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Inherited Thrombocytopenias: Combining High-Throughput Sequencing With Other Relevant Data

Kanjaksha Ghosh, MD, FACP, FRCPath[1], Parizad Patel, MSc[1], Kanchan Mishra, MSc PhD[1], and Kinjalka Ghosh, MD, DNB, MNAMS[2]

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We read with interest the article by Wang et al recently published in *Clinical and Applied Thrombosis/Hemostasis*.[1] Here, the authors have evaluated the role of high-throughput sequencing in 43 apparently familial cases of thrombocytopenia using Drachman[2] review algorithm.

Combining clinical and phenotype and next-generation sequencing (NGS) data, only 15 of 43 cases of inherited platelet disorder, that is about 35% of the cases, could be given clear-cut molecular diagnosis. The authors have taken lot of pains to design and execute their study and tried to standardize the technique taking care of all possible sources of error. Considering the fact that only 8.7%[3] of the patients with possible inherited symptomatic disorders of platelet function eventually gets molecular diagnosis, the results should be considered a quantum jump in the field. The results obtained by the present authors are similar or even better than those described from various European consortiums on platelet disorders.[4-6]

When we closely look at the clinical data of the article under discussion, only 2 of 43 patients had syndromic form of the disease in the form of hearing loss and renal disease, respectively. It is generally believed that when one deals with severe bleeding with congenital platelet defects, clear-cut diagnosis even on molecular pathology is expected in most of the cases. In milder cases of bleeding with inherited disorders of platelet function or even when no bleeding or family history is available, getting a molecular diagnosis becomes difficult. Molecular diagnosis of inherited platelet disorder have progressed from polymerase chain reaction- single strand conformation polymorphism (PCR-SSCP) polymerase chain reaction - conformation sensitive gel electrophoresis (PCR-CSGE) followed by Sanger sequencing and then in the present era beginning with DNA, complementary DNA, and different types of microarray to whole-genome sequencing, to whole-exome sequencing using various NGS platforms.

However, NGS-based data throws lots of challenges in sequence interpretation. Our present software and statistics associated with the NGS data are lagging far behind the amount of information generated through NGS. Moreover, NGS technology is poor in detecting complex structural rearrangements, inversions and large deletion of genes[4] as also repeated mutations and copy number variations.[5] As many of the platelet proteins are adhesion molecules with many repeat nucleotide sequences, chances of such kind of pathologies in platelet genome are high.

Hence, alternative technologies such as multiple ligation-dependent probe amplification or similar such techniques in addition to Sanger sequencing needs to complement NGS techniques. Depth of reading of genes by NGS techniques widely vary and for some genes it can be as low as 70% or lower making the data set incomplete with respect to some of the genes.

Although the American College of Medical Genetics is developing various algorithms to filter out population noise from real genetic changes causing the disease phenotype, we are still far away from the ideal situation. In the present study, 32 (74%) of 43 cases had no family history and many of them had thrombocytopenia or minor bleeding. We don't have the data on mean platelet volume (MPV) and associated changes in complete blood counts and morphological changes in platelets and other formed elements of blood associated with studied platelet defects in many of the patients discussed here. These data are useful as they algebraically summate the results of different genetic changes into meaningful phenotypic variables.

[1] Surat Raktadan Kendra & Research Centre, Surat, Gujarat, India
[2] Department of Clinical Biochemistry, Tata Cancer Hospital, Parel, Mumbai, India

Corresponding Author:
Kanjaksha Ghosh, Surat Raktadan Kendra & Research Centre, Udhna Magdalla Road, Surat 395002, Gujrat, India.
Email: kanjakshaghosh@hotmail.com

It is true that in the present series, all the patients were vetted by hematologists and relevant experts, yet inclusion of complete blood counts, MPV data, and the type of machine in which these data were generated would have given important insight about patients on whom no mutations or likely pathogenic changes were found.

Platelets develop[6] through early and late megakaryopoiesis, proplatelet formation, development of storage granules, and so on. During various steps of development, transcription factor genes, growth factor genes e.g thrombopoietin,/megakaryopoetin ligand (TPO/MPL), genes involved in granule biogenesis and trafficking, cytoskeleton-related genes including glycoprotein, cyclic GMP coupled receptors (GP, GPCR), and other genes take part. As defects at each of the steps may produce a broad range of changes in syndromic and nonsyndromic platelet disorders in the form of morphological changes in the megakaryocytes, platelets, and sometimes in other cellular blood elements, noting them carefully should improve the meaning and interpretation of molecular variation in cognate genes.

Genotype–phenotype correlation is not an exact science particularly so when lots of epistatic–environmental interaction in production of platelets, biogenesis of granules, and cytoskeletons and its phenotype are unknown. The problem become more pressing for the clinician if a patient with a platelet disorder has mutation in Runx or Etv 6 and she/he has to be counseled for regular follow-up for possible future risk of leukemia development. There are other mutations in syndromic form of the disease while initial presentation may only be thrombocytopenia and or platelet dysfunction, but in future, such patient may develop other organ dysfunction such as liver failure, kidney disease, or pulmonary fibrosis. The clinical science of counseling has to develop properly to accommodate such uncertainties without unnecessarily worrying the patient and their family.[5]

In the present study of authors' series, 11 patients were wrongly diagnosed and treated as immune thrombocytopenic purpura (ITP). Hence, they may have been harmed by the treatment. It will be useful to learn whether some of them did undergo splenectomy too. Antiplatelet antibodies may also be present with hereditary platelet disorders such as Glanzmann thrombasthenia; and in ITP patients, also, quite a large number develops antiplatelet antibodies to glycoprotein antigens of platelets. Hence, unless properly investigated, confusions are likely.[7]

When one comes to population screening for rather asymptomatic or mildly symptomatic patients based on machine platelet count showing thrombocytopenia with high or low MPV, challenges in genetic diagnosis becomes formidable.[8] This kind of macrocytic thrombocytopenia is quite common in many populations[8,9] and NGS could substantially be of help to elucidate some of these cases. However, our studies on Bengal macrothrombocytopenia studied through subtractive RNA hybridization had shown more than 20 differently expressed genes and they appear to be very similar to what NGS data are showing for inherited platelet disorders (IPD).[10]

Finally, the present study may form the framework for further studies of IPD both symptomatic and asymptomatic in Asian population. Asian population is extremely heterogenous, yet because of endogamy in large number of such populations, certain kinds of genetic changes in IPD may be more common and fixed in one population than others. For example, Bernard-Soulier syndrome was reported to be quite common in Kerala, state of India, compared to other Indian states.[11] It may so happen that we will come up with few geographically restricted mutations, which can describe majority of IPD in that particular locality similar to what has been described for β thalassemia major.[12]

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ORCID iD

Kanjaksha Ghosh https://orcid.org/0000-0002-0645-6565

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