Atypical hemolytic uremic syndrome targeted re-sequencing study in a South Italian cohort of patients

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OBJECTIVES

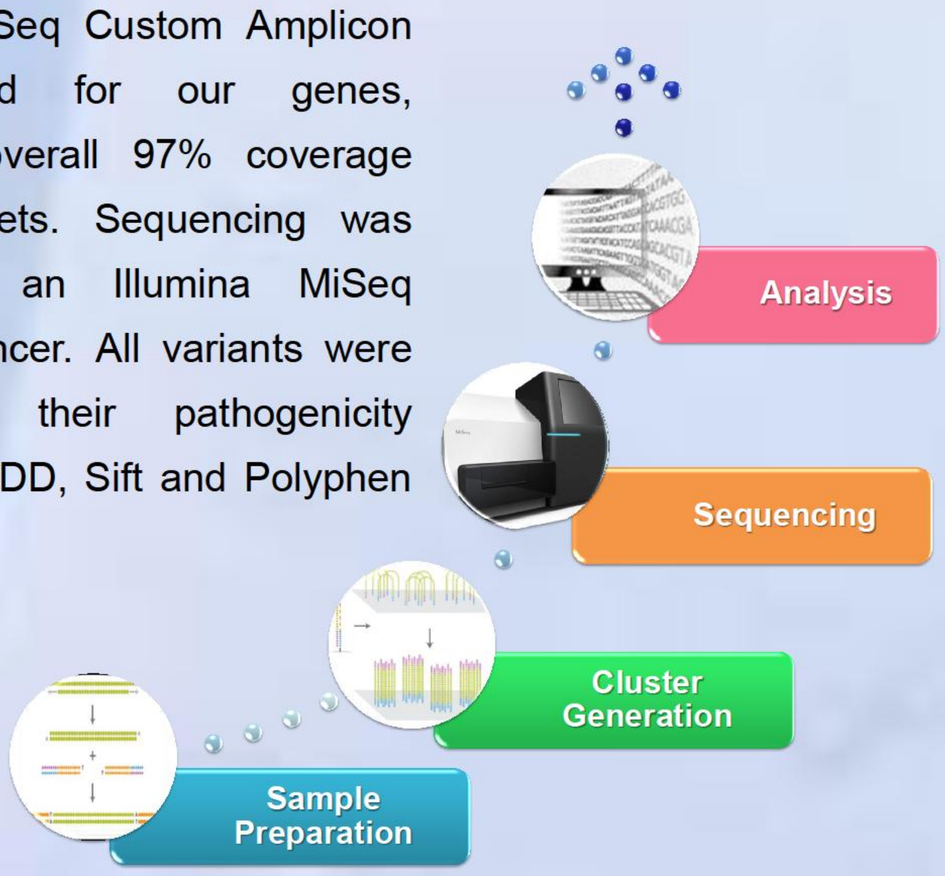
Atypical hemolytic uremic syndrome (aHUS) is a rare disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction. It is a multifactorial disease due to autoimmune or genetic factors leading to deregulated alternative complement pathway activation.
Until now abnormalities in twelve genes are associated to aHUS. However, some aspects of the genotype-phenotype correlations are still unclear.

We performed targeted massively parallel re-sequencing of 12 known and 1 candidate gene in all patients to investigate the role of the genotype in determining the phenotypic spectrum of aHUS and identify novel mutations. The overlapping with other diseases such as Dense Deposits Disease (DDD), C3 glomerulopathy and Age-related Macular Degeneration (AMD), all sharing the alternative complement pathway deregulation, was also investigated.

METHODS

We reconstructed the entire clinical records of the Apulian Region in Italy, enrolling twenty patients and two relatives.

Target enrichment was performed using an Illumina TruSeq Custom Amplicon panel designed for our genes, achieving an overall 97% coverage across all targets. Sequencing was performed on an Illumina MiSeq Desktop Sequencer. All variants were evaluated for their pathogenicity according to CADD, Sift and Polyphen algorithms.



RESULTS

NGS on 20 Patients + 2 relatives

Tested on 7 PATIENTS partially screened with Sanger technology

34 DIFFERENT VARIANTS

Overall, 92.3% of the targets (13 genes) was sequenced at >25X coverage, required for accurate variant calling.
Combination of variants might explain the different phenotypic nuances of our patients, as well as, in some cases, the overlapping of other Complement-related diseases.

SCREENED SUBJECTS	
DISEASE ONSET	
Children (≤18 y)	6
Adults (>18 y)	14
Male/Female	11/9
Recurrences	4
UNDERLYING/ACCOMPANYING CONDITIONS	
Diarrhea/gastroenteritis	7
Upper respiratory tract infections	2
Central nervous system involvement	3
TRIGGERS	
Idiopathic	6
SECONDARY	
Drugs	3
Post-transplant	1
Post-surgery	1
Pregnancy-related HUS	4
Contraceptive pill	1
Glomerulopathy/urinary anomalies	4
Systemic disease	4
OUTCOME	
Complete recovery	2
Chronic kidney disease	6
End Stage Renal Failure	11
Death	1

CONCLUSIONS

Our data suggest that (1) our candidate gene should be added to the list of genes to be screened for aHUS, (2) the overlapping with other complement-related diseases is considerable, often resulting in complex phenotypes, (3) mutations should therefore always be analyzed in combination, never singularly, (4) the high-throughput strategy is the only feasible in this scenario.
This approach would lead to a precise molecular assessment of aHUS patients which is an essential requirement in the management and treatment of the disease, in order to optimize the decision-making process towards them, especially in the perspective of renal transplant.

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