1 after treatment			1.04 (0.33–3.31)	0.94	0.95
Below ULN	2/2/9				
Above ULN	1/8/12	0.24			
Number of adenoma resections		NC	NC	0.5[c]	NC
0	2/6/12				
1	2/6/15				
2–3	0/0/4				

OR, odds ratio; CI, confidence interval; MAF, minor allele frequency; NA, not applicable; NC, not calculated. Association results with $P<0.05$ are marked in bold.
[a]*P* value from Fisher exact test.
[b]*P* value obtained from 100 000 permutation.
[c]*P* value from Pearson χ^2 calculation using 3×3 table (4 degrees of freedom).

after medical treatment (normal versus increased). Body mass index (BMI), age at acromegaly diagnosis and age-adjusted normalized IGF1 levels were used as continuous variables in the linear regression analysis (Table 5). rs642249 had no association with any of these phenotypes. However, rs34037914 was significantly associated with the number of these phenotypes (summarized in Supplementary Figure 1, see section on supplementary data given at the end of this article). Carriers of the rs34037914 T allele were characterized by significantly lower mean age at diagnosis (*P* value = 0.002) and increased BMI value (*P* value = 0.004). Association of the T allele with these variables corresponded best to the additive genetic model (Tables 4 and 5, Supplementary Figure 1, see section on supplementary data given at the end of this article). No association ($P=0.56$) was observed between rs34037914 and increased waist circumference (this measurement was only available for 23 patients) indicating that association of rs34037914 with increased BMI in this group is due to an increase in muscle and bone mass caused by acromegaly rather than to an increase in fat mass. In addition, we tested whether the rs34037914 allele is associated with BMI and stature in the healthy individuals, but we found no differences between the genotype groups (data not shown) for any of the genetic models. Interestingly, the rs34037914 risk (T) allele was absent in the group of patients with observable tumor shrinkage as a result of octreotide or lanreotide treatment, resulting in association between this parameter and presence of the rs34037914 T allele (*P* value = 0.014). Even more intriguing, the presence of the same allele was positively correlated with the number of adenoma resections per patient (*P* value = 0.0001). Among the four patients in the entire study group who had more than one adenoma resection, two were homozygotes for the rs34037914 T allele and one was a carrier of the same allele in the heterozygous state. We also observed an increased rs34037914 T allele frequency in patients who failed to normalize their IGF1 levels compared with those who reached normal IGF1 levels after the SA treatment. Similarly, the mean normalized IGF1 levels were increased in patients with rs34037914 CT and TT genotypes in an additive manner (Table 5). None of these differences, however, reached a statistical significance.

Table 5 Qualitative analysis of SNP association with different phenotypes in acromegaly patients.

	Phenotypes (mean ± S.E.M.)		
Genotype	BMI (kg/m^2)	Age at diagnosis (y)	IGF1 % ULN
SNP: rs34037914			
CC	**28.87 ± 0.80**	**50.43 ± 2.03**	45.40 ± 14.61
CT	**31.35 ± 2.00**	**40.60 ± 3.90**	63.23 ± 30.03
TT	**40.48 ± 1.03**	**28.50 ± 7.50**	80.23 ± 32.28
P value[a]	**0.004**	**0.002**	0.41
P perm[b]	**0.012**	**0.006**	0.81
SNP: rs642249			
GG	31.04 ± 1.06	27.19 ± 1.29	28.33 ± 0.98
GA	48.25 ± 2.33	46.00 ± 3.68	44.50 ± 8.85
AA	40.72 ± 16.08	88.52 ± 19.92	19.93 ± 23.53
P value[a]	0.09	0.51	0.23
P perm[b]	0.23	0.87	0.97

IGF1 % ULN, normalized percentage of ULN (($C_{IGF1} - ULN_{IGF1}$)/ULN_{IGF1} × 100). Association results with $P < 0.05$ are marked in bold.
[a]*P* value from linear regression.
[b]*P* value obtained from 100 000 permutation.

Discussion

In this study, we present a novel and highly significant association of two SNPs, rs34037914 and rs642249, in the SSTR5 gene with acromegaly. This association remains significant after both Bonferroni correction and adjusted permutation tests. Both SSTR2 and SSTR5 are potential candidate genes for an increased risk to develop acromegaly and poor response to SA, due to their important role in controlling GH secretion and somatotroph growth. However, until now there has been a lack of convincing association between genetic variants of any of the SSTRs and acromegaly or resistance to the action of somatostatin or SA (reviewed in (11)). This can be explained in part by the observation that rare SNPs are often not included in genotyping due to low power in studies with a limited number of patients. Thus, to avoid a selection bias, an important issue in genetic studies of rare disease, we performed sequencing of the SSTR5 gene in all available patients and substantial number of controls. We identified four novel polymorphisms (one single nucleotide deletion and three SNPs) that were not previously reported to the SNP databases among the total of 19 polymorphisms (Supplementary Table 2, see section on supplementary data given at the end of this article). The allele frequencies of the previously known SNPs showed a variable prevalence, which was comparable to the allele frequencies reported in databases or previously published reports (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SSTR5&snp=207&search=sstr%205&rf=/home/genecards/current/website/carddisp.pl#snp). The prevalence of three SNPs was significantly different in acromegaly patients compared individually with a sex- and age-matched control group. Our haplotype analysis suggests that the non-synonymous polymorphism rs169068 (Pro335Leu) is not associated with acromegaly. The difference in allele frequencies between cases and controls for this SNP can be explained by strong LD between rs169068 C allele (Pro) and both associated alleles of rs642249 and rs34037914. Interestingly, the C allele of rs169068 has been previously associated with decreased IGF1 (16) in acromegaly patients. Our results may rather suggest an opposite effect due to LD between the C allele and rs34037914 T allele, as the latter has a non-significant tendency toward increased IGF1 levels. Unfortunately, the rs34037914 and rs642249 were not genotyped in a study performed Filopanty *et al*. (16) The C allele of the rs169068 was associated with the presence of bipolar affective disorder in a British but not in a Danish population (24), while there was a lack of association with autism in both Danish and French populations (25). No association was found between this SNP and risk or survival of pancreatic cancer (26). To our best knowledge, the rs34037914 and rs642249 have not been tested previously with respect to acromegaly. The presence of the A allele of rs642249 was associated with increased mean IGF1 levels, but not with risk of breast cancer in the international EPIC study (27). Neither rs34037914 nor rs642249 has shown associations with bipolar affective disorder (24).

The importance of the four SNPs from the SSTR5 gene locus on regulation of IGF1 levels has recently been demonstrated in a large multinational study on samples from the Breast and Prostate Cancer Cohort Consortium (28). One of these SNPs, the rs3751830, was also found in our study, but was not included in the final analysis due to low genotype call rate (Supplementary Table 2, see section on supplementary data given at the end of this article). However, no significant differences were found between the allele frequencies of this SNP and any of the phenotypes studied (data not shown). Data on LD between associated SNPs rs34037914 or rs642249 and other SNPs from the above mentioned study are not available and it is difficult to assess if these SNP may be part of the same haplotype or if they could represent functional SNPs. It is also clear that physiological consequences of the same genetic SSTR5 variants may be very different when a normal pituitary gland is considered compared with pituitary tumors. Unfortunately, the IGF1 levels for the control group were not available in our study, and this would help to assess the effects of these SNPs in on IGF1 regulation in healthy individuals.

rs34037914 shows the strongest association with acromegaly (odds ratio = 3.38, 95% confidence interval = 1.78–6.42) assuming an additive mode of inheritance. We strongly suggest that this is not a false-positive finding since the same rs34037914 allele is associated with a number of independent clinical characteristics in our group of acromegaly patients. This is the first study that reports such a pronounced effect of genetic variants on body mass in the case of

somatotropinomas. The median BMI was 12 kg/m^2 larger in the T allele homozygotes compared with wild-type (wt) homozygotes. Similarly, the median age at diagnosis was 22 years earlier considering the same group of patients. This is in line with previous data obtained in a large international collaborative study showing earlier onset age when investigating germ line mutations in the AIP (15) a gene that initially was associated with familial pituitary adenoma cases (13). According to our data, the T allele of rs34037914 predisposes to increased aggressiveness and post-surgical reoccurrence of pituitary tumors as well as non-responsiveness to antiproliferative effects of SA. The increased BMI in carriers of the T allele, however, indicates that the effect of this polymorphism is not limited to the regulation of tumor cell proliferation, but also affects the systemic GH levels. We did not observe the same effects of rs642249, the other acromegaly associated SNP, on the clinical or hormonal characteristics in our patient group. If not false positive, this may be explained by the smaller effect size of this SNP and a larger cohort of acromegaly patients would be needed to provide conclusive results. Alternatively, the disease predisposing effect linked to rs642249 may not be associated with characteristics available in our study.

It is not clear how the synonymous rs34037914 is connected with functional effects. First, other functional mutations in LD with rs34037914 may exist outside the regions we sequenced. It is plausible that such mutations, or rs34037914, are influencing the expression of SSTR5. Although the unresponsiveness to SA has been clearly associated with low expression of SSTR2 (29–31), the SSTR5 is the most abundantly expressed receptor in somatotropinomas (18, 32, 33) and it plays an important role in mediating the effect of somatostatin. The relative contribution of each of those receptors in the control of GH secretion is still unclear. It is established that both receptors are needed for hormonal regulation since the activation of SSTR2 and SSTR5 results in a synergistic effect on GH release (34–36). Thus, if the rs34037914 or rs642249 is linked to the changes in SSTR5 expression, they may significantly influence the responsiveness of the pituitary tumor cells to somatostatin and its analogs. Genotyping the rs34037914 and rs642249 in patients with known SSTR5 and SSTR2 expression profiles from pituitary tumors would help to test this hypothesis. Another possibility is that the particular sequence variations may induce alternative/*de novo* splicing creating a non-functional receptor protein or receptor with altered functions. It has indeed been shown that SSTR5 is found in two isoforms in pituitary tumors and that these are presumably generated by a splicing of the SSTR5 involving the presence of a cryptic donor and acceptor splice site (37). One of these variants, named SST5TMD4, has been found to be abundantly present in octreotide-resistant somatotropinomas and could interfere with the normal inhibitory response of adenomas to somatostatin (38). rs34037914 is actually located close to the splice site of the other variant SST5TMD5, and it is possible that this polymorphism may be important for this donor splice site formation. Functional consequences of the truncated receptor protein could involve its ability to interact with SSTR5 or SSTR2 leading to non-functional heterodimers. It has been shown previously that SSTR5 can form heterodimers with different GPCRs (reviewed in (39)), including SSTR2 and dopamine D2 receptors heterodimers with enhanced functionality (40, 41).

It is unlikely that changes in the amino acid sequence are responsible for the effects found in our study considering the functional importance of these SNPs. The non-synonymous substitution rs169068 is located in the C-terminal intracellular tail of the receptor causing a proline to leucine change. It has been shown by mutational analysis that the C-terminal domain is involved in the interaction with adenylate cyclase and is important for desensitization and internalization of this receptor (42). A recent study has shown that SSTR5 with leucine at position 335 loses its inhibitory effect on cell proliferation compared with the proline variant (43). However, It is unlikely that this substitution has a major impact on tumor development or drug resistance in our study group, as the proline was actually more frequent among the acromegaly patients (59.4% C allele frequency) while a majority of the general population carry SSTR5 with leucine in this position (56.6% T allele frequency). According to the SNP NCBI database and the HapMap data on European, African, and Asiatic populations (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=169068), the distribution of this variant differs significantly among the ethnic groups. The frequency of the T allele, as far as the HapMap data is considered, varies from 27% in the African American population and 52% in the Caucasian population to 85% in the Japanese and Chinese populations.

The major limitation of this study is the lack of standardized GH and IGF1 measurements for all acromegaly patients that did not allow a full estimation of the influence of these polymorphisms on hormonal regulation and drug responsiveness. Similarly, it was not possible to test the eventual effect of polymorphisms on the level of SSTR5 expression due to the unavailability of the tumor tissue samples. Replication studies with a larger patient size and other ethnic groups would provide additional insight into the associations that we have identified.

In conclusion, we have identified genetic variations in SSTR5 that are strongly associated with acromegaly and several of the clinical characteristics related to this disease. If tested functionally and proven clinically rs34037914 has a potential to become a diagnostic marker of non-invasive tests to determine the prognosis and aggressiveness of somatotropinomas.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-11-0416.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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