VarSearch: Annotating Variations using an e-Genomics Framework

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Abstract: Nowadays experts in the genomics field work with bioinformatics tools (*software*) to generate genomic diagnoses, but the reality is that these solutions do not fully meet their needs. From the perspective of *Information Systems* (IS), the real problems lie in the lack of an approach (i.e., *Software Engineering* techniques) that can generate correct structures for data management. Due to the problems of *dispersion*, *heterogeneity* and the *inconsistency* of the data, understanding the genomic domain is a huge challenge. To demonstrate the advantages of *Conceptual Modeling* (CM) in complex domains -such as *genomics*- we propose "*VarSearch*", a web-based tool for genomic diagnosis that incorporates the *Conceptual Model of the Human Genome* (CMHG) and takes advantage of *Next-Generation Sequencing* (NGS) for ensuring genomic diagnostics that help to maximize the *Precision Medicine* (PM).

1 INTRODUCTION

The study and understanding of the human genome could probably be considered one of the great challenges of our century. Thanks to the advances in NGS (*Next Generation Sequencing*) (Mardis, 2008), there has been considerable growth in the generation of genomic and molecular information. In addition, the interactions that are available with this genomic knowledge have a direct impact on the medical environment and *Precision Medicine* (PM) (Grosso, 2016).

The application of *Conceptual Modeling* (CM) (Olivé, 2007) techniques to the genomic domain now provides solutions and optimizes some of the processes carried out by experts (i.e., in *genetic laboratories* and *hospitals*), and helps to solve the problems that arise in handling the large amounts of information from different sequencing methods. The use of advanced *Information System* (IS) engineering approaches can be useful in this domain due to the huge amount of biological information to be *captured*, *understood* and *effectively* managed. A considerable part of modern Bioinformatics is devoted to the management of genomic data. The existence of a large set of diverse data sources containing large amounts of data in continuous evolution makes it difficult to find convincing solutions (Reyes Román et. al., 2016). When we addressed this problem from the IS perspective, we understood that precise CMs were required to understand the relevant information in the domain and to clearly fix and represent it to obtain an effective data management strategy.

Research and genetic diagnoses are typical examples of the work done by experts -*biologists*, *researchers* or *geneticists*- every day. However, some information is required to perform these tasks. *Where are these data*? Currently, this information is dispersed in genomic repositories including *web sites*, *databanks*, *public files*, etcetera, which are completely *heterogeneous*, *redundant*, and *inconsistent* (containing partial information). In addition, most of these just focus on storing specific information to solve a specific problem (e.g. *YHRD*, designed to store Y chromosome haplotypes from global populations, *https://yhrd.org/*). Due to these characteristics, we are able to estimate the difficulty of experts in finding and manipulating certain genomic information, making this goal almost impossible to achieve. Another relevant factor in the domain is the constant growth and updating of the

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data (i.e., *biological concepts*). The use of standard definitions of concepts is not mandatory, so that sometimes the same term can have different definitions, in which case the meaning of the concept depends on the interpretation given to it by the expert. After studying this situation, we decided to develop a *Genomic Information System* (GeIS) in the form of the *VarSearch* tool (Reyes Román et. al., 2018) for data treatment and management. This system contrasts a set of genomic variations with the information contained in a database that follows the *Conceptual Model of the Human Genome* (CMHG) (Reyes Román et. al., 2016).

Applying GeIS to the bioinformatics domain is a fundamental requirement, since it allows us to structure the *Human Genome Database* (HGDB) with “*curated*” and “*validated*” data. The initial research on applying CM approaches to the human genome was reported in the works of Paton (Bornberg-Bauer and Paton, 2002) and Ram (Ram and Wei, 2004). The main goal in Ram's work was to show the advantages and benefits of using conceptual modeling to compare and search for the protein in 3D (see other related works in (Pastor et. al., 2010)). Reyes et. al. (2016) describes a CMHG which proposes a domain definition at the conceptual level. From this CMHG, we generated a GeIS to support *VarSearch*. The application of CM helps us to better understand and manage the knowledge of the human genome.

This paper is divided as follows: Section 2 describes the design and implementation of the *VarSearch* tool as a GeIS. Section 3 describes two case studies carried out using *VarSearch*. Finally, Section 4 contains our conclusions and outlines future work.

2 DESIGN AND IMPLEMENTATION OF A GeIS

A GeIS can be defined as a system that *collects*, *stores*, *manages* and *distributes* information related to the behavior of the human genome. As mentioned above, the GeIS described here is based on the CMHG. This section deals with the *VarSearch* design stage.

2.1 Design Overview

As outlined at Figure 1, *VarSearch* is based upon the *E-Genomic Framework* (EGF), described in depth in different research papers such as (Roldán et. al., 2014). For the implementation of the tool, a series of steps were carried out to ensure its good performance, as explained below:

a) *Human Genome Database (HGDB)*: The transformation of the model defined for the database schema (*logical model*) was almost automatic, using the Moskitt tool (Muñoz et. al., 2010). In this task, we found two different levels of abstraction in the model. The conceptual model represents the domain from the point of view of scientific knowledge, while the database schema focuses on the efficient storage and retrieval of data. For this reason, the details of the physical representation must be considered to improve the final implementation. It is important to emphasize the integration of the two tables "*Validation*" and "*Curator*" in the DB schema. These tables are not actually part of the knowledge representation of the domain, but are necessary for the development and implementation of the tool.

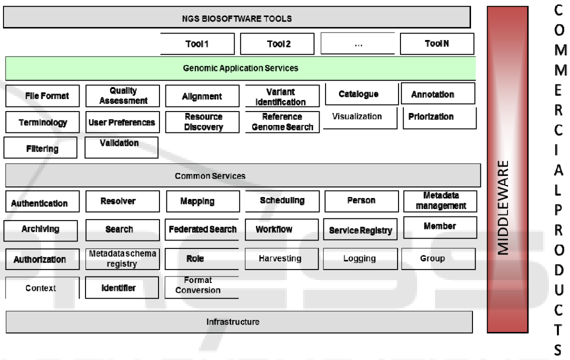


Figure 1: EGF Framework and *VarSearch* (Roldán et. al., 2014).

To load the HGDB the SILE methodology (Reyes and Pastor, 2016) was used, which was developed to improve the loading processes and guarantee the treatment of "*curated data*". SILE was used to perform the “*search*” and “*identification*” of variations associated with a specific disease (a task validated by experts in the genetic domain). When the identified and curated data have been obtained the "*selective loading*” is performed (through the loading module) in the HGDB. The data loaded are then “*exploited*” by *VarSearch*. Some of the diseases (of genetic origin) studied and loaded were *Alcohol Sensitivity*, *Neuroblastoma*, etcetera.

b) *Selection of the different data sources*: For the choice of data sources, we addressed the requirements raised in this first phase of the project. After conducting studies and analysis of various genomic repositories, we selected the following databases: NCBI, dbSNP, UMD and BIC.

NCBI (*https://www.ncbi.nlm.nih.gov/*) is a data source with curated data on structural concepts of

DNA sequencing. From this repository, we extracted information related to *chromosomes*, *genes*, *transcripts*, *exons*... and everything related to the "*Structural view*" of the CMHG. dbSNP (Sherry et. al., 2001), BIC (Szabo et. al., 2000) and UMD (Béroud et. al., 2000) are databases of variations that store curated information on genetic differences between individuals. The main reason for using dbSNP is because it not only focuses on variations of a specific gene or region, but also contains variations related to all chromosomes and updates the information immediately. BIC and UMD were selected because of the requirements of a research group that was collaborating with us in a project (*Future Clinic Project*) focused on "*breast cancer*". This group helped us to test the performance of the GeIS and its associated tool.

c) *Genetic loading module*: For the loading process of the HGDB, a load module was designed to store the data from the previously measured data sources. This load module was developed using an ETL strategy (Zhou et. al., 2011) with three different levels: *extraction*, *transformation*, and *load* (see Figure 2). Each level is completely independent of the others, facilitating and clarifying the design of the system and improving its flexibility and scalability.

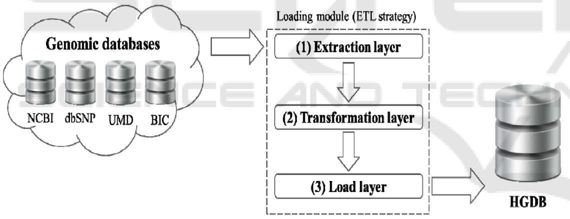


Figure 2: Load module.

As can be seen in Figure 2, all the necessary information is extracted from the source databases in the first layer (1). All this raw unstructured data goes to the second layer (2) where several transformations are made to format the data according to the structure of the database schema. These transformed data are sent to the third layer (3), which communicates directly with the database (following the above-mentioned *SILE methodology* in Task “a”).

2.2 VarSearch Tool

VarSearch is a web application that allows the analysis of variations obtained from the DNA sequenciation of biological samples and which is stored in FASTA or VCF file formats (Claverie and Notredame, 2011). Different users can access the application in private spaces in the HGDB and each user can address his own variations. The validation of variations that they consider relevant can be included. It also offers storage for the users' variations in order to find similarities in the file analysis process.

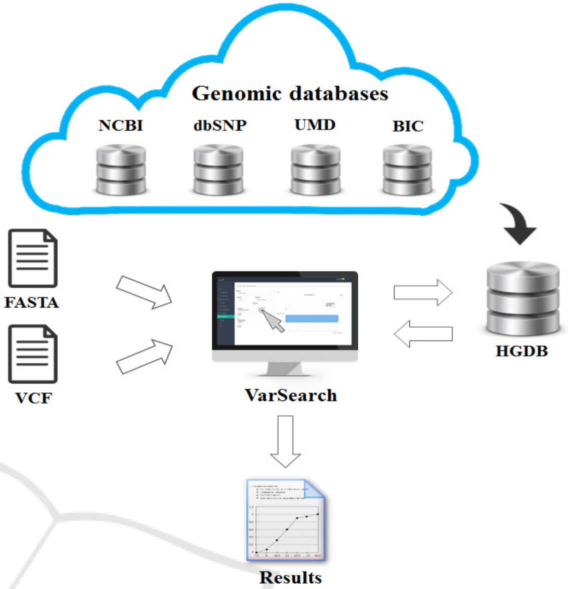


Figure 3: *VarSearch* application.

Another advantage is the inclusion of the information obtained from the data sources, together with the user validations in the database, which is an improvement in performance related to the search for variations. *VarSearch* is capable of finding variations in the database from a provided file (see Figure 3). The variations found are displayed to the user, and any additional information that the file lacks can be calculated and validated. Any variations of the file that have not been found in the database can also be stored. After inserting one or more variations not found in a file -*because they are considered relevant to the user*- and reanalysing this file, these inserted variations will be found in the database and displayed to the user.

The *VarSearch* functionality has been grouped into three main packages: (a) *User management*: a user can act as administrator and control other users, or can create new users and modify or eliminate their Information. (b) *Data load management*: the system allows the user to load the files to be analysed in both VCF and FASTA format (Agliata et. al., 2014), compare the variations in these files to the variations in the HGDB used by *VarSearch*. (c) *Data analysis*: After analysing and verifying the variations in the input files, the user can list the variations and classify them by multiple criteria (*position*, *chromosome*, *existence in the database*, etc.). There is also a series of functionalities related to the login and modification of account information that has not been grouped in any functionality package.

2.2.1 Confidentiality of the Information

As this information is a company's primary resource, *VarSearch* restricts access to it. When a user validates a variation, he can choose a privacy category: (a) *Public content*, if he is willing to share the knowledge with other users, or (b) *Private content*, allowing access only to the owner-user and hidden from other users. All the variations can only be accessed by the user who created them.

2.2.2 VarSearch Architecture

In order to make it accessible to all users, *VarSearch* was designed as a web application with HTML5 technology in a language common to all current browsers. The information is managed by the MySQL database. The *VarSearch* architecture consists of the following elements:

(a) A distributable database based on MySQL (using software tools like: Navicat Enterprise and MySQL Workbench). For the initial validation of this database, we only loaded the information related to chromosomes 13 and 22.

(b) A set of REST services (Haupt et. al., 2014) developed in Java using Hibernate and Jersey, which are deployed on a Tomcat server 7.

(c) A web application, which uses the Bootstrap framework for general organization of the interface and files, together with jQuery to define advanced interface components and invoke REST services.

(d) It also includes a "*mini*" REST service to manage users and roles, which is based on the same architecture and technologies as the other REST services. The data layer is based solely on MySQL (you can see the *VarSearch* architecture represented in Figure 4).

The application entry point is a file with variations detected by a sequencing machine in VCF or FASTA format. With this input the database is searched to detect any variations, additional information on the diseases they may cause and the associated bibliography.

VarSearch users follow this process when working with the tool:

- A VCF file is uploaded from the web.

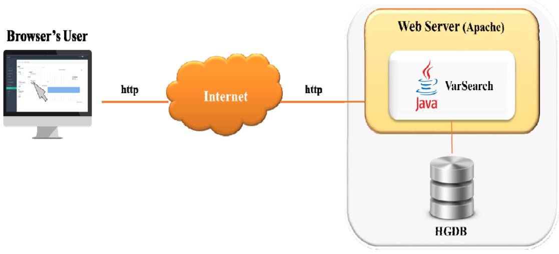


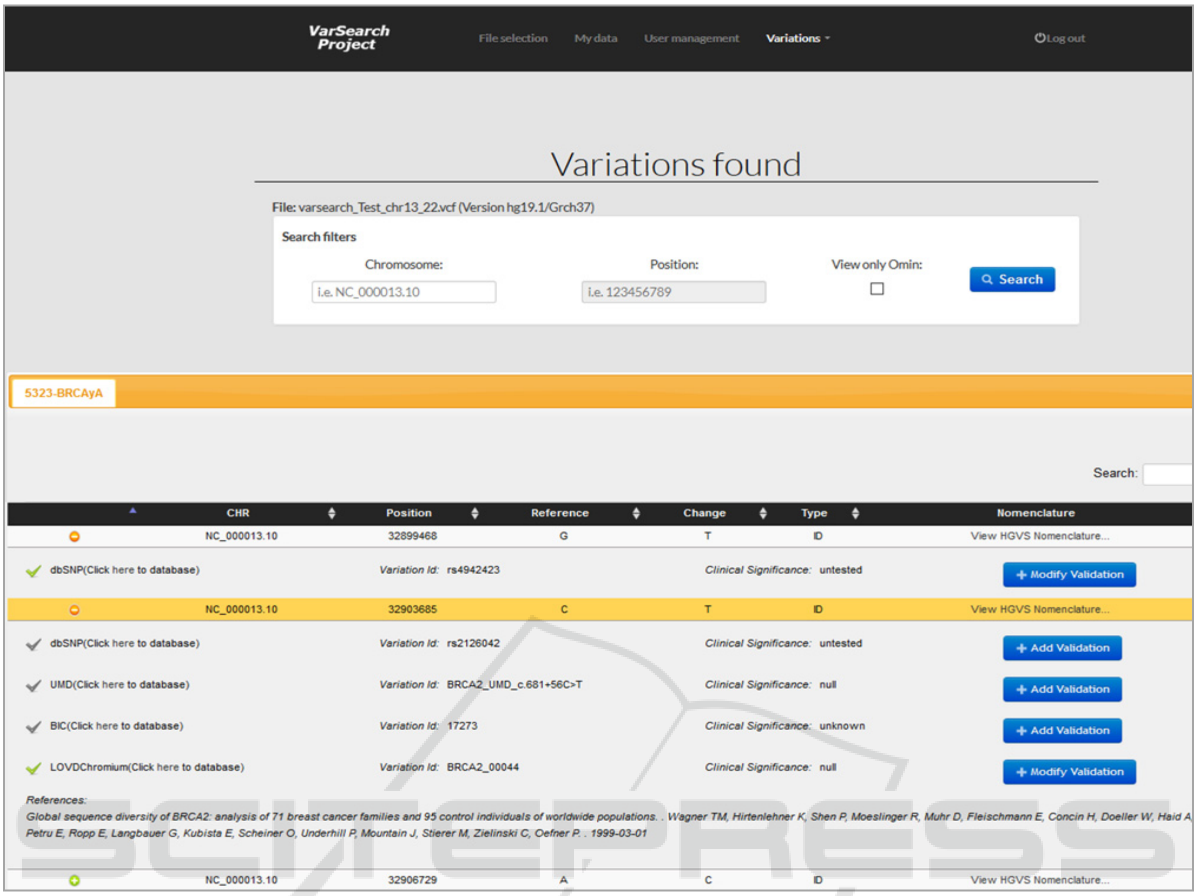
Figure 4: *VarSearch* Architecture.

- The file is then processed and parsed. The entries are shown on an HTML table and the variants of each VCF entry can be seen.

- The variations present in the input file can be annotated against the database and the annotated file is downloaded in *.*xls*, *.*csv* and *.*pdf* format or its contents viewed in another HTML table.

To parse the VCF file and annotate the variants, *VarSearch* relies on *snpEff* and *snpSift* (Tolhuis and Wesselink, 2015) tools, and so well tested libraries are used instead of reinventing the wheel. This also ensures VCF standard support, using ANN files for variant annotation. If another type of information is considered useful for annotation and not covered by the procedure described, *VarSearch* uses the "*INFO*" field to introduce the desired values. As *VarSearch* is based on EGF, new genome annotation files can be quickly integrated by developing the proper parser module, either by a custom development or integrating a third-party tool or library.

All the information associated with the variations found in the HGDB can be obtained. For variations in the lists, user validations can be integrated for future searches with the "*Add Validation*" option. Another advantage of *VarSearch* is user management (new users can be created and edited using the "*User Management*" option). One of the objectives of *VarSearch* is to continue the extension and implementation of all the knowledge defined in the CMHG, such as the treatment of pathways and metabolic routes (Reyes Román et. al., 2017). This tool facilitates the analysis and search for variations, improving the generation of genomic diagnoses associated with diseases of genetic origin. End-users will find the web application easy to use and they are guaranteed security for their data.



Variations found

File: varsearch_Test_chr13_22.vcf (Version hg19.1/Grch37)

Search filters

Chromosome: i.e. NC_000013.10

Position: i.e. 123456789

View only Omin: □

Search

5323-BRCAyA

Search:

▲	CHR	Position	Reference	Change	Type	Nomenclature
⊖	NC_000013.10	32899468	G	T	ID	View HGVS Nomenclature...
✓ dbSNP(Click here to database)		*Variation Id:* rs4942423		*Clinical Significance:* untested		+ Modify Validation
⊖	NC_000013.10	32903685	C	T	ID	View HGVS Nomenclature...
✓ dbSNP(Click here to database)		*Variation Id:* rs2126042		*Clinical Significance:* untested		+ Add Validation
✓ UMD(Click here to database)		*Variation Id:* BRCA2_UMD_c.681+56C>T		*Clinical Significance:* null		+ Add Validation
✓ BIC(Click here to database)		*Variation Id:* 17273		*Clinical Significance:* unknown		+ Add Validation
✓ LOVDChromium(Click here to database)		*Variation Id:* BRCA2_00044		*Clinical Significance:* null		+ Modify Validation

References:
Global sequence diversity of BRCA2: analysis of 71 breast cancer families and 95 control individuals of worldwide populations. . Wagner TM, Hirtenlehner K, Shen P, Moeslinger R, Muhr D, Fleischmann E, Concin H, Doeller W, Haid A, Petru E, Ropp E, Langbauer G, Kubista E, Scheiner O, Underhill P, Mountain J, Stierer M, Zielinski C, Oefner P. . 1999-03-01

⊕	NC_000013.10	32906729	A	C	ID	View HGVS Nomenclature...

Figure 5: Analysis of VCF file using *VarSearch*.

3 CASE STUDIES USING VARSEARCH

To verify *VarSearch* performance, two case studies were carried out. In the first, *VarSearch* was used to analyse a VCF file. The second compared the time spent on searching for variations manually and using the application. To access the application *VarSearch* users must have an account provided by *Gembiosoft* SME (*http://gembiosoft.com/*). After logging in, a file is selected for analysis. *VarSearch* reads all records and transforms them into variations. These transformations depend on the file information: for example, the FASTA files contain a *genetic sequence* (NG), and so require the reference on which the variation is based to be to the "*NG*" sequence. In contrast, VCF files use *positions relative to chromosomes* ("*NC*"). Once the file records have been converted into variations, the next step is to search for these variations in the HGDB.

After the analysis, the "*variations found*" and "*variations not found*" can be differentiated.

- *Found Variations Management*: Found variations are those extracted from the file in which information has been found in the HGDB, which means that this variation has been found in at least one genomic repository. A found variation has much more information than the variation obtained from the file and allows us to calculate and submit detailed information to the user. Having analysed the VCF file, all the variations found are displayed to the user, in each case calculating the HGVS notation, its data source identifier, clinical significance, and the number of validations and databases found together with their bibliographic references.

  This information is calculated for VCF and FASTA; however, VCF variations are sorted by samples. Figure 5 shows the results obtained by analysing a VCF file with a single sample. For this sample (5323-BRCAyA), a number of variations were found with the corresponding information. A variation can have validations made by users.

The validation column corresponds to the number of validations that each variation has and if a validation is private, only the owner will see it. Another *VarSearch* feature is its support for multiple bibliographical references. A variation can be found in different DBs and may contain different bibliographic references.

- *Not found Variations (insertion and treatment)*: The user who is analysing variations may find a variation in the file, which was not found in the database. Using his experience and knowledge he may consider some variations as relevant despite not being found.

  With *VarSearch* the user can insert the not found variations or any variation considered key to the study. If the user has inserted certain variations that had not been found, on reanalysing the file these inserted variations are compared with the variations in the file, showing the similarities. To differentiate the variations of the different repositories from user variations, the results obtained from the user's experience and the results from years of study of different biomedical databases are differentiated.

3.1 Improved Efficiency and Time in Finding Variations with VarSearch

To validate the effectiveness and performance of the proposed software, some experiments were performed to measure efficiency and time. A study was conducted to compare the time spent searching for variations manually with an automatic search of all the repositories mentioned above using *VarSearch*.

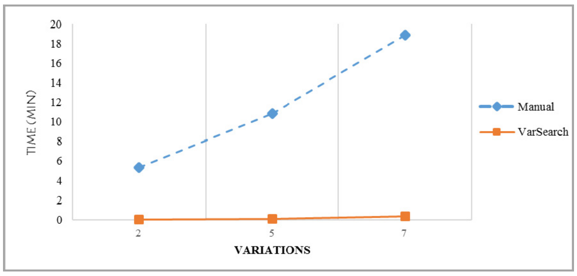


Figure 6: Time optimization.

A manual search of one variation involves detecting the variation in the VCF or FASTA file, a search for the variation in the different databases, and the identification and verification of the variation. *VarSearch* was tested for the time it needed to search for several variations, calculating the time evolution according to the number of variations involved (2, 5 and 7). The results can be seen in Figure 6.

As can be seen in Figure 7, the cost of performing a manual search rises to 5'32 minutes for 2 variations, 10'83 minutes for 5 variations and 18'89 minutes for 7 variations. However, with *VarSearch* the time remains constant at between 2 and 3 seconds for different variations, which confirms its efficient performance. Using this tool thus significantly reduces the time spent on the search for variations. Also, it must be remembered that the manual search process does not calculate additional information for variations. If this information were necessary, the search time would increase significantly, however, with *VarSearch* this time remains constant because this information has already been calculated in the search for variations.

4 CONCLUSIONS

VarSearch is a flexible new analysis framework or web application that provides a powerful resource for exploring both "*coding*" and "*non-coding*" genetic variations. To do this, *VarSearch* integrates VCF format input/output with an expanding set of genome information. *VarSearch* (and other tools built on EGF) will therefore facilitate research into the genetic basis of human diseases.

EGF can also be expected to allow the development of new tools in diverse e-genomics contexts. As genetic laboratories are now oriented to facilitating genetic procedures, web access, usability and feasibility, the definition of different profiles are therefore important goals. All this allows the user to configure the tool according to his specific needs. These necessities include inserting genetic variations and validating its own variations, thus increasing its "*know-how*". *VarSearch* was tested in two different case studies; one focused on the analysis of variations (insert and search) and another to test its search performance.

Future work will be oriented to the implementation of -*haplotypes and statistical factors*- (i.e., frequencies and populations) and improving the next version of *VarSearch* (prototype) for genetic diagnosis. Future research work will also be aimed at the application of Data Quality (DQ) metrics to enhance the HGDB. We also intend to extend the model with studies on the treatment of "*haplogroups*", including subjects with a similar genetic profile who share a common ancestor.

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