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Drug-target network in myocardial infarction reveals multiple side effects of unrelated drugs

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The systems-level characterization of drug-target associations in myocardial infarction (MI) has not been reported to date. We report a computational approach that combines different sources of drug and protein interaction information to assemble the myocardial infarction drug-target interactome network (My-DTome). My-DTome comprises approved and other drugs interlinked in a single, highly-connected network with modular organization. We show that approved and other drugs may both be highly connected and represent network bottlenecks. This highlights influential roles for such drugs on seemingly unrelated targets and pathways via direct and indirect interactions. My-DTome modules are associated with relevant molecular processes and pathways. We find evidence that these modules may be regulated by microRNAs with potential therapeutic roles in MI. Different drugs can jointly impact a module. We provide systemic insights into cardiovascular effects of non-cardiovascular drugs. My-DTome provides the basis for an alternative approach to investigate new targets and multidrug treatment in MI.

M yocardial infarction (MI) and its associated complications represent a major cause of death and health-care costs in the world. Despite significant interventional advances, treatment options rely on a relatively small number of approved drugs, and little is known of underlying interactions between these compounds, targets and complex biological pathways. Moreover, most of these drugs were developed without a deep understanding of their systems-level molecular activity.

Targets and drugs do not interact in isolation and unintended interactions may give rise to undesirable effectiveness and even safety effects on patients. We aim to enhance our understanding of the relationships between known and putative therapeutic compounds and targets used to treat MI patients. We integrate different types of information resources about drugs and proteins by means of a biological network framework to: a. offer an annotated research resource for future investigations, b. investigate biologically meaningful properties of this drug-target interaction network, and c. establish novel associations between drugs, targets and MI-related molecular mechanisms.

Previous research has shown the usefulness of drug-target interaction networks to find inter-disease associations, repurpose drugs and predict side effects[1–6]. However, drug-target interaction network approaches have not been specifically explored in the area of cardiovascular research. Moreover, the majority of published methods from other clinical settings tend to be biased toward approved drugs and their known targets[3,6,7]. This limits the utility of drug-target interaction networks with regard to their capacity to discover new knowledge of potential targets and off-target associations at a proteome- or systems-level. This is particularly evident in clinical domains with relatively small numbers of approved drugs and their interacting targets, as is the case of MI. Furthermore, there is a lack of investigations specific to the MI domain.

In this research we generate and analyze a MI drug-target interactome network (*My-DTome*), which extends beyond the incorporation of approved drugs and primary targets. My-DTome integrates information about clinically relevant drug-drug, drug-target and protein-protein interactions, including those without well-established associations with MI. The resulting network lays the foundation for a more comprehensive visualization of the drug-target interaction landscape. This offers new opportunities for understanding the biological basis of treatment efficacy, safety and patient management. We have developed a network-driven, integrated approach to assemble and analyze My-DTome.
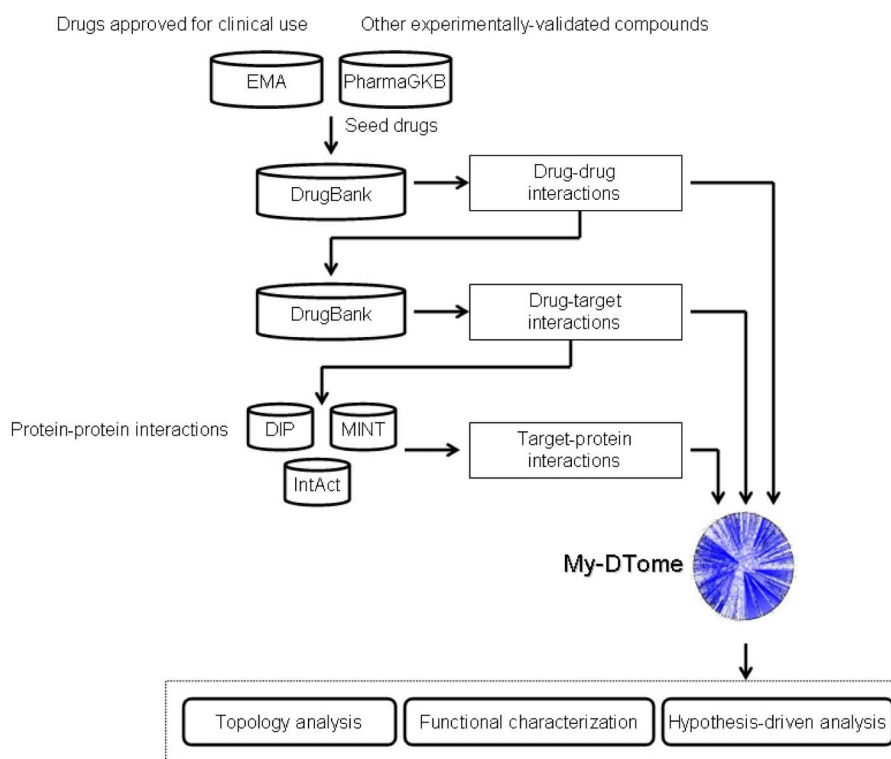
Results

My-DTome generation. To generate My-DTome, first we incorporated all drugs approved by the European Medicines Agency (EMA) (http://www.ema.europa.eu) for use in MI. This drug set was expanded by retrieving other compounds related to MI, as annotated by the Pharmacogenomics Knowledge Base (PharmGKB)[8]. These two sets of MI-related drugs constitute our set of *seed drugs*. This set was used to query drug-drug interactions stored in DrugBank[9]. Next, we obtained the targets for the seed drugs and their interacting drugs. This was followed by the extraction of validated human protein-protein interactions (PPI) for the resulting set of targets from different high-quality, complementary databases. The union of the drug-drug, drug-target and target-protein interactions resulted in My-DTome. In its graphical network format, nodes represent either compounds or proteins and edges encode either drug-drug, drug-protein or protein-protein interactions. Fig. 1 depicts My-DTome network development and analysis phases.

My-DTome: A novel drug-target network in MI. My-DTome network consists of 2097 nodes and 3958 edges, with a highly connected network component comprising 2095 nodes and 3957 edges. We focused on the latter for subsequent analyses (Supporting Information, Dataset 1). Among the edges, there are 1125 drug-target interactions. Fig. 2A shows a global view of My-DTome with colour-coded nodes: seed drugs (green), other drugs (blue), drug targets (orange) and other interacting proteins (grey). The 38 seed drugs (only 10 EMA-approved) display different numbers of interactions with other drugs (715 in total, mean value: 18.82) (Fig. 2B). The anticoagulant warfarin exhibits the highest number of drug interactions. Seed and other drugs also display a rich landscape of interacting targets, whose numbers do not appear to be biased toward seed drugs (Fig. 2C). Among the 38 seed drugs, verapamil has the highest number of targets (17 targets). Clozapine (26 targets) and triazolam (20 targets) are examples of highly connected drugs that are not labeled as seed drugs. The former is used to treat psychiatric disorders, while the latter is a sedative. The
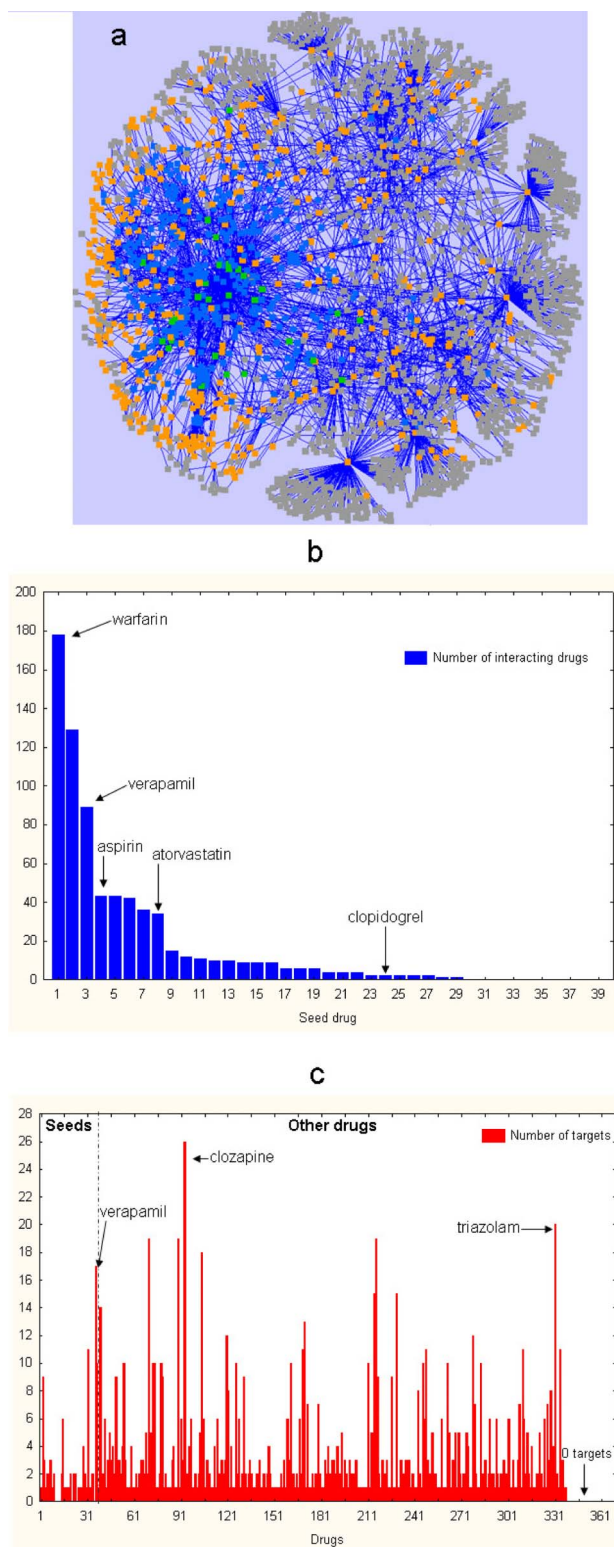
average number of drug targets per seed and other drugs was 2.45 and 3.12 respectively. This further indicates that My-DTome encodes a drug-target space that is not biased toward approved drugs. The availability of My-DTome is by itself a useful compendium that reflects current and potential future drug discovery directions in MI. We next applied different computational and statistical methods to disentangle key global and local properties in this network.

My-DTome: Fundamental global and local properties. Topological analysis of My-DTome may offer insights into biologically relevant connectivity patterns, which may pinpoint highly influential drugs or targets. The distribution of My-DTome node degrees (number of edges per node) follows a power-law distribution (Fig. 3A, power-law exponent (γ) = 1.42, r^2 = 0.84), i.e., most nodes display a small number of interactions, but a few of them are highly connected. Highly connected nodes are referred to as hubs. This also indicates My-DTome interactions were not generated at random and that they may encode clinically relevant associations. Note that if My-DTome represented a randomly-generated network the power-law property would not hold. Figure 3B illustrates the degree distribution from a randomly-generated network with the same number of nodes and edges as My-DTome (r^2 = 0.55). Apart from exhibiting a power-law property, we find that My-DTome is highly modular based on the examination of its clustering coefficient. Its average network clustering coefficient is larger than the average value of 10^3 randomized networks obtained by randomly shuffling the interactions in My-DTome (0.041 vs. 0.039, P < 0.0001, single-sample t-test).

Another fundamental property of network nodes is their capacity to be located in the shortest communication paths between different pairs of nodes in the network. This property defined as *traffic* is measured by computing the "betweenness centrality" of each node in the network (Methods). High traffic nodes are also referred to as network bottlenecks. Different researchers, including us, have demonstrated the potential biological relevance of high traffic nodes with regard to their functional coordinating roles and phenotypic

Figure 1 | My-DTome generation and analysis framework.

Figure 2 | My-DTome: A novel drug-target network in myocardial infarction. (a) Global view. Seed drugs (green), other drugs (blue), drug targets (orange) and other interacting proteins (grey). (b) Distribution of seed drugs and their interacting drugs. (c) Target distribution of My-DTome drugs.
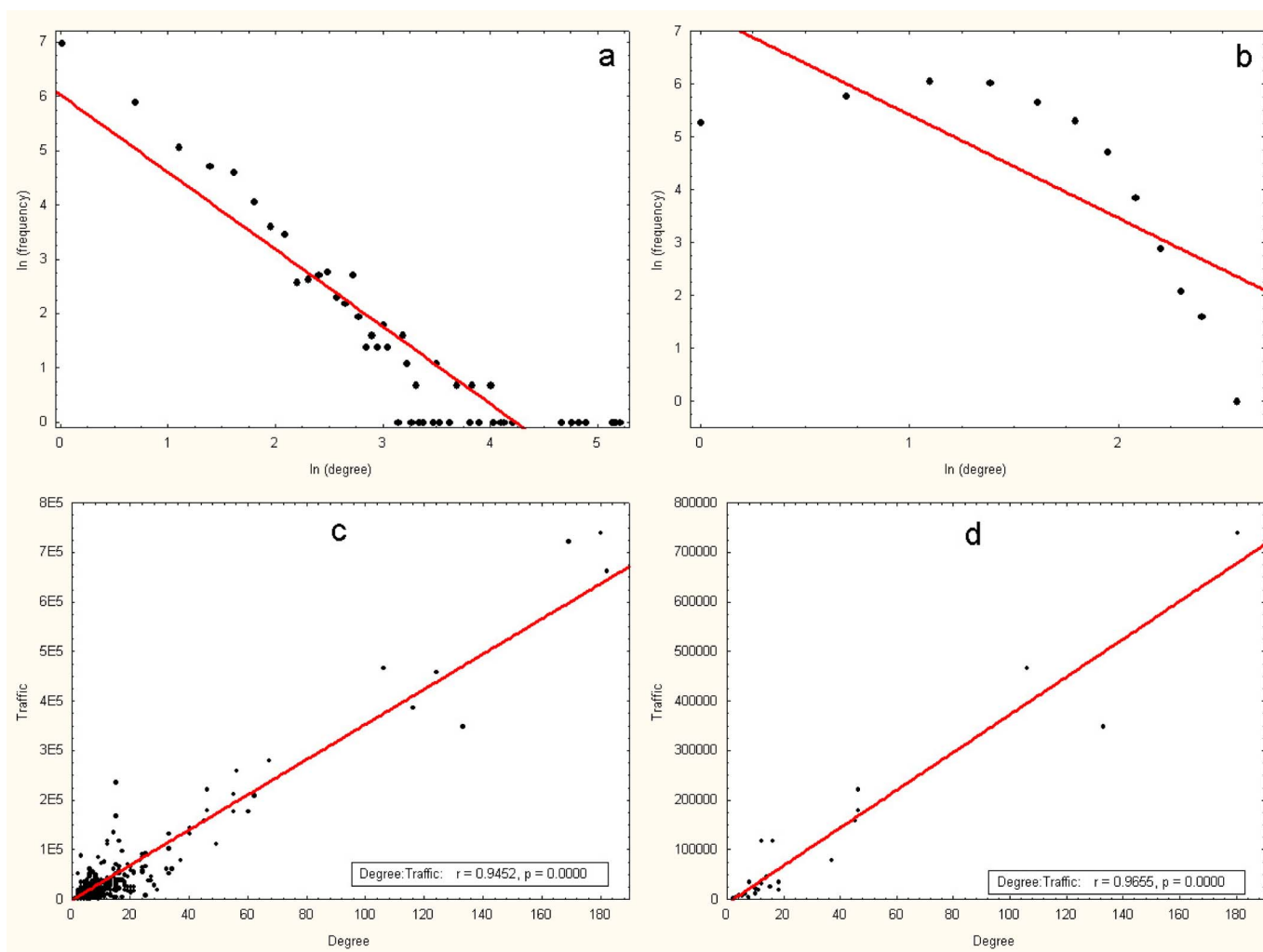
indirect interactions. Different network regions may underlie different biological pathways, processes or cellular localisations, as specified below.

In My-DTome we find that the degree and traffic values of seed drugs are strongly correlated (Fig. 3D). This also implies that a small number of seed drugs, e.g., warfarin, exhibit dual hub-bottleneck properties. This indicates particularly influential roles for such drugs on apparently unrelated targets, drugs and pathways through direct and indirect interactions. One may argue that a strong correlation between traffic and degree reflects a possible bias of available information towards widely-investigated (e.g., approved) drugs in the context of MI. However, this is not the case in My-DTome. We note that unapproved MI drugs, as well as others not included in the seed set, can behave as both hubs and bottlenecks (e.g., acenocoumarol). Moreover, we find that compounds not included in the seed set display either high degree or high traffic values. Examples of hub compounds that are not seed drugs are: clozapine, triazolam, ginkgo biloba, midazolam and clobazam. Drugs such as imatinib, lithium, treprostinil, gefitinib and rifabutin exemplify My-DTome bottlenecks, which are not MI-specified treatments. Furthermore, we find that drug targets that are not directly linked to MI treatment (e.g., ABL1 and EGFR) display both high degree and high traffic values in My-DTome.

My-DTome is a biologically-meaningful modular network. We corroborated the modularity of My-DTome by applying a network clustering algorithm, which have been previously shown to detect groups of highly-connected nodes underlying specific clinical phenotypes and responses[10,13]. In My-DTome we identified a total of 26 modules of highly-interconnected nodes with sizes ranging from 4 (Module 17) to 319 (Module 16) nodes (Figure 4) (Supporting Information, Dataset 2). Other very large modules are Module 25 (277 nodes), Module 13 (245 nodes) and Module 22 (154 nodes). To estimate the potential biological relevance of these modules, we examined each module on the basis of the participation of its targets in specific shared biological processes, cellular localisations, functions and pathways (Supporting Information, Dataset 3).

We detected strong statistical associations between different modules and Gene Ontology (GO)[14] biological processes (17 modules), GO cellular components (16 modules) and GO molecular functions (17 modules) (P = 0.01, Methods). We also observed 18 modules statistically enriched by diverse biological pathways as defined by the KEGG (Kyoto Encyclopedia of Genes and Genomes)[15]. Seven modules did not include proteins with statistically detectable associations with these functional attributes (at P = 0.01). Fig. 5 summarizes this functional characterization of My-DTome modules based on the statistically strongest associations detected. My-DTome modules may significantly alter the normal or pathological operation of diverse biological process ranging from RNA splicing and secretion to wound healing and metabolism. Moreover, drugs in My-DTome can perturb targets with coordinated functional roles in different signalling and regulatory cascades. Many of these alterations can be mainly driven at the extracellular level. Nevertheless, significant functional activity can be detected in the nucleus (Module 22), different complexes (e.g., proteasome, Module 25) and organelles (e.g., autophagic vacuole, Module 11). All approved MI drugs, as well as warfarin and aspirin, were assigned to Module 16, which shows significant involvement in wound healing, sodium channel activity and coagulation cascades. My-DTome proteins also belong to different network modules significantly linked to molecular pathways with relevance in cancer research (e.g., Modules 22 and 23).

My-DTome encompasses evidence that the activity of (or responses to) drugs may be driven by microRNAs (miRNAs): miR-598, miR-451, miR-133b, miR-944, miR-29a and miR-155 (Fig. 5). This was discovered by estimating the statistical enrichment in

effects[10,11,12]. We observe that the distribution of traffic and degree values in My-DTome is strongly correlated (Fig. 3C). This means that My-DTome hubs also tend to encode bottlenecks, and that they can influence different network regions through both direct and

Figure 3 | Fundamental topological properties and relationships. (a) My-DTome degree value distribution. (b) Randomized network degree distribution. (c) Relationship between traffic and degree distributions (r: correlation coefficient). (d) Relationship between traffic and degree distributions in the seed drug set.

miRNA-target associations observed in each module (P = 0.01, Methods). There was no statistically detectable association between miRNAs and the module containing approved MI and other key drugs, such as warfarin and aspirin (Module 16). A significant relationship between miR-944 and Module 13, which contains the seed drug verapamil, is observed. Despite the absence of direct strong relationships between miRNAs and modules containing approved MI drugs, we note that indirect miRNA-mediated regulation may occur via inter-module interactions. For instance, various protein-drug and drug-drug interactions occur between modules 13 and 16 (Supporting Information, Dataset 2).

The role of miR-155 in diffuse large B-cell lymphoma has been reported[16], miR-451 has been linked to glioma cell adaptation[17] and miR-29a can be expressed in neuronal cells[18]. We did not find published associations between miR-598 and cardiovascular disease. Recent research has shown that miR-451 and miR-133b are dysregulated in MI[19,20]. The latter encourages the exploitation of My-DTome to aid in the identification of potential new therapies or targets in MI.

Despite these potentially interesting associations, cautious interpretation is warranted as these findings relied on statistical analysis of known miRNA bindings. Moreover, experimental testing of these hypotheses will be required to support further assessments of potential clinical application.

Approved drugs and other compounds jointly impact diverse clinically-relevant molecular pathways. Multiple drugs can jointly perturb the same module-derived pathways. A more detailed characterization of these relationships may offer a viable strategy to explain side effects, to improve treatment effectiveness and to explore potential drug repositioning. To demonstrate this, we focused on the most highly altered KEGG pathways detected above (Fig. 5) and extracted all the drugs associated with the corresponding modules. Considering intra-module interactions only, we find that 48% of the total number of drugs included in My-DTome (351) is linked to the complement and coagulation cascade. Calcium signalling is shown as the second most targeted pathway (32% of all drugs). The other significantly impacted pathways are each linked to less than 5% of the My-DTome drugs (Fig. 6). Fig. 6 and Supporting Information (Dataset 2) display a more detailed view of (intra-module) relationships between multiple drugs and specific molecular pathways.

Drugs of common use in MI setting: A new look at drug-drug interactions. A closer inspection of drugs commonly applied in the context of MI unfolds a previously unknown diversity of drug-drug interactions. Such potentially clinically relevant associations are inferred from either direct or indirect (including intra- and inter-module) interactions. To illustrate this point, Fig. 7 shows
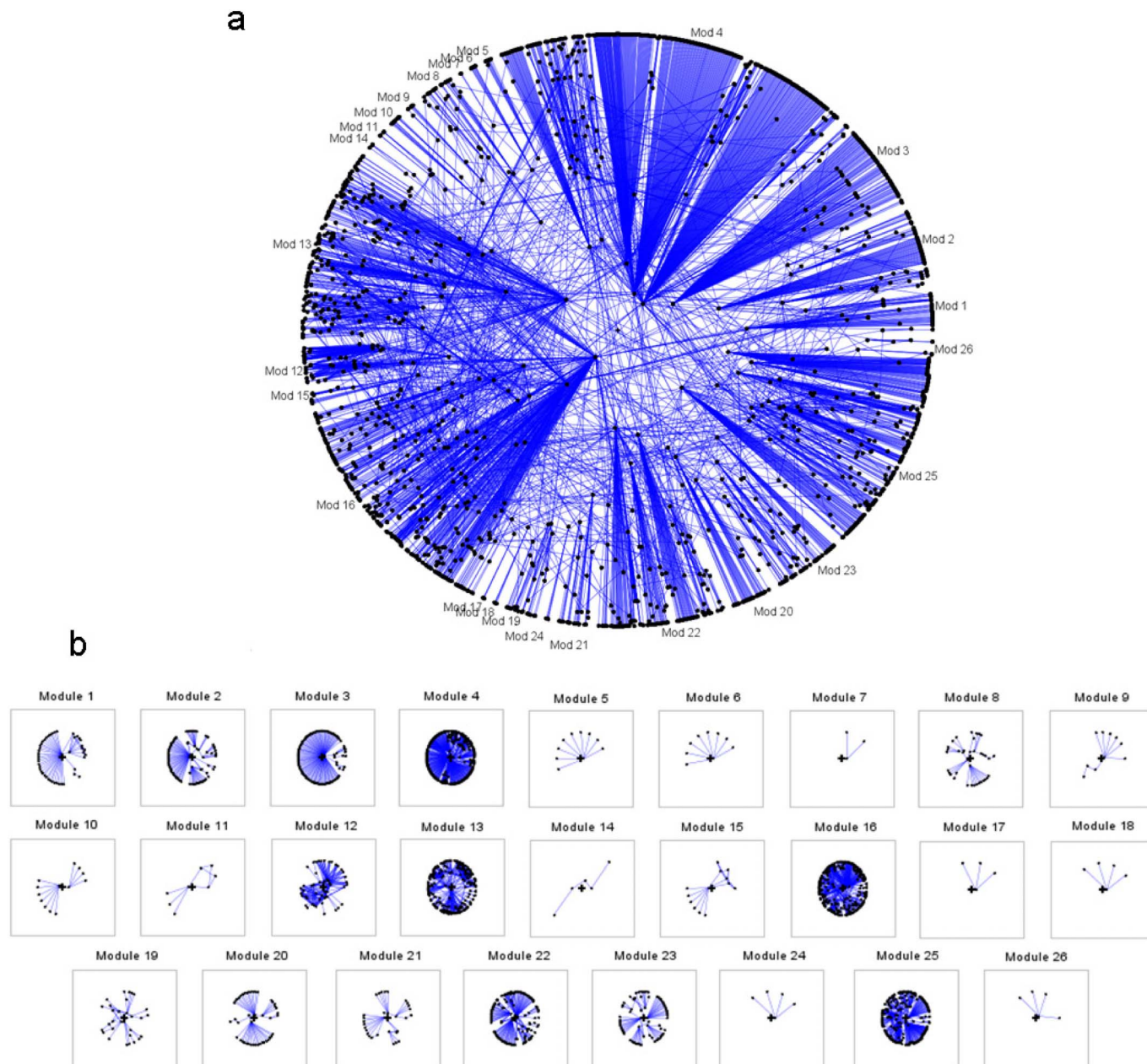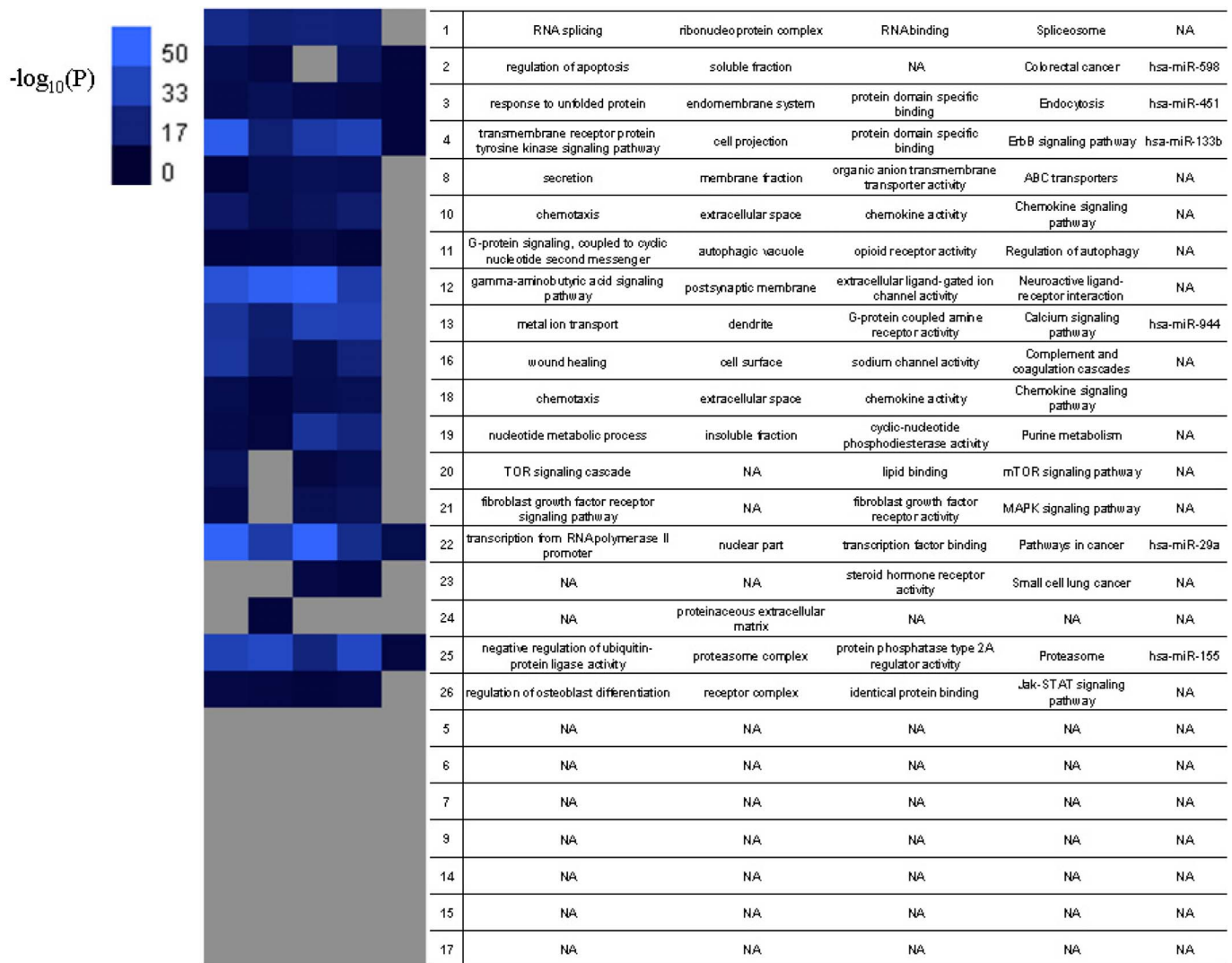
Figure 4 | Modular organisation of My-DTome. (a) Global, modular view of My-DTome. (b)My-DTome modules depicted in isolation.

(intra-module) interactions for aspirin, clopidogrel, warfarin, atorvastatin and verapamil. To facilitate visualisation, partial views of each module may be shown (e.g., clopidogrel). At the intra-module level, these drugs are associated with hundreds of drugs: aspirin, clopidogrel and warfarin each interacting with 169 drugs in the same module, and atorvastatin and verapamil each interacting with 111 drugs (Supporting Information, Dataset 2). If potential inter-module interactions are included, the possible set of drug interactions for these two groups is increased to 240 and 238 drugs respectively. We observe that the sets of drug interactions are in most cases larger than those stored in Epocrates (https://online.epocrates.com/home), a drug information system that is well-known in the medical community. For example, Epocrates Online defines 120 and 201 drug interactions for clopidogrel and verapamil respectively. This includes its contraindicated, avoid/use alternative, monitor/modify Tx and caution advised categories.

Also we observe partial overlaps between these and My-DTome interactions. Nevertheless, we note that the level of commonality between these sources is difficult to accurately estimate as Epocrates includes diverse combinations of single compounds and various commercial versions of the same drug. These findings motivate further investigations of the application of My-DTome to support patient care management.

My-DTome can also provide new perspectives to investigate multidrug therapy. For instance, we find that clopidogrel directly (and indirectly) interacts with drugs that alter clopidogrel responsiveness through competitive inhibition of CYP in the MI setting[21]. My-DTome displays inter-module associations between clopidogrel, atorvastatin, itraconazole and ketoconazole (modules 13 and 16, Supporting Information, Dataset 2), as well as an intra-module relationship with st. John's wort (module 16). None of these associations were included in DrugBank as direct interactions. Although such relationships will require stringent assessments to estimate their clinical relevance, these observations suggest that My-DTome may be exploited to alert clinicians about potential adverse events.

|	BP	CC	MF	KEGG	miRNA
1	RNA splicing	ribonucleoprotein complex	RNA binding	Spliceosome	NA
2	regulation of apoptosis	soluble fraction	NA	Colorectal cancer	hsa-miR-598
3	response to unfolded protein	endomembrane system	protein domain specific binding	Endocytosis	hsa-miR-451
4	transmembrane receptor protein tyrosine kinase signaling pathway	cell projection	protein domain specific binding	ErbB signaling pathway	hsa-miR-133b
8	secretion	membrane fraction	organic anion transmembrane transporter activity	ABC transporters	NA
10	chemotaxis	extracellular space	chemokine activity	Chemokine signaling pathway	NA
11	G-protein signaling, coupled to cyclic nucleotide second messenger	autophagic vacuole	opioid receptor activity	Regulation of autophagy	NA
12	gamma-aminobutyric acid signaling pathway	postsynaptic membrane	extracellular ligand-gated ion channel activity	Neuroactive ligand-receptor interaction	NA
13	metal ion transport	dendrite	G-protein coupled amine receptor activity	Calcium signaling pathway	hsa-miR-944
16	wound healing	cell surface	sodium channel activity	Complement and coagulation cascades	NA
18	chemotaxis	extracellular space	chemokine activity	Chemokine signaling pathway	NA
19	nucleotide metabolic process	insoluble fraction	cyclic-nucleotide phosphodiesterase activity	Purine metabolism	NA
20	TOR signaling cascade	NA	lipid binding	mTOR signaling pathway	NA
21	fibroblast growth factor receptor signaling pathway	NA	fibroblast growth factor receptor activity	MAPK signaling pathway	NA
22	transcription from RNA polymerase II promoter	nuclear part	transcription factor binding	Pathways in cancer	hsa-miR-29a
23	NA	NA	steroid hormone receptor activity	Small cell lung cancer	NA
24	NA	proteinaceous extracellular matrix	NA	NA	NA
25	negative regulation of ubiquitin-protein ligase activity	proteasome complex	protein phosphatase type 2A regulator activity	Proteasome	hsa-miR-155
26	regulation of osteoblast differentiation	receptor complex	identical protein binding	Jak-STAT signaling pathway	NA
5	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA
9	NA	NA	NA	NA	NA
14	NA	NA	NA	NA	NA
15	NA	NA	NA	NA	NA
17	NA	NA	NA	NA	NA

Figure 5 | Functional dissection of My-DTome modules. In heat map: P is the probability associated with the functional enrichment observed in each module (Methods), colours reflect log-transformed values of P. BP: biological process, MF: molecular function, CC: cellular component. NA: No statistically detectable association. Modules are numbered as in Fig. 4. The lighter the colour, the more significant the statistical association.

My-DTome outlines associations between non-cardiovascular drugs and cardiovascular complications. We explored the predictive capacity of My-DTome to help researchers to explain connections between drugs not prescribed in the specific MI setting and major, seemingly unexpected effects. To illustrate this application, we first queried My-DTome with rosiglitazone and celecoxib: antidiabetic and pain relief drugs respectively. Both drugs have been reported to increase the risk of cardiovascular complications, including myocardial infarction[22,23]. Although rosiglitazone was the only drug included in the seed set, it is not surprising to find both drugs in My-DTome because of their known links to MI. However, a closer look at our module-based analysis suggests more detailed and statistically detectable relationships not provided by either DrugBank or PharmGKB. Rosiglitazone and celecoxib were assigned to Modules 13 and 16 respectively.

We found statistically powerful relationships between Module 13 and the regulation of the force of heart contraction (P=0.001, rank: 167th), positive regulation of heart contraction (P= 5.8E-07, rank: 79th) and positive regulation of heart rate (P= 9.3E-06, rank: 105th). Ranks represent significance positions among 212 statistically detectable associations (GO biological processes). We also found strong perturbations in KEGG cardiovascular-specific pathways:

cardiac muscle contraction (P = 2.54E-11, rank: 3rd among 12 statistically detectable associations), dilated cardiomyopathy (P = 1.74E-10, rank: 5th), arrhythmogenic right ventricular cardiomyopathy (P = 2.33E-10, rank: 7th) and hypertrophic cardiomyopathy (P = 1.99E-09, rank: 8th). Module 16 is significantly implicated in vasoconstriction (P = 7.96E-11, ranked 8th among 96 GO biological process perturbations), sodium channel activity (P= 3.1E-08, ranked 1st among 36 GO molecular process perturbations), hypertrophic cardiomyopathy (P = 6.1E-04, ranked 7th among 15 perturbed KEGG pathways) and arrhythmogenic right ventricular cardiomyopathy (P=1.6E-03, ranked 11th among 15 KEGG pathways).

As a second test, we considered non-cardiovascular drugs that were not represented in My-DTome. We focused on fenfluramine and dexfenfluramine, which are appetite suppressants removed from the US drug market and elsewhere by 1997, but still at the centre of legal proceedings in France[24–27]. Various independent studies involving different patient populations have demonstrated the association between exposure to these compounds and cardiovascular complications, such as valvular heart disease and pulmonary arterial hypertension[21,26,27]. Although these drugs were not explicitly included in My-DTome, we found different interaction routes between them and significant perturbations of biological processes and pathways regulating heart function, remodelling and blood pressure (Fig. 8).
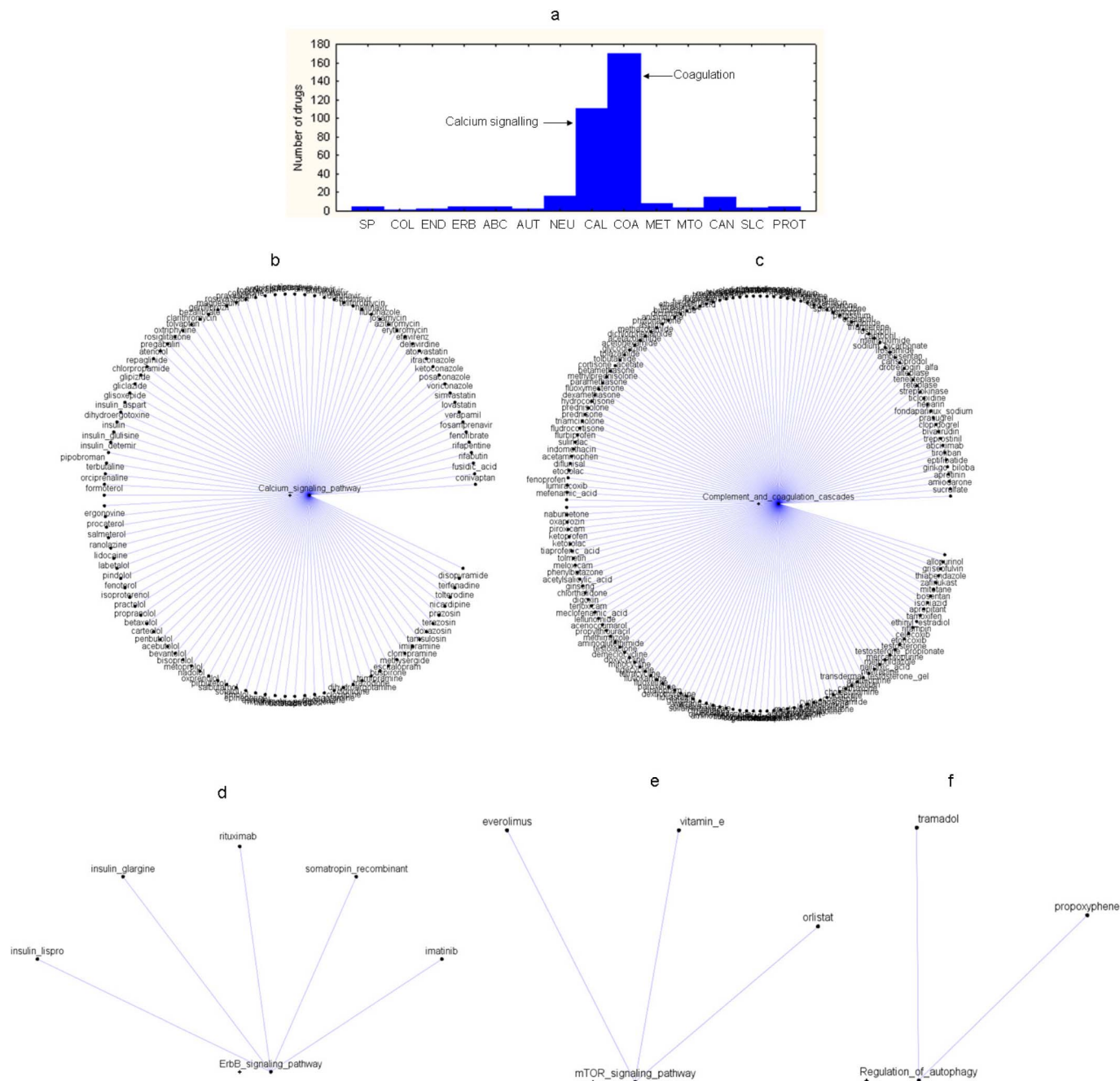
Figure 6 | **Relationships between multiple drugs and specific KEGG pathways.** (a) Distribution of numbers of drug-pathway relationships. SP: spliceosome, COL: colorectal cancer, END: endocytosis, ERB: ErbB signalling pathway, ABC: ABC transporters, AUT: regulation of autophagy, NEU: neuroactive ligand-receptor interaction, CAL: calcium signalling pathway, COA: complement and coagulation cascades, MET: purine metabolism, MTO: mTOR signalling pathway, CAN: pathways in cancer, SLC: small cell lung cancer, PROT: proteasome. From (b) to (f), examples of multiple drugs-pathway interactions: CAL, COA, ERB, MTO and AUT pathways.

Fenfluramine and dexfenfluramine are known to interact with different My-DTome components: HTR1B, HTR2C, HTR2B and SLC6A4, as well as the antidepressant drugs fluoxetine and fluvoxamine[8]. HTR1B, HTR2C and HTR2B are serotonin receptors, while SLC6A4 is a serotonin transporter. Our module-driven My-DTome analysis indicates that these genes are statistically associated with positive regulation of vasoconstriction (HTR2C), regulation of blood vessel size (HTR2C), blood circulation (HTR2B and HTR2C), alcohol metabolic processes (HTR2C) and response to ethanol (HTR1B). These proteins are assigned to My-DTome modules 13 (HTR1B, HTR2C and HTR2B) and 16 (SLC6A4). The drugs fluoxetine and fluvoxamine are members of Module 16. As shown above, these modules are strongly statistically associated with specific biological processes and alterations relevant to serious cardiovascular complications.

Discussion

Based on the integration of information from EMA, PharmaGKB, DrugBank and other public databases, we investigated different network biology methods and tools to contribute: a. My-DTome, a drug-target interaction resource that will assist future translational research in MI, b. new theoretical and clinical insights into the drug-target interaction landscape of MI, c. novel associations between drugs, targets and critical networks with potential clinical value.
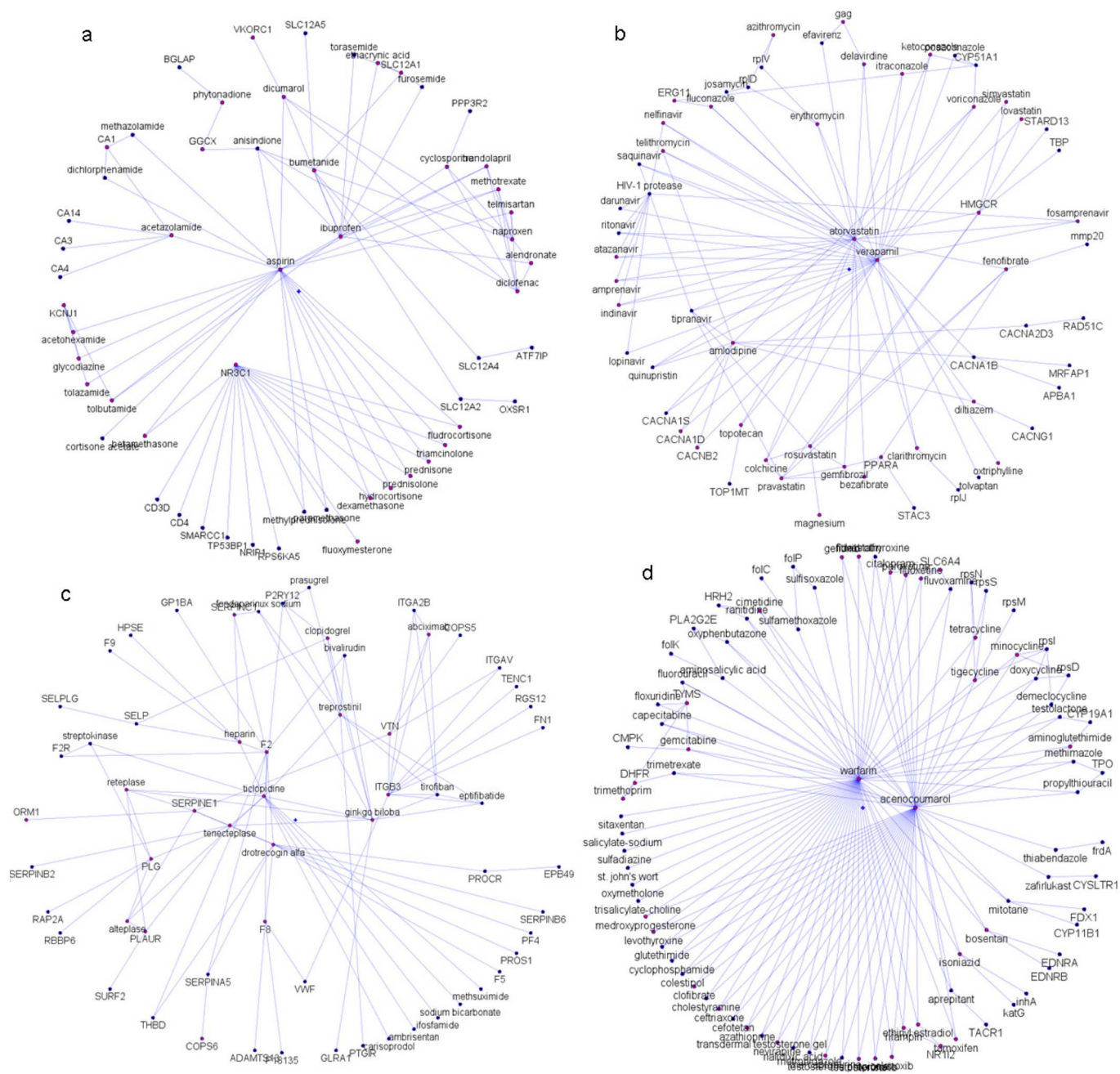
Figure 7 | Visualisation of intra-module interactions between selected drugs of common use in MI setting, other compounds and their targets. From (a) to (d): aspirin, atorvastatin and verapamil, clopidogrel and warfarin respectively. To facilitate visualisation, partial views of each module may be shown (e.g., clopidogrel's module).

Our findings indicate that well-known drugs are not the only critical My-DTome nodes on the basis of topological analysis and the detection of highly perturbed molecular pathways. Apparently unrelated drugs can display comparable network connectivity and functional features as those shown by seed drugs. Our analyses show that most drugs directly perturb calcium signalling and coagulation cascades. This suggests, first, that most known and potentially novel compounds affect clinically-relevant molecular pathways. The finding that the vast majority of these compounds target only two pathways, among the large number of biological pathways involved in the occurrence and outcome of MI, suggests that there is still room to design new drugs targeting so far unexplored pathways. Secondly, this may provide new research routes with regard to novel application of compounds not yet investigated in the specific MI context.

This allowed us to characterize connections between non-cardio-vascular drugs (rosiglitazone, celecoxib and fenfluramine) and major, unwanted cardiovascular effects. Thirdly, it reveals new levels of complexity and activity at a systems-level not formulated prior to this investigation. By data mining My-DTome, we identified several miRNAs that are known and yet-to-be investigated potential novel therapeutic targets for MI treatment. Experimental validation of these associations will be required to further estimate their potential clinical value.

Although our results may be constrained by data incompleteness, they are based on high quality drug and protein interaction annotations. Moreover, the reported modularity-based associations represent statistically detectable patterns. Future My-DTome analyses should directly address the level of ambiguity associated with
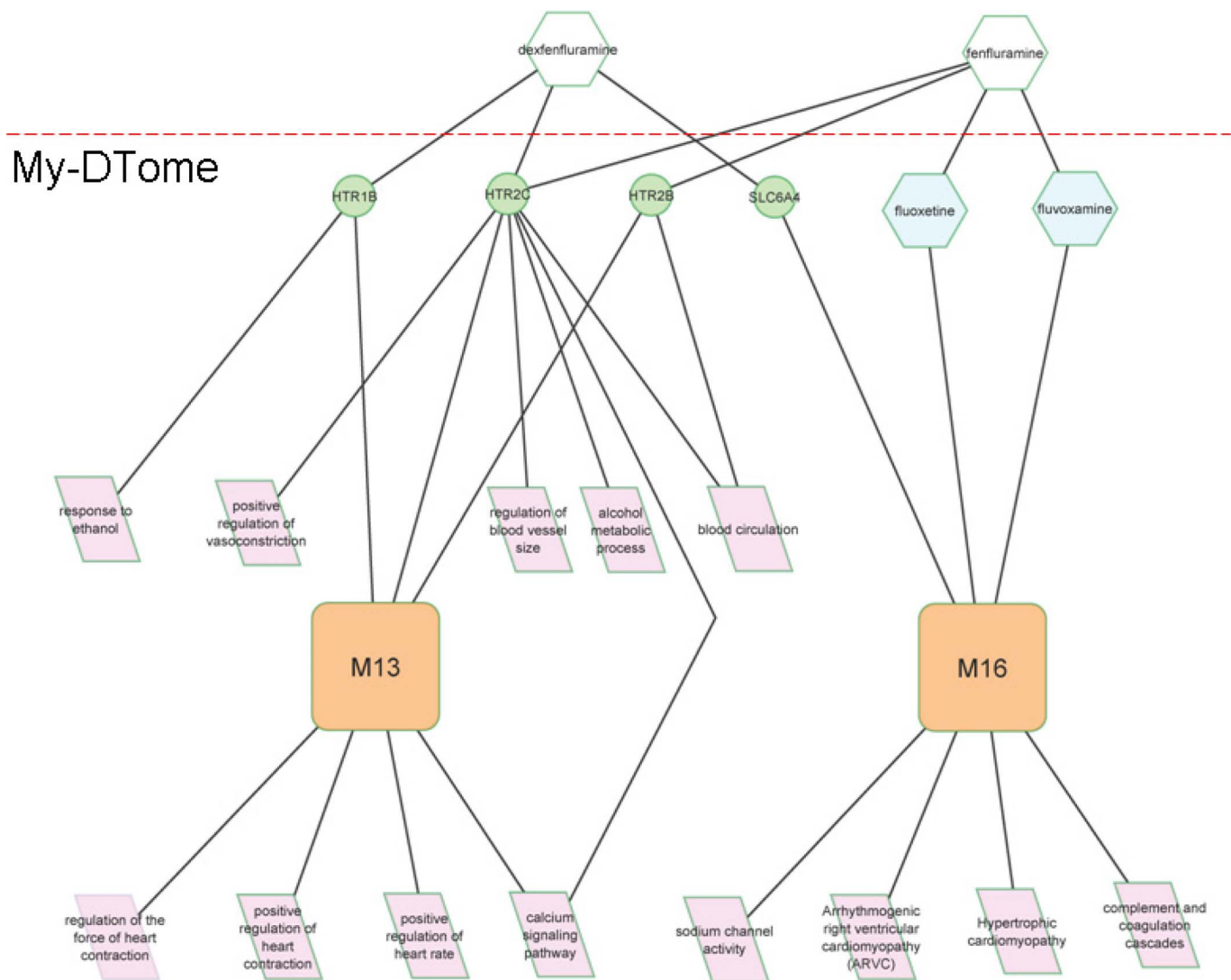
Figure 8 | My-DTome predicts unexpected cardiovascular side effects of appetite suppressants fenfluramine and dexfenfluramine.

drug-drug interactions. More concretely, there is a need to specify the nature of drug-drug interactions, e.g., interaction types and description of supporting evidence. Such a task will naturally be constrained by the availability of this type of information in the original sources.

Our My-DTome investigation supports the notion of applying system-based approaches to elucidating drug and target interactions in the MI context. The prevailing approach based on descriptive views of approved drugs and their interactions cannot adequately characterize the complexity of the therapeutic panorama in MI. This implies reduced opportunities to anticipate unwanted effects and potential novel treatments. We expect new research directions to further address network-driven complexity and therapeutic impact. In the long-term, this will open new possibilities for a more personalised treatment of MI.

Methods

Drug and protein interaction databases. We obtained the seed drugs from the EMA (http://www.ema.europa.eu) and PharmGKB (8) databases (as of 8 December 2010). We considered drugs included in the category of "myocardial infarction" only. Drug-drug and drug-target interactions were downloaded from DrugBank[9]. Target-protein interactions were obtained by integrating experimentally validated interactions in humans from the DIP[28], IntAct[29] and MINT[30] databases. We used them because their good interactome coverage, complementarity and low-error have been demonstrated[31]. We concentrated on immediate direct interactions. All My-DTome associations are available in the Supporting Information (Dataset 1).

Network features and topological analysis. The degree of a node is the number of edges associated with a node. Node traffic is the total number of shortest paths that go through the node, and which connect any two other nodes in the network. Therefore, high traffic nodes can represent "cross-communication" hotspots or "bottlenecks" in the network. The clustering coefficient measures (from 0 to 1) the level of interconnectivity around a node. A node at the centre of a fully-connected sub-network will display a clustering coefficient equal to 1. A network with a higher average clustering coefficient suggests the existence of network modularity. For network comparisons, randomized networks were obtained by randomly shuffling the edges of My-DTome, while keeping unchanged its total number of nodes. My-DTome follows a power-law degree distribution of the form: $D(k) \sim k^{-\gamma}$, k represents the degree values, $D(k)$ is the frequency of nodes with degree k, γ is the power-law exponent and $\sim$ represents proportionality. On a logarithmic scale, γ represents the slope of the line fitted to this distribution (Results).

Module identification and characterization. We identified network modules with a "greedy" network clustering algorithm[13]. This algorithm searches for sub-networks with highly-connected nodes, and the outcome is the partition of the network into a set of disjoint modules that optimize a "modularity score", Q. Q computes the number of edges observed in a putative module in relation to the number of edges that would be obtained by chance, i.e., by randomly pairing the proteins in the putative module. This algorithm has been applied to detect disease-related modules in different network biology investigations[10,13]. Statistical associations between modules and functional annotations (GO terms, KEGG pathways and miRNA targets) were estimated by finding over-representation of annotations in the modules in relation to the other genes in the human genome. This is done by running Fisher's exact tests for 2×2 contingency tables, followed by the (Benjamini & Hochberg method) correction of the P values to account for multiple-hypothesis testing. We concentrated on the analysis of associations with (corrected) P = 0.01 or lower values. miRNA-target associations were mined from miRBase[32].

Statistical analysis and tools. We performed network visualization with Cytoscape[33] and Polar Mapper[13]. Module identification and traffic value estimation were computed with Polar Mapper[13]. Other topological analyses were implemented with Cytoscape plug-ins: NetworkAnalyzer[34] and the Random Network plugin[35]. We conducted functional characterization of modules with the Fatigo tool, under the Babelomics (v4.1) platform[36]. Other statistical analyses were done with the *Statistica* package[37].

1. Arrell, D. K. & Terzic, A. Network systems biology for drug discovery. *Clin Pharmacol Ther* **88**, 120–125 (2010).
2. Dudley, J. T., Schadt, E., Sirota, M., Butte, A. J. & Ashley, E. Drug discovery in a multidimensional world: systems, patterns, and networks. *J Cardiovasc Transl Res* **3**, 438–447 (2010).
3. Keiser, M. J. *et al*. Predicting new molecular targets for known drugs. *Nature* **462**, 175–181 (2009).
4. Kinnings, S. L., Xie, L., Fung, K. H., Jackson, R. M. & Bourne, P. E. The Mycobacterium tuberculosis drugome and its polypharmacological implications. *PLoS Comput Biol* **6**, e1000976 (2010).
5. Yildirim, M. A., Goh, K. I., Cusick, M. E., Barabasi, A. L. & Vidal, M. Drug-target network. *Nat Biotechnol* **25**, 1119–1126 (2007).
6. Zhao, S. & Li, S. Network-based relating pharmacological and genomic spaces for drug target identification. *PLoS One* **5**, e11764 (2010).
7. Takarabe, M., Shigemizu, D., Kotera, M., Goto, S. & Kanehisa, M. Characterization and classification of adverse drug interactions. *Genome Inform* **22**, 167–175 (2010).
8. Klein, T. E. *et al*. Integrating genotype and phenotype information: an overview of the PharmGKB project. Pharmacogenetics Research Network and Knowledge Base. *Pharmacogenomics J* **1**, 167–170 (2001).
9. Knox, C. *et al*. DrugBank 3.0: a comprehensive resource for 'omics' research on drugs. *Nucleic Acids Res* **39**, D1035–1041 (2011).
10. Azuaje, F., Devaux, Y. & Wagner, D. R. Coordinated modular functionality and prognostic potential of a heart failure biomarker-driven interaction network. *BMC Syst Biol* **4**, 60 (2010).
11. Ozgur, A., Vu, T., Erkan, G. & Radev, D. R. Identifying gene-disease associations using centrality on a literature mined gene-interaction network. *Bioinformatics* **24**, i277–285 (2008).
12. Yu, H., Kim, P. M., Sprecher, E., Trifonov, V. & Gerstein, M. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. *PLoS Comput Biol* **3**, e59 (2007).
13. Goncalves, J. P., Graos, M. & Valente, A. X. POLAR MAPPER: a computational tool for integrated visualization of protein interaction networks and mRNA expression data. *J R Soc Interface* **6**, 881–896 (2009).
14. Ashburner, M. *et al*. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* **25**, 25–29 (2000).
15. Kanehisa, M., Goto, S., Furumichi, M., Tanabe, M. & Hirakawa, M. KEGG for representation and analysis of molecular networks involving diseases and drugs. *Nucleic Acids Res* **38**, D355–360 (2010).
16. Eis, P. S. *et al*. Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc Natl Acad Sci U S A* **102**, 3627–3632 (2005).
17. Godlewski, J. *et al*. MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell* **37**, 620–632 (2010).
18. Dostie, J., Mourelatos, Z., Yang, M., Sharma, A. & Dreyfuss, G. Numerous microRNPs in neuronal cells containing novel microRNAs. *RNA* **9**, 180–186 (2003).
19. Bostjancic, E., Zidar, N., Stajer, D. & Glavac, D. MicroRNAs miR-1, miR-133a, miR-133b and miR-208 are dysregulated in human myocardial infarction. *Cardiology* **115**, 163–169 (2010).
20. Zhang, X. *et al*. Synergistic effects of the GATA-4-mediated miR-144/451 cluster in protection against simulated ischemia/reperfusion-induced cardiomyocyte death. *J Mol Cell Cardiol* **49**, 841–850 (2010).
21. Bates, E. R., Lau, W. C. & Angiolillo, D. J. Clopidogrel-drug interactions. *J Am Coll Cardiol* **57**, 1251–1263 (2011).
22. Gislason, G. H. *et al*. Increased mortality and cardiovascular morbidity associated with use of nonsteroidal anti-inflammatory drugs in chronic heart failure. *Arch Intern Med* **169**, 141–149 (2009).
23. Graham, D. J. *et al*. Risk of acute myocardial infarction, stroke, heart failure, and death in elderly Medicare patients treated with rosiglitazone or pioglitazone. *JAMA* **304**, 411–418 (2010).
24. Abenhaim, L. *et al*. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med* **335**, 609–616 (1996).
25. Casassus, B. Drug scandal exposes French regulators to public ire. *Nat Med* **17**, 144 (2011).
26. Connolly, H. M. *et al*. Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med* **337**, 581–588 (1997).
27. Frachon, I. *et al*. Benfluorex and unexplained valvular heart disease: a case-control study. *PLoS One* **5**, e10128 (2010).
28. Salwinski, L. *et al*. The Database of Interacting Proteins: 2004 update. *Nucleic Acids Res* **32**, D449–451 (2004).
29. Aranda, B. *et al*. The IntAct molecular interaction database in 2010. *Nucleic Acids Res* **38**, D525–531 (2010).
30. Ceol, A. *et al*. MINT, the molecular interaction database: 2009 update. *Nucleic Acids Res* **38**, D532–539 (2010).
31. Salwinski, L. *et al*. Recurated protein interaction datasets. *Nat Methods* **6**, 860–861 (2009).
32. Griffiths-Jones, S., Grocock, R. J., van Dongen, S., Bateman, A. & Enright, A. J. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res* **34**, D140–144 (2006).
33. Cline, M. S. *et al*. Integration of biological networks and gene expression data using Cytoscape. *Nat Protoc* **2**, 2366–2382 (2007).
34. Assenov, Y., Ramirez, F., Schelhorn, S. E., Lengauer, T. & Albrecht, M. Computing topological parameters of biological networks. *Bioinformatics* **24**, 282–284 (2008).
35. Random Network Plugin [http://sites.google.com/site/randomnetworkplugin/].
36. Al-Shahrour, F. *et al*. Babelomics: advanced functional profiling of transcriptomics, proteomics and genomics experiments. *Nucleic Acids Res* **36**, W341–346 (2008).
37. Statistica. version 8.0 [http: www.statsoft.com]

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Author contributions

FA conceived the investigation, designed methods, implemented analyses and wrote the manuscript. LZ implemented analyses. DW and YD provided biomedical knowledge feedback. DW, LZ and YD assisted manuscript preparation.

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