The Canadian "National Program for Hemophilia Mutation Testing" database: a ten-year review

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Introduction/ Objective

- Identification of the *F8* or *F9* mutation in Hemophilia A (HA) and B (HB) is important for optimal family counseling, and prediction of risk for inhibitor formation following exposure to replacement therapy.
- The high degree of mutational heterogeneity complicates mutation testing.
- To optimize access to genetic services in Canada, a national genotyping laboratory was established in November 2000 at Queen's University, Kingston.
- Here, we review ten years of the genotyping laboratory's activity.

Methods

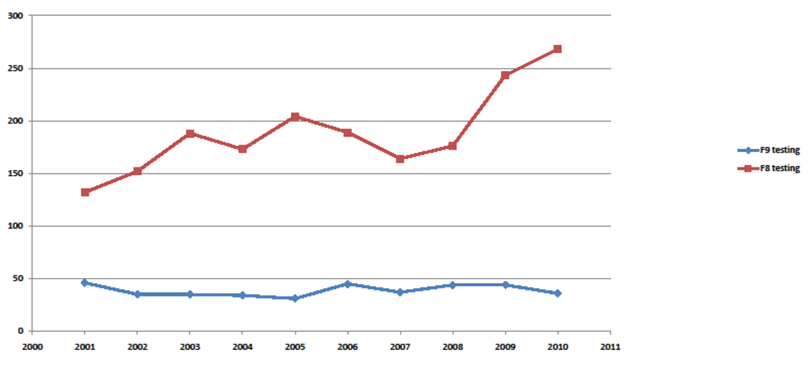
- From November 2000 to March 2011, patients' blood in EDTA or DNA was received from the 26 Canadian HTCs, and genetics clinics.
- In cases of severe HA, Southern Blotting[1] and PCR[2] were carried out to detect intron 22 inversion and intron 1 inversion, respectively.
- Samples were screened with conformation sensitive gel electrophoresis,[3] up to January 2009, after which all samples were subjected to direct sequencing.
- *F8* mutation-negative cases were investigated for type 2N VWD by sequencing of exons 17-21, and 24-27 of the *VWF* gene.

Results

The Activity of the Reference Laboratory

- *F8* gene was analyzed in 1192 males and 787 females. *F9* gene in 271 males and 123 females.
- This represents 48% of the HA and 47% of the HB populations in Canada as compared to the 2011 report of Canadian Hemophilia Registry, found at http://fhs.mcmaster.ca/chr/index.html).
- Demand for genotyping has remained constant over the ten-year period:

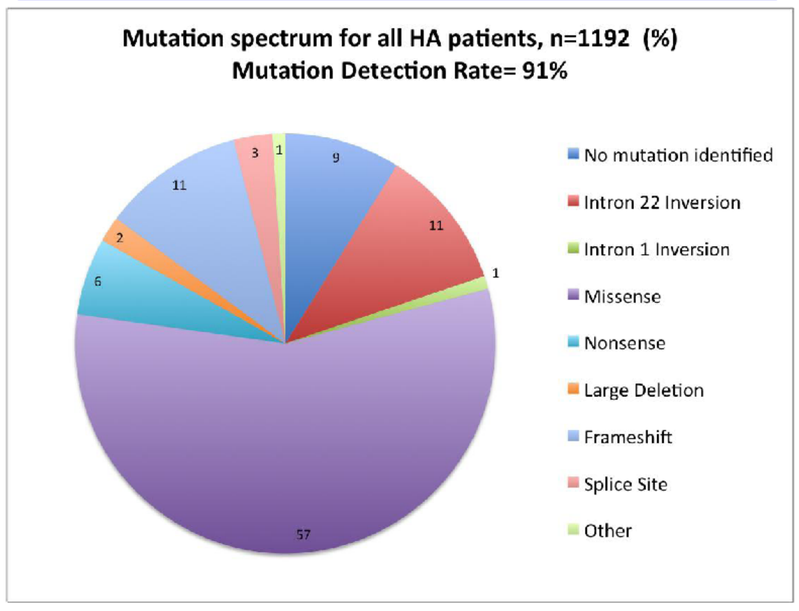
Number of Cases Received per year:



Indications for Genotyping:

	Hemophilia A		Hemophilia B	
Reason for testing	Males, n=1192 (%)	Females, n=787 (%)	Males, n=271 (%)	Females, n=123 (%)
None identified	347 (29)	129 (16)	91 (35)	15 (12)
Family History	294 (25)	36 (5)	69 (25)	9 (7)
Sporadic Case	442 (37)	16 (2)	100 (37)	3 (2)
Case for research	32 (3)	15 (2)	11 (4)	1 (1)
Carrier testing	70 (6)	493 (63)	0	75 (61)
Prenatal or pregnancy	7 (1)	98 (12)	0	20 (16)

Hemophilia A

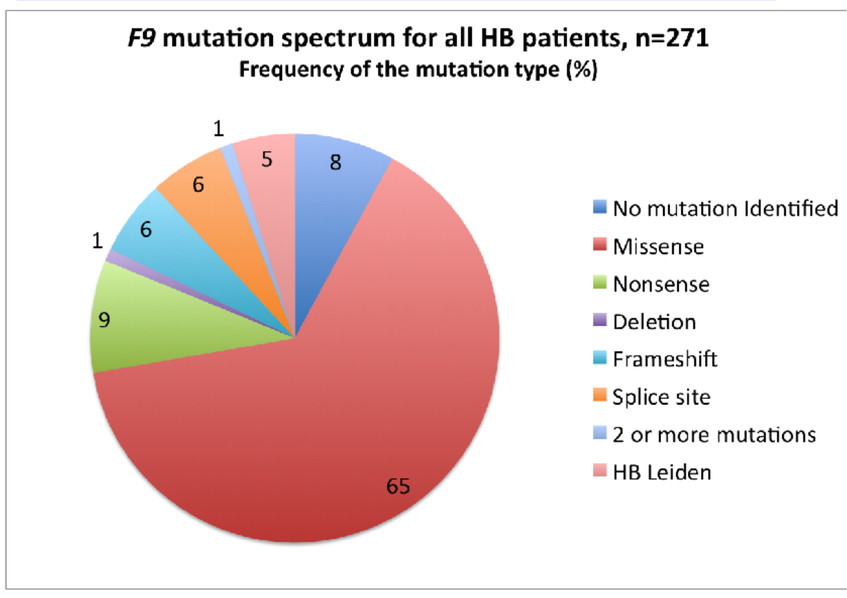


- 398 *F8* unique mutations were identified
- 229 were novel to HADB (Hemophilia A Database found at http://hadb.org.uk/)
- 22% (17 of 78) mutation negative cases were found to have type 2N VWD mutations
- Inhibitors were reported in 8% of cases

Inhibitor Prevalence as per MutationType:

	Canadian Database	Previously reported rates[4-6]
I22I	17	17-28
I1I	0	9-26
Deletion	24	33-55
Frameshift	12	11-16
Nonsense	23	24-31
Missense	4	5-8
Splice Site	9	0-26

Hemophilia B



- 129 *F9* unique mutations were identified
- 36 were novel to International Hemophilia B Database found at http://www.kcl.ac.uk/ip/petergreenhaemBdatabase.html.)
- Inhibitors were reported in 2% of cases (n=4) and associated with the following mutations:

Phenotype	Type of mutation	Nucleotide change	Amino acid change
Severe	Nonsense	30863C➔T	Arg248Stop
Severe	Del from ex A to D		
Severe	Del from ex E to H		
Moderate	Missense	30112C➔T	Ala220Val

Conclusions

- This represents the largest and longest duration experience yet reported for a clinically-driven national hemophilia genotyping program.
- The mutation spectrum for HA and HB is consistent with previously published population studies.
- This large database helps to further define inhibitor risk with certain mutations.
- The mutation detection rates of 91% and 92% for HA and HB are comparable to several previously published reports of mutation databases, but lower than predicted in view of current gene sequencing technology.
- Genotyping of approximately half of the HA and HB populations has now been accomplished in Canada. However, the demand for HB genotying has remained constant while the demand for HA genotyping has increased in the last 2-3 years.

Acknowledgements

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