RESEARCH ARTICLE

Genetic association of *AIP* gene variant c.910C>T with pituitary adenoma-acromegaly patients of Pakistani origin

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Abstract

Objective: To investigate the association of aryl hydrocarbon receptor-interacting protein gene single nucleotide polymorphism (c.910C>T) with acromegaly in pituitary adenoma patients.

Method: The multi-centre cross-sectional study was conducted at the departments of Endocrinology and Neurosurgery, Fatima Memorial Hospital and Jinnah Hospital, Lahore, Pakistan, from February to June, 2023, and comprised healthy controls in group A and patients of pituitary adenoma in group B. Blood samples were used for deoxyribonucleic acid extraction and amplification refractory mutation system-polymerase chain reaction testing. The association of single nucleotide polymorphism was checked using the PLINK tool. Data was analysed using SPSS 27.

Results: Of the 58 subjects, 29(50%) were in control group A. There were 29(50%) subjects in group B; 19(65.5%) females and 10(34.5%) males with mean age 32±9.469 years and median age 30 years (interquartile range: 14 years). Macroadenomas were significantly prevalent 21(72.4%) compared to microadenomas 8(27.6%). Prolactinoma was the most common diagnosis 16(55.2%), followed by acromegaly 8(27.6%). Gender showed a significant association with adenoma size (p=0.015). Single nucleotide polymorphism c.910C>T was not detected.

Conclusion: Pituitary adenoma disproportionately affected females, with macroadenomas being prevalent and having an association with male gender and acromegaly No aryl hydrocarbon receptor-interacting protein gene single nucleotide polymorphism c.910C>T was noted, suggesting population-specific variations.

Keywords: Aryl hydrocarbon receptor-interacting protein, Acromegaly, Pituitary adenoma, Mutation.

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Introduction

The pituitary gland secretes different hormones, such as growth hormone (GH), which helps in growth, and affects the body's metabolism.[1] Acromegaly is a rare hormonal disorder that develops when the pituitary gland produces excessive GH in adults. Pituitary adenomas (PAs) are non-cancerous tumours of the pituitary gland, and can be classified according to the tumour size on radiography; >10mm is macroadenoma and <10mm is microadenoma.[2] In brain tumours, the most commonly found tumours are the PAs, about 15%.[3] In the general population, PA prevalence was found to be one case per 1,000.[4] PAs can arise from various genetic mutations, like *MEN1*,[5] *AIP*,[6] *CDKN1B*,[7] *PRKAR1A*,[8] *GNAS*,[9] *DICER1*.[10]

The aryl hydrocarbon receptor interacting protein (AIP) is composed of 365 amino acids. It belongs to the tetratricopeptide repeat (TPR) family of proteins.[11] *AIP* mutations are an important genetic cause of PAs, particularly somatotroph and lactotroph adenomas. Most importantly, individuals with a family history of pituitary tumours were found to have AIP mutations in familial isolated pituitary adenomas (FIPA) acromegaly.[12] Mutations in the AIP gene are found in 15-20% of FIPA patients, and in hereditary GH-producing adenomas it is found in 40-50%, having penetrance of 13-30%. The most common single nucleotide polymorphism (SNP_ associated with acromegaly was c.910C >T in a study.[6]

In homo sapiens, the location of the AIP gene is on chromosome (Chr) 11 (NC_000011.10) genome assembly GRCh38.p13 (GCF_000001405.40), having a total of 7 exons. At the genomic position 11. 67,490,910-67,490,912 with complementary deoxyribonucleic acid (cDNA) position 910 (GRCh38.p13), with the R304x region being the hotspot for mutations, the variation c.910C>T occurs, and this is highly associated with the disease.[13]

The accurate measurement of disease prevalence and the allocation of appropriate healthcare resources heavily rely on epidemiological data and knowing the exact cause. Early diagnosis could lead to lower rates of morbidity and mortality, and positively impact healthcare costs by preventing the progression of the disease to severe

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stages.[14] To the best of our knowledge, the mutations of the AIP gene or its SNPs have never been studied in Pakistan. The current study was planned to fill the gap by investigating the association of *AIP* gene SNP c.910C>T with acromegaly using PLINK.[15] in Pakistani PA patients.

Materials and Methods

The multi-centre cross-sectional study was conducted at the departments of Endocrinology and Neurosurgery at Fatima Memorial Hospital and Jinnah Hospital, Lahore, Pakistan, from February to June, 2023, and comprised healthy controls in group A and PA patients in group B. After approval from the ethics review committee of Nur International University, Lahore, the sample size was calculated using RaoSoft[16] at level of significance 5% and 90% power with an expected mean value of 68.2 of AIP gene SNP c.910C>T in PA patients.[17] The sample was raised using non-randomised purposive sampling technique from among diagnosed PA patients who were admitted to the relevant departments or had come for follow-up during the study period (Figure 1). Patients with MEN1 or Carney Syndrome, having other malignancies, tuberculosis (TB), or any other major illness were excluded. Healthy controls matched for age and gender were also enrolled.

After taking written informed consent, age and gender were noted. For patients in group B, adenoma size and the exact date when the patients had come to the hospital for the diagnosis were also noted. The patients' family history

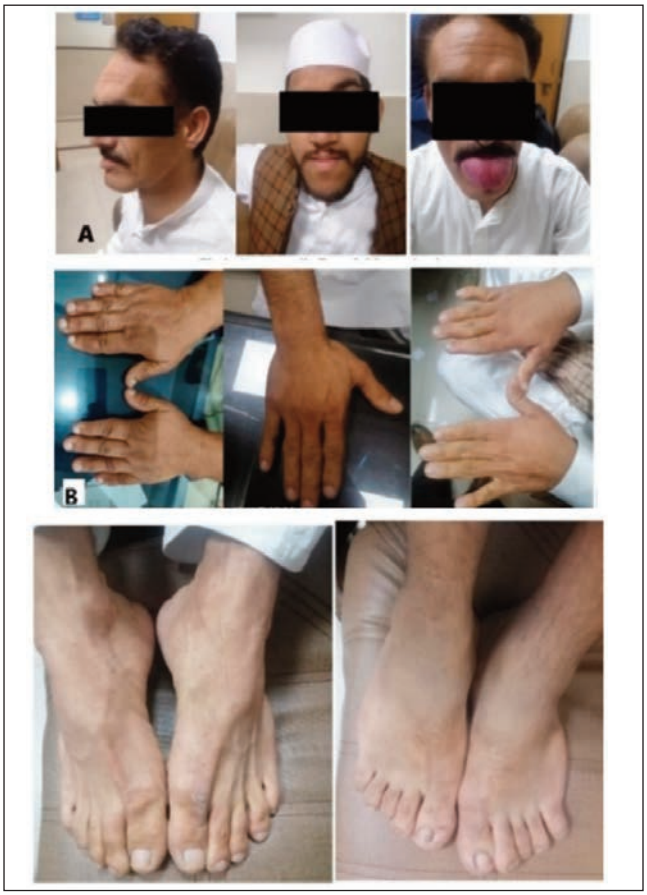

Figure-1: Acromegaly physical features; (a) face, (b) hands, (c) feet.

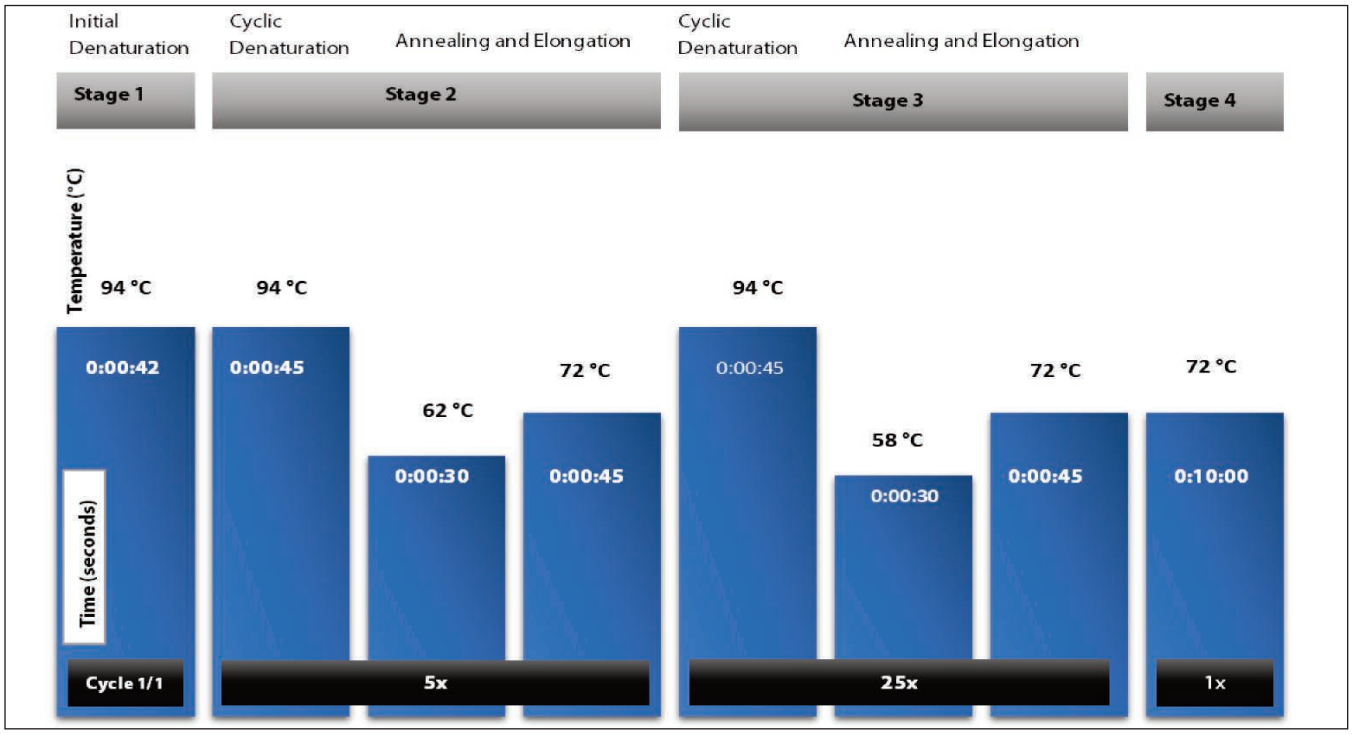

Figure-2: The amplification refractory mutation system-polymerase chain reaction (ARMS- PCR) protocol.

Table-1: Primer sequences and attributes.

Primers	Sequence (5′-3′)	Melting Temp (Tm)	Base Pair Length	Product size in base pairs
RC	GCCTTTATATACACAGAAGCATGACG	62.2	26	196
FN	TGGCGCCTGTGGTGAACC	61.4	18	
FM	CTGGCGCCTGTGGTGAACT	60.5	19	
Forward Internal Control	TAACCCACAGCCTCCTACAC	57.7	20	618
Reverse Internal control	TCAGCATCCTCCTCTGGACT	59.9	20	

RC: Reverse common, FN: Forward normal, FM: Forward mutant

was used to ascertain the familial cause and to rule out MEN1 and Carney Complex.

Blood samples were then collected by a trained phlebotomist. The samples were stored in Ethylenediaminetetraacetic Acid (EDTA) vacutainers in temperature-controlled boxes during transport to the laboratory. In the laboratory, the samples were stored at -20 degrees Celsius till the next procedure was initiated. The manufacturer's (GDSBio)[18] instructions were followed while using a genomic kit for DNA extraction.

NetPrimer[19] was used to create amplification refractory mutation system- polymerase chain reaction (ARMS-PCR) primers for the transcript ID >NM_003977.4 for the process of amplification of wild type and mutant alleles at location c.910C>T in homo sapiens. A total of 6 primers were created (Table 1).

ARMS-PCR was carried out using a thermal cycler (SimpliAmp)[20] in line with literature. The normal and mutant type ARMS Primers, i.e., forward, reverse, and common primers, specific for allele, were used in two independent PCR experiments, one for each sample. In some random samples, as an internal check, the genomic region was concurrently amplified using 2 regular primers. The reaction mixture, which contained 1µL of 50ng/µL genomic DNA, 10mM of each primer, 0.5IU of Thermus aquaticus (Taq) polymerase, 2.5mM $MgCl_2$, 2.5mM deoxynucleotide triphosphates (dNTPs), 1xbuffer and diethylpyrocarbonate (DEPC)-treated water, was produced in a total volume of 12µL. The PCR procedure was used with timings and cycles (Figure 2). The ARMS-PCR protocol[21] was used, and the associations were checked in terms of odds ratio (OR) and Hardy Weinberg Principle[22] using PLINK.[15]

Data was analysed using SPSS 27. Frequencies and percentages were used to express qualitative data, whereas mean±standard deviation were used to express quantitative data. Spearman's correlation test was used to check associations. P<0.05 was considered significant.

Results

Of the 58 subjects, 29(50%) were in control group A. There were 29(50%) subjects in group B; 19(65.5%) females and 10(34.5%) males with mean age 32±9.469 years and median age 30 years (interquartile range [IQR]: 14 years). In group B, 15(51.7%) patients were aged 15-30 years, while 9(30.8%) were aged 31-40 years and five (17.1%) were aged 41-57 years.

Macroadenomas were significantly prevalent 21(72.4%) compared to microadenomas 8(27.6%). Prolactinoma was the most common diagnosis 16(55.2%), followed by acromegaly 8(27.6%) (Table 2). All the 8(100%) acromegaly patients had macroadenoma. All the10(100%) male patients had macroadenomas. Among the group B females, 8(42.1%)

Table-2: Pituitary adenoma with diagnosis and size distribution.

Diagnosis	n (%)	Adenoma Size n (%)	
		Microadenoma	Macroadenoma
Prolactinoma	16 (55.2)	6 (20.7)	10 (34.5)
Acromegaly	8 (27.6)	0 (0)	8 (27.6)
Hypopituitarism	3 (10.3)	2 (6.9)	2 (6.9)
ACTH Dysfunction	1 (3.4)	0 (0)	1 (3.4)
NFPA	1 (3.4)	0 (0)	1 (3.4)
Total	29 (100)	8 (27.6)	21 (72.4)

ACTH: Adrenocorticotropic hormone, NFPA: Non-functioning pituitary adenomas.

Table-3: Correlation of adenoma size with diagnosis, gender and age.

		Adenoma Size
Spearman's rho	**Prolactinoma**	
	Correlation Coefficient	-0.246
	Sig. (2-tailed)	0.198
	n	29
	Acromegaly	
	Correlation Coefficient	0.381*
	Sig. (2-tailed)	0.041
	n	29
	Hypopituitarism	
	Correlation Coefficient	-0.297
	Sig. (2-tailed)	0.118
	n	29
	ACTH Dysfunction	
	Correlation Coefficient	0.117
	Sig. (2-tailed)	0.547
	n	29
	NFPA	
	Correlation Coefficient	0.117
	Sig. (2-tailed)	0.547
	n	29
	Gender	
	Correlation Coefficient	-0.448*
	Sig. (2-tailed)	0.015
	n	29
	Age	
	Correlation Coefficient	0.069
	Sig. (2-tailed)	0.720
	n	29

*Correlation is significant at 0.05 level (2-tailed).

ACTH: Adrenocorticotropic hormone, NFPA: Non-functioning pituitary adenomas.

had microadenomas and 11(57.9%) had macroadenomas.

Acromegaly was significantly correlated with adenoma size (p=0.041). Gender showed a significant association with adenoma size (p=0.015) (Table 3).

In ARMS-PCR, bands of the wild type gene were detected on gel electrophoresis in both groups, while no mutant band was seen in any of the groups (Figure 3). SNP c.910C>T was not detected.

Discussion

In the current study, there was a female dominance in the patient group, which is in contrast tp earlier studies that showed a conspicuous male dominance.[23-25] However, a study in Malta[26] revealed a female-majority sample. The reasons for these variations could be the differences in sampling techniques in specific social, cultural or occupational contexts in which the studies were conducted.

The median age of the patients was 30 years, and the mean age was 32.21±9.469 years. The age range was 15-57 years, indicating a wide spectrum of ages. These findings differ from earlier studies which reported median age in the 40s (rang: 40-50 years).[23-25] This discrepancy highlights potential variations in patient demographics across different studies, and underscores the need for further research. It is noteworthy that in current sample, most patients were of younger ages, which had higher chances of detecting the specific SNP C.910C>T in FIPA patients.[27]

There was a significant prevalence of macroadenomas, while microadenomas constituted only 27.6%. These results align with previous studies.[23-25,28] However, a study in Iceland reported a distinct pattern, with microadenomas being more prevalent than macroadenomas.[29]

The most common diagnosis was prolactinoma (55.2%) in the current study, which aligns with literature.[28] However, a study in Abbottabad, Pakistan, reported thyroid dysfunction as the most common diagnosis in PA patients.30 Acromegaly was the second most prevalent diagnosis in the current study, affecting 27.6% patients, which is in line with previous studies.[28] Hypopituitarism was the next common diagnosis. These overall findings are consistent with literature.[25,31]

Among the microadenomas, 6(75%) were prolactinomas, and 2(25%) were hypopituitarism in the current study.

The association between gender and acromegaly was also checked, and showed no significant association between gender and acromegaly in the patient population under investigation (p=0.295), which is consistent with the literature.[32]

The association between gender and adenoma size was significant (p=0.015), suggesting that males were more likely to have larger adenomas. This finding aligns with existing literature.[33]

There was no patient with microadenoma having acromegaly, whereas eight acromegaly patients had macroadenoma, affirming an association between macroadenomas and the presence of acromegaly. The correlation of adenoma size with acromegaly yielded a correlation coefficient value of 0.381(0.041), suggesting that acromegaly was significantly correlated with adenoma size.

In ARMS-PCR results, SNP c.910C>T was not detected in any of the patients. There could be several reasons. Firstly, it could be due to the small sample size of 29 patients. PAs are relatively rare, and it is challenging to gather a large cohort of patients for genetic studies. Another important reason for having a small sample size was the financial constraints. Additionally, the absence of the SNP c.910C>T in the patients could be due to population-specific variations. Although the c.910C>T is in the hotspot region of the AIP gene, it is known to have mutations.[13,27] Genetic differences between populations can impact the prevalence of certain genetic variations. Expanding the study to include diverse populations may reveal different patterns and shed light on the potential involvement of SNP c.910C>T in PAs. Suggestions for future research include large-scale studies with diverse cohorts, collaboration among research centres, and investigating other genetic variations, including mapping the whole AIP gene and environmental factors.

Conclusion

PAs affected females more compared to males, macroadenomas were predominant, and prolactinomas were the most common PAs, followed by acromegaly in patients of Pakistani origin. The presence of AIP gene SNP c.910C>T was not detected in any of the samples, potentially due to the limited sample size and population-specific variations.

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Author Contribution:

JG: Concept, draft, research planning, wet lab work, statistical analysis, editing and responsible for integrity of research.
SAJ: Design, practical work guidance, proof reading and supervision.
RS: Design, managed troubleshooting of the wet lab, supervision and proof reading.
SI: Sample collection and writing.
HHG: Sample collection and revision.
AH: Sample collection and data analysis.