

Genetic alterations and pathways in patients with Hereditary Angioedema of Unknown Cause (U-HAE)

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ABSTRACT

Objective: Hereditary angioedema (HAE) with normal C1 inhibitor (HAE-nC1-INH), is a genetically complex, rare disease and mutations in *F12*, *ANGPT1*, *PLG*, *MYOF* genes are found in some families with HAE-nC1-INH. However, often a specific mutation cannot be identified and this type is called as hereditary angioedema of unknown cause (U-HAE). Our aim was to identify putative causative genetic alterations and/or pathways by whole exome sequencing in patients with U-HAE.

Patients and Methods: Nine patients from 8 families between the ages of 3 to 63 years with U-HAE and 6 controls were enrolled for the study and whole exome sequencing were performed.

Results: No significant difference was found between the case and control group for the *a priori* suspected set of genes. Variants in the genes; *RAMP2*, *IL6*, *GP1BA*, *CIQBP* were significantly different between U-HAE and control group. Downstream functional analysis found that blood coagulation pathways were enriched in these genes.

Conclusion: Proteins that are not involved in contact pathways may also play a role in U-HAE. These variants need to be replicated in larger cohorts and studied at the functional level to verify our findings.

Keywords: Hereditary angioedema of unknown cause (U-HAE), Whole exome sequencing (WES), Genetic

1. INTRODUCTION

Hereditary angioedema (HAE) is a rare autosomal dominant disease that results in recurrent attacks of swelling and has a highly variable clinical course [1]. There are two well defined types of HAE: HAE due to C1 inhibitor deficiency (C1-INH) and HAE with normal C1-INH deficiency (HAE-nC1-INH). HAE due to C1-INH deficiency is further subdivided into type 1 (low plasma c1INH antigen) and type 2 (normal, but dysfunctional C1INH). Both HAE types are caused by mutations in *SERPING1* gene [2,3].

Hereditary angioedema with nC1-INH is a genetically complex, rare disease first described in 2000. Prevalence is estimated at 1:100.000. Mutations in *F12* gene are seen in approximately

30% of the patients and recently mutations in *ANGPT1*, *PLG* and *MYOF* were shown in some families with HAE-nC1-INH [4]. However, most of the time a specific mutation cannot be shown. There is a need for methods that can identify affected individuals with HAE-nC1-INH with unknown genetic cause (U-HAE) [5,1].

Functional genomics approaches attempt to discover, define and describe function of genes and interactions thereof, making use of the vast data generated by genomic and transcriptomic projects [6]. One initial step into this endeavor, is the discovery of causative genes, in particular for a disease or trait of interest

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via observational studies, where features (e.g. structural variants in the genome) are associated with the traits.

2. PATIENTS and METHODS

After ethics approval (Ankara University Ethics Committee, date: 08/27/2012 number: 13-419-12) and consent forms were taken, 9 patients between the ages of 3 to 63 years with U-HAE were enrolled in the study (Table I). 8 patients were from a Turkish decent and 1 was Irish-American. 2 patients were father and his adult daughter, and the rest belonged to unrelated families. 3 of the index cases were younger than 11 years of age. 6 controls were taken randomly from a sample of patients with congenital heart disease. All index cases presented with recurrent angioedema symptoms and met the criteria for HAE-nC1-INH as described by Cicardi et al [1].

WES analysis, Identification of variants and statistical procedures, bioinformatics analyses.

All patients (controls and cases) underwent to whole-exome sequencing (WES) analysis. The raw sequence files were quality filtered (average Phred score >30, minimum 50X depth of coverage) using FastQC. The resulting sequence files were mapped to human genome (hg19) using Bowtie2 [7] or Burrows-Wheeler Alignment (BWA) tool [8] and downstream analyses were performed using Sam tools [9]. The mapped reads were further used for variant detection using standard bioinformatics workflows of samtools and/or GATK [10]. All detected variants were listed and inspected for their PolyPhen [11] and SIFT [12] scores and further checked in COSMIC database [13]. The variations identified as “benign” were discarded and variations with outcomes as “possibly damaging” and “unknown” were kept.

Construction of Variant profiles per gene

To assess potential association between the U-HAE and structural variants, variant profile for each gene is constructed as follows: for each gene and each sample considered, a binary variable that represents the presence or absence of a structural variation (with suitable PolyPhen outcome) is generated. In doing so, all variations in the selected gene are aggregated. In case there are multiple variations in a gene, the binary variable is still taken as one. This yields variant profiles per gene for case and control groups. The resulting contingency table, taking cases and controls as well as number of variations and non-variations is tested using Fisher's exact test [14] for the null hypothesis that there is no difference in variation profile between the case and control group. $\alpha = 0.05$ is taken as the significance threshold.

Identification of potential candidate genes

Initial gene set, for which a variation profile is generated and tested for significance among groups is constructed based on reported genes for HAE variants and literature search. To expand and identify further potential genes, we used the initial gene set consisting of *F12*, *ACE*, *ADM* and *SERPINA1* as a starting point, and considered genes interacting at protein level (protein-protein interactions) to potentially associate with U-HAE case.

The protein-protein interactions are retrieved from STRING database [15].

Downstream Bioinformatics analyses

The final gene list containing the genes with statistically significant difference in their variation profile between the case and control is further functionally analyzed for their function and GO-terms using The Database for Annotation, Visualization and Integrated Discovery (DAVID) database [16,17] and amiGO tool [18].

3. RESULTS

Characteristics of the Patients

In this study, 9 patients from 8 families and 6 controls with different clinical profile were analyzed. Characteristics of the patients are shown in Table I.

Table I. Characteristics of patients with U-HAE

Patient	Sex	Age at diagnosis	Presenting Symptoms
1	M	33	Lip swelling
2	F	9	Lip and hands swelling
3	M	18	Lip and scrotum swelling
4	F	22	Tongue and lip swelling
5	F	34	Tongue swelling
6	F	7	Lip swelling
7	M	50	Tongue swelling, abdominal pain
8	F	19	Acute abdominal pain with free fluid collection
9	F	28	Hands swelling

Analysis of Variant profiles for F12, ACE, SERPINA1 and ADM

In the first round the initial seed gene list consisting of *F12*, *ACE*, *SERPINA1* and *ADM* are inspected for their variant profiles, different from case to control.

In an attempt to characterize correctly the HAE and making sure that these patients are U-HAE, an additional list of genes was also inspected for their variant profile. This list is composed of genes that are used to characterize the HAE in earlier works. The results, including the seed gene list as well as candidate genes, are given in Figure 1. Only high-quality variants were included in the analysis with possibly damaging and unknown variants based on their polyphen scores. The analysis resulted in no gene with a differential variant profile among case and control groups. Interestingly, no variation was observed for *SERPINA1* and *ADM* genes in the cohort and almost all individuals including control had variation for *F12* ($p = 0.476$) and *ACE* ($p = 0.999$). The lowest among those was the variant profile of *SERPINA1* gene which was only marginally significant ($p = 0.044$) (Figure 1).

Expanding the search space using interactome information

In searching for causative genes in this dataset, we expanded the initial gene set by including the genes whose protein products

were reported to be interacting with the initial set, based on STRING database and selected only the high-confidence pairs (minimum required interaction score > 0.9, Figure 2). The new gene list contained 29 additional genes, for which variation profiles were constructed, tested for significant difference between the case and control groups and found that indeed significant difference was found for this set (p = 0.027).

After seeing that not all genes in the expanded list were different in their variant profile, we focused on genes in the new list that would best differentiate (lowest p-value) the case and control groups, based on the variant profiles. To achieve this, each gene in the expanded list was ranked for their p-value and the final list of genes is given in Table II. Using these 4 genes, p-value was estimated to be 0.023.

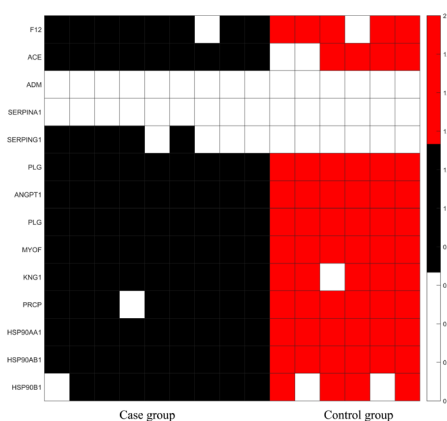


Figure 1. Variant profiles for possibly damaging and unknown variants for case (black) and control (red) groups. Colored spots present the presence of variant in a selected gene in the respective sample and white represents the absence. In this cohort, there are no possibly damaging and unknown variants for ADM and SERPINA1 genes.

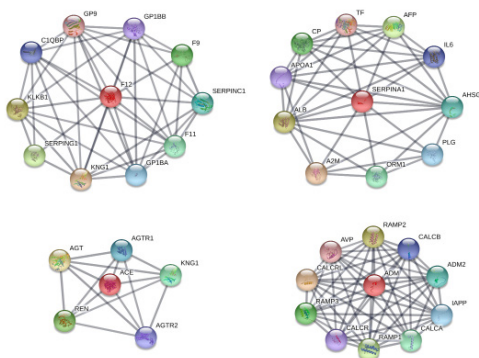
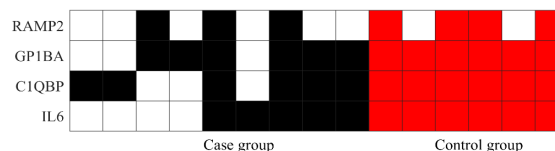


Figure 2. Searching for causative genes U-HAE. The expanded list of genes constructed by considering highest confidence interactions according to STRING database with the initial set of genes. Each network in the figure is centered around one of the genes in the initial set: F12, SERPINA1, ACE, ADM from left to right, top to bottom. The thickness of the lines connecting each protein couple indicates the strength of data support as provided by STRING database.

Table II. Final list of genes proposed in this study, based on statistical association of the variant profile with U-HAE diagnosis state and their variant profiles for case and control groups.

Gene symbol	Gene Name
RAMP2	Receptor Activity Modifying Protein 2
IL6	Interleukin 6
GP1BA	Glycoprotein Ib Platelet Subunit Alpha
C1QBP	Complement C1q Binding Protein



Downstream analysis of the proposed genes

The obtained set of genes were downstream analyzed to be inspected for their (common) function/pathway using GO Enrichment Analysis via amiGO tool. The GO terms overrepresented for the gene list in Table II is given in Table III.

Table III. GO terms overrepresented for the candidate genes in Table I.

GO biological process	FDR corrected p value
blood coagulation, intrinsic pathway (GO:0007597)	3.76E-02
blood coagulation, fibrin clot formation (GO:0072378)	5.54E-02
protein activation cascade (GO:0072376)	4.15E-02
blood coagulation (GO:0007596)	2.95E-02
coagulation (GO:0050817)	2.58E-02
hemostasis (GO:0007599)	2.33E-02
negative regulation of response to external stimulus (GO:0032102)	8.75E-03
regulation of response to external stimulus (GO:0032101)	3.48E-02

FDR: False Discovery Rate, GO: Gene Ontology

4. DISCUSSION

We propose a data-mined set of genes that statistically show variation among U-HAE cases and control groups. Prominent among this set of genes are RAMP2, IL6, GP1BA and C1QBP. RAMP2 can function as an adrenomedullin receptor (ADM) which is a vasoactive peptide and it has been shown that its level increases during C1-INH-HAE attacks together with other vasoactive peptides arginine vasopressin (AVP) and endothelin-1 (ET-1) to counter-balance the actions of excess bradykinin (BK) and terminate the attacks [19]. HAE-nl-C1INH may also be BK mediated as they show a favorable response to BK pathway-targeted medications [20]. The possible effects of RAMP2 in vasoactive peptides may reveal a novel pathophysiological aspect.

Concentrations of interleukin (IL)-1, IL-6, and transforming growth factor (TGF- β) were found to be significantly higher in HAE patients in remission compared with healthy controls. [21]. IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. IL-6 induces excess production of vascular endothelial growth factor (VEGF), leading to enhanced angiogenesis and increased vascular permeability which causes joint swelling and edema. VEGF also interacts with BK to mediate plasma leakage.

Glycoprotein Ibalpha (*GP1BA*) is a platelet surface membrane glycoprotein that functions as a receptor for von Willebrand factor. The binding of VWF to the GP Ib complex initiates signaling events within the platelet that lead to enhanced platelet activation, thrombosis, and hemostasis [22]. Mutations in this gene is associated with Bernard-Soulier syndrome and platelet type Von-Willebrand Disease. This glycoprotein may play a role in U-HAE by platelet activation, but this is another research topic.

Complement component 1q (*CIQBP*) subcomponent binding protein is a multifunctional protein involved in immune response, energy homeostasis of cells as a plasma membrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. It has a role in the mediation of the actions of pro-inflammatory agents, such as high molecular weight kininogen to produce further pro-inflammatory agents [23]. It can play as a mediator in U-HAE since it acts as a bridge between the complement and contact activation system.

.U-HAE is usually misdiagnosed or underdiagnosed due to rarity of the disease and lack of specific biomarkers. This results in mismanagement of this potentially life threatening disease.

Detection of causative genes will help in timely diagnosis of patients and identifying affected family members. It will also allow for understanding the pathophysiology of this disorder, which in turn will lead to better therapeutic options. In our study genes that statistically show variation among U-HAE cases and control group are *RAMP2*, *IL6*, *GP1BA* and *CIQBP*.

Our study contributes to understanding the potential genetic pathways in U-HAE. The next step would be to increase our sample size, do family segregation studies, to extend the work to GWAS or RNA-Seq studies to further our understanding via network analyses and functional (e.g., transcriptomic or low throughput) analyses to understand and confirm the disease modifying effects of these genetic variations.

Compliance with Ethical Standards

Ethical Approval: The study was approved by the Ankara University Ethics Committee (date: 08/27/2012 number: 13-419-12) and consent forms were taken.

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Conflict of interest: The authors declare that they have no conflict of interest to declare

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