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Identification of Putative Drug Targets and Vaccine Candidates for Pathogens Causing Atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory artery disease, responsible for both cardiovascular (heart) and cerebrovascular (brain) stroke with high morbidity and mortality worldwide. Infectious pathogens such as *Chlamydophila pneumoniae*, *Porphyromonas gingivalis* and Helicobacter pylori are shown to be associated with the disease in recent epidemiological studies. Therefore, identification of common drug targets and vaccine candidates against these three pathogens would be vital towards therapy and management of atherosclerosis. *Chlamydophila pneumonia* was selected as a reference organism due to its predominant role in atherosclerosis. Implementing comparative genomic approach, subtractive genomic approach, metabolic pathway analysis, non-homologous gut flora analysis and domain search analysis, 35 common putative drug targets were identified against pathogens of atherosclerosis. Subcellular localization studies were performed and identified UvrABC protein as vaccine candidate. Metabolic pathway analysis has showed that, out of 35 drug targets, 14 enzymes were participating in key pathways linked to pathogen's survival, proliferation and pathogenesis without any alternative mechanism to synthesize the product. The gut microbiota analysis was performed to identify the drug targets which do not affect the microbiota in the humans. Domain search was performed for the identified 14 drug targets using Pfam and SMART databases and protein network analysis was carried out using STRING and Cytoscape v3.2.0. The drug targets and vaccine candidates proposed in the present study would serve as basis to design potent inhibitors and subunit vaccines through *in silico* approach for combating atherosclerosis caused by infectious pathogens.

Keywords: Atherosclerosis; *Chlamydophila pneumonia*; *Poryphromonas gingivali*; *Helicobacter pylori*; Subtractive genomic approach; Drug targets

Introduction

Atherosclerosis is also known as arteriosclerotic vascular disease, a condition where thickening and hardening of the arteries formed by plaque build-up. The clinical significance of atherosclerosis includes extreme fatigue with exertion, swelling in their feet and ankles, ischemia and angina leads to cardiovascular and cerebrovascular stroke [1]. The statistics of the disease revealed that more than 50% of total population is affected with atherosclerosis which is the most serious consideration to be riveted. Recent admiration of atherosclerosis as a chronic inflammatory disease has restored the efforts to examine the role of pathogens causing many infections [2]. Recent literature evidences on atherosclerosis as a chronic inflammatory disease has invigorated many efforts to examine the role played by pathogens in the cause of infections [3].

Three Gram-negative organisms *Chlamydophila pneumoniae*, *Porphyromonas gingivalis* and Helicobacter pylori have attracted most serious consideration in association with atherosclerosis [4-6]. Sero-epidemiologic studies were followed by researchers in which the pathogens were identified in vascular tissue from patients with cardiovascular disease by electron microscopy, PCR, and immuno cytochemical staining. Finding of new effective drugs against atherosclerosis is essential to cure the disease condition. *Chlamydophila pneumoniae* is an obligate, Gram negative, rod shaped bacteria, non-motile, intracellular respiratory pathogen causes infections in humans [1]. Infection by *Chlamydophila pneumoniae* is linked with extra-respiratory diseases of aging and also atherosclerosis.

Porphyromonas gingivalis is an anaerobic, Gram-negative rod shaped bacteria, found in oral cavity, associated with periodontal disease progression including bone and tissue destruction. Similar results were observed in heart and aortic endothelial cells, indicating an association between periodontitis and cardiovascular disease [4]. The infected monocytes are travelled from oral cavity to the respiratory artery wall through saliva, intubation, pro-inflammatory cytokines etc [5]. These infected monocytes are carried throughout the body in arteries by *Chlamydophila pneumoniae* [4]. *Helicobacter pylori* are now incriminated in the pathogenesis of atherosclerosis. The infection is linked to the early stages of coronary atherosclerosis rather than advanced coronary atherosclerosis. Action of *Helicobacter pylori* either directly on vascular cells or via indirectly affects by cytokines and acute-phase proteins at non-vascular sites is incriminated in this acceleration of atherosclerosis [6].

Though there are many works being pursued understanding of the disease, the common drug targets for the three pathogens causing atherosclerosis were not clear till date. Significant attention has been focused on these three pathogens because of their association with atherosclerosis, as evidenced by epidemiological and experimental studies. Literature evidences also reported that the statistical analysis showed 70% of the populations are infected by *Chlamydophila*

pneumoniae infections [2]. Hence, *Chlamydophila pneumoniae* was selected as reference organism in the present study due to its predominant role in pathogenesis of atherosclerosis.

Multi drug resistance is one of reason behind development of diseases caused by the pathogens and also the major reason behind easy proliferation of *Chlamydophila pneumoniae*, *Porphyromonas gingivalis* and *Helicobacter pylori* in the pathogenesis of atherosclerosis [4,5]. Infection arises due to the three pathogens are currently treated with antibiotics such as azithromycin, clarithromycin, levofloxacin, roxitromycin, doxycycline, cefuroxime, cephalothin, piperacillin, amoxicillin, ampicillin, ticarcillin, benzylpencillium, tetracycline, oleandomycin, spiramycine, cindamycin and metronidazole [7,8]. Several vaccines specific to serotypes and serogroups of *Chlamydophila pneumonia* have been reported in literature while no approach had been made yet to identify a common drug target and vaccine candidate against these three selected pathogens involved in pathogenesis of atherosclerosis. Eddens et al., Khalaf et al., Longo-Mbenza et al. reported, poor outcome and drug resistance of existing drug molecules necessitates the implementation of alternative strategy for designing novel drug molecules and vaccine candidates against the pathogens.

The experimental approaches are time consuming, cost effective and also obtain very few results whereas computational approaches were used to identify the putative drug targets. Comparative genomic approach, subtractive genomic approach, metabolic pathway analysis, non-homologous gut flora analysis, domain search analysis, and protein network analysis are extensively used in the prediction of potential drug targets in the three pathogens causing atherosclerosis. In this scenario, potential target must be essential for the growth and survival of the pathogen. Further designing of inhibitors should hinder the function exclusively to pathogen and should avoid the undesirable cross-reactivity with the human proteins. The availability of complete genome sequences of the selected pathogens in combination with Bioinformatics tools and databases is of great assistance in reducing the problem of searching for potential drug targets/vaccine candidates in a large pool of gene/protein polls [9-11]. The present study aims to propose common potential drug targets and vaccine candidates in three pathogens triggering atherosclerosis through a systemic strategy of Bioinformatics tools and protein network analysis.

Materials and Methods

Comparative analysis

Three Gram-negative pathogens, namely, *Chlamydophila pneumonia*, *Porphyromonas gingivalis* and *Helicobacter pylori* were selected for the study [4,5]. *Chlamydophila pneumonia* is one of the most predominant pathogen of atherosclerosis in worldwide [1] therefore, considered as reference organism in the present study. Comparative genomic approach was implemented in the present study to prioritize promising genes that are common in three pathogens. Complete genome and proteome sequences of the three pathogens causing atherosclerosis were selected as subject datasets from J. Craig Venter Institute Comprehensive Microbial Resource (JCVI CMR) for comparative genomic analysis, [12,13]. The minimum identity and similarity were limited to 30% and 50% respectively, to find the common proteins among the selected three pathogens of atherosclerosis [13,14]. Comparative genomic approach was implemented to identify the common genes coding for homologous proteins present in the three pathogens.

Identification of essential and non-homologous gene analysis

The identified common genes from comparative genomic approach were further characterized and verified for their role in the survival of the pathogens. The functions encoded by essential genes were considered as a basis of pathogen existence and thus are expected to vital for the survival of pathogens. The essential genes are likely to be crucial for the bacterial endurance. The common proteins of the selected pathogens were further assured for the essentiality by using database of essential genes (DEG) [15], with a cut off e-value of 10-10 and bit score ≥100. The essential genes were searched for non-homology using National centre for Biotechnology information - basic local alignment search tool (NCBI-BLAST), search against the non-redundant database of the human proteome, with the e-value threshold set at 0.0001 and bit score cut-off at 100. The identified common and essential genes were further considered for the pathway analysis.

Metabolic pathway analysis

The metabolic pathways that play vital role in the function of pathogens were retrieved from the KEGG [16] pathway database. The metabolic pathways of the identified drug targets of three pathogens were compared with the human metabolic pathways. The KEGG analysis also differentiates enzyme and non-enzyme drug targets. The pathways of drug targets were checked and the drug targets having alternative pathways were not considered, because blocking of these drug targets would be ineffective as the product is synthesized by alternative way [13].

Non-homology analysis of drug targets against gut microbiota

Gut microbiota is complex of microbial species that live in the digestive tract of human which are beneficial to humans. The human body carries about 100 trillion microorganisms in intestine which is ten times greater than total number of human cells that belongs to 79 different genera. In the present study drug targets were searched for non-homologous to 79 beneficial human gut microbiota (Supplementary text 1) with the e-value >0.000 with bit score <100 [17,18].

Non-homology analysis of drug targets against domain search

A domain is a conserved part of protein with defined function. The domain architecture of the drug targets were compared with proteins of host (human) using Pfam, which is a large collection of protein families. These domains were also cross checked with SMART database, which is a biological database used for the identification and analysis of protein domains [19]. In addition the identified essential genes were subjected to cluster of orthologous group (COG) database search in order to identify the orthologous proteins [20].

Protein network analysis

STRING is a search tool used for retrieval of interacting Genes/Proteins to construct protein-protein interaction networks. Many functional interactions occur between proteins were core for cellular processing and their systematic characterization helps to provide context in molecular systems biology [21]. STRING database currently covers 5,214,234 genes/proteins from 1133 organisms which are

derived from four sources such as, genomic contexts, high throughput experiments, conserved co expression and literature studies. In the present study drug targets with confidence score more than 0.400 were selected [22,23]. The interactions not more than 50 were also included in the interaction network. Further the network module analysed and visualized for the identified drug targets using Cytoscape v3.2.0. It is a software platform for the visualization and analysis of biological networks [24]. The drug targets those are specific to pathogens and important in the metabolic network of the pathogens were considered as potential drug targets.

Subcellular localization

Subcellular localization of the identified drug targets could be used to obtain information about their potential functions. Subcellular location the potential target differentiates cytoplasmic, periplasmic or inner membrane proteins that are metabolically important as drug targets and outer membrane or extra-cellular proteins as vaccine targets. Broad spectrum candidates were classified as either drug targets or vaccine candidates based on their sub cellular localization using PSORTb [25,26] and further validated with CELLO v2.5 [27].

Drugability of the targets

Drugability of the identified enzyme drug targets were checked using Drug Bank database. The Drug Bank database is a unique Bioinformatics and Cheminformatics resource that combines detailed drug data with comprehensive drug target information. The database contains 7740 drug entries including 1584 FDA-approved small molecule drugs, 157 FDA-approved biotech drugs, 89 nutraceuticals and 6000 experimental drugs. Additionally, 4282 non-redundant protein sequences are linked to these drug entries. Each Drug Card entry contains more than 200 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data. The identified drug targets were further checked for drugability in other pathogens [28].

Results and Discussion

Identification of common drug targets

Chlamydophila pneumonia has 112 gene products, *Porphyromonas gingivalis* has 062 gene products and *Helicobacter pylorus* has 563 gene products. Comparative genomic approach using J. Craig Venter Institute Comprehensive Microbial Resource (JCVI CMR) had revealed 102 common gene products among the three selected pathogens. The subtractive genomic approach allows compiling a large set of common and essential genes present in the pathogens and absent in the host. In turn to define common drug moieties or vaccine candidates are effective towards all the three pathogens. Subtractive genomic approach was performed using DEG-BLAST and NCBI-BLAST to know essential gene products among the 102 common gene products, revealed 61 essential gene products that are vital for pathogens survival. Non-homology towards Homo sapiens (host) were analysed for these 61 essential gene products were selected as drug targets [16]. 35 essential proteins were non-homologous to human. Non-homology towards humans was cross checked so as the inhibitors designed for drug targets were not to interrupt the normal functioning of host proteins. Hence, these 35 proteins were considered as common drug targets against pathogens of atherosclerosis. Among the 35 drug targets UvrABC protein was identified as membrane protein and selected as vaccine candidate against common pathogens of atherosclerosis (Table 1). Outer membrane proteins or ABC transporter proteins that are present on the cell surface acts as a gate way between outer and inner surfaces of the pathogens. Surface proteins are extremely useful in developing prototype vaccine with subsequent reverse vaccinology method because of bacterium's unique structure [29]. Further the identified 35 drug targets were subjected to metabolic pathway analysis.

S.No	Criteria	*C. pneumoniae*	*P. gingivalis*	*H.pylori*
1	Genome size	1.23 Mb	2.44 Mb	1.67 Mb
2	Number of genes	112	62	563
3	Number of gene products	1069	958	469
4	Number of common proteins	102		
5	Number of essential genes	61		
6	Number of human non-homologous	35		
7	Number of vaccine candidate	1		
8	Number of enzymes	17		
9	Enzymes without alternative pathway	14		
10	Number of non-homologous to gut microbiota	14		
11	Number of non-homologous to human protein domains	14		
12	Number of targets involved in protein network analysis	14		

Table 1: Prediction of common drug targets and one vaccine candidate from the selected three pathogens causing atherosclerosis.

Metabolic pathway analysis

KEGG pathway analysis revealed that among 35 drug targets, 17 were enzymes and 18 were non-enzymes. Enzymes are biologically significant than non-enzymes as they catalyzes numerous biochemical reactions that aids for the survival of the pathogens. The active site and the allosteric sites of the enzymes aids in designing of novel inhibitors specific to the target [13]. The pathways of the host and pathogens were downloaded and manually compared to identify pathways unique to the pathogens. Those pathways that were absent in the host and present in the pathogens were selected as unique pathways. Among the 17 enzymes, five were involved in unique pathways namely lipopolysaccharide biosynthesis (four enzymes) and peptidoglycan biosynthesis (one enzyme). The pathways that are not unique to the pathogens were also considered as they confirmed less than 30% identity to humans which will not agitate the human proteins by further designing inhibitors [12]. The remaining twelve enzymes were involved in vital metabolic pathways such as, fatty acid biosynthesis, mevalonate pathway, protein biosynthesis, thiamine metabolism, purine metabolism, homologous recombination, DNA replication, DNA repair, RNA degradation etc (Figure 1) which showed >30% identity towards host. Among the 17 enzymes, three enzymes have alternative pathway to synthesize the product. Hence, 14 enzymes were proposed as potential therapeutic targets for common pathogens of atherosclerosis.

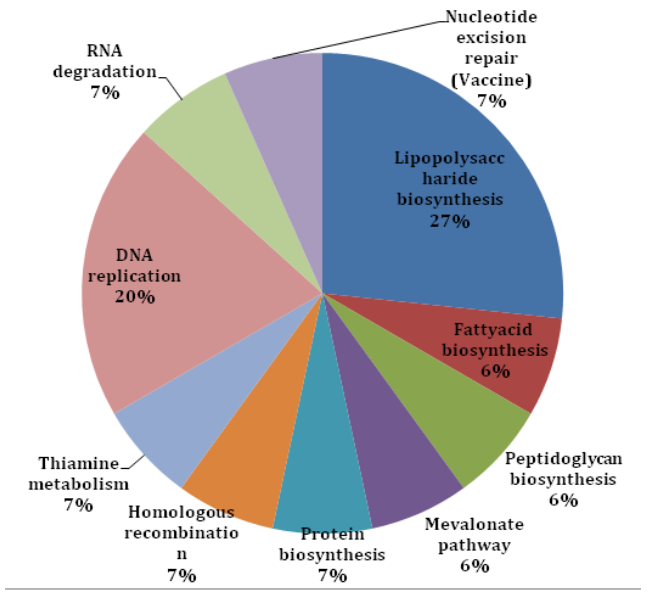

Figure 1: Distribution of identified drug targets in different metabolic pathways.

Gut microbiota analysis

Gut microorganisms benefit the host in many ways such as gleaning the energy from the fermentation of undigested carbohydrates, synthesizing vitamins, subsequent absorption of short-chain fatty acids, degrading xenobiotics etc. The human microflora helps in numerous ways which provide resistance to colonization by pathogenic bacteria and indigenous opportunists by influencing host immune system. Drug designed should neither harm the host enzymes nor hinder the function of enzymes of the gut flora which leads to side effects. Disruption of gut microbiota results in the development of many numerous pathologies in human health [18]. The selected drug targets were compared with the proteome of 79 human gut microbiota enzymes. The identified 14 enzymes of three pathogens were non homologous to gut microbiota and considered as drug targets for designing a drug moieties and one vaccine candidate, that does not hinder the functioning of the enzymes present in human gut microbiota.

Domain search and COG analysis

Domains are conserved part of proteins defined with unique functions and also functional units of that are capable of independent folding. Proposed drug targets of the three pathogens had no domain similarity with the human proteins. The domains that are unique to the common pathogens were alone considered, if the domain was blocked with the drug moiety then the function of the entire protein was lost. These drug targets were considered as potential drug targets against three pathogens which would not further hinder the human enzyme functions. The conservation of the identified 14 enzymes and 1 vaccine candidates were accessed at cluster of orthologous group's database. They were also conserved in many other pathogens, which is believed that the essential genes are conserved among various pathogens such as Streptococcus pneumonia, Neisseria meningitides, *Haemophilus influnzae*, *Staphylococcus aureus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus mitis*, *Bortonella quintana*, *Coxiella burnetti*, *Legionella pneumopholi*, *Brucella melitensis*, *Biovar abartu*, *Leptospira* spp, *Thermus thermophilus*, *Mycobacterium tuberculosis*, *Sulfolobus solfataricus*, *Mycobacterium tuberculosis*, *Escherichia coli*, *Mycobacterium tuberculosis* and *Deinococcus radiodurans* [12,13,30-37].

Protein-protein network analysis

The selected drug targets were examined by protein-protein interaction network analysis. The network analysis of 14 enzymes and 1 vaccine candidate was determined using STRING database. Interactions with score of more than 0.400 and interactions with less than 50 interactors were regarded as potential drug targets. The analysis of STRING database showed that all the proposed drug targets showed interactions with the high confidence scores of 0.900 and with low confidence interactions less than 50 were incorporated in the interaction network [16]. Each network gives particular group of proteins. Hovering over each node will display its annotation and full details of the protein, here network nodes represents proteins. Each node in the network has its own importance. Clustering in STRING has two different parameters to cluster the proteins. KMEANS is the parameter which particularly specifies the number of clusters and MCL is the parameter that indirectly related with the precision of the clustering which is mentioned as 'inflation'. The nodes can be deleted and change in the clustering was noted. Upon deletion of each node manually clustering MCL parameter or KMEANS parameter decrease in clustering coefficient was observed and considered as functionally important protein in the metabolic network. The same results were also observed in the analysis using Cytoscape 3.2.0. High the interaction of the protein it is metabolically important and can act as a potent drug target. The pathogen specific drug targets that are essential for the survival of bacteria are also proved important in the metabolic network and found to be highly interacted with 50

interactions. Hence, all the drug targets were considered as potential targets for the three pathogens causing atherosclerosis.

Vaccine candidate

Nucleotide excision repair mechanism is a DNA repair process which is mainly significant to remove bulk of DNA adducts which is composed of thymine dimmers. Among the 35 drug targets UvrABC system Protein A (uvrA) was identified as common vaccine candidate of the three pathogens causing atherosclerosis. UvrA is a surface exposed protein mainly involved in nucleotide excision repair mechanism (S.No. 15 in Table 2). Hence, identification of epitope driven T-cell candidate for uvrA would be a promising attemptto prevent infections caused by three pathogens of atherosclerosis. The available literature evidences also revealed uvrA as an effective vaccine candidate against many bacterial pathogens like *Escherichia coli, Mycobacterium tuberculosis* and *Deinococcus radiodurans* [29,38,39].

S.No.	UniProt ID	Enzyme	E.C. Number	Gene name	Metabolic Pathway	Subcellular location
1	Q9Z7I4	2-dehydro-3-deoxyphosphooctonate aldolase	2.5.1.55	kdsA	Lipopolysaccharide biosynthesis	Cytoplasmic
2	Q9Z8U9	3-deoxy-manno-octulosonate cytidylyltransferase	2.7.7.38	kdsB	Lipopolysaccharide biosynthesis	Cytoplasmic
3	Q9Z7Q4	Acyl-[acyl-carrier-protein]--UDP-N-acetylglucosamine O-acyltransferase	2.3.1.129	lpxA	Lipopolysaccharide biosynthesis	Cytoplasmic
4	Q9Z7Q2	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosaminedeacetylase	3.5.1.-	lpxC	Lipopolysaccharide biosynthesis	Cytoplasmic
5	Q9Z8P0	3-oxoacyl-[acyl-carrier-protein] synthase 3	2.3.1.180	fabH	Fattyacid biosynthesis initiation and elongation	Cytoplasmic
6	Q9Z706	Phospho-N-acetylmuramoyl-pentapeptide-transferase	2.7.8.13	mraY	Peptidoglycan biosynthesis	Cytoplasmic
7	Q9Z8H0	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	1.17.7.1	ispG	Terpenoid backbone biosynthesis/mevalonate pathway	Cytoplasmic
8	Q9Z6V6	Peptidyl-tRNA hydrolase	3.1.1.29	pth	Protein biosynthesis	Cytoplasmic
9	Q9Z6J9	1-deoxy-D-xylulose-5-phosphate synthase	2.2.1.7	dxs	Thiamine metabolism	Cytoplasmic
10	Q9Z7T3	Crossover junction endodeoxyribonuclease	3.1.22.4	ruvC	Homologous recombination	Cytoplasmic
11	Q9Z7N8	DNA polymerase III subunit alpha	2.7.7.7	dnaE	Homologous recombination , DNA replication, Mismatch repair,	Cytoplasmic
12	Q9Z6W4	DNA primase	2.7.7.	dnaG	DNA replication	Cytoplasmic
13	Q9Z8R4	DNA gyrase subunit A	5.99.1.3	gyrA	DNA replication	Cytoplasmic
14	Q9Z7U4	Transcription termination factor Rho	3.6.4.-	rho	RNA degradation	Cytoplasmic
15	Q9Z985	UvrABC system protein A	-	uvrA	Nucleotide excision repair	Membrane

Table 2: Common drug targets and one vaccine candidate of three pathogens.

Enzymes as drug targets

Since enzymes are biologically significant in regulating the pathogen survival, blocking an enzyme's activity can kill the pathogen. Lipopolysaccharide (LPS) constitutes the lipid portion of the outer leaflet of Gram-negative bacteria, and is essential for survival of bacteria. LPS was also known to be responsible for the variety of biological effects associated with Gram-negative sepsis. 2-dehydro-3-deoxyphosphooctonate aldolase (kdsA) (S.No. 1 in Table 2), 3-deoxy-manno-octulosonate cytidylyltransferase (kdsB) (S.No. 2 in Table 2), Acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase (lpxA) (S. No. 3 in Table 2) and UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosaminedeacetylase (lpxC) (S. No. 4 in Table 2)are playing major role in the lipopolysacharide biosynthesis which is mainly involved in the organization of bacterial outer membrane of Gram-negative bacteria [40-42]. The enzymes kdsA, kdsB, lpxA and lpxC were also reported as putative drug targets against pathogens such as *Leptospira, Enterococcus faecalis, Streptococcus mitis, Bortonella quintana, Coxiella burnetti, Legionella pneumopholi, Brucella melitensis, Biovar abartu, Staphylococcus aureus* [12,13]. Raetz et al. and Taylor et al. were reported that biosynthesis of lipopolysaccharide is blocked when the enzymes lpxA and lpxC are mutated or removed and which leads to destruction of outer membrane of the bacteria [43]. Hence, designing potential inhibitors for kdsA, kdsB, lpxA and lpxC can halt the lipopolysacharide biosynthesis pathway which is unique to pathogens and might be exploited for therapeutic intervention in the pathogens of atherosclerosis.

Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that form a mesh-like layer outside the plasma membrane of all bacteria, forming the cell wall. Phospho-N-acetylmuramoyl-pentapeptide-transferase (mraY) (S. No. 5 in Table 2) is one of the drug target mainly involved in the peptidoglycon biosynthesis which is unique and important for the survival of pathogens. MraY also plays a major role in cell shape and cell wall biosynthesis [44]. Hence, selecting mraY as a potential biological target for designing inhibitor would also dissolve the structural

integrity, flexibility and rigidity of the cell wall and expose the pathogens to osmotic lysis.

Fatty acid biosynthesis is an important metabolic pathway mainly carried out in cytosol of the pathogens. 3-oxoacyl-[acyl-carrier-protein] synthase 3 (fabH) (S. No. 6 in Table 2) is one of the enzyme drug target mainly involved in fatty acid biosynthesis. It catalyzes the condensation reaction of fatty acid synthesis by the addition to an acyl acceptor of two carbons from malonyl-ACP. Substrate specificity of fabH determines the biosynthesis of branched-chain and/or straight-chain of fatty acids [45]. FabH catalyses two stages such as acetyl Co-A condensation and malonyl-ACP decarboxylase reaction, blocking the ping-pong reaction of fabH will prevent the fatty acid biosynthesis. The enzyme is also reported as potential drug target against *Streptococcus pneumonia, Neisseria meningitides, Haemophilys influnzae, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mitis, Bortonella quintana, Coxiella burnetti, Legionella pneumopholi, Brucella melitensis and Biovar abartus* [13,45].

Mevalonate pathway is an important cellular metabolic pathway essential for the biosynthesis of molecules used in processes such as cell membrane maintenance in many bacteria. It is important for the production of dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP). The enzyme 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (ispG) (S.No. 7 in Table 2) is an oxido-reductase converts 2C-methyl-D-erythritol 4-cyclodiphosphate (ME-4cPP) into 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate in mevalonate biosynthesis. Rekittke et al. also reported that obstructing the ispG enzyme procession of *Thermus thermophilus* would be inhibited [46]. In the present study, ispG was defined as molecular drug target against pathogens of atherosclerosis which is mainly involved mevalonate pathway blocking of this leads to destruction of cell membrane of pathogens.

Protein synthesis refers to the process whereby biological cells generate new proteins. It is balanced by the loss of cellular proteins via degradation or export. Peptidyl-tRNA hydrolase (pth) (S.No. 8 in Table 2) is an enzyme target which is mainly involved in the protein synthesis of pathogens which catalyzes the reaction UDP-N-acetylmuramoyl-L-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine and undecaprenyl phosphate affords UMP and N-acetylmuramoyl-L-alanyl-D-glutamyl-L-lysyl-D-alanyl-D-alanine-diphosphoundecaprenol as reaction products. Pth was also identified as molecular drug target in pathogens like *Escherichia coli, Mycobacterium tuberculosis and Sulfolobus solfataricus* [47]. Blocking of pth directs to obstruct protein synthesis in the pathogens like *Escherichia coli, Mycobacterium tuberculosis* and *Sulfolobus solfataricus* [48] hence, it was proposed as putative drug target for atherosclerosis.

1-deoxy-D-xylulose-5-phosphate synthase (dxs) (S.No. 9 in Table 2) is an enzyme that catalyses the first step of thiazole biosynthesis. Condensation reaction of dxs between 2nd and 3rd carbon atoms of pyruvate and glyceraldehyde 3-phosphate to yield 1-deoxy-D-xylulose-5-phosphate (DXP) is involved in metabolic intermediate of thiamine metabolism [49]. Xiang et al., reported that dxs was conserved and ubiquitous among microbes like *Escherichia coli* and *Deinococcus radiodurans* which can further benefit the rational drug design [50]. Many of the organisms use thiamine but thiamine can only synthesised by bacteria, fungi etc. Hence, by designing novel inhibitors for dxs enzyme formation of thiamine can be stopped which is essential for growth and survival of bacteria.

Homologous recombination was pairing between two complementary single strands coming from two distinct homologous duplexes, with the formation of hetero duplexes joined by a four-way junction essential for the cell division. Crossover junction endodeoxyribonuclease (ruvC) (S.No. 10 in Table 2) is an enzyme that resolves holiday junction intermediates in genetic recombination. It is mainly involved in homologous recombination, genetic transformation, conjugation, transduction or F-duction which play a major role for the growth and survival of the organism in extreme conditions [51]. Therefore inhibiting the enzyme can hinder the mechanisms of homologous recombination which is vital for the cell division of pathogens. The enzyme ruvC was also reported as drug target in pathogens of bacterial meningitis like *Streptococcus pneumonia, Neisseria meningitides, Haemophilus influnzae* and *Staphylococcus aureus* [45].

DNA replication is theprocess of making an identical copy of DNA, using existing DNA as a template for the synthesis of new DNA strands. A complex network of interacting proteins and enzymes like DNA helicase, DNA polymerase, DNA clamp, single strand binding proteins, DNA gyrase, DNA ligase, DNA primase etc are required for DNA replication. DNA polymerase III subunit alpha (dnaE) (S.No. 11 in Table 2), DNA primase (dnaG) (S.No. 12 in Table 2) and DNA gyrase subunit A (gryA) (S.No. 13 in Table 2) are also involved in homologous recombination, mismatch repair and DNA repair. Replication of pathogens can be hindered by blocking dnaE, dnaG and gryA enzymes which further ceases the proliferation of pathogens. These enzymes were also endured as drug targets against bacterial pathogens like *Escherichia coli* [52], *Mycobacterium tuberculosis* [53], *Mycobacterium smegmatis* [54-56] and also identified as common drug target in pathogens causing bacterial meningitis like *Streptococcus pneumonia*, *Neisseria meningitides*, *Haemophilus influnzae*, *Staphylococcus aureus* [45]. Therefore, dnaE, dnaG and dryA were proposed as molecular drug targets against pathogens triggering atherosclerosis.

Transcription termination factor (rho) (S.No. 14 in Table 2) facilitates termination of transcription by a mechanism that involves rho binding to the nascent RNA by the activation of Rho's RNA-dependent ATPase activity and releases the mRNA from the DNA template. The cellular process that completes DNA-templated transcription and the phosphodiester bonds were ceased resulting in the RNA-DNA hybrid dissociation and DNA was released by RNA polymerases. Rho is mainly involved in the transcription that targets hundreds of transcription units in the pathogens, by inhibiting this enzyme we can obstruct the transcription mechanism followed by protein synthesis and can detain the proliferation of pathogens in atherosclerosis. The literature evidences proved that rho was also identified as a crucial drug target in Mycobacterium tuberculosis [57,58].

Prediction of subcellular localization

Computational prediction of subcellular localization provides a quick and inexpensive means for gaining insight into protein function, verifying experimental results, annotating newly sequenced bacterial genomes and detecting potential cell surface/secreted drug targets [25]. The analysis of PSORTb revealed that 14 enzymes were cytoplasmic and uvrA protein was a membrane protein. Similar results were also observed using CELLO v2.5 [27].

Druggable target prioritization

Fourteen common drug targets were submitted to DrugBank database and identified that the drug targets were identified as druggable targets in other pathogens like *Streptococcus pneumonia, Neisseria meningitides, Haemophilus influenzae, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mitis, Bortonella quintana, Coxiella burnetti, Legionella pneumopholi, Brucella melitensisand Biovar abartu Leptospira, Thermus thermophilus, Mycobacterium tuberculosis, Sulfolobus solfataricus, Escherichia coli and Deinococcus radiodurans* [12,13,43] (Table 2).

Grossman et al., Rossi et al. and White et al. were reported uvrA as an effective vaccine candidate against pathogens like *Escherichia coli, Mycobacterium tuberculosis* and *Deinococcus radiodurans* [57,58]. Hence, the supporting evidences in the literature reported that uvrA protein as common vaccine candidate against three pathogens causing atherosclerosis. Munikumar et al. were reported that enzymes fabH, dnaG, ruvC and dnaE as potential drug targets in four pathogens bacterial meningitis such as *Streptococcus pneumonia*, *Neisseria meningitides, Haemophilus influnzae* and *Staphylococcus aureus* [43]. Priyadarshini et al. was also reported fabH as a putative drug target against eight pathogens Staphylococcus aureus, *Enterococcus faecalis, Streptococcus mitis, Bortonella quintana, Coxiella burnetti, Legionella pneumopholi, Brucella melitensis and Biovar abartu* of infective endocardritis [13]. Umamaheswari et al. were identified kdsA, kdsB, lpxA, lpxC as potential drug targets against leptospirosis in the pathogen *Leptospira* spp [12]. Rekittke et al. were identified ispG as putative drug target against pathogen *Thermus thermophilus* [30]. Pth was reported as molecular drug target in pathogens like *Mycobacterium tuberculosis* and *Sulfolobus solfataricus* by Kumar et al. [31]. Mitra et al. and D'Heygère et al. has identified rho as a crucial drug target in Mycobacterium tuberculosis [55,56]. The selected 14 drug targets were found to be involved in vital pathways of pathogen such as lipopolysaccharide biosynthesis, peptidoglycan biosynthesis etc and targeting these enzymes can arrest the proliferation of pathogens causing atherosclerosis.

The identified 14 common drug targets in the present study affirmed to essential for the survival of pathogens, non-homologous to humans gut microbiota, human domains and also involved in protein-protein network. Lipopolysaccharide biosynthesis, peptidoglycan biosynthesis, fatty acid biosynthesis, mevalonate pathway, protein biosynthesis, amino acid biosysnthesis, thiamine metabolism, purine metabolism, DNA replication, DNA repair, RNA degradation, homologous recombination and nucleotide excision repair mechanism were particularly well represented in the pool of identified enzyme drug targets. Fourteen enzyme targets identified in the present study were cytoplasmic enzymes. As no common vaccine candidate is available for the three pathogens causing atherosclerosis till date, an attempt was made through this study and uvrA protein was proposed as common vaccine candidate against three pathogens. Vaccine candidate identified in this study was a membrane protein which is involved in nucleotide excision repair mechanism. These drug/vaccine targets are involved in key regulatory pathways of *Chlamydophila pneumonia, Porphyromonas gingivalis* and *Helicobacter pylori*. The common targets in the present study were also identified as drug targets and vaccine candidate in other bacterial pathogens [12,13,29-36] representing a promising broad-spectrum of targets that could be entered into drug/vaccine design pipelines. These drugs/vaccines would affect the pathogen's systems without interfering the host's essential biology. Due to their collective and intertwined roles of the identified fourteen drug targets and one vaccine candidate represents a panel of broad functional relevance in drug discovery research and would bring new possibility in developing powerful drug molecules for the treatment regimen in pathogenesis of atherosclerosis.

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