Clinical Exome Sequencing Study Of Growth Hormone Secreting Pituitary Tumors

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INTRODUCTION

The **tumor microenvironment (TME)** may provide a useful framework for understanding the heterogeneous behavior of **growth hormone (GH) secreting pituitary tumors**. Although the interest in TME in somatotropinomas has increased exponentially over the last few decades, there is limited elucidation of its mechanisms, particularly in relation to genes expression involved in its regulation.

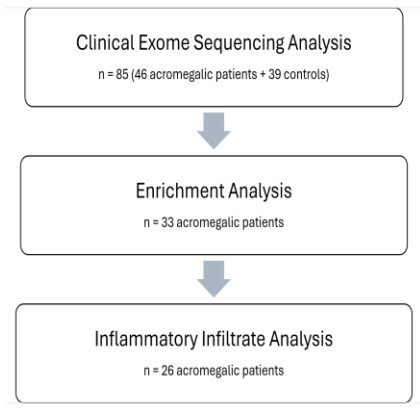
AIM

This study aims to investigate germline genetic variants in patients with acromegaly through **clinical exome sequencing (CES) analysis** and to explore a possible correlation with molecular and histological characteristics of GH-PitNETs, including TME immune cells.

METHODS

A retrospective, observational, single centre study was conducted. 85 subjects, of which **46 patients diagnosed with acromegaly and 39 controls**, were included in this study. After DNA extraction, CES was performed and genomic alterations were detected, classified and filtered using a dedicated bioinformatics pipeline. We excluded benign and probably benign variants according to the ACMG criteria, and variants with a minor allele frequency of more than 0.05 in the international databases. The remaining variants were subjected to the ENSEMBL Impact classification. The Metascape database was employed to perform pathway enrichment analyses, based on Gene Ontology biological process and KEGG Pathway for the targets.

Figure 1: *Panel representing the selection process of patients with acromegaly and controls.*



RESULTS

In our cohort, CES analysis identified **5759 unique variants** in patients with GH-secreting pituitary tumors. No predominant variants were identified in our cohort of somatotropinomas, but **a missense variant of uncertain significance (VUS) in the KCNJ12 gene (c.1289 A>G, p.Glu430Gly, rs5021699)** was found to be more frequent in patients with **invasive tumor (P-value = 0.017)** and in those with a **higher Ki-67% (P-value = 0.033)**, suggesting an increased proliferation of tumor cells in patients with this genetic alteration.

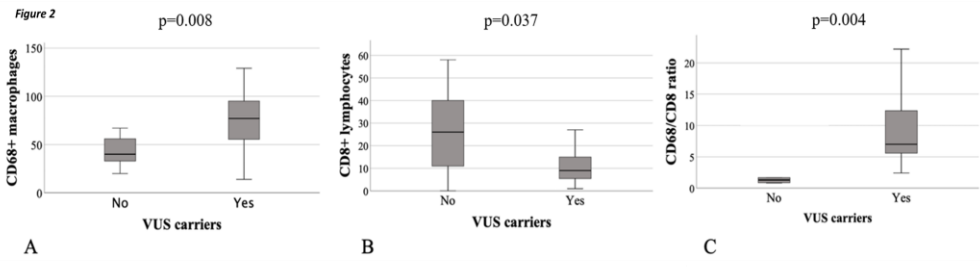
Moreover, 33 patients with acromegaly (72% of our cohort) showed the presence of at least one pathogenic variant in at least one of the following genes: **FANCD2, SPTA1, TYRO3, and ZNF335**. The enrichment pathway analysis of mutated genes was performed and showed that these genes were included in the same genetic pathway called **"regulation of lymphocyte activation"** (GO:0051249).

Inflammatory infiltrate was analyzed in histological samples in 26 patients. A significantly **higher number of CD68+ macrophages (P-value = 0.008), a lower number of CD8+ T lymphocytes (P-value = 0.037)** and **a higher CD68+ macrophages/ CD8+ T-lymphocytes ratio (P-value = 0.004)** were observed in patients with pathogenic variants of genes of "regulation of lymphocyte activation" pathway.

Table 1: *A missense variant of uncertain significance (VUS) in the KCNJ12 gene was found to be significantly more frequent in patients with invasive tumor and in those with higher Ki-67%.*

Gene	Variant (RefSeq)	dbSNP	Clinical Features		Carriers	Wild Type	Total	P-value
KCNJ12	c.1289A>G (NM_021012.5)	rs5021699	**Invasiveness**	yes	24	4	46	**0.017***
				no	9	9		
KCNJ12	c.1289A>G (NM_021012.5)	rs5021699	**Ki-67 percentage**	High	20	4	34	**0.034***
				Low	4	6		

Figure 2A: *Box-plot representing the number of CD68+ macrophages infiltering somatotropinomas in patients carriers of VUS of "regulation of lymphocyte activation" genes.*
Figure 2B: *Box-plot representing the number of CD8+ T-lymphocytes infiltering somatotropinomas in patients carriers of VUS of "regulation of lymphocyte activation" genes.*
Figure 2C: *Box-plot representing the CD68+/CD8+ ratio in somatotropinomas in patients carriers of VUS of "regulation of lymphocyte activation" genes.*



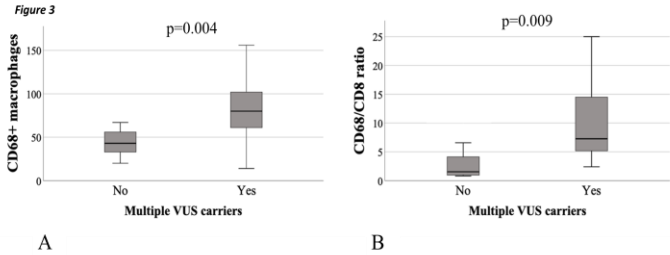
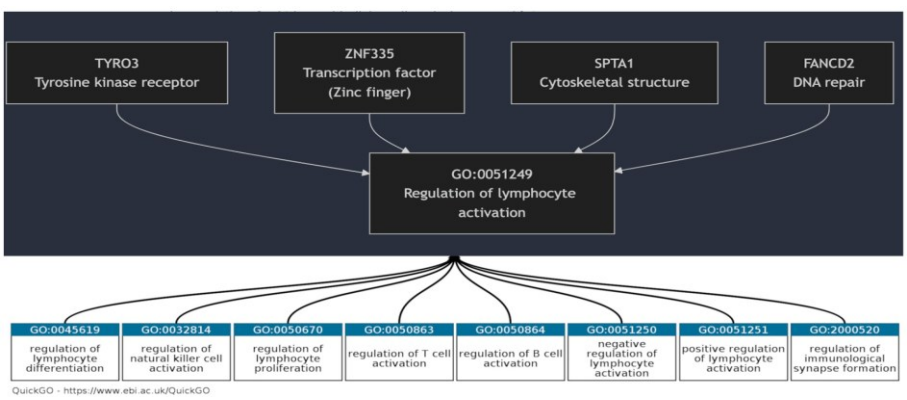


Figure 3A: *Box-plot representing the number of CD68+ macrophages infiltering somatotropinomas in patients carriers of multiple VUS of "regulation of lymphocyte activation" genes.*
Figure 3B: *Box-plot representing the CD68+/CD8+ ratio in somatotropinomas in patients carriers of multiple VUS of "regulation of lymphocyte activation" genes.*

Figure 3: *Overview of the functional associations between four candidate genes and the biological processes defined by GO:0051249 ("Regulation of lymphocyte activation"). The upper panel displays the network of genes (TYRO3, ZNF335, SPTA1, FANCD2) identified through STRING analysis, while the lower panel illustrates the corresponding GO terms related to lymphocyte activation. This integrated representation highlights the potential regulatory role of these genes in modulating immune cell activation.*



CONCLUSIONS

This study provides, for the first time, a comprehensive analysis by CES of germline variants in a cohort of GH-secreting pituitary tumors and offers new insights into the genetic basis of the TME in somatotropinomas, suggesting that genetics may influence immune cells infiltration in acromegaly.

CONTACT INFORMATION

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