Phenotypic classification of the mutations in coagulation factor IX segregate to different locations in its protein structure

Pavithra M. Rallapalli[1], Edward G. Tuddenham[2], Keith Gomez[2] and Stephen J. Perkins[1]

[1]Research Department of Structural and Molecular Biology, Division of Biosciences, University College London, London WC1E 6BT

[2] The Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free Hampstead NHS Trust, London NW3 2QG, UK;

Introduction

Coagulation factor IX (FIX), a zymogen synthesised in the liver, is a single-chain, vitamin K-dependent plasma glycoprotein. It is an important component of the intrinsic system of the coagulation cascade. Defects in the *F9* gene leads to Haemophilia B, a gender-linked recessive coagulation disorder with an incidence of approximately 1 in 50,000, and occur almost exclusively in males. Haemophilia B has attracted much recent interest because of the development of ground-breaking gene therapy methods for this at UCL by Nathwani et al.

Objective

To develop an interactive database in which the *F9* mutations are presented in searchable formats, and viewed in conjunction with the FIX protein structure and sequence along with the clinical phenotypes for Haemophilia B.

Methods

- Literature searches to collate all previously known and novel FIX mutations.
- Homology modelling and energy minimisation to create the human FIX structure starting from the crystal structure of porcine FIX.
- Use of PHP, MySQL, XML, Perl and Jmol tools for the database and web-interface, and for statistical, structural and sequence analyses.

Novel Mutations

Of the 1094 unique mutations, 121 unique mutations are novel and not published since the last update of the Haemophilia B mutation database in 2004. There are also 42 (3%) multiple mutations within F9 comprising of 40 double, 1 triple and 1 quadruple mutants, an additional total of 87 individual mutations.

Data Set

Arg3	Asn48	Asp93	Leu130	Ala192	Val227	Ala279	Ile334	Thr381	Ile428
Ile7	Ser49	Gly94	Asp131	Thr194	Val228	Gly280	Cys335	Cys382	Ser430
Glu10	Gly50	Asp95	Cys134	Asp198	Gly229	His282	Ile336	Leu383	Trp431
Ile17	Lys51	Gln96	Ile136	Tyr201	Gly230	Ile284	Ala337	Arg384	Gly432
Cys18	Leu52	Cys97	Asn138	Val202	Glu231	Glu286	Lys339	Ser385	Glu433
Leu19	Glu53	Glu98	Asn138	Glu208	Ala233	Glu288	Glu340	Thr386	Glu434
Leu20	Glu54	Pro101	Gly139	Thr209	Gly236	His289	Tyr341	Lys387	Cys435
Gly21	Phe55	Cys102	Arg140	Ser217	Gln237	Thr290	Thr342	Thr389	Ala436
Tyr22	Gln57	Leu103	Cys141	Gln219	Phe238	Glu291	Ile344	Ile390	Lys440
Leu23	Gly58	Asn104	Glu142	Ser220	Pro239	Gln292	Phe345	Tyr391	Tyr441
Leu24	Leu60	Gly105	Gln143	Asp223	Trp240	Arg294	Leu346	Asn392	Gly442
Ala26	Glu61	Gly106	Cys145	Phe224	Gln241	Val296	Lys347	Asn393	Ile443
Cys28	Arg62	Ser107	Asn147	Arg226	Val243	Arg298	Gly349	Met394	Tyr444

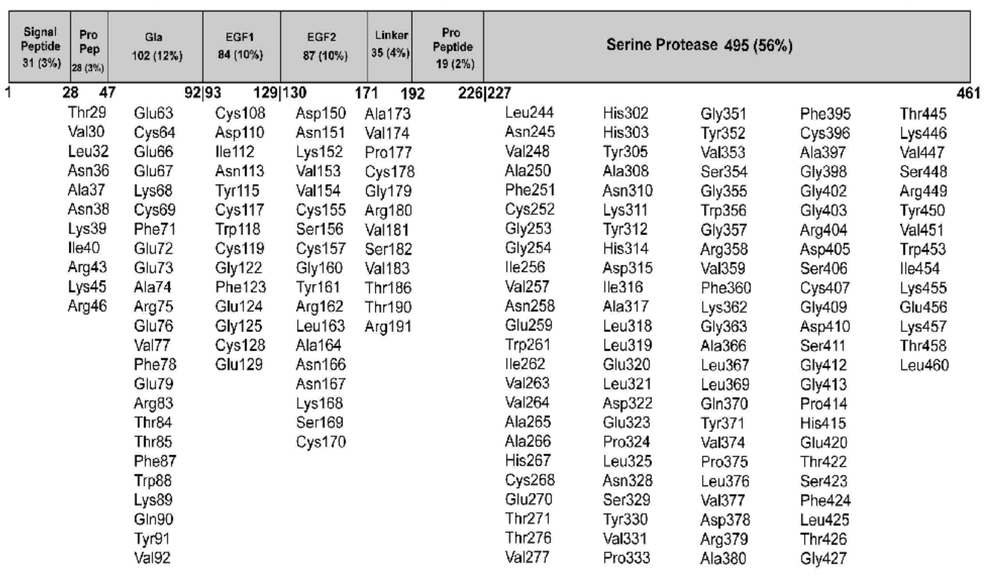


Figure 1: The distribution of 319 unique mutations on the exonic region of F9 associated with Haemophilia B

Statistics

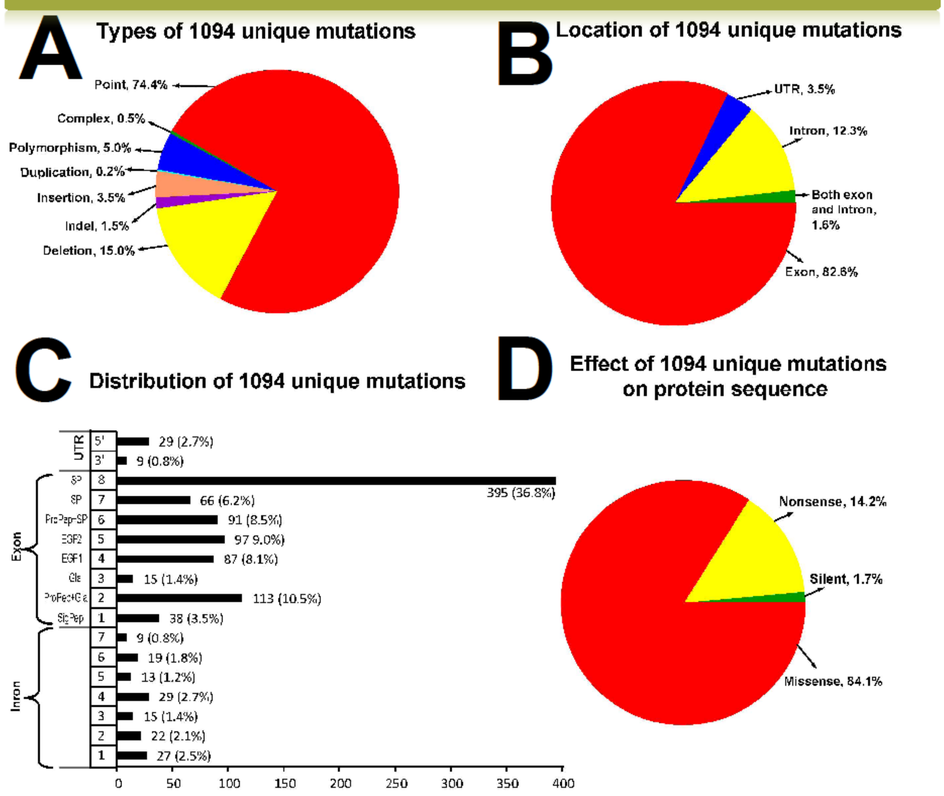
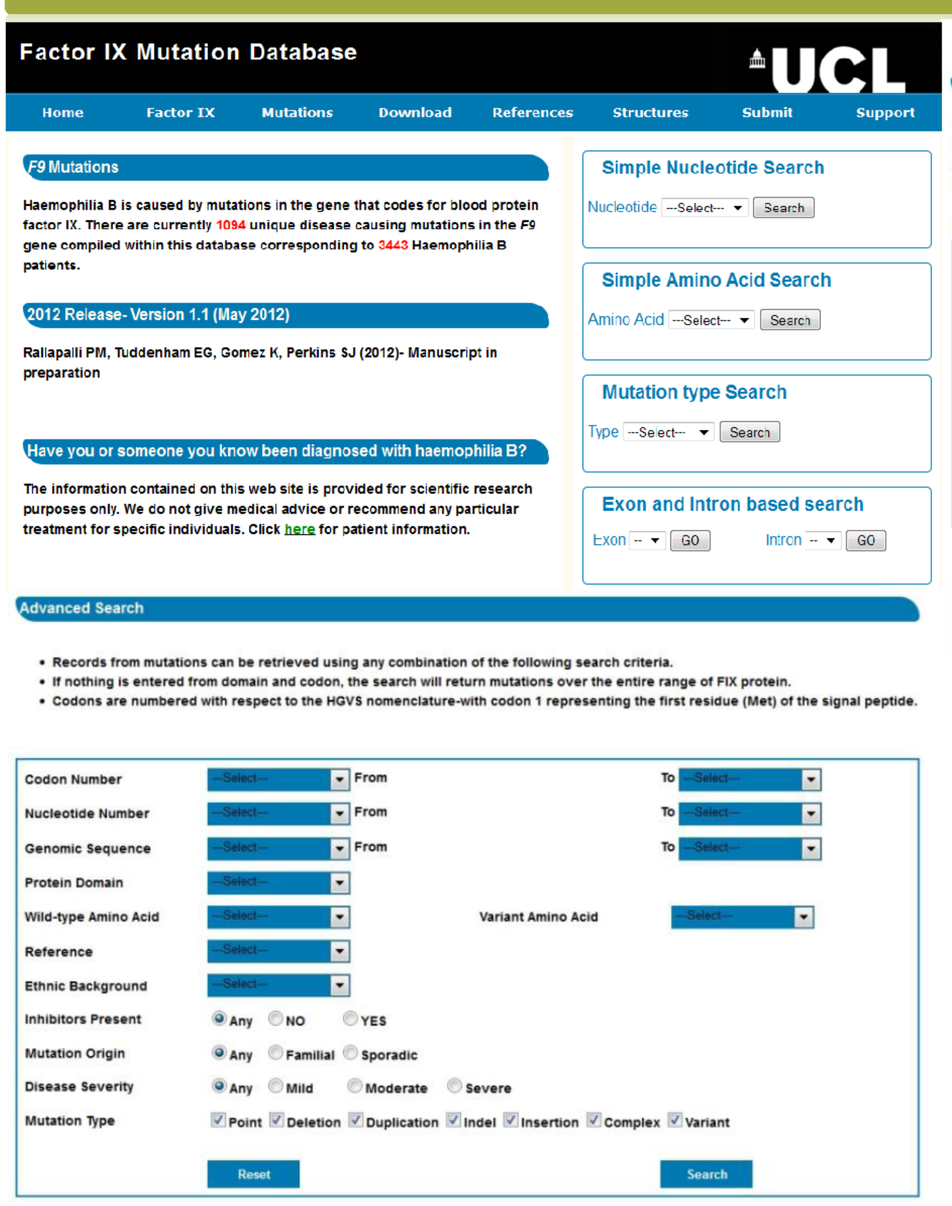


Figure 2: Statistics of the FIX mutations available in the newly-updated Factor IX mutation database.

www.factorix.org



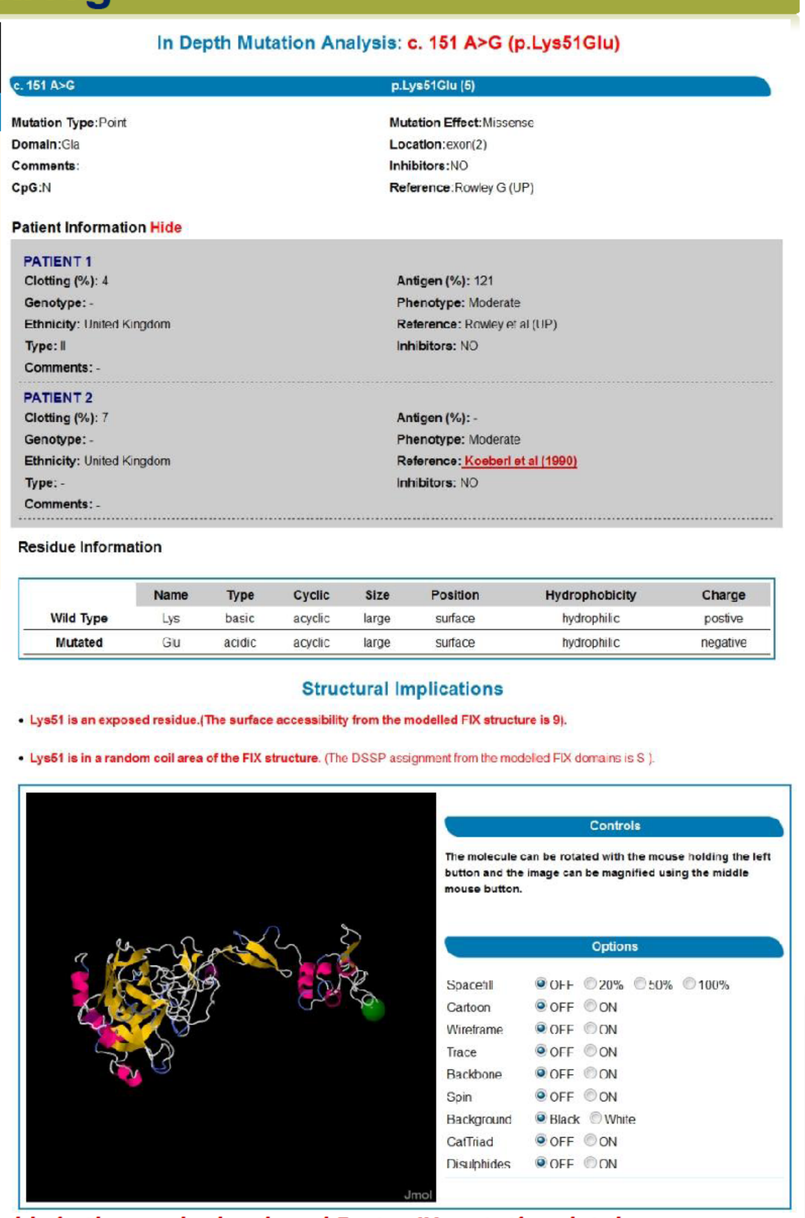


Figure 5: Screenshots of the various mutational analysis options available in the newly-developed Factor IX mutation database.

Database work flow

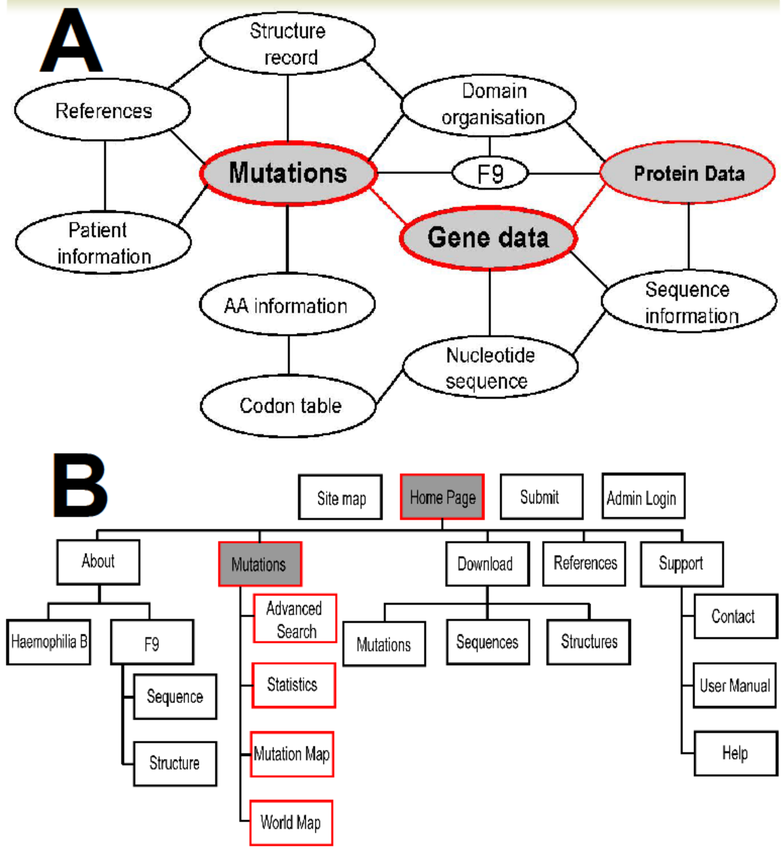


Figure 3: Organisation of the Haemophilia B mutation database. (A) General architecture of the backend database. (B) Network of the webpages inside the Factor IX mutation database.

Phenotype and location of mutations

Haemophilia B-associated mutations have been reported for 319 out of the 461 residues in FIX, with activity and antigen levels reported for 164 of these. Of the 150 unique mutations in the four domains with known phenotypes, 18% (26) are quantitative type I mutations, 68% (102) are qualitative type II mutations, and 14% (22) are unclassified. Note that the SP domain is formed as two subdomains, between which lies the catalytic active site cleft. In this, 46 of the 64 type II mutations (blue) are located in subdomain 2, and the majority of these lead to severe haemophilia B. In contrast, 13 of the 21 type I mutations (yellow) are in the subdomain 1 of the SP domain.

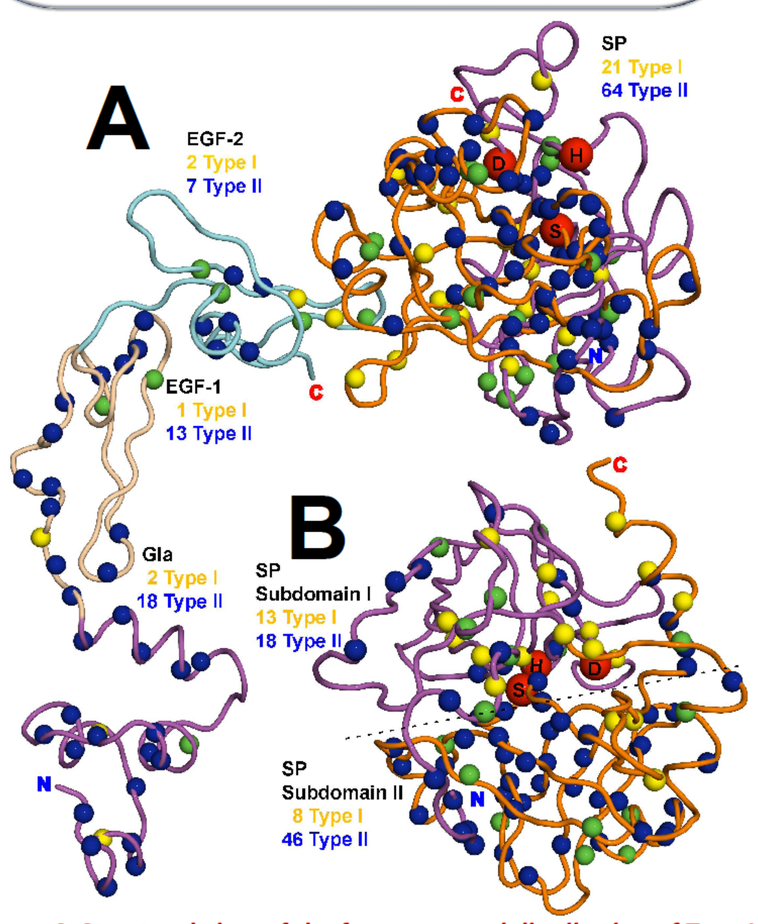


Figure 4: Structural view of the frequency and distribution of Type I and Type II phenotypic mutations within the four domain regions of human FIX protein.

Conclusion

By mapping the mutations with a 3D structural model for FIX, we can now correlate the phenotype and severity of the FIX mutations with their location in the FIX structure. The type II phenotype is dominant in the small Gla, EGF-1 and EGF-2 domains, and in the subdomain II of the catalytic SP domain. Curiously the type I phenotype is dominant in the subdomain I of the SP domain. This finding implies that most of the FIX structure is involved with functional interactions, while subdomain I is prone to protein misfolding or degradation.

Acknowledgement

We thank Pfizer and the Special Trustees of the Royal Free Hospital and the Katharine Dormandy Trust for Haemophilia and Related Disorders for their support.

DOI: 10.3252/pso.eu.WFH2012.2012

Poster presented at: WFH 2012 World Congress Paris, France Sunday, July 8 - Thursday, July 12

PosterSessionOnline.com