Proposal for a new therapy for drug-resistant malaria using *Plasmodium* synthetic lethality inference ☆

Sang Joon Lee [a,*], Eunseok Seo [a], Yonghyun Cho [b]

[a] Center for Biofluid and Biomimic Research, Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology (POSTECH), San 31, Hyoja Dong, Namgu, Pohang 790-784, Republic of Korea
[b] Samsumg SDS, Trade Center ASEM Tower, Samseong 1-dong, Gangnam-gu, Seoul 135-798, Republic of Korea

ARTICLE INFO

ABSTRACT

Many antimalarial drugs kill malaria parasites, but antimalarial drug resistance (ADR) and toxicity to normal cells limit their usefulness. To solve this problem, we suggest a new therapy for drug-resistant malaria. The approach consists of data integration and inference through homology analysis of yeast–human–*Plasmodium*. If one gene of a *Plasmodium* synthetic lethal (SL) gene pair has a mutation that causes ADR, a drug targeting the other gene of the SL pair might be used as an effective treatment for drug-resistant strains of malaria. A simple computational tool to analyze the inferred SL genes of *Plasmodium* species (malaria parasites *Plasmodium falciparum* and *Plasmodium vivax* for human malarial therapy, and rodent parasite *Plasmodium berghei* for *in vivo* studies of human malarias) was established to identify SL genes that can be used as drug targets. Information on SL gene pairs with ADR genes and their first neighbors was inferred from yeast SL genes to search for pertinent antimalarial drug targets. We not only suggest drug target gene candidates for further experimental validation, but also provide information on new usage for already-described drugs. The proposed specific antimalarial drug candidates can be inferred by searching drugs that cause a fitness defect in yeast SL genes.

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Malaria is one of the most serious causes of morbidity and mortality in tropical regions. The World Health Organization recently estimated that malaria causes about 240 million bouts of illness and around 0.86 million deaths annually (http://www.who.int/mediacentre/factsheets/fs094/en/print.html, world health organization malaria fact sheet No. 94, 2010). Several *Plasmodium* species (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*) have been identified to be responsible for a significant number of human malaria infections in Southeast Asia. Among these species, *P. falciparum* is the most severe and responsible for about 90% of malaria deaths (Cox-Singh et al., 2008), predominantly in Africa (Eisenstein, 2012).

P. vivax has a considerably lower mortality rate but a far greater geographical distribution. It causes widespread malaria outside Africa, mainly afflicting Asia and the Americas (Price et al., 2007). The human malaria parasite *P. vivax* is responsible for 25–40% of the annual bouts of malaria worldwide. Although infection by the parasite is seldom fatal, such infection induces severe, debilitating clinical symptoms and often causes relapses months after a primary infection is cleared. Despite being a major human pathogen, studies on *P. vivax* are very limited compared with *P. falciparum* because *P. vivax* is not contagiously propagated in the laboratory and *in vitro* culture is not amenable (Carlton et al., 2008).

The major problem with the currently available antimalarial drugs is that some malaria parasites have continuously developed resistance to these drugs. Several special treatments for specific malarial strains have been introduced. However, the parasites have become increasingly resistant to conventional antimalarial drugs, thereby increasing morbidity and mortality. Numerous malarial drugs kill malaria parasites, but antimalarial drug resistance (ADR) and toxicity to normal cells limit their usefulness. Such emerging drug resistance and a lack of effective vaccines require urgent discovery of new antimalarial drugs and vaccines against malarial parasites (Anderson, 2009; Huthmacher et al., 2010). Most malaria researchers are eager to identify and act on drug target

* Corresponding author. Tel.: +82 54 279 2169; fax: +82 54 279 3199.
E-mail address: sjlee@postech.ac.kr (S.J. Lee).

genes. However, ideal drug and vaccine targets are not easy to determine. Currently, the systematic disruption of every gene in *Plasmodium* is technically difficult. A computational approach to prioritizing potential drug targets was recently established (Yeh et al., 2004). As massive screening methods, bioinformatics and computational approaches play predominant roles in drug design. Nevertheless, a novel strategy is required to overcome the technological limitations encountered by the current single-drug approach for effective treatments (Fernández et al., 2009).

We therefore investigated targeting synthetic lethal (SL) gene pairs as an alternative antimalarial drug therapy. When the mutation of either component of a gene pair is not lethal but the mutation of both leads to death or a significant decrease in the fitness of the organism, the two genes are called synthetic lethal (Conde-Pueyo et al., 2009). Fig. 1 represents the rationale for SL applications in antimalarial therapy. Given that malarial parasites exhibit various types of drug resistance, the identification of proper SL gene partners may be helpful in finding specific antimalarial drug targets. Such identification provides new insights into the design of a selective antimalarial therapy by exploiting SL partners that have ADR genes. By filtering out the SL targets that have analogues of the human proteome, minor damage is expected in the human cell, as shown in the conceptual schematics of selective antimalarial therapy (Fig. 1). Therefore, this method would be helpful in dramatically overcoming the limitations in the design of a suitable malarial drug. However, large-scale screenings for SL gene pairs have been performed mostly in yeast (Forsburg, 2001; Pan et al., 2004; Ooi et al., 2006; Boone et al., 2007), and to a lesser extent in *Caenorhabitis elegans* (Jorgensen and Mango, 2002; Baugh et al., 2005; Lehner et al., 2006) and other organisms. Screening research on *Plasmodium* SL gene pairs has yet to be accomplished.

Consequently, we investigated *Plasmodium* SL gene pair candidates in the present study using inference methods. Fig. 2 shows the basic concept of searching for drug targets for antimalarial therapy. SL screening in yeast is often used to identify the genes involved in cell polarity, secretion, DNA repair, and the cell cycle
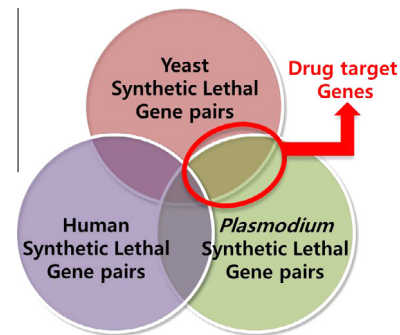

Fig. 2. Information diagram showing the basic concept of searching for drug targets for use in malarial therapy and a new SL therapy for the elimination of antimalarial drug-resistant *Plasmodium*. Information diagram of the basic concept of a drug target search for malarial therapy. In this study, the phylogenetic inference of synthetic lethal (SL) genes extracted from yeast and malaria parasites was utilized for pharmacologic purposes because most existing SL data are restricted to yeast screenings. SL pairs with human orthologs were not considered.

(Tong et al., 2001, 2004). Considering the high conservation of genome integrity and cell cycle-related genes from yeast to higher organisms (Yuen et al., 2007), a massive screening for yeast SL interactions may provide information on using SL inference to search for novel malarial therapies. The present study aimed not to discuss the general inference method for the SL network from one organism to another, but to present the rationale for drug design by supplying suitable *Plasmodium* SL gene pairs that can be used as a potential basis for future pharmacologic tests. Another objective was to provide an excellent framework for handling very large genetic systems. In particular, studies on genetic diseases (Goh et al., 2007; Platzer et al., 2007), gene targets (Yildirim et al., 2007), and SL networks (Boone et al., 2007; Paladugu et al., 2008; Chipman and Singh, 2009) have contributed to the understanding of these systems as a whole. In the present study, network
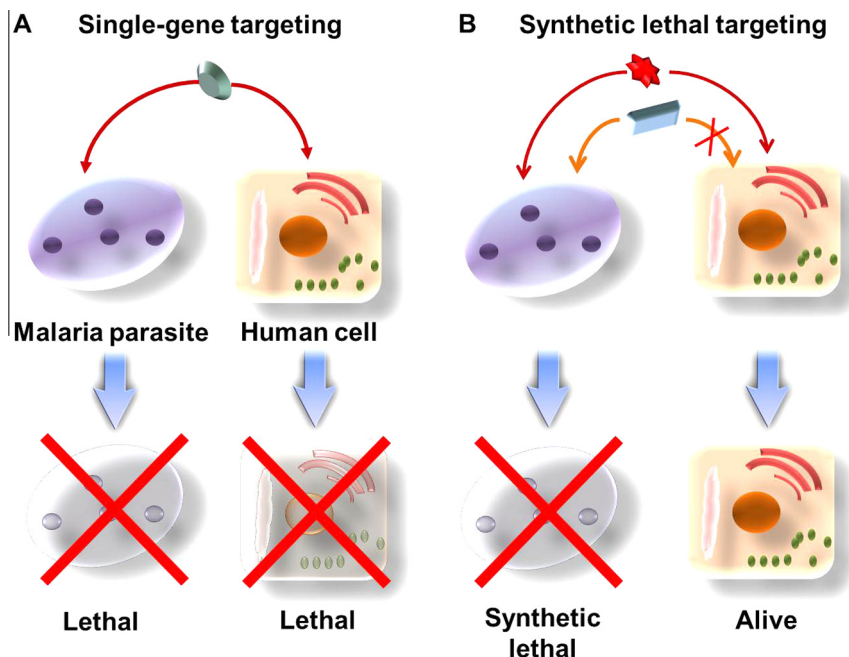
Fig. 1. Rationale for SL applications in antimalarial therapy. Rationale for synthetic lethality applied in the design of novel antimalarial therapies. Two genes are synthetic lethal (SL) if the mutation of either gene alone is compatible with viability; the mutation of both leads to death. (A) Single gene targeting is more likely to express toxicity in both the malaria parasite and the human cell. (B) If one gene of the SL pair is mutated and the other SL partner is not mutated, the human cells would not die. However, if both SL partners are inactivated in the malaria parasite, the parasite is selectively damaged.

framework data from several databases were integrated to increase the reliability of the supplied candidates.

2. Materials and methods

Potential antimalarial drug target genes were identified by integrating whole biological information from many databases. Fig. 3 illustrates the methodology used for data collection and the selection of potential antimalarial drug target genes. The phylogenetically inferred *Plasmodium* SL gene network was reconstructed by using phylogenetic analysis and database manipulation to search for potential antimalarial drug targets. The databases used include BioGRID (Biological General Repository for Interaction Datasets) (http://thebiogrid.org) for yeast SL genes, KEGG SSDB (Kyoto Encyclopedia of Genes and Genomes Sequence Similarity DataBase) (http://www.genome.jp/kegg), KEGG OC (Ortholog Cluster) (http://www.genome.jp/tools/oc) (Nakaya et al., 2013), PlasmoDB Version 9.1 (http://plasmodb.org), and Gene DB (http://www.genedb.org) for *Plasmodium* genes. The inferred *Plasmodium* SL networks were constructed using Cytoscape (version 2.8.1) (Shannon et al., 2003).

2.1. Inference of Plasmodium SL genes from yeast SL genes

The list of yeast SL gene pairs was obtained from the 'BIOGRID-ALL-3.1.72.tab2' data source provided by the BioGRID database. The collected data on yeast genetic screens included synthetic lethality. The yeast SL network was constructed using the yeast SL pairs. Then, three *Plasmodium* species (*P. falciparum*, *P. vivax*, and *P. berghei*) SL gene networks were inferred from the yeast SL gene data. *Plasmodium*–yeast gene ortholog data were obtained from the ortholog information in the KEGG SSDB. Among the yeast genes (14,548 genes in total), approximately 29% were SL genes (4,284 genes). *Plasmodium* gene information, such as gene expression levels and stages, were obtained from the PlasmoDB version 9.1 (PlasmoDB: http://plasmodb.org/plasmo/webcite, Aug. 31, 2012).

2.2. Extraction of ADR genes from inferred Plasmodium SL genes

We identified eight *P. falciparum* ADR genes (*PfABC*, *PfCRT*, *PfDHFR-TS*, *PfDHPS*, *PfMT*, *PfMDR1*, *PfMDR2*, and *PfNHE1*) through a systematic literature survey (Wilson et al., 1989; Foote et al., 1990; Wellems et al., 1990; Cowman et al., 1991, 1994; Peel et al., 1993, 1994; Zalis et al., 1993; Rubio and Cowman, 1996; Reed et al., 2000; Mutabingwa et al., 2001; Sibley et al., 2001; Giancarlo et al., 2009; Briolant et al., 2012). By phylogenetic inference, four ADR genes (*PfDHFR-TS*, *PfMDR1*, *PfMDR2*, and *PfNHE1*) were discovered to contain the inferred SL genes. *PfDHFR-TS* exhibits a homologous relation with *YBR205W* and *YOR074C*. Pfmdr1 has a homologous relation with *YKL209C*, *YGR281W*, *YKL188C*, and *YLL048C*. *PfMDR2* has a homologous relation with *YMR301C*. *PfNHE1* has a homologous relation with *YHR081W* and *YDR456W*. Detailed information is included in Supplementary file 1.

2.3. Search for Plasmodium genes without similarity to human genes

We searched for information about protein similarities in the Protein Data Bank (PDB) provided by PlasmoDB. *Plasmodium* genes which do not have protein similarities to human genes were selected. The protein structure data in the PDB have been used actively in the studies of protein function, evolution, and structure prediction (Bernstein et al., 1977; Noguchi and Akiyama, 2003). Information on the *Plasmodium* genes which do not have human orthologs was obtained from the KEGG OC database. Detailed information is included in Supplementary file 2.

2.4. Search for drug candidates

Fitness data from the Yeast Fitness DB (http://fitdb.stanford.edu, 2012) (Hillenmeyer et al., 2008, 2010) on the yeast homologous genes of the selected *Plasmodium* genes were searched to find new drugs for the clinical treatment of malaria. A list of drug candidates was obtained through the top three experiments that gave rise to a fitness defect in yeast genes by heterozygous or homozygous deletion. To utilize the yeast drugs provided by the FitDB, we conjectured the possibility of evolutionary conservation between the yeast genes and *Plasmodium* homologs by identifying protein similarities in the PDB. From the list of drug candidates, we selected the final drug candidates by filtering out the compounds which do not exist in the DrugBank 3.0 database (http://www.drugbank.ca) (Knox et al., 2011). A detailed report is available in Supplementary file 3.

2.5. Statistical analysis of gene function

The networks associated with gene ontology (GO) annotation (GOA) terms were statistically analyzed using BINGO 2.44
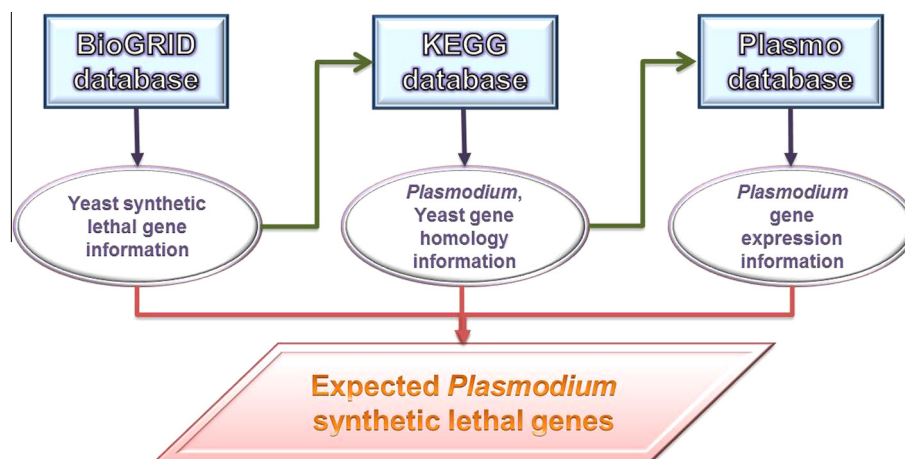
Fig. 3. Schematic diagram of the synthetic lethal (SL) gene identification methodology. Biological information from different databases including BioGRID, KEGG, and PlasmoDB were integrated. To search for potential antimalarial drug targets, the inferred *Plasmodium* SL gene network was reconstructed by phylogenetic analysis and database manipulation. The databases employed in this study were BioGRID for yeast SL genes, as well as KEGG and PlasmoDB for *Plasmodium* genes.

Cytoscape plugging (Maere et al., 2005). BINGO is a useful tool for determining statistically over-represented GO categories in a set of genes or subgraphs of a biological network. *P. falciparum* GOA data were obtained using BINGO. The GO biological process, molecular functions, and cellular components were selected from the ontology list, whereas *P. falciparum* was selected from the organism list. Finally, the over-represented GO categories were analyzed using Cytoscape. The gene functions of the inferred *P. falciparum* SL genes, as well as the ADR genes and their first neighbors, were analyzed from the inferred *P. falciparum* gene networks using BINGO.

The significance of their cellular components, molecular functions, and biological processes was evaluated using a hypergeometric test, in which the Benjamini and Hochberg false discovery rate with a *P* value less than 0.05 was used as the minimum cutoff threshold. Using this method, only 608 genes out of a total of 1,423 inferred *P. falciparum* SL genes were found to be associated with GOA terms. Only 23 genes out of 50 ADR genes and their first neighbors extracted from the inferred *P. falciparum* gene network were associated with GOA terms. The extracted list of genes related to specific GOA terms is available in Supplementary file 4. In addition, we analyzed the gene functions of the genes selected ADR genes and their first neighbors from the inferred *Plasmodium* gene network using the *Plasmodium* genomics resource in the PlasmoDB. A detailed report including the list of genes related to specific GOA terms is available in Supplementary files 2 and 3.

3. Results

3.1. Inference of Plasmodium SL genes from yeast SL genes

We inferred human and *Plasmodium* SL gene pairs from yeast SL gene pairs. A total of 4284 yeast SL genes were obtained from the BioGRID database (version 3.1.72, http://thebiogrid.org). We then inferred the *Plasmodium* (*P. falciparum*, *P. vivax*, and *P. berghei*) SL gene pairs from the yeast SL gene pairs using the KEGG database. The inferred *P. falciparum*, *P. vivax*, and *P. berghei* SL networks are composed of 1536 nodes and 7600 links, 1449 nodes and 6458 links, as well as 1299 nodes and 7608 links, respectively.

3.2. Extraction of ADR genes from inferred Plasmodium SL genes

Due to mutations in ADR genes, some antimalarial are not effective in certain patients. Through a literature survey, we identified eight ADR genes: *P. falciparum* ABC Transporter (*PfABC*), *P. falciparum* putative metabolite/drug transporter (*PfMT*), *P. falciparum* dihydropteroate synthase (*PfDHPS*), *P. falciparum* multidrug resistance 1 (*PfMDR1*), *P. falciparum* multidrug resistance 2 (*PfMDR2*), *P. falciparum* sodium/hydrogen exchanger 1 (*PfNHE1*), *P. falciparum* dihydrofolate reductase-thymidylate synthase (*PfDHFR-TS*), and *P. falciparum* chloroquine-resistance transporter gene (*PfCRT*) (Wilson et al., 1989; Foote et al., 1990; Wellems et al., 1990; Cowman et al., 1991; Peel et al., 1993, 1994; Zalis et al., 1993; Cowman et al., 1994; Rubio and Cowman, 1996; Reed et al., 2000; Mutabingwa et al., 2001; Sibley et al., 2001; Giancarlo et al., 2009; Briolant et al., 2012). Four genes (*PfMDR1*, *PfMDR2*, *PfDHFR-TS*, and *PfNHE1*) among the eight *P. falciparum* ADR genes are inferred as *Plasmodium* SL genes.

3.3. Extraction of of ADR genes' SL partners

The SL partners of the ADR genes were extracted from the yeast SL gene pairs. Fig. 4A and B show typical combinations of *Plasmodium* SL pairs inferred from the yeast SL pairs. Each entry in the homology data file contains the gene's evolutionary history, which

corresponds to a gene tree diverging from a common ancestor (Conde-Pueyo et al., 2009). In this study, the gene conservation relations in yeast and *Plasmodium* are categorized into three types. The simplest is the one-to-one relation between yeast and *Plasmodium* genes (orthologous relation). However, evolutionary duplication events lead to two alternative types; one yeast gene has more than one *Plasmodium* homolog (one-to-*m* relation), and vice versa (*n*-to-one relation). The *n*-to-*m* relation corresponds to the case where *n Plasmodium* genes are homologs of *m* yeast genes (Sonnhammer and Koonin, 2002). The *Plasmodium* genes selected in this study have one-to-one and one-to-*n* (*Plasmodium* to yeast) relations. These relations include auto-loop genes (marked *x* in Fig. 4) as SL partners; when an SL pair was composed of the same single *Plasmodium* gene, we defined it as an 'auto-loop gene'. We discovered four auto-loop genes in *P. falciparum*, one in *P. vivax*, and two in *P. berghei* from the inferred SL networks composed of ADR genes and their SL partners. The auto-loop nodes are represented as yellow background genes in Supplementary file 1. In this study, the SL pairs containing auto-loop genes were excluded from the list of drug target candidates.

3.4. Networks consisting of ADR genes and their SL partners

Fig. 4C shows the networks composed of four ADR genes and their inferred SL partners as inferred from the yeast SL gene networks. Detailed information is given in Supplementary files 1. The inferred SL networks composed of ADR genes and their SL partners have 50 nodes and 86 links in *P. falciparum*, 17 nodes and 27 links in *P. vivax*, as well as 15 nodes and 19 links in *P. berghei*. Octagons denote ADR-related nodes and circles indicate their SL partners. The links connecting the ADR genes are depicted as blue lines. *PfNHE1*, *PfMDR1*, *PfMDR2* and *PfDHFR-TS* are four representative ADR genes inferred as SL genes. Red indicates the *PfMDR1* gene and its inferred SL partners, yellow represents the *PfDHFR-TS* gene and its inferred SL partners, green indicates the *PfMDR2* gene and its inferred SL partners, and blue indicates the *PfNHE1* gene and its inferred SL partners. *PF11_0192* (orange circle) is the inferred SL partner of the *PfMDR1* (red octagon) and *PfDHFR-TS* (yellow octagon) genes. Four ADR genes and their 46 SL partners were extracted from the inferred *P. falciparum* SL gene network. Meanwhile, 4 ADR genes and 13 SL partners were selected from the inferred *P. vivax* network, whereas four ADR genes and 11 partners were extracted from the inferred *P. berghei* network. Detailed functional information on the drug target candidates selected from the PlasmoDB are summarized and provided in Table 1 and Supplementary file 2. In this study, we selected genes which do not have protein similarities between *Plasmodium* genes and their human orthologs. Interestingly, almost all of the selected genes are connected to interrelated biological functions, such as ATPase activity, ATP binding, and ion transportation.

In Table 1, we compare the GO terms of yeast SL genes with that of the *Plasmodium* SL genes inferred from yeast SL gene pairs. The GO terms of *PFD0830w*, *PVX_089950*, and *PB000415.02.0* (*Plasmodium* homologous genes of ADR gene- *PfDHFR-TS*) match the GO terms of *YOR074C* (yeast homolog gene of *PFD0830w*, *PVX_089950*, and *PB000415.02.0*) well. Their biological process is the dTMP biosynthetic process and their molecular function is thymidylate synthase activity. The GO terms of the biological process, molecular function, and cellular component of *PVX_118100* (*P. berghei* homologous genes of ADR gene: *PfMDR2*) and *YMR301C* (yeast homologous gene of *PVX_118100*) are particularly well matched. Their biological process is transmembrane transport and their molecular function is ATPase activity. In addition, the cellular component is an integral factor to the membrane, and the GO terms of the biological process (glutathione biosynthetic process) and the molecular function (glutamate–cysteine ligase activity)
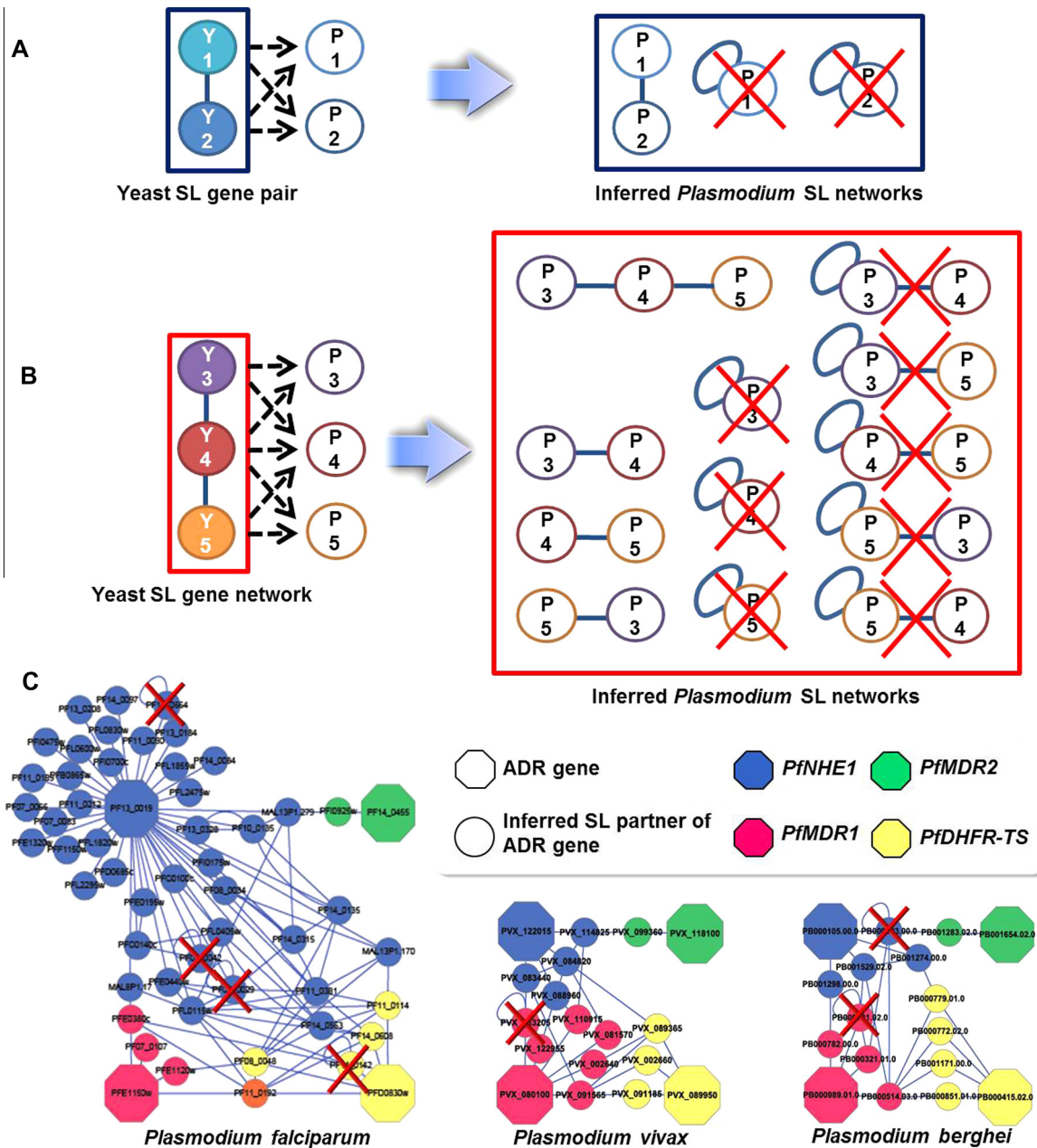
Fig. 4. Networks of ADR genes and their SL partners extracted from the inferred *Plasmodium* SL genes. *Plasmodium* SL gene pairs were inferred from yeast SL pairs. In this network, nodes represent genes and the link between them indicates an SL interaction. When both are simultaneously mutated, an SL condition is satisfied. Gene conservation between *Plasmodium* and yeast can be categorized into two types of relations. The simplest case is a one-to-one relation between *Plasmodium* and yeast genes (orthologous relation). However, duplication events during evolution cause a one-to-n relation in which two or more yeast genes are homologous to one *Plasmodium* gene. (A) For example, the one-to-one case corresponds to one yeast gene having one *Plasmodium* homolog; the SL pairs Y1 and Y2 correspond to SL pair P1 and P2. In the one-to-n case, several yeast genes have the same *Plasmodium* homolog; SL pairs Y1 and Y2 can correspond to only one gene between P1 and P2. In this case, an autolink (auto-loop) is formed in the network. (B) For yeast SL networks represented by Y3, Y4, and Y5, various relations such as one-to-one, n-to-one, auto-loop, and SL pair-containing auto-loop genes can be combined. (C) Networks of the four inferred ADR genes in the *Plasmodium* species. When two SL yeast partners are phylogenetically related to a single *Plasmodium* gene, an auto-link appears in the SL network. Single gene targeting is possibly more dangerous than double gene targeting. Therefore, we eliminated the auto-loop nodes from the drug target candidates and denoted them as red X's. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of *PVX_099360* (*PVX_118100*'s neighbor gene) and *YJL101C* (yeast homologous gene of *PVX_099360*) match each other. In Table 2, *PVX_099360*, which is the neighbor gene of *PVX_118100* (ADR gene: *PfMDR2*), is a putative gamma-glutamylcysteine synthetase. *YJL101C* is a yeast homolog gene of *PVX_099360*. In addition, using the PlasmoDB, we investigated the similarities of the protein chains between the yeast and its plasmodium. *YJL101C* and *PVX_099360* were seen to exhibit protein similarities to glutamate–cysteine ligase with an identity of 32% and a P value of 7.6×10^{-64}.

3.5. Prediction of antimalarial drug targets

To narrow down the list of drug target candidates, we examined the developmental stages in which *Plasmodium* genes were expressed (Supplementary file 1). In addition, we searched for the protein similarities within the protein databank (PDB) provided by the PlasmoDB. These ADR genes and their SL partners extracted from the inferred *Plasmodium* SL gene networks can serve as alternative antimalarial drug targets for malaria patients with ADR genes. In addition, we conjectured the possibility of evolutionary

Table 1
Selected antimalarial drug target genes and their GO terms.

Inferred *Plasmodium* SL genes	*Plasmodium* GO terms	Yeast homolog genes of *Plasmodium*	Yeast GO terms
PB000415.02.0 (ADR gene: *PfDHFR-TS*)	Biological Process: dTMP biosynthetic process, glycine biosynthetic process, one-carbon metabolic process, nucleotide biosynthetic process, oxidation-reduction process; Molecular Function: dihydrofolate reductase activity, thymidylate synthase activity	YOR074C	Biological Process: dTMP biosynthetic process; Molecular Function: thymidylate synthase activity; Cellular Component: nucleus
PB000779.01.0 (*PB000415.02.0*'s neighbor gene)	Molecular Function: nucleic acid binding, DNA binding, helicase activity, ATP binding	YDR334W	Biological Process: chromatin remodeling, histone exchange; Molecular Function: structural molecule activity; Cellular Component: Swr1 complex, nucleus
PFD0830w (ADR gene: *PfDHFR-TS*)	Biological Process: dTMP biosynthetic process, glycine biosynthetic process, one-carbon metabolic process, nucleotide biosynthetic process, oxidation-reduction process; Molecular Function: dihydrofolate reductase activity, thymidylate synthase activity	YOR074C	Biological Process: dTMP biosynthetic process; Molecular Function: thymidylate synthase activity; Cellular Component: nucleus
PF08_0048 (*PFD0830w*'s neighbor gene)	Molecular Function: nucleic acid binding, DNA binding, helicase activity, ATP binding, ATP-dependent helicase activity	YDR334W	Biological Process: chromatin remodeling, histone exchange; Molecular Function: structural molecule activity; Cellular Component: Swr1 complex, nucleus
PVX_089950 (ADR gene: *PfDHFR-TS*)	Biological Process: dTMP biosynthetic process, glycine biosynthetic process, one-carbon metabolic process, nucleotide biosynthetic process, oxidation–reduction process; Molecular Function: dihydrofolate reductase activity, thymidylate synthase activity	YOR074C	Biological Process: dTMP biosynthetic process; Molecular Function: thymidylate synthase activity; Cellular Component: nucleus
PVX_089365 (*PVX_089950*'s neighbor gene)	Molecular Function: nucleic acid binding, DNA binding, helicase activity, ATP binding	YLR085C	Biological Process: chromatin remodeling, histone exchange; Molecular Function: nucleosome binding; Cellular Component: Swr1 complex, cytoplasm
PVX_118100 (ADR gene: *PfMDR2*)	Biological Process: transport, transmembrane transport; Molecular Function: ATP binding, ATPase activity, nucleoside-triphosphatase activity; Cellular Component: integral to membrane	YMR301C	Biological Process: cellular iron ion homeostasis, transmembrane transport; Molecular Function: ATPase activity, coupled to transmembrane movement of substances; Cellular Component: integral to membrane, mitochondrial inner membrane, mitochondrion
PVX_099360 (*PVX_118100*'s neighbor gene)	Biological Process: glutathione biosynthetic process; Molecular Function: glutamate–cysteine ligase activity	YJL101C	Biological Process: glutathione biosynthetic process, response to cadmium ion, response to hydrogen peroxide; Molecular Function: glutamate–cysteine ligase activity; Cellular Component, cytoplasm, intracellular

In this table, we can see detailed functional information on the drug target candidates. We selected genes which do not have protein similarities between *Plasmodium* genes and their human orthologs. Interestingly, almost all of the selected genes are connected to interrelated biological functions, such as ATPase activity, ATP binding, and ion transportation. In this table, we compare the GO terms of yeast SL genes with that of *Plasmodium* SL genes inferred from yeast SL gene pairs. In addition, through PlasmoDB, we investigated similarities to Protein Data Bank chains between the yeast gene and its *Plasmodium* homolog gene. Although these are examples of similarity in the biological process, molecular function, or cellular component of *Plasmodium* evolutionary conserved from yeast, their synthetic lethality might conserve in *Plasmodium* from yeast in the same vein.

conservation between the yeast genes and *Plasmodium* homologs using the same identification process involving protein similarities. We obtained the five inferred SL partners (marked by red loops in Fig. 5) having the inferred *Plasmodium* SL gene networks which have protein similarities among yeast orthologs and *Plasmodium* orthologs. In the three networks, the filtered *Plasmodium* SL pairs (ADR genes and their inferred SL partners) do not include human homologous genes. The filtered *Plasmodium* SL pairs also do not have human orthologs in the KEGG OC database.

3.6. Analysis of biological function of selected drug targets

We carried out a statistical analysis on the biological function of the selected drug targets using the BINGO 2.44 program (Maere et al., 2005). BINGO has been used to determine which GO categories are statistically over-represented in a set of genes or a subset of biological networks. We obtained only *P. falciparum* GO data because the program provides information on the GO of *P. falciparum* genes among the *Plasmodium* species. Fig. 6 shows the relationship

between the biological processes of the *PFE0195W* and *PF13_0019* genes as a typical example of GO term analysis. These genes are the ADR genes and their first neighbors extracted from the inferred *P. falciparum* SL networks. Information on the biological functions of other species (*P. vivax* and *P. berghei*) can be obtained from the PlasmoDB. We analyzed the biological processes, molecular functions, and cellular components of the inferred *P. falciparum* SL genes, the inferred ADR genes, and their SL partners. Detailed information, including the GO terms, is available in Supplementary files 2–4. Table 1 summarizes the biological functions of the selected drug target genes analyzed using the PlasmoDB.

3.7. New malarial drug candidates

The five selected drug target gene pairs are listed in Table 2 and depicted in Fig. 5. According to the information obtained through the identification of protein similarities, the selected SL genes do not have human homologous genes. The drug candidates for the selected drug target genes were inferred from the Yeast Fitness

Table 2
Suggested antimalarial drug target genes and possibility of evolutionary conservation of yeast synthetic lethal genes.

Plasmodium ADR gene	Plasmodium ADR gene's inferred SL partner	Saccharomyces cerevisiae Similarities to Protein Data Bank Chains (ADR gene's inferred SL partner)
PB000415.02.0 (ADR gene: PfDHFR-TS)/bifunctional dihydrofolate reductase-thymidylate synthase, putative	PB000779.01.0 (PB000415.02.0's neighbor gene)/Snf2-related CBP activator, putative	Chromo domain-containing protein 1 (% of PlasmoDB protein Covered: 42;% Identity: 32; P-value: 2.5×10^{-78})
PFD0830w (ADR gene: PfDHFR-TS)/bifunctional dihydrofolate reductase-thymidylate synthase	PF08_0048 (PFD0830w's neighbor gene)/Snf2-related CBP activator, putative	Chromo domain-containing protein 1 (% of PlasmoDB protein Covered: 34;% Identity: 33; P-value: 1.5×10^{-75})
PF13_0019 (ADR gene: PfNHE1)/sodium/hydrogen exchanger, Na, H antiporter	MAL13P1.170 (PF13_0019's neighbor gene)/nucleotidyltransferase, putative	Poly(A) RNA polymerase protein 2 (% of PlasmoDB protein Covered: 32; % Identity: 31; P-value: 5.2×10^{-29})
PVX_089950 (ADR gene: PfDHFR-TS)/bifunctional dihydrofolate reductase-thymidylate synthase 1, putative	PVX_089365 (PVX_089950's neighbor gene)/helicase, putative	Chromo domain-containing protein 1 (% of PlasmoDB protein Covered: 32;% Identity: 35; P-value: 3.5×10^{-75})
PVX_118100 (ADR gene: PfMDR2)/multidrug resistance protein 2, putative	PVX_099360 (PVX_118100's neighbor gene)/gamma-glutamylcysteine synthetase, putative	Glutamate–cysteine ligase (% of PlasmoDB protein Covered: 63;% Identity: 32; P-value: 7.6×10^{-64})

The list of selected SL gene pairs is shown in this table. Potential antimalarial drug targets (inferred SL partners for ADR genes) are suggested for future experimental validation. All SL genes represented in this table do not have any human homologous gene. For application to yeast drugs provided by the FitDB, we conjectured the possibility of evolutionary conservation between the yeast genes and Plasmodium homologs by identifying similarities in protein chains in the Protein Data Bank. The five drug target genes are also shown in Fig. 5.
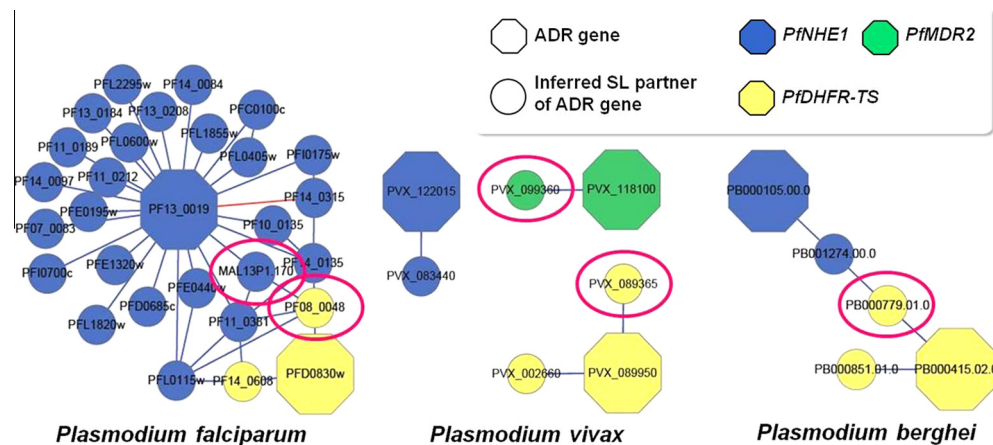

Fig. 5. Inferred Plasmodium SL gene networks to select antimalarial drug targets. To narrow down the list of drug target candidates, potential antimalarial drug target genes are suggested for future experimental validation. The resulting network was filtered by identifying protein similarities in the PDB provided by the PlasmoDB. In three networks, all the filtered inferred Plasmodium SL pairs (ADR genes and their inferred SL partners) do not include human orthologs except for some ADR genes such as PFD0830w, PVX_089950, and PB000415.02.0. These ADR genes and their SL partners extracted from the inferred Plasmodium SL gene network can serve as alternative antimalarial drug targets for malaria patients with ADR genes. In addition, we conjectured the possibility of evolutionary conservation between the yeast genes and Plasmodium homologs through the identification of protein similarities. We then obtained the five inferred SL partners (marked by red loops) having the inferred Plasmodium SL gene networks, which exhibit protein similarities among the yeast orthologs and Plasmodium orthologs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

DB and the results are summarized in Table 3. We searched the fitness data for the yeast homologs of the selected Plasmodium SL genes to find new drugs for clinical malaria treatment.

All Plasmodium genes summarized in Tables 1–3 are not human orthologs except for ADR genes such as PFD0830w, PVX_089950 and PB000415.02.0. Hence, the selective mutation of the SL genes might have strong therapeutic potential in malaria elimination.

4. Discussion

In the present study, a methodology based on the SL relation of genes is proposed to select drug targets and drug candidates for human antimalarial therapy and for experimental validation. A simple computational tool was established to identify SL genes as proper drug targets by analyzing the inferred SL genes of Plasmodium species (P. falciparum and P. vivax for human malarial therapy, and P. berghei for an in vivo experimental model). The inferred Plasmodium SL genes can be used to search for drug targets

for effective antimalarial therapy through in vitro or in vivo experiments. However, in vitro experiments on P. vivax are not easy to perform. P. vivax can only be cultured in vitro for short periods (at least partially due to this parasite's preference for infecting short-lived reticulocytes) and drug studies must therefore be performed at sites where P. vivax is readily available. In addition, genetic manipulation of this parasite is virtually impossible (Eisenstein, 2012). The lack of good experimental models is another limiting factor. Alternatively, the inferred P. berghei target genes can be used for in vivo tests. Commercial drug candidates can eliminate malaria parasites, but they are toxic for people with mutating human genes.

The incomplete effectiveness of a specific antimalarial drug in a certain patient results from the mutation of one of the ADR genes. To ensure successful therapy for these patients, the first neighbors of the mutated ADR gene should be the target of antimalarial therapy such as chemical therapy with SL gene-specific drugs. If the mutation of the ADR genes coincides with the mutation of their SL partners, the malaria parasites would die. However, definitive
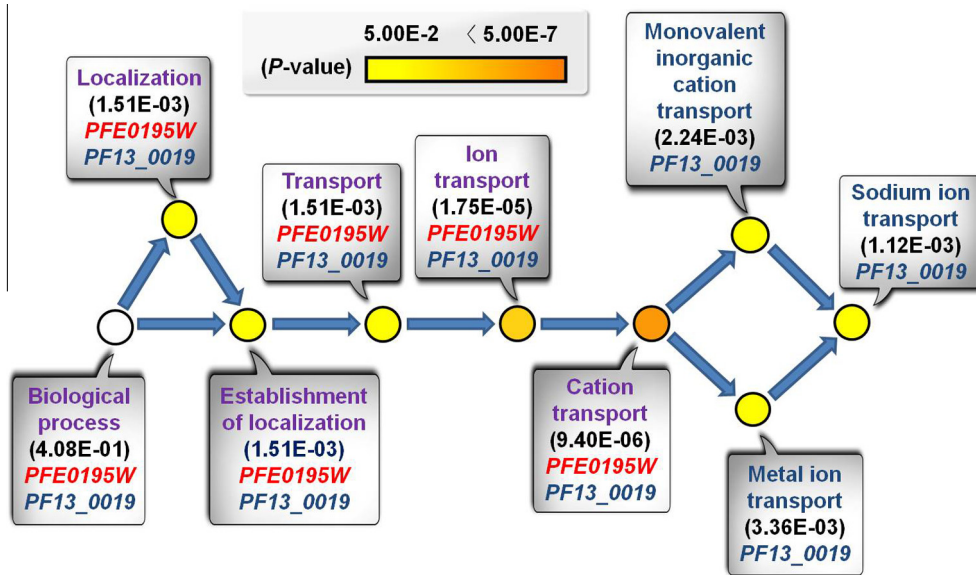
Fig. 6. Relationship between the biological processes of the *PFE0195W* and *PF13_0019* genes. We conducted statistical GO analysis on the biological function of the selected antimalarial drug target genes using BINGO 2.44. Using this GO term network (BINGO graph), the biological process of the *PFE0195W* and *PF13_0019* genes were compared. The BINGO graph visualizes the GO categories that are statistically over-represented in the context of the GO hierarchy. The *PFE0195W* and *PF13_0019* genes are the ADR genes and their first-neighbor genes inferred from yeast SL pairs. The gene pair is one of the inferred SL gene pairs that contain the most potential malaria drug target genes. For each circle, the GO term related to the biological process and the *P* value on the two corresponding genes are specified. The *P* value is a good indicator for the prominence of a given functional category; white, yellow, and orange denote the different *P* value scales. The uncolored nodes are not over-represented; they are the parents of over-represented categories at downstream (biological process in white box). Yellow nodes represent GO categories that are significantly over-represented. For more significant *P* values, the saturation of the node color becomes denser (color legend panel). Blue arrows denote the stage and direction of biological processes. The GO term and *P* value are shown in each white box. Purple represents the biological processes that are related only to the two genes. Monovalent inorganic cation transport, metal ion transport, and sodium ion transport are biological processes that are related only to the *PF13_0019* gene. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
New malarial drug candidates.

Inferred *Plasmodium* SL genes (Description)	Yeast homologs of *Plasmodium*	Heterozygous knockout drug (Description)	Homozygous knockout drug (Description)
PB000415.02.0 (ADR gene: *PfDHFR-TS*)	YOR074C	5-Fluorouridine (antineoplastic), floxuridine (antiviral)	x
PB000779.01.0 (*PB000415.02.0*'s neighbor gene)	YDR334W	Caspofungin (antifungal)	Mechlorethamine (alkylating)
PFD0830w (ADR gene: *PfDHFR-TS*)	YOR074C	Methotrexate (antineoplastic antimetabolite), 5-fluorouridine (antineoplastic), floxuridine (antiviral)	x
PF08_0048 (*PFD0830w*'s neighbor gene)	YDR334W	Caspofungin (antifungal)	Mechlorethamine (alkylating)
PF13_0019 (ADR gene: *PfNHE1*)	YHR081W	Miconazole (antifungal), fluconazole (antifungal), fenpropimorph (antifungal)	x
MAL13P1.170 (*PF13_0019*'s neighbor gene)	YOL115W	5-fluorouracil (antineoplastic antimetabolite), 5-fluorouridine (antineoplastic)	x
PVX_089950 (ADR gene: *PfDHFR-TS*)	YOR074C	Methotrexate (antineoplastic antimetabolite), 5-fluorouridine (antineoplastic), floxuridine (antiviral)	x
PVX_089365 (*PVX_089950*'s neighbor gene)	YLR085C	Alverine citrate (psychoactive)	Mechlorethamine (alkylating), busulfan (alkylating)
PVX_118100 (ADR gene: *PfMDR2*)	YMR301C	Fluconazole (antifungal)	x
PVX_099360 (*PVX_118100*'s neighbor gene)	YJL101C	Itraconazole (antifungal), methotrexate (antineoplastic antimetabolite)	x

In future antimalarial drug research, a couple of the best candidates must be selected from the list, and they should be experimentally demonstrated to have the desired effect on the parasite. Drug target candidates were finally selected from ADR genes and their SL partners containing inferred *Plasmodium* (*P. falciparum*, *P. vivax*, and *P. berghei*) SL genes. We analyzed the fitness data for the yeast homologs of the selected *Plasmodium* genes to find new drugs for clinical malaria treatment. The right column of this table shows which drugs obtained from the top three experiments can be searched in the Drug Bank database.

results are impossible to obtain because of the long evolutionary history and different architectures of yeast and *Plasmodium* genomes.

The evolutionary conservation of SL pairs is controversial (Tarailo et al., 2007; Yuen et al., 2007; Dixon et al., 2008; Tischler et al., 2008), although a significant conservation of SL interactions between eukaryotes has been studied (Dixon et al., 2008). Not all

yeast SL gene pairs are conserved in distantly related organisms. However, previous studies have reported SL genes conserved from yeast. For instance, the evolutionary conserved gene *MHO1* is synthetic lethal with *PLC1* (Schlatter et al., 2012).

P-type ATPase 3 seems to have no merit as a drug target because the gene has been proposed as a target by other researchers (Rozmajzl et al., 2001). Therefore, we regarded the *PfATP3*

(*PFE0195w*) gene as a drug target under special conditions, such as when its SL partner mutates.

The drug may have an adverse effect on *Plasmodium* but not on human cells. To avoid such unwanted side effects on human hosts, parasitic enzymes should be targeted instead of homologous human enzymes (Yeh et al., 2004). If the homologs of drug target genes exist in humans, the enzyme would neither be essential to the human host nor have different inhibitory effects because of their different protein structure and regulation.

Potential drug target genes have to be expressed in some or all of the developmental stages of parasites. We do not know the developmental stage at which the effect of the drug therapy would appear. However, we conjectured that the therapeutic effect would be better when the expressed SL genes in some of the developmental stages of the parasites are simultaneously targeted. Provisional drug targets can be examined computationally and experimentally to extract these features. Experimental analyses can be performed under *in vitro* and *in vivo* conditions for *Plasmodium*, but such experimental studies are costly and laborious. Comparisons between the present predictions with such experimental data would be particularly interesting.

Homology data provide important information that can be used to predict the functions of unknown proteins of closely related species. A large number of gene or protein sequences are stored in many databases, but the functions of the majority of these proteins are unknown. An experimental analysis for all of them is almost impossible (Itoh et al., 2004). Thus, computational methods to predict protein functions are commonly adopted, and homology searching is the most efficient and prevalent means (Itoh et al., 2004). Interestingly, all of the selected genes express protein products with interrelated biological functions, such as ATPase and ion transportation. Thus, ADR genes and their partners with these functions can reasonably be expected to give SL interactions. In this study, we conjectured that the key metabolic pathways, housekeeping functions, and repertoire of predicted membrane transporters were highly conserved between the *P. vivax* and *P. falciparum* proteomes (Gardner et al., 2002). Consequently, the two species had similar metabolic potentials (Carlton et al., 2008). We surveyed the fitness data for the homolog yeast genes of the selected genes to find new drugs for clinical malaria treatment. In this survey, the yeast chemogenomic assays were used to predict its therapeutic potential in malaria (Hillenmeyer et al., 2010). The proposed drug candidates were searched in the DrugBank 3.0 database, which contains comprehensive information on the target diseases, proteins, genes, and organisms on which these drugs act. Researchers can obtain helpful information from the DrugBank database to verify a specific antimalarial therapy for the proposed antimalarial drug candidates. Some of the drug candidates in Table 3, such as methotrexate, fluoxuridine, mechlorethamine, fluorouracil, alverine, busulfan, and miconazole, have influence on humans. If these drugs are to be used as antimalarial drugs, the severity of their side effects should be checked. A drug can bind to a human protein even if it is not a close analogue of the parasite target, as long as the two have a strong similarity in their ligand-binding domains. Among the list of drug candidates in Table 3, caspofungin and itraconazole are worthwhile for drug screening because there is no report on toxicity or effect on the human organism for these two drugs. No significant lethality was observed when itraconazole was administered orally to mice and rats. Therefore, based on previous studies, we suggest a new methodology for antimalarial therapy. This methodology provides not only new potential gene candidates for further experimental tests and for drug design by supplying suitable *Plasmodium* SL gene pairs that can be used as a potential basis for future pharmacologic tests, but also information on new usage for existing drugs.

5. Conclusions

Taking a specific antimalarial drug is not always effective for some patients because of the mutation of ADR genes in malaria parasites. To solve this problem, we proposed a novel approach for searching for pertinent antimalarial drug targets based on the identification of SL pairs of proteins with ADR genes. We suggested a new concept of antimalarial therapy using data integration and inference by homology analysis of yeast–human–*Plasmodium*. Potential antimalarial drug targets and drug candidates were suggested for future experimental validation. ADR genes and their first neighbors extracted from the inferred *Plasmodium* SL gene networks can be used as alternative antimalarial drug targets for a malaria parasite with ADR genes. The inference of SL gene pairs in *Plasmodium* and humans was described based on yeast SL gene data. Yeast SL interaction data as well as homologous information on *Plasmodium*, human, yeast, and ADR genes were integrated to provide a list of candidate genes for malarial therapy. The proposed candidates can be used as drug targets in antimalarial therapy for future experimental validation. If one gene of a *Plasmodium* SL gene pair has a mutation that causes ADR, the drug targeted at another gene can be used for effective therapy with largely reduced injury to human cells.

The *Plasmodium* cells can be killed, while keeping the human cells intact by mutating or blocking the SL partner of the mutated gene that causes ADR. SL gene pairs have been used to kill malaria parasites selectively and leave human cells unharmed. The present SL screening analysis and the use of highly curated databases are very helpful for discovering proper drugs and identifying correct gene targets in antimalarial research. In conclusion, the present computational approach is inexpensive and very simple to adopt. The approach also has strong potential in the preparation of chemical therapy with SL gene-specific chemical drugs or gene therapy and can help identify suitable drug candidates with apparent antimalarial function for clinical malaria treatments.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpddr.2013.06.001.

References

Anderson, T., 2009. Mapping the spread of malaria drug resistance. PLoS Med. 6 (4), e1000054.

Baugh, L.R., Wen, J.C., Hill, A.A., Slonim, D.K., Brown, E.L., Hunter, C.P., 2005. Synthetic lethal analysis of *Caenorhabditis elegans* posterior embryonic patterning genes identifies conserved genetic interactions. Genome Biol. 6 (5), r45.

Bernstein, F.C., Koetzle, T.F., Williams, G.J., Meyer Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T., Tasumi, M., 1977. The Protein Data Bank: a computer-based archival file for macromolecular structures. J. Mol. Biol. 112 (3), 535–542.

Boone, C., Bussey, H., Andrews, B.J., 2007. Exploring genetic interactions and networks with yeast. Nat. Rev. Genet. 8 (6), 437–449.

Briolant, S., Bogreau, H., Gil, M., Bouchiba, H., Baret, E., Amalvict, R., Rogier, C., Pradines, B., 2012. The F423Y mutation in the pfmdr2 gene and mutations N51I, C59R, and S108N in the pfdhfr gene are independently associated with

pyrimethamine resistance in *Plasmodium falciparum* isolates. Antimicrob. Agents Chemother. 56, 2750–2752.

Carlton, J.M., Adams, J.H., Silva, J.C., Bidwell, S.L., Lorenzi, H., Caler, E., Crabtree, J., Angiuoli, S.V., Merino, E.F., Amedeo, P., Cheng, Q., Coulson, R.M., Crabb, B.S., Del Portillo, H.A., Essien, K., Feldblyum, T.V., Fernandez-Becerra, C., Gilson, P.R., Gueye, A.H., Guo, X., Kang'a, S., Kooij, T.W., Korsinczky, M., Meyer, E.V., Nene, V., Paulsen, I., White, O., Ralph, S.A., Ren, Q., Sargeant, T.J., Salzberg, S.L., Stoeckert, C.J., Sullivan, S.A., Yamamoto, M.M., Hoffman, S.L., Wortman, J.R., Gardner, M.J., Galinski, M.R., Barnwell, J.W., Fraser-Liggett, C.M., 2008. Comparative genomics of the neglected human malaria parasite *Plasmodium vivax*. Nature 455 (7214), 757–763.

Chipman, K., Singh, A., 2009. Predicting genetic interactions with random walks on biological networks. BMC Bioinformatics 10, 17.

Conde-Pueyo, N., Munteanu, A., Solé, R.V., Rodríguez-Caso, C., 2009. Human synthetic lethal inference as potential anti-cancer target gene detection. BMC Syst. Biol. 3, 116.

Cowman, A.F., Karcz, S., Galatis, D., Culvenor, J.G., 1991. A P-glycoprotein homologue of *Plasmodium falciparum* is localized on the digestive vacuole. J. Cell Biol. 113, 1033–1042.

Cowman, A.F., Galatis, D., Thompson, J.K., 1994. Selection for mefloquine resistance in *Plasmodium falciparum* is linked to amplification of the pfmdr1 gene and cross-resistance to halofantrine and quinine. Proc. Natl. Acad. Sci. USA 91, 1143–1147.

Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J., Singh, B., 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin. Infect. Dis. 46, 165–171.

Dixon, S.J., Fedyshyn, Y., Koh, J.L.Y., Prasad, T.S.K., Chahwan, C., Chua, G., Toufighi, K., Baryshnikova, A., Hayles, J., Hoe, K.L., Kim, D.U., Park, H.O., Myers, C.L., Pandey, A., Durocher, D., Andrews, B.J., Boone, C., 2008. Significant conservation of synthetic lethal genetic interaction networks between distantly related eukaryotes. Proc. Natl. Acad. Sci. USA 105 (43), 16653–16658.

Eisenstein, M., 2012. Drug development: holding out for reinforcements. Nature 484, s16–s18.

Fernández, A., Crespo, A., Tiwari, A., 2009. Is there a case for selectively promiscuous anticancer drugs? Drug Discov. Today 14 (1–2), 1–5.

Foote, S.J., Kyle, D.E., Martin, R.K., Oduola, A.M., Forsyth, K., Kemp, D.J., Cowman, A.F., 1990. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. Nature 345, 255–258.

Forsburg, S.L., 2001. The art and design of genetic screens: yeast. Nat. Rev. Genet. 2 (9), 659–668.

Gardner, M.J., Hall, N., Fung, E., White, O., Berriman, M., Hyman, R.W., Carlton, J.M., Pain, A., Nelson, K.E., Bowman, S., Paulsen, I.T., James, K., Eisen, J.A., Rutherford, K., Salzberg, S.L., Craig, A., Kyes, S., Chan, M.S., Nene, V., Shallom, S.J., Suh, B., Peterson, J., Angiuoli, S., Pertea, M., Allen, J., Selengut, J., Haft, D., Mather, M.W., Vaidya, A.B., Martin, D.M., Fairlamb, A.H., Fraunholz, M.J., Roos, D.S., Ralph, S.A., McFadden, G.I., Cummings, L.M., Subramanian, G.M., Mungall, C., Venter, J.C., Carucci, D.J., Hoffman, S.L., Newbold, C., Davis, R.W., Fraser, C.M., Barrell, B., 2002. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature 419, 498–511.

Giancarlo, A.B., Patrick, G.B., Stephen, A.W.D.L., 2009. Mechanisms of antimalarial drug resistance. In: Mayers, D.L. (Ed.), Antimicrobial Drug Resistance. Humana Press, New York, pp. 561–574.

Goh, K.I., Cusick, M.E., Valle, D., Childs, B., Vidal, M., Barabási, A.L., 2007. The human disease network. Proc. Natl. Acad. Sci. USA 104 (21), 8685–8690.

Hillenmeyer, M.E., Fung, E., Wildenhain, J., Pierce, S.E., Hoon, S., Lee, W., Proctor, M., St Onge, R.P., Tyers, M., Koller, D., Altman, R.B., Davis, R.W., Nislow, C., Giaever, G., 2008. The chemical genomic portrait of yeast: uncovering a phenotype for all genes. Science 320 (5874), 362–365.

Hillenmeyer, M.E., Ericson, E., Davis, R.W., Nislow, C., Koller, D., Giaever, G., 2010. Systematic analysis of genome-wide fitness data in yeast reveals novel gene function and drug action. Genome Biol. 11 (3), r30.

Huthmacher, C., Hoppe, A., Bulik, S., Holzhütter, H.G., 2010. Antimalarial drug targets in *Plasmodium falciparum* predicted by stage-specific metabolic network analysis. BMC Syst. Biol. 4, 120.

Itoh, M., Akutsu, T., Kanehisa, M., 2004. Clustering of database sequences for fast homology search using upper bounds on alignment score. Genome Inform. 15 (1), 93–104.

Jorgensen, E.M., Mango, S.E., 2002. The art and design of genetic screens: *Caenorhabditis elegans*. Nat. Rev. Genet. 3 (5), 356–369.

Knox, C., Law, V., Jewison, T., Liu, P., Ly, S., Frolkis, A., Pon, A., Banco, K., Mak, C., Neveu, V., Djoumbou, Y., Eisner, R., Guo, A.C., Wishart, D.S., 2011. DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. Nucleic Acids Res. 39, D1035–D1041.

Lehner, B., Crombie, C., Tischler, J., Fortunato, A., Fraser, A.G., 2006. Systematic mapping of genetic interactions in *Caenorhabditis elegans* identifies common modifiers of diverse signaling pathways. Nat. Genet. 38 (8), 896–903.

Maere, S., Heymans, K., Kuiper, M., 2005. Bingo: a cytoscape plugging to assess over-representation of gene ontology categories in biological networks. Bioinformatics 21 (16), 3448–3449.

Mutabingwa, T., Nzila, A., Mberu, E., Nduati, E., Winstanley, P., Hills, E., Watkins, W., 2001. Chlorproguanil-dapsone for treatment of drug- resistant falciparum malaria in Tanzania. Lancet 358, 1218–1223.

Nakaya, A., Katayama, T., Itoh, M., Hiranuka, K., Kawashima, S., Moriya, Y., Okuda, S., Tanaka, M., Tokimatsu, T., Yamanishi, Y., Yoshizawa, A.C., Kanehisa, M., Goto, S., 2013. KEGG OC: a large-scale automatic construction of taxonomy-based ortholog clusters. Nucleic Acids Res. 41, D353–D357.

Noguchi, T., Akiyama, Y., 2003. PDB-REPRDB: a database of representative protein chains from the Protein Data Bank (PDB) in 2003. Nucleic Acids Res. 31 (1), 492–493.

Ooi, S.L., Pan, X., Peyser, B.D., Ye, P., Meluh, P.B., Yuan, D.S., Irizarry, R.A., Bader, J.S., Spencer, F.A., Boeke, J.D., 2006. Global synthetic-lethality analysis and yeast functional profiling. Trends Genet. 22, 56–63.

Paladugu, S.R., Zhao, S., Ray, A., Raval, A., 2008. Mining protein networks for synthetic genetic interactions. BMC Bioinformatics 9, 426.

Pan, X., Yuan, D.S., Xiang, D., Wang, X., Sookhai-Mahadeo, S., Bader, J.S., Hieter, P., Spencer, F., Boeke, J.D., 2004. A robust toolkit for functional profiling of the yeast genome. Mol. Cell. 16 (3), 487–496.

Peel, S.A., Merritt, S.C., Handy, J., Baric, R.S., 1993. Derivation of highly mefloquine-resistant lines from *Plasmodium falciparum* in vitro. Am. J. Trop. Med. Hyg. 48, 385–397.

Peel, S.A., Bright, P., Yount, B., Handy, J., Baric, R.S., 1994. A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (pfmdr) of *Plasmodium falciparum* in vitro. Am. J. Trop. Med. Hyg. 51, 648–658.

Platzer, A., Perco, P., Lukas, A., Mayer, B., 2007. Characterization of protein-interaction networks in tumors. BMC Bioinformatics 8, 224.

Price, R.N., Tjitra, E., Guerra, C.A., Yeung, S., White, N.J., Anstey, N.M., 2007. Vivax malaria: neglected and not benign. Am. J. Trop. Med. Hyg. 77, 79–87.

Reed, M.B., Saliba, K.J., Caruana, S.R., Kirk, K., Cowman, A.F., 2000. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum*. Nature 403, 906–909.

Rozmajzl, P.J., Kimura, M., Woodrow, C.J., Krishna, S., Meade, J.C., 2001. Characterization of P-type ATPase 3 in *Plasmodium falciparum*. Mol. Biochem. Parasitol. 116 (2), 117–126.

Rubio, J.P., Cowman, A.F., 1996. The ATP-binding cassette (ABC) gene family of *Plasmodium falciparum*. Parasitol. Today 12, 135–140.

Schlatter, I.D., Meira, M., Ueberschlag, V., Hoepfner, D., Movva, R., Hynes, N.E., 2012. MHO1, an evolutionarily conserved gene, is synthetic lethal with PLC1; Mho1p has a role in invasive growth. PLoS One 7 (3), e32501.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13 (11), 2498–2504.

Sibley, C.H., Hyde, J.E., Sims, P.F., Plowe, C.V., Kublin, J.G., Mberu, E.K., Cowman, A.F., Winstanley, P.A., Watkins, W.M., Nzila, A.M., 2001. Pyrimethamine–sulfadoxine resistance in *Plasmodium falciparum*: what next? Trends Parasitol. 17, 582–588.

Sonnhammer, E.L.L., Koonin, E.V., 2002. Orthology, paralogy and proposed classification for paralog subtypes. Trends Genet. 18 (12), 619–620.

Tarailo, M., Tarailo, S., Rose, A.M., 2007. Synthetic lethal interactions identify phenotypic "interologs" of the spindle assembly checkpoint components. Genetics 177 (4), 2525–2530.

Tischler, J., Lehner, B., Fraser, A.G., 2008. Evolutionary plasticity of genetic interaction networks. Nat. Genet. 40 (4), 390–391.

Tong, A.H., Evangelista, M., Parsons, A.B., Xu, H., Bader, G.D., Pagé, N., Robinson, M., Raghibizadeh, S., Hogue, C.W., Bussey, H., Andrews, B., Tyers, M., Boone, C., 2001. Systematic genetic analysis with ordered arrays of yeast deletion mutants. Science 294 (5550), 2364–2368.

Tong, A.H.Y., Lesage, G., Bader, G.D., Ding, H., Xu, H., Xin, X., Young, J., Berriz, G.F., Brost, R.L., Chang, M., Chen, Y., Cheng, X., Chua, G., Friesen, H., Goldberg, D.S., Haynes, J., Humphries, C., He, G., Hussein, S., Ke, L., Krogan, N., Li, Z., Levinson, J.N., Lu, H., Ménard, P., Munyana, C., Parsons, A.B., Ryan, O., Tonikian, R., Roberts, T., Sdicu, A.M., Shapiro, J., Sheikh, B., Suter, B., Wong, S.L., Zhang, L.V., Zhu, H., Burd, C.G., Munro, S., Sander, C., Rine, J., Greenblatt, J., Peter, M., Bretscher, A., Bell, G., Roth, F.P., Brown, G.W., Andrews, B., Bussey, H., Boone, C., 2004. Global mapping of the yeast genetic interaction network. Science 303 (5659), 808–813.

Wellems, T.E., Panton, L.J., Gluzman, I.Y., Rosario, V.E., Gwadz, R.W., Walker-Jonah, A., Krogstad, D.J., 1990. Chloroquine resistance not linked to mdr-like genes in a *Plasmodium falciparum* cross. Nature 345, 253–255.

Wilson, C.M., Serrano, A.E., Wasley, A., Bogenschutz, M.P., Shankar, A.H., Wirth, D.F., 1989. Amplification of a gene related to mammalian mdr genes in drug-resistant *Plasmodium falciparum*. Science 244, 1184–1186.

Yeh, I., Hanekamp, T., Tsoka, S., Karp, P.D., Altman, R.B., 2004. Computational analysis of *Plasmodium falciparum* metabolism: organizing genomic information to facilitate drug discovery. Genome Res. 14, 917–924.

Yildirim, M.A., Goh, K.I., Cusick, M.E., Barabási, A.L., Vidal, M., 2007. Drug-target network. Nat. Biotechnol. 25, 1119–1126.

Yuen, K.W.Y., Warren, C.D., Chen, O., Kwok, T., Hieter, P., Spencer, F.A., 2007. Systematic genome instability screens in yeast and their potential relevance to cancer. Proc. Natl. Acad. Sci. USA 104 (10), 3925–3930.

Zalis, M.G., Wilson, C.M., Zhang, Y., Wirth, D.F., 1993. Characterization of the pfmdr2 gene for *Plasmodium falciparum*. Mol. Biochem. Parasitol. 62, 83–92.