HUMAN MUTATION 29(11), 1337–1341, 2008

DATABASES

A Database to Support the Interpretation of Human Mismatch Repair Gene Variants

Jianghua Ou,[1,2] Renée C. Niessen,[1] Jan Vonk,[1] Helga Westers,[1] Robert M.W. Hofstra,[1] and Rolf H. Sijmons[1]*

[1]*Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands;* [2]*Third Department of Tumor Surgery, XinJiang Tumor Hospital, XinJiang Medical University, People's Republic of China*

For the Mutation Pathogenicity Special Issue

Germline mutations in the mismatch repair (MMR) genes *MLH1, MSH2, MSH6,* or *PMS2* can cause Lynch syndrome. This syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominantly-inherited disorder predominantly characterized by colorectal and endometrial cancer. Truncating MMR gene mutations generally offer a clear handle for genetic counseling and allow for presymptomatic testing. In contrast, the clinical implications of most missense mutations and small in-frame deletions detected in patients suspected of having Lynch syndrome are unclear. We have constructed an online database, the Mismatch Repair Gene Unclassified Variants Database (www.mmruv.info), for information on the results of functional assays and other findings that may help in classifying these MMR gene variants. Ideally, such mutations should be clinically classified by a broad expert panel rather than by the individual database curators. In addition, the different MMR gene mutation databases could be interlinked or combined to increase user-friendliness and avoid unnecessary overlap between them. Both activities are presently being organized by the International Society for Gastrointestinal Hereditary Tumours (InSiGHT; www.insight-group.org). Hum Mutat 29(11), 1337–1341, 2008.

KEY WORDS: MLH1; MSH2; MSH6; PMS2; MLH3; mismatch repair; mutation database; Lynch syndrome; hereditary nonpolyposis colorectal cancer; HNPCC; functional assay

INTRODUCTION

Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is an autosomal dominantly-inherited cancer syndrome and is associated with a strongly increased risk of developing colorectal and endometrial cancer and, to a lesser extent, a range of other tumors, including cancer of the small bowel, stomach, ovaries, renal pelvis, ureter, brain, and sebaceous glands. Germline mutations in the mismatch repair (MMR) genes *MLH1* (MIM# 120436), *MSH2* (MIM# 609309), *MSH6* (MIM# 600678), or *PMS2* (MIM# 600259) can cause Lynch syndrome. Germline mutations of the *MLH3* gene (MIM# 603495), another MMR gene, have been reported as well, but their contribution to Lynch syndrome is less clear [Wu et al., 2001; Korhonen et al., 2008]. Lynch syndrome–associated tumors are characterized by DNA MMR deficiency, which can be demonstrated by the presence of microsatellite instability (MSI) in the vast majority of cases. Usually, these tumors have physically or functionally lost the wild-type allele of the MMR gene mutated in the germline, which results in absence of immunohistochemical staining of the MMR protein in question [Lynch et al., 2006].

The detection of truncating MMR gene mutations in patients suspected of having Lynch syndrome generally offers a good clinical handle for diagnosis and genetic counseling and allows for presymptomatic testing in relatives. In contrast, the clinical implications of most missense mutations and small in-frame deletions are unclear (unclassified variants [UVs]). A significant proportion of DNA variations found in Lynch syndrome (suspected) patients are such UVs: 32%, 18%, and 38% for *MLH1*, *MSH2*, and *MSH6*, respectively [Peltomäki and Vasen, 2004]. Investigators and the clinicians confronted with these UVs would be assisted by online resources that store information on these types of variants [Greenblatt et al., 2008]. MMR variant databases already exist: the MMR Genes Variant Database (www.med.mun.ca/MMRvariants) [Woods et al., 2007]; the Leiden Open source Variation Database (LOVD) format (chromium.liacs.nl/LOVD2/home.php) [Fokkema et al., 2005]; the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) mutation database (www.insight-group.org) [Peltomäki and Vasen, 2004]; and the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) [Stenson et al., 2008] are probably the most widely consulted for these genes. Their developers and curators do an impressive job of cataloging published MMR gene variants, as well as unpublished MMR gene

Received 18 March 2008; accepted revised manuscript 15 August 2008.

*Correspondence to: Rolf H. Sijmons, M.D., Ph.D., Department of Genetics, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail: r.h.sijmons@medgen.umcg.nl

Grant sponsor: Department of Genetics of the University Medical Center Groningen; Grant sponsor: Dutch Cancer Society; Grant number: RUG 2002-2678; Grant sponsor: European Union (MMR-Related Cancer Project; LIFESCIHEALTH-5); Grant number: proposal no. 018754.

DOI 10.1002/humu.20907
Published online in Wiley InterScience (www.interscience.wiley.com).

variants (depending on the database in question). However, these databases do not report in great detail the functional aspects and other findings that may help in clinically classifying these MMR variants. Therefore, we set out to build an online database, the Mismatch Repair Gene Unclassified Variants Database (www.mmruv.info), that particularly focuses on these details.

DATABASE STRUCTURE

We searched the literature published in English through Entrez PubMed (www.ncbi.nlm.nih.gov/sites/entrez) using sets of keywords to identify publications on the functional and in silico analyses of human MMR gene mutations and we selected for missense mutations and small in-frame deletions. The reference lists of publications found through this approach were searched for additional relevant papers. From the selected papers, we extracted details on the type of mutation and cross-checked this information whenever possible with the mentioned other MMR gene variant databases. We subsequently extracted details on observed clinical phenotype, family history, results of tumor analysis with respect to MSI testing and immunohistochemical staining for the MMR proteins, frequency of the variants detected in controls, segregation of the mutation within the family, evolutionary conservation, type of functional assays and in silico tests used, and outcome of those analyses. Only those mutations with reported results of functional and/or in silico testing were subsequently included in the database. Variant nomenclature was used as proposed by the Human Variome Project (report by J. den Dunnen, available at www.hgvs.org/mutnomen). Reference sequences used were as listed in the following GenBank (www.ncbi.nlm.nih.gov/GenBank) entries: NM_000249 for *MLH1*, NM_000251 for *MSH2*, NM_000179 for *MSH6*, NM_000535 for *PMS2*, and NM_001040108 for *MLH3*. Depending on the information available, the outcome of each of the functional tests or in silico analyses was labeled with a certain degree of pathogenicity, ranging from nonpathogenic to pathogenic. This labeling was given for research purposes only at this stage, using the interpretation published in the original reports and our expert opinion rather than any formal algorithm. Because, in our opinion, unclassified variants should be clinically classified by a broad and officially recognized expert panel rather than by individual database curators, we have deliberately not provided an overall pathogenicity classification for the variants. We refer to the discussion section of this work for the approach toward such clinical classification. For each variant, if possible, we constructed a link (URL) to the corresponding entry in the MMR Genes Variant Database at the LOVD platform (chromium.liacs.nl/LOVD2/home.php).

The data thus collected were stored first in individual fields in an offline Microsoft Access (Microsoft, Redmond, WA) relational database and subsequently ported to a Microsoft SQL Server online environment. Interfaces for data retrieval by external users and for editing the database were programmed using ASP.NET (Microsoft). A copy of our database is presently being converted to the LOVD format [Fokkema et al., 2005] to test possibilities for linking with the InSiGHT mutation database and the MMR Genes Variant Database. which have already been converted to this format, and also for more easily gaining insight in which gene variants are and which are not shared between these three databases.

FEATURES

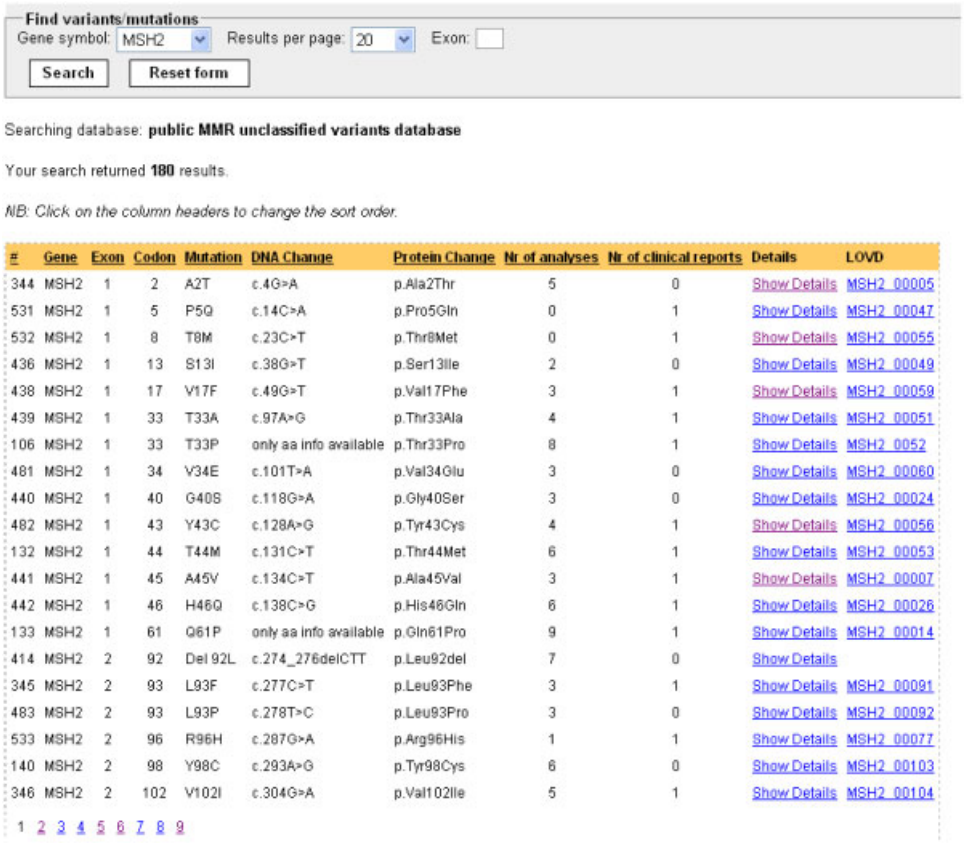
At the time of submission of this work, the database contained information on 513 variants (308 for *MLH1*, 180 for *MSH2*, 15 for *MSH6*, 3 for PMS2 and 7 for *MLH3*). The types of functional studies and their outcome published for these mutations have recently been reviewed in detail elsewhere [Ou et al., 2007]. Based on the functional data, more than one-half of these UVs are probably pathogenic, underlining the clinical importance of studying these UVs [Ou et al., 2007].

The database can be searched at www.mmruv.info through a user interface as shown in Figure 1. The results and print layout can be sorted in many ways by simply clicking on the column headers. The number of functional tests and clinical reports are shown for each of the individual variants. Details can be displayed by clicking on the "Show Details" button (example shown in Fig. 2). New data can be added to the database by the curators through a range of editing tools, but also by external users, after a check by the curators, through a special online form. At the time of submission of this work, only data from peer-reviewed papers had been included in our database. The option of data submission by external users has primarily been developed for researchers that want to assist us in adding their already published data to our database. Presently we will not consider including unpublished data because of the obvious quality control issues. However, we will consider the inclusion of data on additional mutations that have been generated by a research group using the exact methods already reported in one of their peer-reviewed publications. In all cases, unpublished data will be clearly labeled as such in our database. Not all laboratories and clinicians may have access to research groups that perform functional analysis of MMR gene variants. We have therefore added an option to our website by which users can request testing for a particular UV. We will contact these users to discuss details of the mutation and the phenotype, and the laboratory that could be approached for such testing.

DISCUSSION

We have constructed an online database for information on functional assays and other findings, including outcome of in silico testing, that may help in classifying missense variants and small in-frame deletions of the Lynch syndrome–associated MMR genes. For genetic counseling and medical management of the families in which these variants have been detected, it is important that the pathogenicity of these variants is understood. Classifying these variants is notoriously difficult as has recently been reviewed and discussed in an international workshop at the International Agency for Research on Cancer (IARC) in Lyon [Plon et al., 2008]. Functional assays may produce contradictory results [Couch et al., 2008]; segregation may not easily be studied due to lack of DNA from affected relatives in Lynch syndrome–suspected families [Goldgar et al., 2008]; the number of control chromosomes analyzed in regionally- or ethnically-matching populations may be small [Goldgar et al., 2008]; and reported clinical and tumor phenotypes may lack detail [Hofstra et al., 2008]. For many MMR gene UVs, data are incomplete [Lucci-Cordisco et al., 2006].

Before evidence can be weighed and variants classified, data should be available for that purpose; many challenges exist with respect to databasing (i.e., the process of storing, managing, and manipulating large amounts of information/data). Keeping up to date with published reports requires effective and efficient literature-mining techniques. For this purpose, we are in contact with researchers from the Department of Computer Science and Software Engineering at the National Information and Communications Technology (ICT) Australia (NICTA), Victoria Research Laboratory, University of Melbourne (Melbourne, Australia), who may be able to improve our methods of mining relevant literature.



Find variants/mutations

Gene symbol: MSH2 Results per page: 20 Exon:

Search Reset form

Searching database: **public MMR unclassified variants database**

Your search returned **180** results.

NB: Click on the column headers to change the sort order.

#	Gene	Exon	Codon	Mutation	DNA Change	Protein Change	Nr of analyses	Nr of clinical reports	Details	LOVD
344	MSH2	1	2	A2T	c.4G>A	p.Ala2Thr	5	0	Show Details	MSH2_00005
531	MSH2	1	5	P5Q	c.14C>A	p.Pro5Gln	0	1	Show Details	MSH2_00047
532	MSH2	1	8	T8M	c.23C>T	p.Thr8Met	0	1	Show Details	MSH2_00055
436	MSH2	1	13	S13I	c.38G>T	p.Ser13Ile	2	0	Show Details	MSH2_00049
438	MSH2	1	17	V17F	c.49G>T	p.Val17Phe	3	1	Show Details	MSH2_00059
439	MSH2	1	33	T33A	c.97A>G	p.Thr33Ala	4	1	Show Details	MSH2_00051
106	MSH2	1	33	T33P	only aa info available	p.Thr33Pro	8	1	Show Details	MSH2_0052
481	MSH2	1	34	V34E	c.101T>A	p.Val34Glu	3	0	Show Details	MSH2_00060
440	MSH2	1	40	G40S	c.118G>A	p.Gly40Ser	3	0	Show Details	MSH2_00024
482	MSH2	1	43	Y43C	c.128A>G	p.Tyr43Cys	4	1	Show Details	MSH2_00056
132	MSH2	1	44	T44M	c.131C>T	p.Thr44Met	6	1	Show Details	MSH2_00053
441	MSH2	1	45	A45V	c.134C>T	p.Ala45Val	3	1	Show Details	MSH2_00007
442	MSH2	1	46	H46Q	c.138C>G	p.His46Gln	6	1	Show Details	MSH2_00026
133	MSH2	1	61	Q61P	only aa info available	p.Gln61Pro	9	1	Show Details	MSH2_00014
414	MSH2	2	92	Del 92L	c.274_276delCTT	p.Leu92del	7	0	Show Details	
345	MSH2	2	93	L93F	c.277C>T	p.Leu93Phe	3	1	Show Details	MSH2_00091
483	MSH2	2	93	L93P	c.278T>C	p.Leu93Pro	3	0	Show Details	MSH2_00092
533	MSH2	2	96	R96H	c.287G>A	p.Arg96His	1	1	Show Details	MSH2_00077
140	MSH2	2	98	Y98C	c.293A>G	p.Tyr98Cys	6	0	Show Details	MSH2_00103
346	MSH2	2	102	V102I	c.304G>A	p.Val102Ile	5	1	Show Details	MSH2_00104

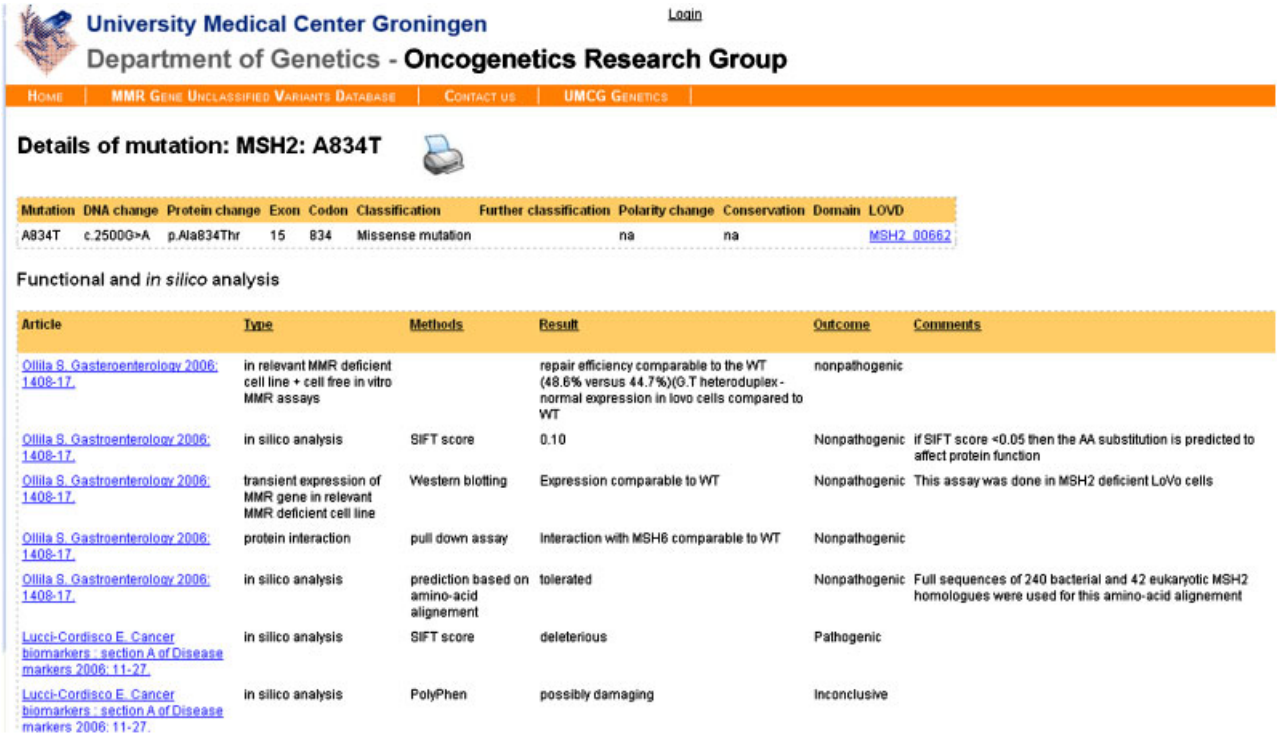
1 2 3 4 5 6 7 8 9

FIGURE 1. **User interface of the search engine of our database. Example of part of the search results for variants of the *MSH2* gene. For each variant the following details are shown: local database identifier (#), gene name, exon and codon number, amino acid change, DNA change, predicted protein change, number of functional tests and/or in silico analyses for this variant in the database, number of papers in the database reporting on the variant in patients and controls, a link to the details of the tests and clinical reports, and, if available, a link to the corresponding entry in the MMR Genes Variant Database at the LOVD platform chromium.liacs.nl/LOVD2/home.php. [Color figure can be viewed in the online issue, which is available at http://www.interscience.wiley.com.]**

Getting *unpublished* data into a central database is another challenge. Several incentives should be developed to stimulate local laboratories or national laboratory professional societies to submit their data to international online databases; for example, inclusion as electronic publication in PubMed for mutations submitted to databases. National, regional, or local legislation and rules with respect to privacy and medical confidentiality may prohibit clinicians and DNA laboratories from submitting detailed phenotypic information on their patients and their families with UVs or other types of mutations. Another issue is interpreting the co-occurrence of multiple MMR gene UVs in individual patients. We and others have observed several of these co-occurrences of missense mutations in series of patients tested because of suspected Lynch syndrome. Most information in databases is listed for single variants, and adjustments should be made to allow information storage and retrieval for combinations of these variants. Needless to say, functional analysis of these combinations of variants is a challenge in itself.

Methods are needed to weigh available evidence in a structured way. One step in this process would be comparative analysis of the available evidence. For example, computational methods based on comparative sequence and or protein structure to classify UVs are not necessarily in agreement [Tavtigian et al., 2008]. Several studies have demonstrated that in silico predictions of *MLH1* and *MSH2* splicing defects can be unreliable and should be complemented by in vivo studies (which may even reveal tissue-dependent splicing) whenever possible [Auclair et al., 2006; Lastella et al., 2006]. If the in silico predictions are concordant, however, then predictive value is much improved, as has been recently been demonstrated by Chan et al. [2007], who compared the outcome of these type of computational methods with the outcome of functional analysis for *MLH1* and *MSH2*.

For *BRCA1* and *BRCA2* variants, Easton et al. [2007] recently developed an algorithm to predict pathogenicity, which takes into account personal and family history of cancer, segregation of the variant in families, co-occurrence with known deleterious mutations, position of the mutation in functional domains, evolutionary conservation, and predicted splice-site involvement. Variants predicted to be pathogenic based on the clinical data also were likely to have high conservation, and more likely to affect splicing and to be located in particular protein domains [Easton et al., 2007]. In another comparative study on *BRCA1* mutations, Lovelock et al. [2007] compared the results from a multifactorial likelihood analysis incorporating evolutionary conservation, segregation in families, co-occurrence with known pathogenic mutations, and histopathology with the outcome of functional analysis. This likelihood analysis was shown to improve classification of some variants, whereas conflicting results from functional analysis were present in others [Lovelock et al., 2007]. Approaches like these may prove of value in the classification of MMR gene variants. They may, for example, help in testing and further developing scoring systems like that of Barnetson et al. [2008], who recently published a system to classify *MLH1*, *MSH2*, and *MSH6* UVs. These authors noted the lack of and contradictory



University Medical Center Groningen
Department of Genetics - Oncogenetics Research Group

Login

HOME | MMR GENE UNCLASSIFIED VARIANTS DATABASE | CONTACT US | UMCG GENETICS

Details of mutation: MSH2: A834T

Mutation	DNA change	Protein change	Exon	Codon	Classification	Further classification	Polarity change	Conservation	Domain	LOVD
A834T	c.2500G>A	p.Ala834Thr	15	834	Missense mutation		na	na		MSH2_00662

Functional and *in silico* analysis

Article	Type	Methods	Result	Outcome	Comments
Ollila S. Gasteroenterology 2006; 1408-17.	in relevant MMR deficient cell line + cell free in vitro MMR assays		repair efficiency comparable to the WT (48.6% versus 44.7%)(G.T heteroduplex - normal expression in lovo cells compared to WT	nonpathogenic	
Ollila S. Gastroenterology 2006; 1408-17.	in silico analysis	SIFT score	0.10	Nonpathogenic	if SIFT score <0.05 then the AA substitution is predicted to affect protein function
Ollila S. Gastroenterology 2006; 1408-17.	transient expression of MMR gene in relevant MMR deficient cell line	Western blotting	Expression comparable to WT	Nonpathogenic	This assay was done in MSH2 deficient LoVo cells
Ollila S. Gastroenterology 2006; 1408-17.	protein interaction	pull down assay	interaction with MSH6 comparable to WT	Nonpathogenic	
Ollila S. Gastroenterology 2006; 1408-17.	in silico analysis	prediction based on amino-acid alignement	tolerated	Nonpathogenic	Full sequences of 240 bacterial and 42 eukaryotic MSH2 homologues were used for this amino-acid alignement
Lucci-Cordisco E. Cancer biomarkers : section A of Disease markers 2006; 11-27.	in silico analysis	SIFT score	deleterious	Pathogenic	
Lucci-Cordisco E. Cancer biomarkers : section A of Disease markers 2006; 11-27.	in silico analysis	PolyPhen	possibly damaging	Inconclusive	

FIGURE 2. **Part of the details screen for the *MSH2* variant A834 T. The original screen, which in addition to more functional and in silico data also summarizes the associated clinical reports, is too large to be shown here. na = data not yet available. [Color figure can be viewed in the online issue, which is available at http://www.interscience.wiley.com.]**

results of functional data and discordant in silico predictions of effects on splicing and protein function of UVs that had been detected in a large series of colorectal cancer patients. Most of the weight in their scoring system is attributed to absence of the UVs in controls, cosegregation of the UV with disease in families, loss of expression of the relevant MMR gene, and presence of tumor MSI in the tumor. Ideally, MMR gene UV mutations should be clinically classified by a broad expert panel. Such a panel and the professional organization behind it might also carry more weight in directing additional research to fill in the data that are needed to classify particular mutations and possibly apply for funding that is needed to support that research.

Lynch syndrome is not a rare disorder; with the increasing availability of mutation analysis, a substantial contribution to the already known pool of unclassified MMR gene variants can surely be expected. The need for clinical classification of these variants will likewise increase. Within the community of the International Society for Gastrointestinal Hereditary Tumours (InSiGHT; www.insight-group.org) there is a strong motivation to address these issues. InSiGHT activities include interconnecting or integrating the existing MMR gene databases and encouraging laboratories and clinicians to add unpublished mutations to those databases. InSiGHT has also recently brought together a group of experts in its new MMR gene variant interpretation committee (chair, Prof. Maurizio Genuardi), which aims at reviewing evidence for variant pathogenicity, working toward a classification algorithm, and ultimately providing clinical classification for each of the MMR gene UVs. These joint activities also act as a pilot project of the Human Variome Project (www.humanvariomeproject.org) [Cotton et al., 2007], and their outcome may help those interested in other disorders and genes to develop their own strategies of addressing the problem of gene UVs.

ACKNOWLEDGMENTS

We thank Jan Herman Veldkamp (www.aardworm.com) for his assistance in developing the website. We thank Professor Finlay Macrae, secretary of InSiGHT (www.insight-group.org) for his comments on the original version of this manuscript. Part of the research for this database was financially supported by a grant from the Dutch Cancer Society (RUG 2002-2678). The technical development of the website was financially supported by a grant from the European Union (MMR-Related Cancer Project; LIFE-SCIHEALTH-5, proposal no. 018754). Curation of this database is structurally financially supported by the Department of Genetics of the University Medical Center Groningen.

REFERENCES

Auclair J, Busine MP, Navarro C, Ruano E, Montmain G, Desseigne F, Saurin JC, Lasset C, Bonadona V, Giraud S, Puisieux A, Wang Q. 2006. Systematic mRNA analysis for the effect of MLH1 and MSH2 missense and silent mutations on aberrant splicing. Hum Mutat 27:145–154.

Barnetson RA, Cartwright N, van Vliet A, Haq N, Drew K, Farrington S, Williams N, Warner J, Campbell H, Porteous ME, Dunlop MG. 2008. Classification of ambiguous mutations in DNA mismatch repair genes identified in a population-based study of colorectal cancer. Hum Mutat 29:367–374.

Chan PA, Duraisamy S, Miller PJ, Newell JA, McBride C, Bond JP, Raevaara T, Ollila S, Nyström M, Grimm AJ, Christodoulou J, Oetting WS, Greenblatt MS. 2007. Interpreting missense variants: comparing computational methods in human disease genes CDKN2A, MLH1, MSH2, MECP2, and tyrosinase (TYR). Hum Mutat 28:683–693.

Cotton RG 2006 Human Variome Project; Appelbe W, Auerbach AD, Becker K, Bodmer W, Boone DJ, Boulyjenkov V, Brahmachari S, Brody L, Brookes A, Brown AF, Byers P, Cantu JM, Cassiman JJ, Claustres M, Concannon P, Cotton RG, den Dunnen JT, Flicek P, Gibbs R, Hall J, Hasler J, Katz M, Kwok PY, Laradi S, Lindblom A, Maglott D, Marsh S,

Masimirembwa CM, Minoshima S, de Ramirez AM, Pagon R, Ramesar R, Ravine D, Richards S, Rimoin D, Ring HZ, Scriver CR, Sherry S, Shimizu N, Stein L, Tadmouri GO, Taylor G, Watson M. 2007. Recommendations of the 2006 Human Variome Project meeting. Nat Genet 39:433–436.

Couch FJ, Rasmussen L, Hofstra R, Monteiro AANM, Greenblatt MS, de Wind N; IARC Unclassified Genetic Variants Working Group. 2008. Assessment of functional effects of unclassified genetic variants. Hum Mutat 29:1314–1326.

Easton DF, Deffenbaugh AM, Pruss D, Frye C, Wenstrup RJ, Allen-Brady K, Tavtigian SV, Monteiro ANA, Iversen ES, Couch FJ, Goldgar DE. 2007. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the *BRCA1* and *BRCA2* breast cancer–predisposition genes. Am J Hum Genet 81:873–883.

Fokkema IF, den Dunnen JT, Taschner PE. 2005. LOVD: easy creation of a locus-specific sequence variation database using an "LSDB-in-a-box" approach. Hum Mutat 26:63–68.

Goldgar DE, Easton DF, Byrnes GB, Spurdle AB, Iversen ES, Greenblatt MS; IARC Unclassified Genetic Variants Working Group. 2008. Integration of various data sources for classifying uncertain variants into a single model. Hum Mutat 29:1265–1272.

Greenblatt MS, Brody LC, Foulkes W, Genuardi M, Hofstra R, Plon S, Sijmons RH, Sinilnikova OM, Spurdle AB; IARC Unclassified Genetic Variants Working Group. 2008. Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. Hum Mutat 29:1273–1281.

Hofstra RW, Spurdle AB, Eccles D, Foulkes WD, de Wind N, Hoogerbrugge N, Hogervorst FBL; IARC Unclassified Genetic Variants Working Group. 2008. Tumor characteristics as an analytic tool for classifying genetic variants of uncertain clinical significance. Hum Mutat 29:1292–1303.

Korhonen MK, Vuorenmaa E, Nyström M. 2008. The first functional study of MLH3 mutations found in cancer patients. Genes Chromosomes Cancer 47:803–809.

Lastella P, Surdo NC, Resta N, Guanti G, Stella A. 2006. In silico and in vivo splicing analysis of MLH1 and MSH2 missense mutations shows exon- and tissue-specific effects. BMC Genomics 7:243.

Lovelock PK, Spurdle AB, Mok MTS, Farrugia DJ, Lakhani SR, Healey S, Arnold S, Buchanan D; kConFab Investigators, Couch FJ, Henderson BR, Goldgar DE, Tavtigian SV, Chenevix-Trench G, Brown MA. 2007. Identification of BRCA1 missense substitutions that confer partial functional activity: potential moderate risk variants? Breast Cancer Res 9:R82.

Lucci-Cordisco E, Boccuto L, Neri G, Genuardi M. 2006. The use of microsatellite instability, immunohistochemistry and other variables in determining the clinical significance of MLH1 and MSH2 unclassified variants in Lynch syndrome. Cancer Biomark 2:11–27.

Lynch HT, Boland RC, Gong G, Shaw TG, Lynch PM, Fodde R, Lynch JF, de la Chapelle A. 2006. Phenotypic and genotypic heterogeneity in the Lynch syndrome: diagnostic, surveillance and management implications. Eur J Hum Genet 14:390–402.

Ou J, Niessen RC, Lützen A, Sijmons RH, Kleibeuker JH, de Wind N, Rasmussen LJ, Hofstra RMW. 2007. Functional analysis helps to clarify the clinical importance of unclassified variants in DNA mismatch repair genes. Hum Mutat 28:1047–1054.

Peltomäki P, Vasen H. 2004. Mutations associated with HNPCC predisposition—update of ICG-HNPCC/INSiGHT mutation database. Dis Markers 20:269–276.

Plon SE, Eccles DM, Easton DF, Foulkes W, Genuardi M, Greenblatt MS, Hogervorst FBL, Hoogerbrugge N, Spurdle AB, Tavtigian S; IARC Unclassified Genetic Variants Working Group. 2008. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 29:1282–1291.

Stenson PD, Ball E, Howells K, Phillips A, Mort M, Cooper DN. 2008. Human Gene Mutation Database: towards a comprehensive central mutation database. J Med Genet 45:124–126.

Tavtigian SV, Greenblatt MS, Lesueur F, Byrnes GB; IARC Unclassified Genetic Variants Working Group. 2008. In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat 29:1327–1336.

Woods MO, Williams P, Careen A, Edwards L, Bartlett S, McLaughlin JR, Banfield Younghusband H. 2007. A new variant database for mismatch repair genes associated with Lynch syndrome. Hum Mutat 28:669–673.

Wu Y, Berends MJW, Sijmons RH, Mensink RGJ, Verlind E, Kooi KA, van der Sluis T, Kempinga C, van der Zee AGJ, Hollema H, Buys CHCM, Kleibeuker JH, Hofstra RMW. 2001. A role for *MLH3* in hereditary nonpolyposis colorectal cancer. Nat Genet 29:137–138.