The Factor VIII Mutation Database on the World Wide Web: The Haemophilia A Mutation, Search, Test and Resource Site

HAMSTeRS Update (version 3.0)

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ABSTRACT

The HAMSTeRS WWW site was set up in 1996 in order to facilitate easy access to, and aid understanding of, the causes of haemophilia A at the molecular level; previously, the first and second text editions of the database have been published in Nucleic Acids Research. This report describes the facilities originally available at the site and the recent additions which we have made to increase its usefulness to clinicians, the molecular genetics community and structural biologists interested in factor VIII. The database (version 3.0) has been completely updated with easy submission of point mutations, deletions and insertions via e-mail of custom-designed forms. The searching of point mutations in the database has been made simpler and more robust, with a concomitantly expanded real-time bioinformatic analysis of the database. A methods section devoted to mutation detection has been added, highlighting issues such as choice of technique and PCR primer sequences. Finally, a FVIII structure section gives access to 3D VRML (Virtual Reality Modelling Language) files for any user-definable residue in a FVIII A domain homology model based on the crystal structure of human caeruloplasmin, together with secondary structural data and a sound+video animation of the model. It is intended that the general availability of this model will assist both in interpretation of causative mutations and selection of candidate residues for in vitro mutagenesis. The HAMSTeRS URL is http://europium.mrc.rpms.ac.uk.

INTRODUCTION

Coagulation factor VIII (FVIII) is an essential cofactor for the activation of factor X by factor IXa (1). The FVIII gene (F8C) contains 26 exons and spans 186 kb of DNA (2). Deleterious mutations in the FVIII gene have been demonstrated to reduce either expression or activity of the FVIII protein and thus cause haemophilia A (3) an X-linked bleeding disorder affecting ~1 in 5000 males (4).

The Internet, and more specifically sites coded in the graphics-oriented hypertext markup language (HTML), is commonly called the World Wide Web (WWW). Advances in HTML, WWW servers and browsers have been made rapidly in recent years to the point where many academic users rely on WWW both to search for relevant information and to make available their own data resources. The advantages of such a system include instant access, user-definable database interaction and the ability to update databases in real time, continuous error correction, inclusion of unpublished data and expansion of categories of information in response to users’ requests. It is clear, therefore, that this new means of communication provides an ideal vehicle through which to publish an interactive database of nucleotide substitutions, deletions, insertions and rearrangements of the factor VIII gene.

METHODS

World Wide Web site construction

Additional HTML 2.0 code was written using a Silicon Graphics (Mountain View, CA) Indigo2 workstation. Since the site has been coded to exploit the many useful features of HTML 2.0, for best results it is recommended that the site is accessed through Netscape V2.02 (Netscape URL: http://home.netscape.com) or later; earlier versions or other browsers may give unpredictable results.

All CGI scripts were coded in C, and the home page image files were generated using SGI Showcase. The FVIII A domain homology model (5) was generated using Homology and Insight II (Biosym/MSI, Cambridge, UK), based on the crystal structure for human caeruloplasmin (6); individual VRML files corresponding to display of each residue in the model were generated using Biosym Command Language (BCL) and converted to VRML images using a patch for Insight II available from Biosym/MSI (http://www.msi.com/marketing/life/products/InsightII/vrml); VRML files were subsequently visualized using SGI WebSpace. The animation was created using a BCL script in Insight II to generate individual frames, which were captured as RGB images.

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Figure 1. Home page of HAMSTeRS Version 3.0, showing the function icons.

before being imported into SGI MovieMaker to create a Silicon Graphics format movie. Finally, this was converted to Quicktime format (.mov file extension) with JPEG compression and 16-bit sound using SGI MediaConvert.

Hardware

HAMSTeRS is served from europium, a Silicon Graphics Indigo² workstation running IRIDX 5.3 and the CERN WWW server software httpd (V3.0, obtainable from CERN at http://www.cern.ch/ExpSupport/ ) and delivered to outside users via the Hammersmith Hospital 100 MB fibreoptic line.

RESULTS

Features available at the HAMSTeRS WWW site

All of the functions of the database may be accessed from the icons of the home page, or alternatively by clicking the text links below these icons (Fig. 1).

The What’s New page summarizes recent changes and upcoming features. The Review (7) contains a concise overview of the molecular genetics of haemophilia A, updated from the original version described earlier (8) to include all published and unpublished mutations submitted to HAMSTeRS; a glossary of terms to aid the lay reader has also been included. Submission page: three new tailored forms allow users to submit newly discovered insertions, deletions and point mutations directly via e-mail to the site. The Search page has been greatly modified to improve both ease of use and powerful database searching of point mutations using a Boolean search function (AND/OR) with an extensive range of search parameters (exon and codon number, nucleotide sequence, amino acid change, laboratory test values, clinical severity, inhibitor status and reporting group), while insertions and deletions are accessible as HTML-formatted tables: as an example, Figure 2 shows the output from a search of the point mutations database for all records of mild haemophilia with mutations in exon 23 (using the Boolean AND function). An updated bioinformatic analysis of features of the point mutations has also been introduced. A further new feature has been to provide listings of all mutations reported subsequent to the last printed version in 1994 (9).

A FVIII Structure Model page offers the opportunity to inspect and download various representations of a homology model of the A domains of FVIII (5) based on the crystal structure of human caeruloplasmin (6). First, for each FVIII A domain residue in the model users may view a VRML file with the user-specified sidechain rendered in CPK spheres and the rest of the model drawn as an α-carbon trace coloured according to domain. Each of the files is <100 KB in size and thus amenable to easy downloading: viewing is possible on PCs by use of Live3D (the VRML plugin for Netscape), by WebSpace mounted on Silicon Graphics workstations, or by other proprietary software. Tables of secondary structural data calculated with INSIGHT II (structural feature, i.e. helix/sheet/tum/coil, accessible sidechain area and fractional solvent accessibility) are provided for each A domain. Secondly, a larger VRML ribbon image of the FVIII A domain model (1.3 MB) is available to download, although a powerful graphics workstation with
Z-plane buffering may be necessary to display it (e.g. SGI, IBM R6000 or UltraSparc 2). Thirdly, the co-ordinates of the model in PDB format will be available to download; PC-based viewers such as Roger Sayle’s RASMOL 2.5 (available by anonymous FTP from src.doc.ic.ac.uk in directory packages/rasmol) and MSI WebLab (available from http://www.msi.com/weblab ) may be used to view the model, while there are many UNIX-based molecular graphics packages available for workstations. Lastly, a 60s Quicktime format animated sound+video file (9 MB) has been constructed to demonstrate the overall features of the model.

A Methods page has now been added which details the various approaches to FVIII mutation screening by PCR-based methods (10), including detailed method descriptions, cDNA restriction maps and primer sequences for amplification of exons 1–26. The Links page has been expanded to increase further the site’s interest and usefulness.

Figure 3 shows a montage of several of the features of HAMSteRS V3.0.

**Combined use of the database with the FVIII A domain model**

In the absence of a crystal structure, the FVIII A domain model constitutes the best available representation of the 3D structure of the A domains of FVIII. To aid in structure/function investigations of FVIII (including site-directed mutagenesis), it is possible to map selected data from the point mutation database on to the FVIII model. For example, Figure 4 shows the distribution of residues (white), missense mutation of which leads to CRM+ve haemophilia A (where dysfunctional FVIII is secreted normally), over the three domains of the model: while critical residues abound at or near the FIXa-binding site in A2 (purple) (12) and at interdomain interfaces (particularly A1–A2), the body of the A1 domain appears to lack functional importance (as defined by the lack of such residues). Thus, the database and model may be used together to probe the relationship between structure and other aspects of FVIII (for example, secretion defects indicated by CRM–ve reports).

**CONCLUSIONS**

The continuing development of the HAMSteRS WWW site is driven both by the need to provide accurate and fully up-to-date information for users in the fields of FVIII molecular genetics and structure/function, and the desire to explore more speculative areas as exemplified by the FVIII homology model. We hope that the improvements to the site represented by the A domain model coupled with powerful means to search the point mutations database will be of assistance to workers who study the factor VIII gene and protein in both normal and disease states.
Figure 3. Montage of some of the key features of HAMSTeRS. Top left, the point mutation database query form, showing the possible search fields; top right, the review of FVIII molecular genetics; centre of screen, part of the methods page showing PCR primer listings for Exon 1; bottom left, a VRML image of the FVIII A domain model with residue 100 (alanine) in CPK displayed by SGI WebSpace; bottom centre, part of the Bioinformatics output generated from the point mutations; bottom right, the FVIII animation (displayed by SGI MoviePlayer).

CITATION

Users of HAMSTeRS are asked to cite this article in their publications, including the URL (http://europium.mrc.rpms.ac.uk).

DISTRIBUTION

The Haemophilia A Mutation Search, Test and Resource Site (HAMSTeRS) may be accessed via the World Wide Web at http://europium.mrc.rpms.ac.uk.

Users without WWW access may download the basic database tables by anonymous FTP (ftp.ebi.ac.uk/pub/databases/hamsters) from the European Bioinformatics Institute database collection (11), although the most recently updated versions will be only available through the WWW site. For those with no Internet access, text versions of the main mutation tables may be obtained from G.K-C.

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Figure 4. Mapping of FVIII residues causing CRM+ve haemophilia A onto a ribbon representation of the FVIII A domain model. A1 domain, red; A2, blue; A3, green. Haemophilia-implicated residues marked in white, FIXa interaction sites (12,13) in purple.

REFERENCES