Variant discovery in patients with Mendelian vascular anomalies by next-generation sequencing and their use in patient clinical management

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ABSTRACT

Objective: An accurate "molecular" diagnosis and classification of similar but distinct diseases is sometime challenging but often crucial for the definition of the appropriate patient medical management and treatment as well as for genetic counseling and risk assessment in families. The advent of next-generation sequencing (NGS), which analysed all known disease-associated genes in parallel in a cost- and time-effective manner, eased this process of disease definition and also for vascular anomalies that are a heterogeneous group of vascular tumors and congenital circulatory malformations and often characterized by overlapping phenotypes.

Methods: We designed a NGS-based screening of the 25 currently most prevalent genes identified in patients with vascular anomalies with Mendelian inheritance and applied this panel to study the DNA of 150 patients affected with vascular anomalies for autosomal recessive and autosomal dominant variants and to analyse the paired blood and DNA from intralesional biopsy specimens in 17 patients for somatic unbalance. Results were confirmed with Sanger sequencing.

Results: We identified 14 pathogenic variants in 13 of 150 patients. Eight variants were previously reported as a disease-causing variant, and six were new. In 55 additional probands we detected 75 variants with unknown significance. Moreover, a previously reported somatic variant was detected in five of 17 available tissue biopsy specimens.

Conclusions: Our results show that many genes can cause a wide variety of syndromic and nonsyndromic disorders, confirming that genetic testing by NGS is the approach of choice to diagnose heritable vascular anomalies, especially, but not only, when an intralesional biopsy specimen is available. The identification of the causative genes and the possibility of tracing somatic variants in tissues provide important information about etiology, patient clinical management, and treatment, and it could highlight otherwise unsuspected clinical situations. (J Vasc Surg 2018;67:922-32.)

Clinical Relevance: The prompt and correct identification of the causative gene variant in those uncertain phenotype or complex cases of patients affected by vascular anomalies is of inestimable value in order to provide the appropriate clinical management, monitoring, and treatment of patients. Genetic testing by next-generation sequencing of blood DNA or tissue DNA could be fundamental in helping clinicians determine the right disease and take the appropriate therapeutic decision. The identification of variants could provide prognostic or therapeutic information, directing a personalized patient care with development of specific small-molecule therapies, with the aim of increasing efficacy of traditional therapeutic methods.

Vascular anomalies with Mendelian inheritance are a heterogeneous group of circulatory alterations, charac terized by morphologic-structural or functional defects, or both, of varied nature, severity, and extent.[1,2] They comprise vascular tumors (hemangiomas) and vascular malformations. Hemangiomas are benign, highly proliferative lesions involving aberrant localized growth of capillary endothelium that grows during the first year of age and then spontaneously regress over time. Most hemangiomas occur sporadically, but some families with autosomal dominant inheritance have been reported.[3]

Vascular malformations are rare and affect ~0.3% of the population.[4] They are subdivided depending on the type(s) of vessel(s) affected.[5] Most are sporadic (ie, without family history), but familial cases exist transmitted as an autosomal recessive or dominant trait. Sporadic forms usually present with a single lesion, whereas more lesions are observed in familial cases. In addition, evidence for a few genes is also accumulating to support a paradominant model of inheritance in which development of lesions depends on the combination of a germline hereditary variants and a somatic second-hit. Somatic variants have been identified in venous, cerebral cavernous, and glomuvenous malformations.[3]

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For some lesions, the clinical features are often characteristic, but in some cases there may be atypical clinical or radiologic findings, and a definitive diagnosis may be difficult, requiring histologic evaluation of tissue samples.[6] Incorrect nomenclature and misdiagnoses are indeed frequently experienced by patients with vascular anomalies because they may present overlapping phenotypes and can occur in association with other symptoms (ie, in syndromes) or with high variability and extension. In addition, despite being present since birth, the anomalies do not immediately become evident; thus, they are often inappropriately evaluated and managed.

During the last 10 years, major advances have been made in identifying the genetic bases of vascular anomalies, shedding light on the fact that variants in different genes could give rise to the same malformation and that variants in the same gene can originate in different phenotype. A more accurate "molecular" diagnosis, being available thanks to the advances obtained in next-generation sequencing (NGS) techniques, has allowed for a better and more rapid understanding of the deregulated downstream pathways that could be targeted for therapy and to defining better recommendations for patient monitoring to early identify risks of health problems.

In addition, the availability of a genetic test can help in the classification of those complex clinical cases or in the evaluation of a presymptomatic phenotype and can give people more information for making decisions about their health and their family's health. Therefore, in this study we aimed to analyze in parallel all of the genes known to be involved in vascular anomalies with Mendelian inheritance in a large cohort of 150 Italian patients affected by vascular malformation or hemangiomas and enrolled in a single hospital. Genomic DNA was analyzed by a NGS using a Illumina (San Diego, Calif) custom-made oligonucleotide probe library to study a panel of 25 genes. In addition, we sequenced 17 intralesional biopsy specimens to test for the presence of somatic variants, because accumulating evidence supports the notion that the analysis of tissue samples may provide prognostic or therapeutic information, directing a personalized patient care with the development of specific small-molecule therapies.[7,8]

ARTICLE HIGHLIGHTS

- **Type of Research:** Single center prospective cohort study
- **Take Home Message:** DNA of 150 patients with vascular anomalies was sequenced by next generation sequencing identifying 89 variants. DNA from biopsy specimens identified the pathogenic variant in five of 17 patients.
- **Recommendation:** Genetic variants can be determined in a number of patients with congenital vascular lesions, and could provide prognostic or therapeutic information, directing a personalized patient care with the aim of increasing efficacy of traditional treatments.

METHODS

Study patients. This study enrolled 150 patients after clinical assessment of congenital vascular malformations or hemangiomas conducted at the Clinical Institute Humanitas, Castellanza (VA), Italy. Enrollment criteria were three of the following: (1) family history with Mendelian inheritance, (2) neonatal or congenital appearance of lesions, (3) presence of vascular anomalies with exclusion of those induced by teratogen of chemical, physical, or biologic origin (ie, those patients presenting vascular anomalies that could be attributed, as assessed during anamnesis, to treatment with a teratogenic drug were excluded from the study), (4) whenever diagnostic criteria for each disease (as defined by Genereview or Orphanet) were met. The study excluded patients affected by lymphedema.

All patients received genetic counselling to explain the risks and benefits of genetic testing, signed a written informed consent, including the authorization to use anonymized genetic results for research and publication, and were asked to provide a blood sample. For 17 patients, one intralesional biopsy specimen was available in addition to their blood DNA for screening.

Sample processing. Genomic DNA from blood samples was extracted following standard procedures using the MagPurix Blood DNA Extraction Kit (Zinexts Life Science, New Taipei City, Taiwan) and was extracted from biopsy samples using the MagPurix Tissue DNA Extraction Kit.

Custom panel design. A custom-made oligonucleotide probe library was designed to capture all coding exons and flanking exon/intron boundaries (±15 bp) of 114 genes known to be associated with a large group of cardiovascular and lymphatic diseases from the literature or databases (Human Gene Mutation Database Professional [Qiagen, Redwood City, Calif], Online Mendelian Inheritance in Man [OMIM; Johns Hopkins University, Baltimore, Md], Orphanet [Paris, France], National Center for Biotechnology Information GeneReviews, National Center for Biotechnology Information PubMed [Bethesda, Md], and specific databases). The 25 genes known to be involved in vascular malformation (Table I) were included in the analyzed panel. The genomic coordinates of the probes are provided in Supplementary Table I (online only). The DNA probe set, complementary to the target regions (GRCh37/hg19), was designed using the specific online tool, Illumina DesignStudio (Nextera Rapid Capture Custom Assay Technology; http://designstudio.illumina.com/Home/SelectAssay/), and was