The Hemophilia Center of Western Pennsylvania

Discrepancy of *F8* and *F9* gene variant classifications between clinical laboratories

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Introduction and Objectives:

Despite the *F8* and *F9* genes being well-conserved, not all variants in these genes are pathogenic. Targeted, familial genetic testing for variants which are not pathogenic may lead to inaccurate risk assessment and genetic counseling for relatives. Therefore, variant classification is a critical element for determining a genetic test result's clinical utility.

There are multiple professional guidelines and standards that exist for clinical genetic test results, including recommendation to classify an identified sequence variant according to a 5-tier system (Figure 1) and, more recently, a suggested classification process utilizing various types of supporting evidence.[1-3] However, discrepancy of variant classification between laboratories has been demonstrated in other genetic diseases.[4]

We review the experience of our Hemophilia Treatment Center (HTC) with patients who have discrepant *F8* and *F9* variant classifications between clinical laboratories, as well as our approaches for addressing this potential clinical dilemma.

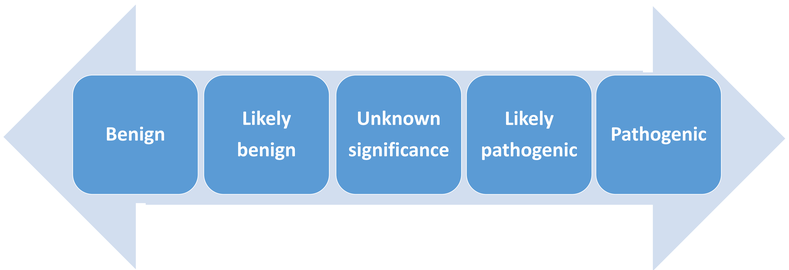

Figure 1. Five-tier system for classifications of sequence variants [2,3]

Methods and Materials:

Patients with hemophilia A or B from our HTC who had a genetic test result, including a variant classification, from more than one clinical laboratory were identified. Individuals identified to have one of the following well-defined F8 and F9 variants were excluded: *F8* intron 22 inversion, *F8* intron 1 inversion, *F9* Amish founder mutation.[5-7] Classification and supporting lines of evidence for test results were compared for each individual.

Results:

Twelve patients were identified with ten unique variants. Two variants were each identified in two patients, not known to our HTC to be related. Eligible test results were from 3 different laboratories.

Comparisons of variant classifications are detailed in Table 1.

- Classifications were discrepant for seven of twelve individuals (58.3%).
- All discrepant variants were missense mutations.

Differing lines of evidence between laboratories are outlined in Table 2.

- Eleven individuals (91.7%) had at least one different line of evidence documented between their two laboratory reports.

DISCREPANT VARIANT CLASSIFICATIONS	F8 (n=2)	F9 (n=5)	Total (n=7)
Classifications			
Uncertain significance, pathogenic	1	3*	4*
Likely pathogenic, pathogenic	1	2^	3^
Mutation type			
Missense	2	5*^	7*^
Severity			
Severe	1	0	1
Moderate	0	4*^	4*^
Mild	1	1	2
CONSISTENT VARIANT CLASSIFICATIONS	**F8 (n=2)**	**F9 (n=3)**	**Total (n=5)**
Classification			
Pathogenic	2	2	4
Uncertain significance	0	1	1
Mutation type			
Missense	1	0	1
Nonsense	0	1	1
Frameshift	1	1	2
Non-coding	0	1	1
Severity			
Severe	0	3	3
Moderate	1	0	1
Mild	1	0	1

Table 1. Summary of discrepant and consistent variant classifications among individuals with clinical genetic testing through more than one laboratory. *,^ Denotes that two individuals were identified to have the same variant.

Case	Evidence provided by Lab A but not Lab B	Evidence provided by Lab B but not Lab A
1[D]	population data, computational and predictive data	n/a
2^[D]	n/a	population data, computational and predictive data, segregation data
3*[D]	population data	functional data
4[C]	population data, functional data	n/a
5^[D]	n/a	population data, computational and predictive data, segregation data
6[C]	population data, computational and predictive data	n/a
7[D]	n/a	population data, computational and predictive data
8[D]	population data, computational and predictive data, segregation data	n/a
9*[D]	n/a	population data
10[C]	population data, computational and predictive data	n/a
11[C]	population data	computational and predictive data, segregation data
12[C]	n/a	n/a

Table 2. Summary of lines of evidence for variant classification differing between laboratories. Lab A/B randomly assigned for each case. *, ^ cases identified to have the same variant. [C], [D] cases with consistent and discrepant classification between laboratories, respectively.

Conclusions:

Variant classification, which may impact a test result's clinical utility, can be laboratory-dependent. To remedy this potential clinical dilemma, our HTC has found it helpful to understand a laboratory's internal processes for variant classification, as well to collaborate on a case-by-case basis with personnel from the laboratory of record. Existing guidelines may help to improve consistency of variant classifications across laboratories; however, it will remain critical for ordering providers to understand variant classification concepts in order to ensure correct diagnosis and interpretation of test results for patients and at-risk family members.

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