POS0354 IDENTIFICATION OF SOMATIC MUTATIONS IN PATIENTS WITH ANCA-ASSOCIATED VASCULITIS

Keywords: Vasculitis, Genetics/Epigenetics

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Background: Growing evidence reveals a pathological role of somatic mutations in various autoimmune diseases, such as the mutation in *UBA1* in VEXAS syndrome, *CARD11* and *KLHL6* in cryoglobulinemic vasculitis, or *STAT3* in Felty's syndrome[1]. Somatic mutations might also be involved in the pathogenesis of ANCA-associated vasculitis (AAV), which typically manifests in middle-aged and elderly individuals.
Objectives: We aimed to identify somatic mutations in patients with AAV.
Methods: We collected whole blood and obtained peripheral blood mononuclear cells (PBMCs) and neutrophils from patients with AAV in active-disease status (n=16), as well as from patients with other autoimmune diseases (n=8) as disease controls, and healthy subjects (n=10). In addition, we collected these specimens from 12 out of the 16 patients with AAV after remission induction. We performed RNA sequencing (RNA-seq) on the obtained cells and whole genome sequencing (WGS) on DNA extracted from the whole blood. Somatic mutations were detected by comparing the RNA and DNA sequences[2].
Results: After stringent quality control, we identified 108 somatic mutations across 16 patients in active-disease status. The mean coverage of RNA-Seq at the mutation site was 100.9 ± 367.8 ×, and that of WGS was 14.4 ± 4.3 ×, while the mean allele fraction was 22.9 ± 20.5%. One or more mutations were detected in each of the 15 (93.8%) patients. The median mutation count of each patient was 4.0, which was not significantly different from disease controls or samples after remission induction. We mapped one gene to each of the 108 mutations, resulting in 95 genes in total. Mutations for six of the 95 genes were observed in two or more patients, and two of them were related to the ubiquitin system. Of the 108 mutations, 37 were missense, and 20 were predicted to be deleterious (combined annotation-dependent depletion Phred score > 20). Among the 20 mutations, the *HIST2H2AC* mutation (NM_003517: p.L86P) in neutrophils was observed in two patients. To evaluate the functional outcome of the 20 mutations, we analysed the data on knocking out the corresponding genes using CRISPR in K562 cells[3]. The results showed that the expression of genes related to antigen presentation was increased in *HEATR1* and *RPL18* knockouts. When we followed up with 12 of the 16 patients after remission induction, 81.4% of the somatic mutations were no longer detected. Additionally, 91.7% of the deleterious mutations disappeared.
Conclusion: We found somatic mutations with potential pathological effects in some patients with AAV exhibiting active-disease status. Notably, the majority of these mutations were not detected after remission induction.
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POS0355 CLINICAL TRAIT-SPECIFIC GENETIC ANALYSIS IN BEHÇET'S DISEASE IDENTIFIES NOVEL LOCI ASSOCIATED WITH OCULAR AND NEUROLOGICAL INVOLVEMENT

Keywords: Behcet's disease, Genetics/Epigenetics

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Background: Behçet's disease is a complex inflammatory vasculitis with a broad spectrum of clinical manifestations.
Objectives: The purpose of this study was to investigate the genetics underlying specific clinical features of Behçet's disease in a group of patients with > 5 years of follow up.
Methods: A total of 436 patients with Behçet's disease from Turkey were studied. Genotyping was performed using the Infinium ImmunoArray-24 BeadChip. After imputation and quality control measures, logistic regressions adjusting for sex and the first five principal components were performed for each clinical trait using a case-case genetic analysis approach. A weighted genetic risk score was calculated for each clinical feature.
Results: Genetic association analyses of previously identified susceptibility loci in Behçet's disease revealed a genetic association between ocular lesions and *HLA-B/MICA* (rs116799036: OR=1.85, 95% CI=1.35-2.52, p-value=$1.1x10^{-4}$). The genetic risk score was significantly higher in Behçet's disease patients with ocular lesions compared with those without ocular involvement, and is explained by the genetic variation in the HLA region. New genetic loci predisposing to specific clinical features in Behçet's disease were suggested when genome-wide variants were evaluated. The most significant associations were observed in ocular involvement with *SLCO4A* (rs6062789: OR=0.41 (95% CI=0.30-0.58), p-value=$1.92x10^{-7}$), and neurological involvement with *DDX60L* (rs62334264: OR= 4.12 (95% CI 2.34 to 7.24), p-value = $8.85x10^{-7}$).
Conclusion: Our results emphasize the role of genetic factors in predisposing to specific clinical manifestations in Behçet's disease, and might shed additional light into disease heterogeneity, pathogenesis, and variability of Behçet's disease presentation across populations.
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POS0356 EVALUATION OF FC GAMMA RECEPTOR (FCΓR/ FCGR) & CYP GENETIC POLYMORPHISM AND ITS PHARMACOGENOMIC CORRELATION WITH RITUXIMAB AND CYCLOPHOSPHAMIDE RESPONSE IN ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODY ASSOCIATED VASCULITIS

Keywords: Remission, Vasculitis, Genetics/Epigenetics

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Background: The antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV) are three discrete entities – Granulomatosis with polyangiitis (GPA), Microscopic polyangiitis (MPA), and Eosinophilic granulomatosis with polyangiitis (EGPA). They present with a plethora of signs and symptoms resulting in significant burden of mortality and morbidity. There is huge lacuna in the knowledge of relationship between various genes affecting pharmacogenetics of AAV and its implications in context of therapeutic complications. We thus intend to evaluate genotypic polymorphism in FcγR family & CYP genes affecting pharmaco-biological response of Rituximab and Cyclophosphamide respectively in AAV patients undergoing induction treatment.
Objectives: To study the single nucleotide polymorphism (SNP) of the FcγR family (FcγRIIIA, FcγRIIA, FcγRIIB) in patients receiving Rituximab (RTX) and the SNPs of CYP2C19*2 (rs4244285), CYP2B6 (rs321137) polymorphisms in patients receiving Cyclophosphamide (CYC) using polymerase chain reaction. Additionally, to correlate the genetic polymorphism of AAV patients with Rituximab and Cyclophosphamide treatment response.
Methods: This study was a prospective cohort study in which AAV patients undergoing induction treatment were enrolled from Jan 2021 to Dec 2022 in Clinical Rheumatology Department at PGIMER Chandigarh, India. Functional SNPs for FcγR(FcγRIIb 695T>C, FcγRIIIa 559T>G and FcγRIIa 519G>A) and

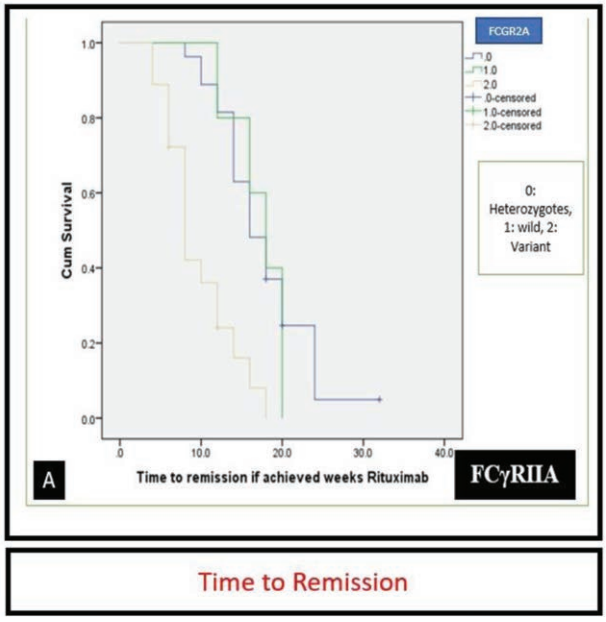
CYP enzymes (CYP2C19 681G>A, CYP2B6 1459C>T) were assesed by Sanger sequencing of PCR amplified genomic DNA. The end points were to detect associations between the tested SNPs and status of remission at six months and complete remission at end of the study.

Results: In this study we recruited ninty seven patients of AAV in Indian population. Table 1 summarises the baseline clinical characterisitics of the study population. FcγRIIA variant (v) allele frequency was more than wild allele (W) frequency in the study population. The time to achieve remission was significantly lower (mean:9.22 weeks) in FcγRIIA variant genotype polymorphism (FcγRIIa 519AA) in comparison to wild and heterozygotes variants (mean: 17.25 weeks) of FcγRIIA at site 131 (519G>A), as shown in Figure1. There were no significant difference observed in CYP polymorphism and cyclophosphamide response.

Table 1. Clinical and demographic characteristics of 97 AAV patients

Characteristic	N (%) or mean
Sex	
Male	38 (39%)
Female	59 (61%)
Male: Female Ratio	1:1.55
Mean age at diagnosis (years) ±SD	42.10 ± 15.57
Patient profile	
Newly diagnosed	31
Follow-up	66
AAV Subtypes	
GPA	82
MPA	8
EGPA	7
Signs and symptoms, n(%)	
Constitutional	76 (78.3%)
Sino-nasal	62 (63.9%)
Auditory	36 (37%)
Pulmonary	71 (73.2%)
Renal	52 (53.6%)
GIT	14 (14.4%)
CVS	12 (12.37%)
Skin	20 (20.6%)
CNS	23 (23.7%)
PNS	24 (24.7%)
Eyes	42 (43.2%)
Outcomes characteristics	
Number of patients achieving Remission (at 6 months of induction)	88
Number of patients achieving Complete Remission (at time of analysis)	40

Figure 1: Effect of SNP of FcγR2A at site 131 (519G>A); 0: heterozygotes(519 GA), 1: wild (519GG), 2: variant(519 AA)



Conclusion: This study is one of the first to evaluate the pharmacogenomic profile of AAV in Indian population. We observed highly significant association of SNPs of FcγRIIa 131 site variant/519AA genotype with early remission and complete remission after induction treatment with rituximab. This data could further help in tailored treatment of AAV patients and may also serve as a prognostic marker.

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POS0357

A NOVEL MUTATION IN ALPL GENE CAUSING ADULT AUTOSOMAL DOMINANT HYPOPHOSPHATASIA IN A FAMILY OF SOUTHERN SPAIN: PHENOTYPE CHARACTERIZATION

Keywords: Genetics/Epigenetics, Rare/orphan diseases, Bone diseases

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Background: Tissue non-specific alkaline phosphatase (TNSALP) is essential for bone and tooth extracellular matrix mineralization. The gene encoding TNSALP (ALPL) is located on the long arm of chromosome 2. More than 400 ALPL variants exist. Low enzymatic activity of serum alkaline phosphatase (ALP) due to gene mutations leads to hypophosphatasia (HPP), a heterogeneous clinical condition ranging from asymptomatic or mild adult forms to severe perinatal disease. Adult's HPP most typical features are stress fractures and early loss of dentition.

Objectives: To describe the clinical phenotype and laboratory features in a family suffering from HPP in which a new gene variant was detected.

Methods: A descriptive observational study of a family belonging to the Virgen de Valme Hospital area (Seville) was conducted through a primary identification of an index case with low level of ALP in whom a novel ALPL gene mutation was found. Members from three family generations were screened for low level of ALP to select patients meeting laboratory criteria for HPP. To assess family segregation, phenotype and clinical relevance of this mutation, genetic analysis of those with confirmed low ALP and a selection of members with normal ALP levels was done. Epidemiological, clinical, laboratory and genetic study variables were collected. A descriptive quantitative analysis of the data was performed.

Results: We studied 16 members corresponding to three generations of the same family suspected to have familial hypophosphatasia. The index case was a 57-year-old male with chronic pain, problems with dentition and previous history of fractures. ALP screening found 8/16 (50%) patients with low level. Genetic testing was performed for 7/16 patients and 3/16 with low and normal ALP levels, respectively. A familial carrier variant c.786A>G heterozygotic single-nucleotide polymorphism (SNP) in the ALPL gene was found in all patients with low ALP (Graph 1). Clinical and laboratory features for the seven positive carriers are shown in Table 1. Most fractures were due to non-minor trauma. Chronic arthralgia was the main articular complaint. All laboratory values were within normal range, ruling out secondary causes of HPP. Only one patient presented with high pyridoxal-5'-phosphate (PLP).

Conclusion: We present a new autosomal dominant inheritance mutation in the ALPL gene that causes HPP. Variable clinical expressivity is observed. Dentition problems and recurrent fractures predominate, associated with chronic pain. Early diagnosis and genetic counselling may be important, although further studies and a larger population are required to establish the definitive pathologic relevance of this variant.