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101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Characterizing Hereditary Hemolytic Anemias in a Hispanic Cohort Using Next Generation Sequencing

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Background:
Hereditary hemolytic anemias (HHA) are a heterogeneous group of nonimmune disorders characterized by increased red blood cell (RBC) destruction due to abnormalities of hemoglobin stability, defects of RBC metabolism, and disorders of RBC hydration. Although uncommon, they comprise important causes of anemia in children and adults by causing chronic or episodic hemolysis. Clinical, laboratory, and genetic heterogeneity characterize this group of disorders and make recognition challenging. The advent of next generation sequencing (NGS) has made accurate genetic diagnoses attainable, with an increasing number of studies reporting clinical utility in HHA. For patients with suspected HHA, multigene testing is available incorporating an NGS panel associated genes. However, there is limited data on the use of multigene NGS to characterize HHA in the Hispanic population. Herein, we describe results from NGS testing of a pilot cohort referred for HHA investigation to hematology clinics along the US/Mexico border.

Methods:
Pediatric and adult hematology clinics at Texas Tech University in El Paso, TX collaborated for testing on HHA referral and clinical case review. The diagnostic panel (AnemiaID/PerkinElmer/Revvity®) included 51 genes encoding RBC cytoskeletal proteins, membrane transporter, RBC enzymes, and certain bilirubin metabolism genes. The complete coding region, splice site junctions, and, where appropriate, deep intronic or regulatory regions were covered. Targeted gene capture and library construction for NGS were performed using a Whole Blood and Saliva kit and 150 base pair paired-end sequencing was done on Illumina® NGS systems at target average coverage of 80×. NGS output data were summarized descriptively for all patients and clinically correlated for diagnosis.

Results:
In this pilot cohort, 11 patients were tested during September 2022 to June 2023, age ranging 7-75 years, with females accounting for 64% of the group. Findings are reported in the Table below; Pathogenic variants leading to a definitive diagnosis were identified in 8 of 11 cases (73%). The most frequently mutated genes were *SPTA1* followed by *PIEZO1*, *SPTB*, *RHAG*, *GSR*, *SLC4A1* and *TPI1*. Approximately 58% of the mutations were reported as novel. Diagnoses included, most commonly, Hereditary Spherocytosis followed by Hereditary Pyropoikilocytosis and Congenital Dyserythropoietic Anemia. Compound heterozygosity leading to complex interactions was noted in 4 cases (36%), involving *SPTA1* low expression alleles *in trans* (alpha-LELY and alpha-LEPRA) in 2 cases.

Conclusion:
Hereditary hemolytic anemia in the Hispanic population is characterized by complex molecular interactions and a high prevalence of novel mutations in RBC cytoskeleton/enzyme genes. NGS has clinical utility and should be considered for accurate diagnosis. Further research into the molecular basis and genetic spectrum of HHA in the Hispanic population is needed.

Disclosures No relevant conflicts of interest to declare.

Table 1: Results of next generation sequencing to evaluate hereditary hemolytic anemias in a Hispanic cohort

Age	Sex	Peripheral blood smear and examination	Next- generation sequencing	*In silico* meta-analysis	Clinical diagnosis
7	F	Spherocytosis, Splenomegaly	*SPTA1* missense mutation;	Pathogenic;	Hereditary spherocytosis
			PIEZO1 missense mutation	VUS	
9	M	Spherocytosis, Anisocytosis, Splenomegaly	*SPTA1* α-LEPRA allele;	Pathogenic;	Hereditary spherocytosis
			SPTA1 duplication (null);	Pathogenic;	
			PIEZO1 missense mutation;	Likely pathogenic;	
			PIEZO1 missense mutation;	VUS	
			TPI1 missense mutation	VUS;	
23	F	Macrocytosis, Anisocytosis	No reportable sequence / copy number variants identified	-	Hemolytic Anemia, unspecified
26	M	Spherocytosis, Splenomegaly	*SEC23B* Exon 2 point mutation;	Pathogenic;	Congenital Dyserythropoietic Anemia
			SEC23B Exon 10 point mutation	Pathogenic	
27	F	Elliptocytosis, Schistocytosis, Anisocytosis	*SPTB* Exon 23 point mutation	Benign	Hemolytic Anemia, unspecified
27	F	Microcytosis, Anisopoikilocytosis	*UGT1A1* promoter mutation	Pathogenic	Gilbert Syndrome
29	F	Schistocytosis, Anisopoikilocytosis, Thrombocytopenia, Ovalocytosis	*UGT1A1* promoter mutation	Pathogenic	Gilbert syndrome
39	F	Anisopoikilocytosis	No reportable sequence / copy number variants identified	-	Hemolytic anemia, unspecified
45	M	Poikilocytosis, Hepatosplenomegaly	*SPTA1* missense mutation;	Pathogenic;	Hereditary Pyropoikilocytosis
			SPTA1 α-LELY allele;	Pathogenic;	
			PIEZO1 indel mutation;	Pathogenic;	
			RHAG missense	VUS	
52	M	Spherocytosis, Hepatosplenomegaly Thrombocytopenia	*SPTA1* missense mutation	Inconclusive	Hereditary spherocytosis
75	F	Anisopoikilocytosis, Ovalocytosis, Thrombocytopenia, Splenomegaly	*GSR* Exon 4 missense mutation;	Inconclusive;	Hereditary Spherocytosis
			SLC4A1 missense mutation	Pathogenic	

Figure 1

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