Paired Exome Sequencing Reveals Recurrent Germline Variants in Patients with Idiopathic Erythrocytosis

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Mario Mauri, Elena Elli, Deborah D'Aliberti, Ilaria Crespiatico, Miriam Nava, Luca Massimino, Geeta G. Sharma, Diletta Fontana, Sara Redaelli, Silvia Bombelli, Sara Pelucchi, Roberto Perego, Alberto Piperno, Carlo Gambacorti-Passerini, Rocco Piazza

Objectives

Erythrocytosis is a condition characterized by an increase in the red cell mass and hemoglobin levels in presence of an intrinsic defect of the erythroid cell (primary erythrocytosis, PE), or related to extrinsic causes unrelated to erythrocyte development (secondary erythrocytosis) and both condition can be further classified as either congenital or acquired. The most common cause of acquired PE is Polycythemia Vera, which is caused by somatic, activating mutations of JAK2. Little is known about Polycythemia cases where no JAK2 variants can be detected and no other causes can be identified. This condition has been defined as idiopathic erythrocytosis (IE).

Here we analyzed a group of 32 carefully selected adult IE patients by paired blood/buccal-DNA exome-sequencing to identify recurrent somatic mutations responsible for the onset of the disease.

Methods

Exome sequencing was performed using Illumina paired-end sequencing combined with SureSelect-V6 enrichment assays. Somatic and germline variant calling was performed with dedicated bioinformatic pipelines. In vitro validation was performed on K562 cell-line models stably expressing selected germline mutations.

Results

Strikingly, no evidence of somatic variants could be found in the entire group. This finding opened-up the possibility of an underlying genetic disorder responsible for the abnormal production of erythrocytes, hence functionally connected with congenital erythrocytosis but characterized by adult onset and limited penetrance.

To perform a combined analysis of the genetic events occurring in patients sharing a similar phenotype, we developed GeneticLens, a bioinformatic tool dedicated to the extraction of common germline variant data

from patients' groups. This approach allowed us to reveal significant enrichment ($p < 0.05$; FDR < 0.1) in ontologies involved in erythrocyte development such as JAK-STAT signaling, Iron metabolism and Hypoxia pathways and to identify germline variants likely implicated in erythrocyte development: JAK2-N1108S, JAK2-G571S, JAK2-I982L, JAK3-V722I, HFE-C282Y, HFE-H63D, HIF1A-P582S, EPAS1-P540L and EPAS1-F374Y.
K562 cell-line models of these germline mutations support the hypothesis of their involvement in erythrocyte proliferation acting as regulators of key pathways modulating STAT5 signaling axis, EPO production and likely iron bioavailability.

Conclusions

Taken globally our data suggest that a large fraction of JAK2-negative IE that are currently considered as bona fide somatic diseases are instead germline disorders. We expect this to have profound impact on how we treat these disorders paving the way to clinical trials focused on the definition of a proper treatment and, at the same time, it also suggests the opportunity of supporting these patients with dedicated genetic counselling.