# Not in the genotype: can unexplained hemophilia A result from "micro(RNA) management"?

Micro RNAs (miRNAs), first discovered in 1993, are noncoding RNA sequences ranging between 17 and 25 bp in length. They regulate genes posttranscriptionally, primarily acting to fine-tune gene expression, although recent research suggests that miRNAs may also serve as master regulators depending on the context, as in neural development and function,[1] intracellular signaling pathways,[2] glomerular function and homeostasis,[3] and so on. The most recent update of the miRBase online database (http://mirbase.org/, updated March 12, 2018) lists 1917 annotated hairpin precursors and 2654 mature sequences of miRNAs found in the human genome[4] that are believed to regulate over 60% of human protein coding genes. miRNA biogenesis follows multiple well-coordinated steps involving two RNaseIII enzymes: Drosha, which initiates processing of the long primary transcript into pre-miRNA in the nucleus, and Dicer, which cleaves the pre-miRNA subsequently exported into the cytoplasm, liberating the mature miRNA duplex.[5] Together with Argonaute proteins, the mature miRNA forms the RNA-induced silencing complex and targets messenger RNAs (mRNAs), leading to inhibition of translation and/or degradation of transcript.[6]

Given the pivotal role miRNAs play in cellular processes such as development, differentiation, proliferation, and apoptosis, any dysregulation of miRNAs is likely to perturb cellular physiology and thereby contribute to disease development. Not surprisingly, deficiencies or excesses of miRNAs have been linked to many human disorders, including cancer,[7] neurodegenerative disorders,[8] autoimmune diseases,[9] diabetes,[10] cardiovascular diseases,[11] and so on. Furthermore, miRNAs offer great potential as diagnostic and prognostic biomarkers[12] in addition to being suitable targets for therapeutic intervention.[13]

The role of miRNAs in blood coagulation, despite their ubiquitous involvement in most biologic processes, is still poorly understood, but a few recent reports[14–16] have sought to broaden our horizons, especially regarding the regulation of expression of hemostatic factors. "Clinical Manifestation of Hemophilia A in the Absence of Mutations in the *F8* Gene That Encodes FVIII: Role of Micro RNAs" by Jankowska and colleagues[17] in this issue of TRANSFUSION is another valuable addition to the growing knowledge base in this field. In this promising study, the investigators have attempted to unravel the mechanism behind the mild and moderate clinical phenotype of hemophilia A (HA) observed in two patients, part of a small subset (<2%) of patients with HA with no attributable causative mutation(s) in their *F8* genes. Among eight dysregulated miRNAs, they were able to identify two, hsa-miR-374b-5p and hsa-miR-30c-5p, that were significantly up regulated in both patients. Both miRNAs are known to be expressed in human liver and are predicted to bind to target sites in the 3′ untranslated region (UTR) of *F8* mRNA. In vitro overexpression followed by inhibitor studies in cells constitutively expressing factor VIII (FVIII) demonstrated a "fine-tuning" role for these two miRNAs in regulating *F8* gene expression.

As the authors acknowledge, their observation is more hypothesis-generating than a definitive proof that the up regulation of the two miRNAs unequivocally causes the HA phenotype in these patients. In a recent publication, Nourse et al.[16] identified 52 specific miRNA interactions with 11 key hemostatic associated genes including the *F8* gene by using in vitro RNA affinity purification in conjunction with next-generation sequencing. Interestingly, neither miR-374b-5p nor miR-30c-5p were among the many miRNAs functionally validated to interact with the 3′ UTR of the *F8* gene as analyzed by transfection studies in the human hepatic cell line HuH-7. These seemingly contradictory findings only reinforce the extremely complex nature of miRNA-mediated regulation of the hemostatic system that may occur at different levels of action. The regulatory interactions could be either direct between one or several miRNAs with the corresponding mRNAs or indirect via miRNA targeting of other elements such as receptors, transcription factors, and/or other intermediate proteins.

Jankowska and colleagues[17] were able to narrow down to the two miRNAs based on three computerized prediction algorithms. However, a relatively large number of potential mRNA targets exist for any single miRNA. The dozens of available online prediction tools[18] rely on complementarity of evolutionarily conserved seed regions spanning a mere six nucleotides, resulting in a high number of false matches. Therefore, a reliable in silico prediction search might require the use of several different algorithms. Such predictions are typically not cell specific and do not take biologic context into account, including genetic events such as RNA editing or alternative splicing that might affect miRNA binding to its target mRNA.[19] The robustness of future studies will depend on the development of tools that can accurately predict the complex modulation occurring within a particular tissue or cell.

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Hemostasis reflects a delicate balance between pro- and anticoagulant and profibrinolytic mechanisms, and any disturbance could result in either bleeding or thrombosis. The 52 functionally confirmed interactions involving 40 miRNAs and 11 hemostatic genes identified by Nourse et al.[16] included cooperative miRNA regulation of key procoagulant (F7, F8, F11, FGA, FGG, and KLKB1), anticoagulant (SERPINA 10, PROZ, SERPIND 1, and SERPINC 1), and fibrinolytic (PLG) components. Jankowska and colleagues[17] carried out a methodical genetic and clinical characterization of the two patients with HA in terms of their procoagulant parameters, but seemed to have overlooked the anticoagulant and fibrinolytic components in their analysis. Any comprehensive follow-up study in the future is unlikely to be complete without the inclusion of these parameters. In addition, the data presented by Jankowska and colleagues in the current report and the intriguing lack of overlap with some of the key findings from their previously published work[20] raises important questions about the likely influence of ethnic and/or geographic factors on miRNA profiles and their regulation of FVIII gene expression.

Unlike in cancer,[21] little is known about the associations between miRNA single-nucleotide polymorphisms (SNPs) and hemostasis or thrombosis. A recent report[22] based solely on in silico prediction suggested that an SNP (c.8728 A > G rs1050705, position 1672 of the mRNA) found in the 3′ UTR of the *F8* gene in patients with HA likely affects putative miRNA binding and acts as a potential modifier of the HA phenotype. Further analysis of a larger subset of patients with HA with more reliable in silico prediction tools backed up by functional assays could shed more light on the miRNA-mediated regulation of coagulation and thrombosis.

In conclusion, the report by Jankowska and colleagues[17] lays a solid groundwork for more robust investigations in the future into the complex regulation of the hemostatic system by miRNAs in general and the finely balanced expression of the *F8* gene in particular. More comprehensive studies are likely to fill in the knowledge gaps and address some of the critical issues discussed here.

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