Network-Based Computational Approach to Identify Delineating Common Cell Pathways Influencing Type 2 Diabetes and Diseases of Bone and Joints

MOHAMMAD ALI MONI[1,2]**, M. BABUL ISLAM**[3]**, MD REZANUR RAHMAN**[4]**,
MD RASHED-AL-MAHFUZ**[5]**, MD ABDUL AWAL**[6]**,
SHEIKH MOHAMMED SHARIFUL ISLAM**[7]**,
MD. NURUL HAQUE MOLLAH**[8]**, AND JULIAN M. W. QUINN**[2]

[1]Faculty of Medicine and Health, School of Medical Sciences, The University of Sydney, Sydney, NSW 2006, Australia
[2]Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[3]Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi 6205, Bangladesh
[4]Department of Biochemistry and Biotechnology, School of Biomedical Science, Khwaja Yunus Ali University, Sirajgonj 6751, Bangladesh
[5]Department of Computer Science and Engineering, University of Rajshahi, Rajshahi 6205, Bangladesh
[6]Electronics and Communication Engineering Discipline, Khulna University, Khulna 9208, Bangladesh
[7]Faculty of Health, School of Exercise and Nutrition Sciences, Deakin University, Geelong, VIC 3220, Australia
[8]Bioinformatics Laboratory, Department of Statistics, Rajshahi University, Rajshahi 6205, Bangladesh

Corresponding authors: Mohammad Ali Moni (mohammad.moni@sydney.edu.au) and Julian M. W. Quinn (j.quinn@garvan.org.au)

This work was supported by the University of Sydney Fellowship Award.

ABSTRACT Developing type 2 diabetes (T2D) can increase patient risk of developing other common diseases and exacerbate their severity, including diseases that affect bone and joints. Such comorbidity interactions are hard to study in detail by traditional endocrinological methods. Thus, we developed tissue transcript analytical approaches to identify common pathways through which these diseases can interact. We examined RNAseq and microarray transcript datasets from studies of T2D and chronic bone and joint diseases, namely rheumatoid arthritis (RA), osteoarthritis (OA), juvenile idiopathic arthritis (JIA) and low peak bone density, a key osteoporosis (OP) determinant. These datasets contained data from affected individuals and matched controls. Differentially expressed genes (DEGs) for each condition were compared with T2D DEG. Overlapping DEGs (i.e., those common to T2D and a bone or joint condition) were subjected to gene enrichment by pathway analyses and by gene ontology methods, and the results were evaluated by using SNP-disease linkage (dbGaP) and gene-disease association (OMIM) databases that indicate gene involvement in pathologies. By examining gene targets of transcription factors (TFs) and microRNA (miRNAs), we also constructed DEG-TF and DEG-miRNA interactions networks for analysis. We identified strong candidate genes in common pathways, notably including SYK, UCP3, ROR1, PPARG, BUB1, AKT2, ADCY2 and CCR5. The DEG-TF network and DEG-miRNA interactions network analyses revealed a number of TFs (GATA2, FOXC1, USF2, YY1, E2F1, JUN, RELA, CREB1, TFAP2A, NFB1) and miRNAs (mir-335-5p, mir-16-5p, mir-26b-5p, mir-124-3p, mir-218-5p, mir-98-5p, mir-29b-3p, mir-3135b, mir-29c-3p, mir-1-1) that can regulate the identified DEGs at the transcriptional and post-transcriptional levels. Thus this data-driven approach has enabled identification and validation of regulatory factors and cell pathways by which T2D may influence bone and joint conditions, which may suggest new ways to interfere with the pathogenic processes involved.

INDEX TERMS T2 diabetes, network-based approach, joint diseases, bone diseases.

I. INTRODUCTION

Type 2 diabetes (T2D) affects hundreds of millions of people and has become an enormous clinical problem with serious

attendant vascular disease issues, including heart disease, strokes, retinopathy and peripheral ischemia. The high incidence of T2D and its tendency to affect the function of many organs makes it important to determine how T2D interacts with co-morbidities, i.e., other diseases suffered by the same individual at the same time [1]. This is an important issue

The associate editor coordinating the review of this manuscript and approving it for publication was Derek Abbott.

for the management of the co-morbidities and in determining whether a T2D therapy may affect those co-existing conditions. T2D-associated features of particular concern include increased body weight and fat composition, chronic secretion of inflammatory hormones and high circulating levels of glucose and glycated proteins that cause vascular damage [1], [2]. In addition there are the local cellular effects of insulin resistance itself. These affect diseases of bone and joints, the commonest of which include osteoporosis, osteoarthritis and inflammatory types of arthritis such as rheumatoid arthritis (RA) [3]. It is not well understood how the development and progression of these diseases are influenced by T2D. Disease interactions are typically studied by endocrinological approaches, such as methods that focus on T2D-associated cell secretions, of high serum glucose and of glycation product. Here, we employed a computation-based approach that utilized gene expression data, seeking to identify any gene pathways that are common to T2D and these bone and joint diseases. While not all processes occur at the same time (e.g., T2D may influence the early stage of development of a bone disease but not later stages), nevertheless gene expression is profoundly affected by the pathological processes and reveals information about pathways shared by the diseases. We therefore identified pathways that overlap in T2D and bone/joint diseases and so may have clinical utility. This approach may also identify important pathways that are relevant to a range of other diseases.

Osteoporosis (OP) is a disorder that weakens the bone and makes it vulnerable to low trauma fracture. The hallmark of OP is low bone mineral density (BMD), identified by X-ray absorptiometry [4]. After early adulthood, BMD of individuals decline (as in other mammalian species), and many factors aggravate this over time, such as diminishing sex hormone levels as well as circulating inflammatory hormones. An important determinant of OP in old age is peak the BMD level attained during early adulthood, a high point from which BMD declines. T2D also greatly increases risk of fracture, even though it does not always affect BMD, which may reflect bone incorporation o T2D glycation products that affect bone material strength [5], [6]. Bone health can also be impaired by T2D medications such as rosiglitazone. Thus, new approaches to identify such overlapping problems due to shared common pathways will greatly inform treatment. A similar approach may be useful in considering how T2D interacts with joint diseases.

RA is an inflammatory joint condition with painful joint swelling, synovial membrane hyperplasia and local bone damage, destroying the local bone and joint architecture to cause severe pain and impaired mobility. An important treatment option is the use of anti-inflammatory drugs. T2D is a risk factor for inflammatory arthritis and, conversely, RA is a risk factor for T2D, with disease modifying anti-rheumatic drugs ameliorating T2D [7], [8]. Other inflammatory forms of arthritis such as juvenile idiopathic arthritis (JIA) are also major clinical challenges [9]. Osteoarthritis (OA) is the most common joint disease but is not typically

inflammation-associated at presentation (although inflammation is a significant influence in early stages of OA development), rather it involves progressive damage and erosion of joint cartilage [10], [11]. This can be induced by joint damage or instability and high mechanical load. Surprisingly, meta-analysis studies clearly implicate T2D as an independent risk factor for OA and in rodent models of OA untreated T2D results in much more severe OA joint that non-diabetic controls [12]. As with OP, while T2D interactions with OA, JIA and RA have been studied, we lack any description of cell pathways common to their presentation and pathophysiologies.

To address these issues we studied transcript datasets to identify pathways in cells and tissues that are commonly affected by both T2D and diseases of bone and joints. Utilizing computational analysis of global transcriptomes, we identified and characterized the gene expression profiles seen in T2D and in RA, OA, OP and JIA from their common gene expression patterns. We cross-compared this data with pathways previously identified and validated in publicly available resources such as dbGaP and Online Mendelian Inheritance in Man (OMIM), as well as protein-protein interaction (PPI) datasets [13], [14]. Our network-based approach thus enabled us to identify common pathways with pathological potential that may influence the progression of bone and joint diseases.

II. MATERIALS AND METHODS
A. OVERVIEW OF ANALYTICAL APPROACH
The schematic diagram in Fig. 1 summarises our methodology which constitutes a quantitative systematic approach that can be used to evaluate comorbidity interaction using a variety of gene expression datasets. This methodology comes in gene expression analytics that are validated using
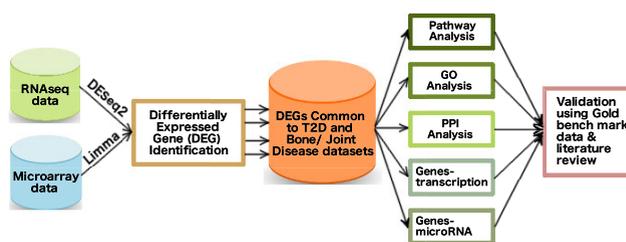
FIGURE 1. The schematic diagram of the network-based methodology employed in this study. The high-throughput transcriptomics datasets (RNA-Seq and/or microarray) of T2D, RA, OA, OP, JIA were obtained from Gene Expression Omnibus and EBI ArrayExpress databases. The differential expression analysis was performed on the transcriptomics analysis. The DEseq2 package was utilized to identify differentially expressed genes (DEGs) from RNA-Seq and Limma package was used to identify DEGs from microarray genes expression datasets. Then, we screened those DEGs which were mutually expressed in T2D and RA, OA, OP, JIA. These identified DEGs were further subjected to clarify the biological significance using gene ontology, pathways analysis. The protein-protein interactions analysis around the proteins encoded by the common/mutual DEGs were studied to identify the key signaling molecules termed "hub protein" by topological measures. The regulatory biomolecules consisting of transcription factors (TFs) and microRNAs (miRNAs) were identified from the DEGs-TFs and DEGs-miRNAs network analysis.

signal pathway information, Gene Ontology (GO) data, disease-gene associations as well as protein-protein interaction data. These allow not only the identification of possible pathways components common to comorbidities but information as to any known pathogenic potential of these pathways.

B. DATASETS EMPLOYED IN THIS STUDY

To investigate molecular pathways common to T2D and RA, OA, OP, and JIA, we first analyzed global transcriptome analyses (RNAseq) and gene expression microarray datasets. We, therefore, collected raw data from the Gene Expression Omnibus of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/geo/) and EBI array express (https://www.ebi.ac.uk/arrayexpress/). We selected 5 large human gene expression datasets to use in this study; these datasets had accession numbers E-MTAB-5060, GSE114007, GSE55457, GSE7158, and E-GEOD-71595. E-MTAB-5060 is an RNAseq dataset obtained from pancreatic islet cell studies of healthy and T2D individuals. GSE114007 is an RNAseq dataset derived from OA and normal human knee articular cartilage. GSE55457 is an Affymetrix DNA array dataset from a study of normal, OA and RA synovial tissues; the OA data in this set was not employed as OA primarily affects articular cartilage. GSE7158 is an Affymetrix DNA array-based study of blood cells from outlier high and low peak bone mass females aged 20-45; while these were not OP sufferers at the time of transcript analysis the latter individuals are at greater risk of OP as they age compared to the former group. It should be noted that the original study (and dataset generation) focused on individuals at the key age period as they undergo bone changes that later affect their bone health. E-GEOD-71595 was from an RNAseq study of CD4+ cells obtained from auto-immune/inflammatory type JIA patients and healthy controls.

C. ANALYSIS METHODS

Employing RNAseq and DNA microarrays technologies to perform global transcriptome analyses, gene expression profiles of T2D with that in our RA, OA, OP and JIA datasets were compared. These datasets were derived from studies that compared pathological and normal tissue s to identify DEGs that are associated with the respective disease. Our analytical pipeline employed the original unprocessed datasets with DESeq2 [15] and Limma [16] R Bioconductor packages which were designed for RNAseq and microarray data, respectively. In order to avoid issues inherent in comparison of gene expression data that is derived from different platforms and experimental systems, we normalized and calibrated data from all the samples (diseased or control) by Z-score transformation (Z_{ij}) for each disease-gene expression matrix using:

$$Z_{ij} = \frac{g_{ij} - \text{mean}(g_i)}{\text{SD}(g_i)} \tag{1}$$

where SD is a standard deviation, g_{ij} is the expression value of gene i in sample j. Such a transformation enables comparisons of gene expression across tissue samples and across disease categories. We thus used a Studentised t-test statistic to identify those genes that with altered expression associated with the individual disease states. Thus, the data were $\log_2$-transformed to determine differential expression, then unpaired t-tests performed used to reveal statistical differences in expression of those genes expressed in patients vs control (normal) tissue samples. Genes showing significant differences were then selected then further filtered using their observed log-fold change. Thus, genes of interest were those showing 1 $\log_2$ change, with a *p*-value in the t-tests of $< 5 \times 10^{-2}$ adjusted for multiple testing using the estimate of false discovery rate.

To investigate associations between gene expression and disease state, we used a neighborhood-based benchmarking and topological approaches [17]. We constructed gene-disease networks (GDNs) in which network nodes are either diseases or genes, forming a network that can be viewed as a bipartite graph. In this schema, two diseases are considered to have a connection when sharing at least one significantly dysregulated gene. Taking a particular set of human diseases D and a set of genes G, we attempt to determine whether gene $g \in G$ is associated with disease $d \in D$. If G_i and G_j, the sets containing genes that are significantly altered in their expression (both up- and down-regulated) and are respectively associated with diseases i and j, then the number of shared expression-altered genes (n_{ij}^g] associated with both diseases i and j is as follows:

$$n_{ij}^g = N(G_i \cap G_j] \tag{2}$$

Co-occurrence is a parameter that refers to how many shared dysregulated genes (i.e., DEGs) are in the GDN. Common neighbors are identified using the Jaccard Coefficient method [18], [19], where edge prediction scores for the node pair is:

$$E(i, j) = \frac{N(G_i \cap G_j]}{N(G_i \cup G_j]} \tag{3}$$

where E refers to the set of all edges. We then estimate the disease comorbidity associations for these datasets by employing two of our R software packages "comoR" [20] which can computes and predicts novel estimators of the disease comorbidity associations and "POGO" [21] which computes the association disease comorbidity risks and patient stratification.

Thus, in order to reveal the pathways active in T2D that are also found in the OA, RA, OP-related and JIA conditions, we employed pathway and gene ontology analysis with DAVID bioinformatics (*https* : //*david* − *d.ncifcrf.gov/*) and the KEGG pathway database [22]. In addition, we constructed protein-protein interaction (PPI) networks for the disease-pair datasets by means of information derived from the STRING resource [string-db.org]. We then incorporated two gold-benchmark curated disease datasets, OMIM

in order to provide proof of principle for the network-based approach described here.

D. DEG-TF INTERACTION NETWORK ANALYSIS: REGULATORY TRANSCRIPTION FACTORS

To determine whether common transcriptional regulatory elements of these DEGs exist, we investigated the transcription factors (TFs)-DEGs interactions network utilizing publicly available JASPAR database [23]. The network was subjected to topological analysis via using NetworkAnalyst [24]. We selected 10 TFs with the highest topological matrix (degree) as the regulatory TFs.

E. DEG-MIRNA INTERACTION NETWORK ANALYSIS: REGULATORY MICRORNAS

We also analyzed the DEGs-miRNAs interactions network utilizing experimentally verified miRNAs-target gene interactions from TarBase [25] and miRTarBase [26] database respectively to identify miRNAs that regulate the DEGs as the post-transcriptional level. The interactions were retrieved via NetworkAnalyst [23]. The top miRNAs with highest topological matrix degree were selected as the regulator of the identified DEGs.

III. RESULTS

A. DEG ANALYSIS OF DATASETS

DEGs were analyzed in the human RNAseq and microarray datasets selected, performing comparisons between disease T2D, RA, OP/low peak bone mass, OA and JIA tissue; these were performed using DESeq2 and Limma (Bioconductor packages). For each dataset DEG thus identified were statistically analyzed by the R Bioconductor packages noted below. DEGs were defined as genes with false discovery rate (FDR) under 0.05 and more than log 2-fold increase or decrease in gene expression The numbers of unfiltered DEG that were identified were, respectively, 1290 for T2D, 393 for OP, 2013 for OA, 833 for RA and 1003 for the JIA datasets.

We also performed comparison analysis to identify the common significant genes across T2D and bone and the different joint diseases. We observed T2D shares 5, 16, 4 and 19 significantly up-regulated genes and 34, 40, 4 and 9 significantly down-regulated genes with RA, OA, OP and JIA conditions respectively. To identify statistically significant associations between T2D and the other conditions, we constructed an up- and down-regulation diseasome relationship network that was centerd on T2D where two diseases are comorbid when one or more DEGs associated with both diseases are found (see Figure 1a and 1b). 2 particular significant genes, PLAU and GJA1 (which were, respectively, a serine protease and plasminogen activator, and a gap junction connector protein), are commonly up-regulated in T2D, OA and JIA, while 2 significant genes (BIRC3 and MX1, respectively an anti-apoptosis factor and a poorly understood interferon induced gene) are commonly up-regulated in T2D,

RA and JIA tissues, and 1 significant gene (GAP43, a growth cell plasticity associated factor) is commonly up-regulated in T2D, RA and OA tissues. It was interesting to note that, only 1 gene (ELL2, an RNA polymerase gene) was commonly down-regulated among T2D, RA and OA.

DEG common to T2D and the bone and joint condition datasets (OA, RA, JIA and OP-related) were identified, and these are summarised graphically in Figs 2A and B, which shows genes with, respectively elevated and reduced expression. Note that some genes (e.g., PLAU and GAP43) are
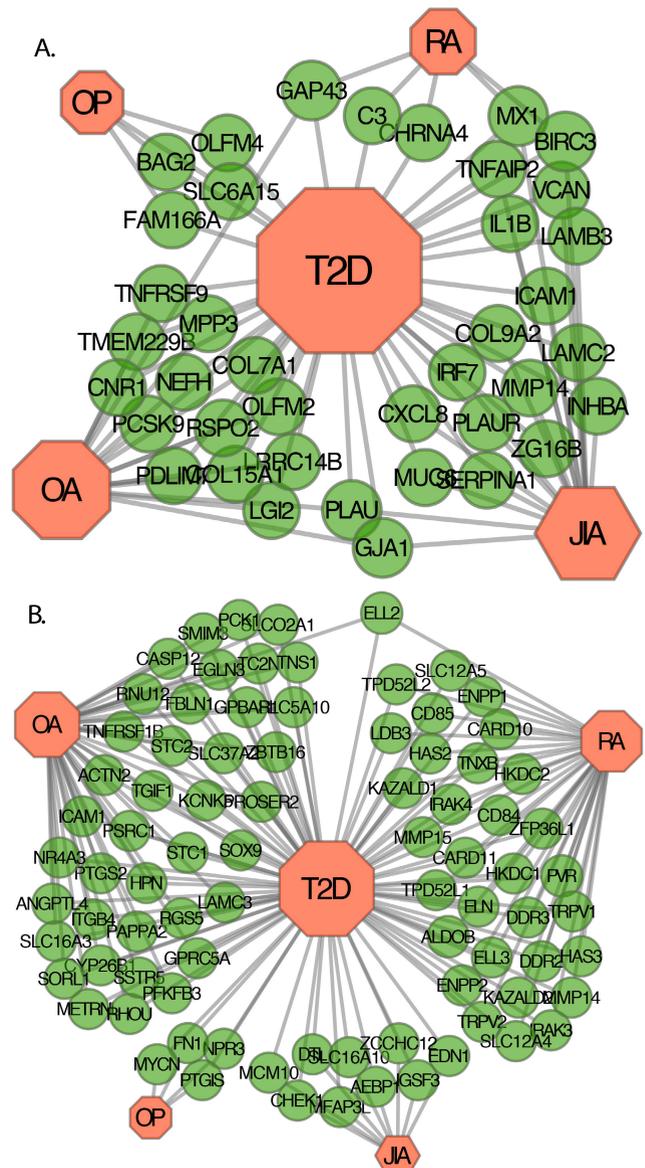
FIGURE 2. Identification of DEG in comparisons of T2D tissue with OP, OA, RA and JIA. A) DEG with increased transcript levels and B) decreased transcript levels in T2D tissues compared to the bone and joint diseases. In the connection diagrams T2D forms the center of a connection web with the bone and joint diseases placed on the periphery and significant DEG indicated (green circles), with connecting lines linking the datasets undergoing comparison. In these figures the green circles are used to represent genes and red hexagons are used to represent diseases. The size of the hexagons is the proportion of the number of the associated genes.

found in more than one common dataset and are drawn with more than one edge.

B. FUNCTIONAL ENRICHMENT OF DEG SETS COMMON TO T2D AND BONE/JOINT DISEASES

We performed pathway and gene ontology analyses with the DEG sets with the DAVID online bioinformatics portal. For pathways we used KEGG data enrichment for T2D vs RA, T2D vs OA, T2D vs JIA and T2D vs OP. To combine transcriptome and proteome analyses, we went on to perform a regulation analysis to gain more insight into pathways associated with these common DEGs. In addition, we could use this approach to predict links to pathologically dysregulated pathways. These pathway analyses were performed using the KEGG pathway database *(http://www.genome.j/kegg/pathway.html)* and functional annotation analysis tool DAVID-v-6.8 *(http://niaid.abcc.ncifcrf.gov)* to identify over-represented pathway groups among the DEGs, as well as to put them into functional categories. Pathways that were identified as significantly enriched among the common DEGs (FDR <0.05) were filtered using a manual approach to include only genes with known relevance to the diseases concerned. These outcomes, summarised in Table 1, included a number of relevant and significant pathways. For example in OA we found hormonal response pathway genes that included TNF, NF-κB, PPAR-gamma and TGF-β pathways, many of which were shared with JIA.

To obtain better insights into the pathways that we identified, the enriched common DEG sets were processed using a gene ontology approach employing EnrichR *(http://amp.pharm.mssm.edu/Enrichr/)* which identifies biological processes that are related. The list of processes identified in this way were curated to reveal those with a known involvement in bone and joint diseases and T2D. These processes and the associated genes are summarised in Table 2. We found a variety of pathways that notably included mineralization, glucose metabolism and BMP functions that are relevant to bone physiology.

C. PROTEIN-PROTEIN INTERACTION (PPI) ANAYSIS TO IDENTIFY COMMON SUB-NETWORKS

Altered expression of proteins in a sub-network may identify such sub-networks as dysfunctional, at least in the context of these diseases. Indeed, multiple diseases may arise from the malfunction of a protein complex. Thus, two diseases may be related if sharing one or more protein sub-network. After identifying DEGs that are involved in pathways and processes common to T2D and bone/joint diseases of interest to our study, we looked for any evidence for previously identified sub-networks, based on previously reported PPI. With these enriched common disease-gene sets, we made PPI networks by the web-based visualization package STRING [27]. Sub-networks seen in T2D vs the bone/joint diseases are summarised in Fig. 3. Gene clusters were determined from the Markov cluster algorithm (MCL); we noted that many

TABLE 1. KEGG pathway analyses to identify pathways common to T2D and the bone and joint conditions revealed by the commonly expressed genes. These include significant pathways common to T2D and A) RA B) OA C) OP and D) JIA.

A. Common significant pathways of T2D and RA

KEGG ID	Pathway	Genes in the pathway	adj. p-value
hsa04064	NF-kappa B signaling pathway	IRAK4;CARD11;BIRC3	6.74E-04
hsa00051	Fructose and mannose metabolism	HKDC1;ALDOB	1.60E-03
hsa04668	TNF signaling pathway	MMP14;BIRC3	1.76E-02
hsa00030	Pentose phosphate pathway	ALDOB	3.23E-02
hsa04930	Type II diabetes mellitus	HKDC1	4.51E-02
hsa04910	Insulin signaling pathway	HKDC1	4.98E-02

B. Common significant pathways of T2D and OA

KEGG ID	Pathway	Genes in the pathway	adj. p-value
hsa05014	Amyotrophic lateral sclerosis (ALS)	CASP12;TNFRSF1B;NEFH	3.94E-04
hsa04064	NF-kappa B signaling pathway	PLAU;PTGS2;ICAM1	2.26E-03
hsa04668	TNF signaling pathway	TNFRSF1B;PTGS2;ICAM1	3.63E-03
hsa03320	PPAR signaling pathway	ANGPTL4;PCK1	1.60E-02
hsa04920	Adipocytokine signaling pathway	TNFRSF1B;PCK1	1.65E-02
hsa04152	AMPK signaling pathway	PFKFB3;PCK1	4.73E-02
hsa04350	TGF-beta signaling pathway	TGIF1	4.83E-02
hsa05323	Rheumatoid arthritis	ICAM1	4.86E-02
hsa05145	Toxoplasmosis	LAMC3	4.93E-02

C. Common significant pathways of T2D and OP

KEGG ID	Pathway	Genes in the pathway	adj.p-value
hsa00590	Arachidonic acid metabolism	PTGIS	2.45E-02
hsa04512	ECM-receptor interaction	FN1	3.23E-02
hsa04510	Focal adhesion	FN1	4.80E-02
hsa05202	Transcriptional misregulation in cancer	MYCN	4.98E-02
hsa04933	AGE-RAGE signaling pathway in diabetic complications	FN1	3.97E-02
hsa01100	Metabolic pathways	PTGIS	4.95E-02

D. Common significant pathways of T2D and JIA

KEGG ID	Pathway	Genesin the pathway	adj.p-value
hsa04064	NF-kappa B signaling pathway	CXCL8;PLAU;IL1B;BIRC3;ICAM1	1.76E-07
hsa04668	TNF signaling pathway	EDN1;MMP14;IL1B;BIRC3;ICAM1	4.08E-07
hsa04933	AGE-RAGE signaling pathway in diabetic complications	EDN1;CXCL8;IL1B;ICAM1	1.14E-05
hsa04621	NOD-like receptor signaling pathway	CXCL8;IL1B;BIRC3	6.84E-05
hsa04932	Non-alcoholic fatty liver disease (NAFLD)	CXCL8;IL1B	1.88E-02
hsa04010	MAPK signaling pathway	IL1B	3.02E-02
hsa04350	TGF-beta signaling pathway	INHBA	4.81E-02
hsa04540	Gap junction	GJA1	4.92E-02
hsa04380	Osteoclast differentiation	IL1B	4.99E-02

TABLE 2. GO identification of biological processes that are common to T2D and the bone and joint diseases. KEGG pathway-enriched genesets were used for GO studies to identify processes that are in common between these pathologies. GO terms were curated to identify those relevant to the bone and joint function and pathology. Example pathways genes and pathway adjusted p-values are indicated.

A. Common significant GOs of T2D and RA

GO ID	Pathway	Genes in the pathway	adj. p-value
GO:0030278	regulation of ossification	ENPP1;DDR2	2.36E-03
GO:0030500	regulation of bone mineralization	ENPP1;DDR2	4.01E-03
GO:0007249	I-kappaB kinase/NF-kappaB signaling	CARD11; BIRC3; IRAK4	5.16E-03
GO:0071356	cellular response to tumor necrosis factor	HAS2; BIRC3; ZFP36L1	5.56E-03
GO:0006821	chloride transport	SLC12A4; SLC12A5	8.27E-03
GO:0032869	cellular response to insulin stimulus	ENPP1; ZFP36L1	1.79E-02
GO:0010829	negative regulation of glucose transport	ENPP1	2.02E-02
GO:0046890	regulation of lipid biosynthetic process	C3	4.23E-02

B. Common significant GOs of T2D and OA

GO ID	Pathway	Genes in the pathway	adj. p-value
GO:0060348	bone development	CYP26B1;STC1; HPN;SOX9	4.32E-03
GO:0060349	bone morphogenesis	CYP26B1	4.66E-02
GO:0045923	regulation of fatty acid metabolic process	NR4A3	4.92E-02
GO:0048705	skeletal system morphogenesis	CYP26B1	4.59E-02
GO:0030326	embryonic limb morphogenesis	CYP26B1	4.36E-02
GO:0048762	mesenchymal cell differentiation	SOX9	4.12E-02
GO:0045778	positive regulation of ossification	ZBTB16	4.83E-02
GO:0045598	regulation of fat cell differentiation	ZBTB16	4.54E-02
GO:0071773	cellular response to BMP stimulus	SOX9	4.91E-02
GO:0030177	regulation of Wnt signaling pathway	RSPO2;SOX9	4.75E-02
GO:0043122	regulation of I-kappaB kinase/NF-kappaB signaling	GJA1	4.88E-02

C. Common significant GOs of T2D and OP

GO ID	Pathway	Genes in the pathway	adj.p-value
GO:0033688	regulation of osteoblast proliferation	NPR3	6.78E-03
GO:0010605	negative regulation of macromolecule metabolic process	MYCN	4.89E-02
GO:0032088	regulation of NF-kappaB transcription factor activity	PTGIS	3.27E-02
GO:0019221	cytokine-mediated signaling pathway	FN1	4.72E-02

D. Common significant GOs of T2D and JIA

GO ID	Pathway	Genes in the pathway	adj.p-value
GO:0016264	gap junction assembly	GJA1	9.76E-03
GO:0071260	cellular response to mechanical stimulus	IL1B;CHEK1	2.13E-03
GO:0043122	regulation of I-kappaB kinase/NF-kappaB signaling	GJA1;IL1B; BIRC3	2.88E-03
GO:0045833	negative regulation of lipid metabolic process	IL1B	2.76E-02
GO:0042476	odontogenesis	INHBA	4.72E-02
GO:0010803	tumor necrosis factor-mediated signaling pathway	BIRC3; CXCL8	4.69E-02
GO:0030509	BMP signaling pathway	ZCCHC12	4.87E-02
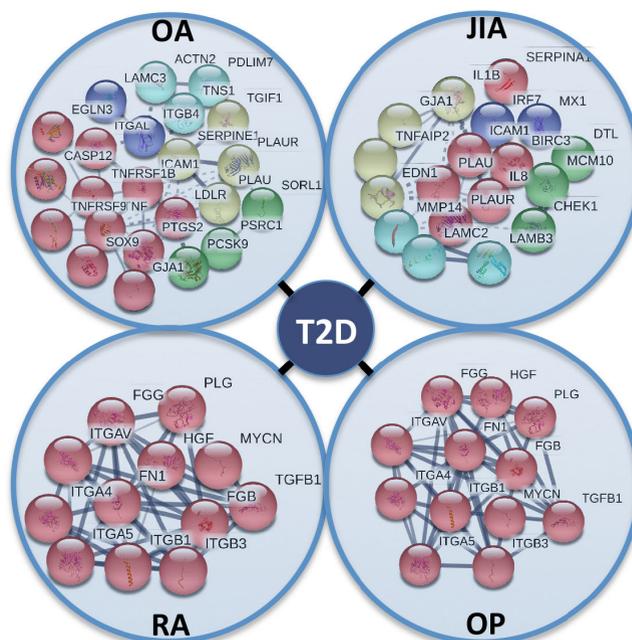
FIGURE 3. Protein-protein interaction (PPI) network of the five diseases that share protein sub-networks. These include significant pathways common to T2D and OP, RA, JIA and OA as indicated. Genes were identified by STRING software tools. Color indicates MCL analysis clusters of genes.

PPI sub-networks contained genes within one cluster, which is indicated in Fig. 3 in red. This analysis suggests PPI sub-networks do exist in our enriched genesets, confirming the existence of relevant functional pathways.

D. CO-MORBIDITY ANALYSIS AND IDENTIFICATION OF SIGNIFICANT PATHWAY MARKER GENES

Significant pathways relevant to the common disease gene datasets are displayed in Fig. 4A with gene pathway links shown; here we have considered genes identified in T2D DEG and their connections with bone/joint disease DEG sets. Considering both the dysregulated gene expression seen in T2D and gene-disease associations, we set up gene-disease associations networks (GDN) in order to explore the pathology-shared genes and comorbidity networks (see Fig. 4B). Beginning with from a bipartite graph we constructed biologically relevant network projections and multi-relational gene-disease networks where the nodes are either diseases or genes and edges indicate gene-disease association. This type of bipartite graph contains two disjointed sets of nodes; one of these relates to known genetic disorders and the other to genes identified as significant for T2D. Information related to disorders, disease genes (and their associations) that we employed were obtained from the OMIM and dbGaP. The GDN, nodes represent disease or genes such that two diseases are connected to each other where they share a gene with variants (usually single nucleotide polymorphisms) that have previously been demonstrated to be associated with both diseases (see Fig. 4B). The number of such interlinked

A.

B.
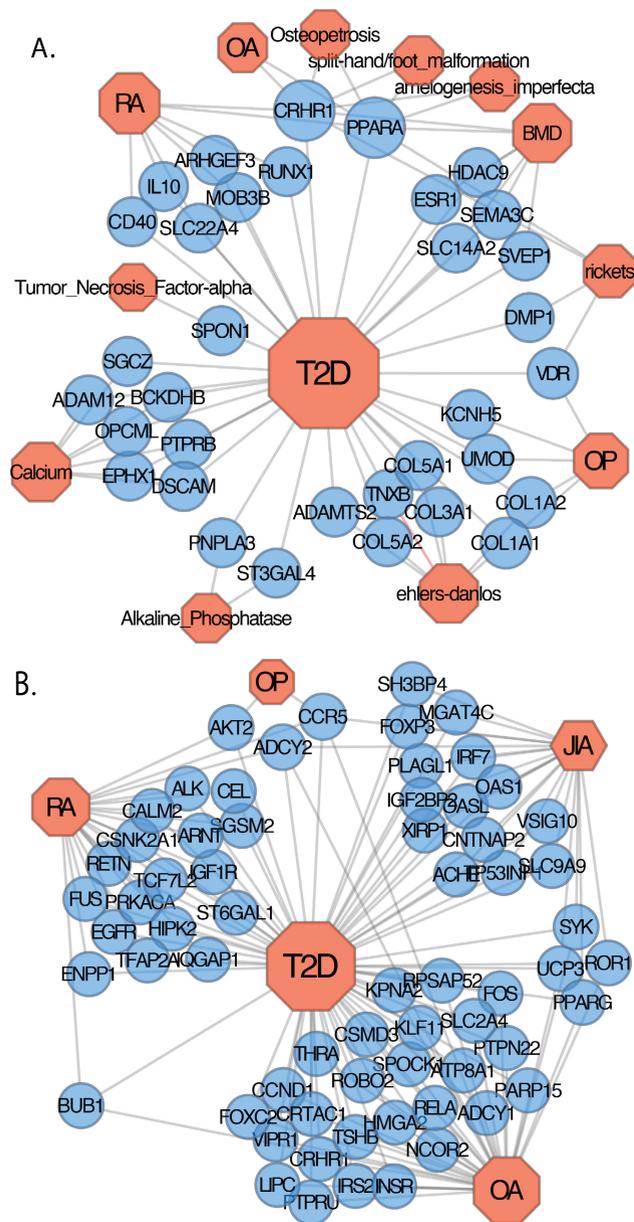
FIGURE 4. Genes significant in T2D that are potential markers of bone and joint diseases. A) Enriched datasets from our analyses with links to processes and pathways revealed by our analysis. Gene names are in small circles; color indicates membership of clusters evident from MCL analysis B) Genes identified from OMIM and dbGaP databases using single nucleotide polymorphism association with diseases. The graphic summarises these genes that are also found in our analysis of T2D and the bone/joint conditions. In these figures the blue circles are used to represent genes and red hexagons are used to represent diseases. The size of the hexagons is the proportion of the number of the associated genes.

genes between T2D were typically between 2 and 25, with several being linked to more than one bone/joint disease, notably SYK, UCP3, ROR1 and PPARG (OA and JIA), BUB1 (OA and RA), AKT2, ADCY2 and CCR5 (RA and OP), ADCY2 and CCR5 (OA and OP) and CCR5 and ADCY2 (RA and JIA). These overlapping-set genes may be of particular priority for further study although their functions do not currently appear to overlap.

E. IDENTIFICATION OF TRANSCRIPTIONAL AND/OR POST-TRANSCRIPTIONAL REGULATORS

We studied the TFs-DEGs and miRNAs-DEGs networks to identify regulators of the DEGs at transcriptional and post-transcriptional levels (see Figure 5 and 6). The statistical analysis of the topological parameters revealed TFs (GATA2, FOXC1, USF2, YY1, E2F1, JUN, RELA, CREB1, TFAP2A, NFB1) and miRNAs (mir-335-5p, mir-16-5p, mir-26b-5p, mir-124-3p, mir-218-5p, mir-98-5p, mir-29b-3p, mir-3135b, mir-29c-3p, mir-1-1) as the principal regulators of the DEGs which were identified as shared by T2D and bone and joint conditions.
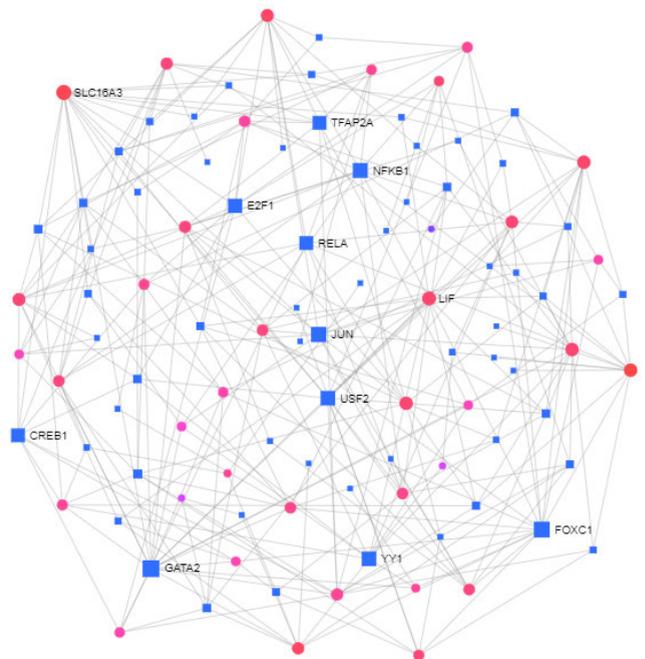
FIGURE 5. Differentially expressed genes-transcription factors interaction network analysis. The experimentally verified interaction data were obtained from JASPAR database. The blue squares represent transcription factors and circles represent genes. The area of the squares and circles are proportional to topological measures degree. The larger squares (higher degree) representing the strong interactions with differentially expressed genes.

IV. DISCUSSION

Our study helps to fill a significant gap in our knowledge about how T2D may be influencing the development of OA, RA, OP and JIA. Rather than using a directly mechanistic or endocrinological approach we looked for genes that appeared dysregulated in T2D and in one or more of the bone and joint diseases, using this information to give clues to identify aberrantly acting pathways and control mechanisms that might otherwise not be suspected to play a role in these diseases. This line of inquiry starts with an agnostic approach that employs a sequence of bioinformatics steps to analyze widely available resources and data and can easily be applied to a range of possible co-morbidities. The power and quality of the analysis can over time be further improved and
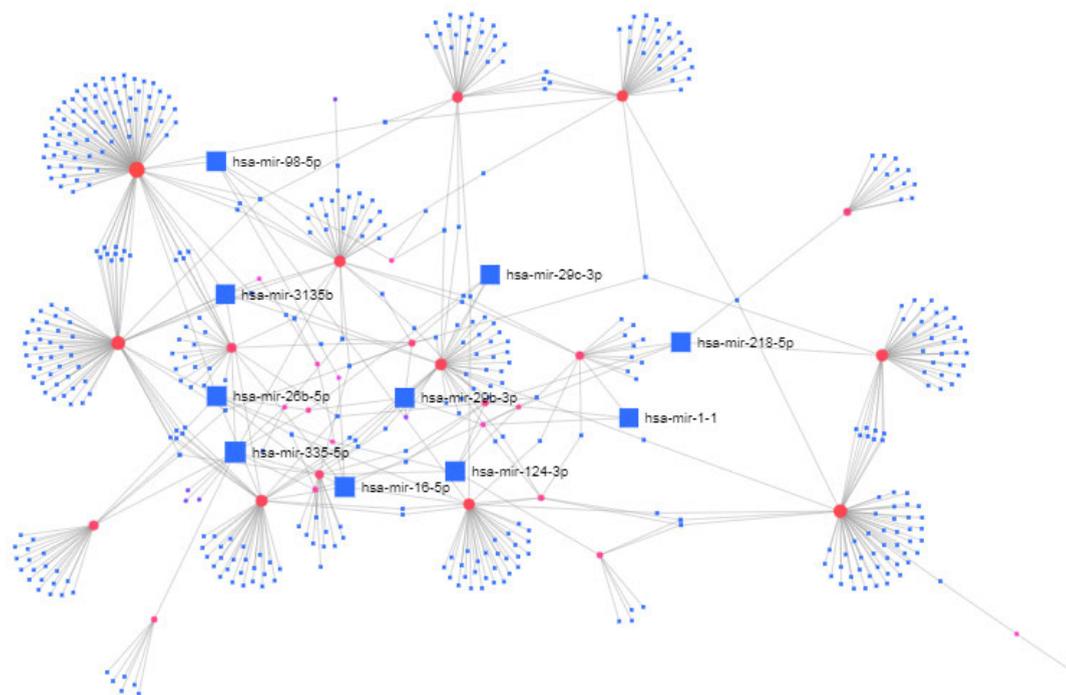
FIGURE 6. Differentially expressed genes-microRNA interaction network analysis. The experimentally verified interaction data were retrieved from TarBase and miRTarBase database. The blue squares represent microRNAs and circles represent genes. The areas of the squares and circles are proportional to topological measures degree. The larger squares (higher degree) representing the strong interactions with differentially expressed genes.

augmented as more large disease-relevant gene expression datasets become available.

Using the combined analysis of transcriptomic, genetic, PPI, pathway and GO data, our disease network revealed a number of new putative disease relationships that has not been identified from earlier studies and analyses, These have the potential to inform and direct further clinical and biochemical studies of these co-morbidities. Our approach, in effect, takes advantage of the complexity that is usually such a barrier to conventional co-morbidity studies. Our underlying hypothesis is that once we construct a list of a reasonably large proportion of disease-related genes and pathways, we will be able to predict the susceptibility of individuals to other diseases using molecular biomarkers found using this approach. Combined with genetic data (e.g., SNP data) such information will be needed as a key element in developing accurate prognostic medicine. Our results here indicate that combining molecular and population-level data can provides insights that can lead to the generation of new hypotheses about the mechanisms that underlie disease development and comorbidity interactions. In addition, this approach can provide important information relevant to overlaps in medication that may have implications for patient care, and provide suggestions as to why these diseases are seen commonly as interacting comorbidities. This is known problem in particular for T2D, whose development is associated with worse outcomes for RA patients, an interaction seen in pre-clinical rodent models as well [12].

Gene activity is regulated both at the transcriptional and at the post-transcriptional level. Thus, to provide deeper insights into the regulatory patterns of the identified genes, we analyzed the TFs-DEGs and miRNAs-DEGs networks. TFs drive the transcription of genes and may do so coordinately across genes with related functions. In contrast, miRNAs are particularly powerful regulators of transcript levels at the post-transcriptional level, although it should be noted that there are other classes of non-coding RNAs that are less potent and less well characterized that also affect transcript levels post-transcriptionally. We thus used targets of TF and miRNA to identify their targets among the DEGs that were involved in T2D and bone and joint diseases. Among the identified TFs, GATA2, FOXC1, USF2, YY1, E2F1, and JUN were previously identified as the regulators of DEGs in our previous report on Alzheimer's diseases by a network-based approach [28]. GATA2 is also implicated in early-onset coronary artery disease [29]. The dysregulation of FOXC1 is involved in skeletal abnormalities [30]. The USF2 is also involved in diabetic nephropathy [31]. Dysregulation of YY1 in the liver causes insulin resistance, dyslipidemia etc and has also been proposed as targeting hepatic YY1 an important target for insulin-resistant diabetes [32]. It has been suggested that E2F1 promotes the hyperglycemia during diabetes [33]. The JUN is involved in inflammatory and bone disease [34]. T2D is involved with dysregulated NFKB1 [35]. The involvement of the RELA is not found in the literature in T2D and bone and joint disease.

CREB1 polymorphism is associated with T2D risk in the Chinese population [36].

The genes that we found to commonly show dysregulation in T2D and with OP, RA, JIA and OA included some with known influences on T2D (or related metabolic phenomena) and the bone and joint diseases, but for some the common link was not clear. For example, UCP3 codes for a mitochondrial 'uncoupling factor' that affects cell metabolism; it uncouples oxidative phosphorylation from ATP synthesis, which can result in heat generation and can protect the mitochondria from oxidative stress. Mutations in UCP3 are associated with obesity [37] and indeed insulin regulate UCP3 expression [38]. UCP3 is not characterized as affecting bone but has been linked to cartilage cell survival which is clearly crucial for joints and may indirectly affect bone density [39], [40]. SYK codes for a tyrosine kinase enzyme that is essential for embryo development and crucial to immune system function, notably B cells that are involved in many aspects of inflammation, however, only indirect links of SYK with bone physiology are known [41]. ROR1 codes for another tyrosine kinase of poorly understood function but is involved with cell response to hormones. However, ROR1 has known influences on bone forming cells although a role in joint biology and diabetes is unclear [42], although there is data linking ROR1 to adipogenesis processes in vitro [43]. PPARG is a crucial nuclear receptor that is the target of glitazone compounds used to treat T2D, but also plays an important role in connective tissue function, especially bone osteoblast and smooth muscle formation [44], [45]. BUB1 protein has an important role in cell division (mitosis) as a checkpoint regulator, so this (and functions in cell death regulation) could lead to many effects on bone and joint tissues [46]. AKT2 is a kinase enzyme regulator involved in cell signals; its function is strongly linked to T2D due to its involvement with insulin signaling, but it also plays a well established role in regulating the activity of bone forming cells [47]. CCR5 encodes a receptor that mediates cell response to a secreted chemokine factor, and it is particularly involved in immune cell inter-communication and in macrophages that regulate inflammation and bone formation. CCR5 blocking compounds have been developed as HIV-1 therapies [48]. It has recently been found to have unexpected roles in bone cells important in osteoporosis and joint damage [49]. A role has been suggested in diabetes relating to lipid formation. Lastly, ADCY2 encodes an adenyl cyclase enzyme that generates cyclic AMP, which is a crucial intracellular signaling molecule induced by many regulatory hormones important to bone [50] and metabolism [51]. In sum, these genes indicate a range of ways that T2D and the bone and joint diseases may interact, although in some cases further work is needed to establish a firm link. Clearly there must be other shared pathways that are not evident from our analysis, since it is unlikely that they will all be detectable in the datasets available, but the genes we identified, listed above, show a mix of well and poorly characterized disease links that might be expected.

miRNAs are short 20-22 nucleotide long non-coding RNAs which regulate expression of gene transcripts (mRNAs) at post-transcriptional level. In this way, the miRNAs regulate the level of protein expression by inhibiting the mRNAs levels. There is evidence indicating the high potential of miRNAs as biomarkers for complex diseases [28], [52]–[60]. Among the miRNA post-transcriptional regulators of the identified DEGs, it has been shown that mir-335-5p increases insulin resistance and suppresses pancreatic Îš cell secretion [61]. Moreover, overexpression of mir-335-5p can perturb insulin secretion [62]. Evidence suggests mir-16-5p is deregulated in retinal cells during hyperglycemia [63]. The significant overexpression of mir-16-5p was observed in hyperglycemia and euglycemia conditions in coronary heart disease [64]. It has been shown that circulating mir-26b-5p is dysregulated in T2D compared to control samples [65]. Moreover, significantly reduced expression of mir-26b-5p was observed in metformin treatments in T2D patients [66] indicating its involvement in pathogenesis, and its possible therapeutic targeting in the treatment of T2D. Involvement of the mir-124-3p in the progression and development of T2D has seen previously [67]. The involvement of the mir-218-5p, mir-98-5p, and mir-3135b with T2D and bone disease, however, has not been reported in the literature. However, overexpression of mir-29b-3p in T2D and in diarrhea-predominant irritable bowel syndrome is seen [68], and it has been shown that diabetes affects tissue expression of mir-29c-3p [69].

V. LIMITATIONS OF THE STUDY

Our analysis was performed using publicly available mRNA expression, so it was limited by what is currently accessible. We performed analyses of DEGs for use in gene enrichment approaches and cell signal pathway and Gene Ontology (GO) data; we were unable therefore to detect of DEGs that may be highly variable in their response to disease conditions between individuals, and we are limited by the current knowledge of cellular pathways. It should also be noted that pathway and GO analyses a large number of categories obtained were reduced by manual curation, making the approach not completely agnostic. While not difficult or time consuming, developing more analytical semantic methodologies to tackle this would better facilitate this approach and reduce operator bias as we have undertaken previously [20]. In addition to transcript analyses, we also found evidence for disease processes in the PPI data. This provided some confirmatory evidence for pathways identified through consideration of cell proteins and their interactions. However, this approach is necessarily incomplete as our understanding of and cataloging of PPI is an early stage due to the enormous number of protein motifs that exist.

VI. CONCLUSIONS

Our study demonstrates that an integrated approach to analyzing gene expression and gene function data can uncover novel relationships between diseases, potentially lead to new insights into the pathways that influence these conditions.

We identified pathways that were common to T2D and the bone and joint conditions and identified ten genes. Their potential for pathogenic involvement was validated by evidence of disease-associated SNPs located in (or close to) them as well as the use of gene-disease association databases. The candidate genes identified were SYK, UCP3, ROR1, PPARG, BUB1, AKT2, CCR5, and ADCY2. The present study also identified significant TFs (GATA2, FOXC1, USF2, YY1, E2F1, JUN, RELA, CREB1, TFAP2A, NFB1) as well as miRNAs with the potential to regulate these pathways (namely mir-335-5p, mir-16-5p, mir-26b-5p, mir-124-3p, mir-218-5p, mir-98-5p, mir-29b-3p, mir-3135b, mir-29c-3p, mir-1-1). These factors have known disease involvement although many have not been validated in the interaction between T2D and the diseases examined here. Disease interaction pathways are very important since it indicates pathways of particular significance to the individual diseases, outside from a comorbidity context. Thus, the methods we used and pathway information identified show new ways to detect pathogenic mechanisms in common and complex diseases.

REFERENCES

[1] M. N. Haidar, M. B. Islam, U. N. Chowdhury, M. R. Rahman, F. Huq, J. M. Quinn, and M. A. Moni, "Network-based computational approach to identify genetic links between cardiomyopathy and its risk factors," *IET Syst. Biol.*, Oct. 2019, doi: 10.1049/iet-syb.2019.0074.

[2] K. C. Howladar, M. S. Satu, A. Barua, and M. A. Moni, "Mining significant features of diabetes mellitus applying decision trees: A case study in bangladesh," *BioRxiv*, Jan. 2018, Art. no. 481994.

[3] P. Liò, N. Paoletti, M. A. Moni, K. Atwell, E. Merelli, and M. Viceconti, "Modelling osteomyelitis," *BMC Bioinf.*, vol. 13, no. 14, p. S12, 2012.

[4] P. Alejandro and F. Constantinescu, "A review of osteoporosis in the older adult: An update," *Rheumatic Disease Clinics North Amer.*, to be published.

[5] S. A. Abdulameer, S. A. S. Sulaiman, M. A. A. Hassali, K. Subramaniam, and M. N. Sahib, "Osteoporosis and type 2 diabetes mellitus: What do we know, and what we can do," *Patient Preference Adherence*, vol. 6, p. 435, Jun. 2012.

[6] I. Goldshtein, A. M. Nguyen, S. Ish-Shalom, J. M. Chandler, G. Chodick, and V. E. A. Shalev, "Epidemiology and correlates of osteoporotic fractures among type 2 diabetic patients," *Arch. Osteoporosis*, vol. 13, no. 1, p. 15, 2018.

[7] J. Nicolau, T. Lequerré, H. Bacquet, and O. Vittecoq, "Rheumatoid arthritis, insulin resistance, and diabetes," *Joint Bone Spine*, vol. 84, no. 4, pp. 411–416, 2017.

[8] N. Sakib, U. N. Chowdhury, M. B. Islam, J. M. Quinn, and M. A. Moni, "A system biology approach to identify the genetic markers to the progression of parkinson's disease for aging, lifestyle and type 2 diabetes," *BioRxiv*, Jan. 2018, Art. no. 482760.

[9] J. Guzman, T. Kerr, L. M. Ward, J. Ma, K. Oen, A. M. Rosenberg, B. M. Feldman, G. Boire, K. Houghton, and P. Dancey, "Growth and weight gain in children with juvenile idiopathic arthritis: Results from the reacch-out cohort," *Pediatric Rheumatology*, vol. 15, no. 1, p. 68, 2017.

[10] Y. Krishnan and A. J. Grodzinsky, "Cartilage diseases," *Matrix Biol.*, vols. 7–72, pp. 51–69, Oct. 2018.

[11] M. H. Rahman, S. Peng, C. Chen, P. Lio, and M. A. Moni, "Genetic effect of type 2 diabetes to the progression of neurological diseases," *BioRxiv*, Jan. 2018, Art. no. 480400.

[12] A. Courties and J. Sellam, "Osteoarthritis and type 2 diabetes mellitus: What are the links," *Diabetes Res. Clin. Pract.*, vol. 122, pp. 198–206, Dec. 2016.

[13] J. S. Amberger and A. Hamosh, "Searching online mendelian inheritance in man (omim): A knowledgebase of human genes and genetic phenotypes," *Current Protocols Bioinf.*, vol. 58, no. 1, pp. 1–2, 2017.

[14] U. N. Chowdhury, S. Ahmad, M. B. Islam, F. Huq, J. M. Quinn, and M. A. Moni, "Network-based identification of genetic factors in ageing, lifestyle and type 2 diabetes that influence in the progression of alzheimer's disease," *BioRxiv*, Jan. 2019, Art. no. 482844.

[15] M. I. Love, W. Huber, and S. Anders, "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2," *Genome Biol.*, vol. 15, no. 12, p. 550, 2014.

[16] M. E. Ritchie, B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, and G. K. Smyth, "Limma powers differential expression analyses for rna-sequencing and microarray studies," *Nucleic Acids Res.*, vol. 43, no. 7, p. e47, 2015.

[17] H. Xu, M. A. Moni, and P. Liò, "Network regularised cox regression and multiplex network models to predict disease comorbidities and survival of cancer," *Comput. Biol. Chem.*, vol. 59, pp. 15–31, Dec. 2015.

[18] M. A. Moni and P. Liò, "Network-based analysis of comorbidities risk during an infection: Sars and hiv case studies," *BMC Bioinf.*, vol. 15, no. 1, p. 1, 2014.

[19] M. A. Moni, H. Xu, and P. Lio, "Cytocom: A cytoscape app to visualize, query and analyse disease comorbidity networks," *Bioinformatics*, vol. 31, no. 6, pp. 969–971, 2014.

[20] M. A. Moni and P. Liò, "comoR: A software for disease comorbidity risk assessment," *J. Clin. Bioinf.*, vol. 4, no. 1, p. 1, 2014.

[21] M. A. Moni and P. Liò, "How to build personalized multi-omics comorbidity profiles," *Frontiers Cell Developmental Biol.*, vol. 3, p. 28, Jun. 2015.

[22] M. Kanehisa, S. Goto, Y. Sato, M. Furumichi, and M. Tanabe, "Kegg for integration and interpretation of large-scale molecular data sets," *Nucleic Acids Res.*, vol. 40, no. D1, pp. D109–D114, 2011.

[23] A. Khan, O. Fornes, A. Stigliani, M. Gheorghe, J. A. Castro-Mondragon, R. van der Lee, A. Bessy, J. Cheneby, S. R. Kulkarni, and G. Tan, "Jaspar 2018: Update of the open-access database of transcription factor binding profiles and its Web framework," *Nucleic Acids Res.*, vol. 46, no. D1, pp. D260–D266, 2017.

[24] J. Xia, E. E. Gill, and R. E. Hancock, "Networkanalyst for statistical, visual and network-based meta-analysis of gene expression data," *Nature Protocols*, vol. 10, no. 6, p. 823, 2015.

[25] P. Sethupathy, B. Corda, and A. G. Hatzigeorgiou, "Tarbase: A comprehensive database of experimentally supported animal microrna targets," *Rna*, vol. 12, no. 2, pp. 192–197, 2006.

[26] S.-D. Hsu, F.-M. Lin, W.-Y. Wu, C. Liang, W.-C. Huang, W.-L. Chan, W.-T. Tsai, G.-Z. Chen, C.-J. Lee, and C.-M. Chiu, "miRTarBase: A database curates experimentally validated microrna–target interactions," *Nucleic Acids Res.*, vol. 39, no. 1, pp. D163–D169, 2010.

[27] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, and K. P. Tsafou, "String v10: Protein–protein interaction networks, integrated over the tree of life," *Nucleic Acids Res.*, vol. 43, no. D1, pp. D447–D452, 2014.

[28] M. R. Rahman, T. Islam, B. Turanli, T. Zaman, H. M. Faruquee, M. M. Rahman, M. N. H. Mollah, R. K. Nanda, K. Y. Arga, E. Gov, and M. A. Moni, "Network-based approach to identify molecular signatures and therapeutic agents in alzheimer's disease," *Comput. Biol. Chem.*, vol. 78, pp. 431–439, Feb. 2019.

[29] J. J. Connelly, T. Wang, J. E. Cox, C. Haynes, L. Wang, S. H. Shah, D. R. Crosslin, A. B. Hale, S. Nelson, and D. C. Crossman, "Gata2 is associated with familial early-onset coronary artery disease," *PLoS Genet.*, vol. 2, no. 8, p. e139, 2006.

[30] M. Yoshida, K. Hata, R. Takashima, K. Ono, E. Nakamura, Y. Takahata, T. Murakami, S. Iseki, T. Takano-Yamamoto, and R. Nishimura, "The transcription factor foxc1 is necessary for ihh–Gli2-regulated endochondral ossification," *Nature Commun.*, vol. 6, p. 6653, Mar. 2015.

[31] S. Wang, "Role of upstream stimulatory factor 2 in diabetic nephropathy," *Frontiers Biol.*, vol. 10, no. 3, pp. 221–229, 2015.

[32] F. Verdeguer S. M. Blättler, J. T. Cunningham, J. A. Hall, H. Chim, and P. Puigserver, "Decreased genetic dosage of hepatic yin yang 1 causes diabetic-like symptoms," *Molecular Endocrinology*, vol. 28, no. 3, pp. 308–316, 2014.

[33] A. Giralt, P.-D. Denechaud, I. C. Lopez-Mejia, B. Delacuisine, E. Blanchet, C. Bonner, F. Pattou, J.-S. Annicotte, and L. Fajas, "E2f1 promotes hepatic gluconeogenesis and contributes to hyperglycemia during diabetes," *Mol. Metabolism*, vol. 11, pp. 104–112, May 2018.

[34] R. Zenz, R. Eferl, C. Scheinecker, K. Redlich, J. Smolen, H. B. Schonthaler, L. Kenner, E. Tschachler, and E. F. Wagner, "Activator protein 1 (fos/jun) functions in inflammatory bone and skin disease," *Arthritis Res. Therapy*, vol. 10, no. 1, p. 201, 2008.

[35] A. S. Andreasen, M. Kelly, R. M. G. Berg, K. Møller, and B. K. Pedersen, "Type 2 diabetes is associated with altered nf-κb dna binding activity, jnk phosphorylation, and ampk phosphorylation in skeletal muscle after lps," *PloS ONE*, vol. 6, no. 9, pp. 1–8, 2011.

[36] Y. Xu, R. Song, W. Long, H. Guo, W. Shi, S. Yuan, G. Xu, and T. Zhang, "Creb1 functional polymorphisms modulating promoter transcriptional activity are associated with type 2 diabetes mellitus risk in chinese population," *Gene*, vol. 665, pp. 133–140, Jul. 2018.

[37] G. Argyropoulos, A. M. Brown, S. M. Willi, J. Zhu, Y. He, M. Reitman, S. M. Gevao, I. Spruill, and W. T. Garvey, "Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes," *J. Clin. Invest.*, vol. 102, no. 7, pp. 1345–1351, 1998.

[38] W. Tang, S. Tang, H. Wang, Z. Ge, D. Zhu, and Y. Bi, "Insulin restores ucp3 activity and decreases energy surfeit to alleviate lipotoxicity in skeletal muscle," *Int. J. Mol. Med.*, vol. 40, no. 6, pp. 2000–2010, 2017.

[39] H. Watanabe, J. Bohensky, T. Freeman, V. Srinivas, and I. M. Shapiro, "Hypoxic induction of ucp3 in the growth plate: Ucp3 suppresses chondrocyte autophagy," *J. Cellular Physiol.*, vol. 216, no. 2, pp. 419–425, 2008.

[40] J.-Y. Kim, S.-H. Park, J. M. Baek, M. Erkhembaatar, M. S. Kim, K.-H. Yoon, J. Oh, and M. S. Lee, "Harpagoside inhibits rankl-induced osteoclastogenesis via syk-btk-plcγ2-ca2+ signaling pathway and prevents inflammation-mediated bone loss," *J. Natural Products*, vol. 78, no. 9, pp. 2167–2174, 2015.

[41] R. Rezzonico, A. Schmid-Alliana, G. Romey, I. Bourget-Ponzio, V. Breuil, V. Breittmayer, S. Tartare-Deckert, B. Rossi, and H. Schmid-Antomarchi, "Prostaglandin e2 induces interaction between hslo potassium channel and syk tyrosine kinase in osteosarcoma cells," *J. Bone Mineral Res.*, vol. 17, no. 5, pp. 869–878, 2002.

[42] Y. Liu, R. A. Bhat, L. M. Seestaller-Wehr, S. Fukayama, A. Mangine, R. A. Moran, B. S. Komm, P. V. Bodine, and J. Billiard, "The orphan receptor tyrosine kinase ror2 promotes osteoblast differentiation and enhances ex vivo bone formation," *Mol. Endocrinology*, vol. 21, no. 2, pp. 376–387, 2007.

[43] B. Sánchez-Solana, J. Laborda, and V. Baladrón, "Mouse resistin modulates adipogenesis and glucose uptake in 3t3-l1 preadipocytes through the ror1 receptor," *Mol. Endocrinology*, vol. 26, no. 1, pp. 110–127, 2012.

[44] M. Yang, A. Arai, N. Udagawa, L. Zhao, D. Nishida, K. Murakami, T. Hiraga, R. Takao-Kawabata, K. Matsuo, and T. Komori, "Parathyroid hormone shifts cell fate of a leptin receptor-marked stromal population from adipogenic to osteoblastic lineage," *J. Bone Mineral Res.*, to be published.

[45] W. Ahsan, "The journey of thiazolidinediones as modulators of ppars for the management of diabetes: A current perspective," *Current Pharmaceutical Des.*, vol. 25, no. 23, pp. 2540–2554, 2019.

[46] I. Leontiou, N. London, K. M. May, Y. Ma, L. Grzesiak, B. Medina-Pritchard, P. Amin, A. A. Jeyaprakash, S. Biggins, and K. G. Hardwick, "The bub1-tpr domain interacts directly with mad3 to generate robust spindle checkpoint arrest," *Current Biol.*, vol. 29, no. 14, pp. 2407–2414, 2019.

[47] S. Kaneshiro, K. Ebina, K. Shi, C. Higuchi, M. Hirao, M. Okamoto, K. Koizumi, T. Morimoto, H. Yoshikawa, and J. Hashimoto, "Il-6 negatively regulates osteoblast differentiation through the SHP2/MEK2 and SHP2/AKT2 pathways *in vitro*," *J. Bone Mineral Metabolism*, vol. 32, no. 4, pp. 378–392, 2014.

[48] P. Steinberger, J. Andris-Widhopf, B. Bühler, B. E. Torbett, and C. F. Barbas, "Functional deletion of the ccr5 receptor by intracellular immunization produces cells that are refractory to ccr5-dependent hiv-1 infection and cell fusion," *Proc. Nat. Acad. Sci. USA*, vol. 97, no. 2, pp. 805–810, 2000.

[49] J.-W. Lee, A. Hoshino, K. Inoue, T. Saitou, S. Uehara, Y. Kobayashi, S. Ueha, K. Matsushima, A. Yamaguchi, and Y. Imai, "The hiv co-receptor ccr5 regulates osteoclast function," *Nature Commun.*, vol. 8, no. 1, p. 2226, 2017.

[50] P. W. Ho, A. S. Chan, N. J. Pavlos, N. A. Sims, and T. J. Martin, "Brief exposure to full length parathyroid hormone-related protein (pthrp) causes persistent generation of cyclic amp through an endocytosis-dependent mechanism," *Biochem. Pharmacol.*, vol. 169, Nov. 2019, Art. no. 113627.

[51] Y. Meng, Y. Guan, W. Zhang, Y.-E. Wu, H. Jia, Y. Zhang, X. Zhang, H. Du, and X. Wang, "Rna-seq analysis of the hypothalamic transcriptome reveals the networks regulating physiopathological progress in the diabetic GK rat," *Sci. Rep.*, vol. 6, Sep. 2016, Art. no. 034138.

[52] M. A. Moni, H. K. Rana, M. B. Islam, M. B. Ahmed, H. Xu, M. A. M. Hasan, Y. Lei, and J. M. Quinn, "A computational approach to identify blood cell-expressed parkinson's disease biomarkers that are coordinately expressed in brain tissue," *Comput. Biol. Med.*, vol. 113, Oct. 2019, Art. no. 103385.

[53] M. Rahman, T. Islam, M. Shahjaman, T. Zaman, H. M. Faruquee, M. A. H. M. Jamal, F. Huq, J. M. Quinn, and M. A. Moni, "Discovering biomarkers and pathways shared by alzheimer's disease and ischemic stroke to identify novel therapeutic targets," *Medicina*, vol. 55, no. 5, p. 191, 2019.

[54] M. A. Moni and P. Lio', "Genetic profiling and comorbidities of zika infection," *J. Infectious Diseases*, vol. 216, no. 6, pp. 703–712, 2017.

[55] M. R. Rahman, T. Islam, F. Huq, J. M. Quinn, and M. A. Moni, "Identification of molecular signatures and pathways common to blood cells and brain tissue of amyotrophic lateral sclerosis patients," *Informat. Med. Unlocked*, vol. 16, Jan. 2019, Art. no. 100193.

[56] M. A. Hossain, T. A. Asa, F. Huq, J. M. Quinn, and M. A. Moni, "A network-based approach to identify molecular signatures and comorbidities of thyroid cancer," in *Proc. Int. Joint Conf. Comput. Intell.* Singapore: Springer, 2020, pp. 235–246.

[57] M. R. Rahman, T. Islam, M. Shahjaman, J. M. Quinn, R. D. Holsinger, and M. A. Moni, "Identification of common molecular biomarker signatures in blood and brain of alzheimers disease," *BioRxiv*, Jan. 2019, Art. no. 482828.

[58] M. A. Hossain, T. A. Asa, M. R. Rahman, and M. A. Moni, "Network-based approach to identify key candidate genes and pathways shared by thyroid cancer and chronic kidney disease," *Informat. Med. Unlocked*, vol. 16, Jan. 2019, Art. no. 100240.

[59] M. R. Rahman, T. Islam, T. Zaman, M. Shahjaman, M. R. Karim, F. Huq, J. M. Quinn, R. D. Holsinger, and M. A. Moni, "Identification of biomarkers and pathways to identify novel therapeutic targets in alzheimer's disease: Insights from a systems biomedicine perspective," *BioRxiv*, Jan. 2019, Art. no. 481879.

[60] M. R. Rahman, T. Islam, E. Gov, B. Turanli, G. Gulfidan, M. Shahjaman, N. A. Banu, N. Mollah, N. Haque, and K. Y. Arga, "Identification of prognostic biomarker signatures and candidate drugs in colorectal cancer: Insights from systems biology analysis," *Medicina*, vol. 55, no. 1, p. 20, 2019.

[61] X.-W. Tang and Q.-X. Qin, "miR-335-5p induces insulin resistance and pancreatic islet β-cell secretion in gestational diabetes mellitus mice through VASH1-mediated TGF-β signaling pathway," *J. Cellular Physiol.*, vol. 234, no. 5, pp. 6654–6666, 2019.

[62] J. Duan, X.-L. Qian, J. Li, X.-H. Xiao, X.-T. Lu, L.-C. Lv, Q.-Y. Huang, W. Ding, H.-Y. Zhang, and L.-X. Xiong, "miR-29a negatively affects glucose-stimulated insulin secretion and min6 cell proliferation via cdc42/β-catenin signaling," *Int. J. Endocrinology*, vol. 2019, Aug. 2019, Art. no. 5219782.

[63] E.-A. Ye and J. J. Steinle, "miR-15b/16 protects primary human retinal microvascular endothelial cells against hyperglycemia-induced increases in tumor necrosis factor alpha and suppressor of cytokine signaling 3," *J. Neuroinflammation*, vol. 12, no. 1, p. 44, 2015.

[64] Y. Jiang, N. Liu, B. Xue, J. Hou, C. Lin, J. Ren, and J. Liu, "Circulating microrna profiles differ between hyperglycemia and euglycemia in coronary heart disease patients," *BioMed Res. Int.*, vol. 2017, Oct. 2017, Art. no. 9192575.

[65] T. S. Assmann, M. Recamonde-Mendoza, B. M. De Souza, and D. Crispim, "Microrna expression profiles and type 1 diabetes mellitus: Systematic review and bioinformatic analysis," *Endocrine Connections*, vol. 6, no. 8, pp. 773–790, 2017.

[66] İ. H. Demirsoy, D. Y. Ertural, Ş. Balci, Ü. çinkir, K. Sezer, L. Tamer, and N. Aras, "Profiles of circulating mirnas following metformin treatment in patients with type 2 diabetes," *J. Med. Biochem.*, vol. 37, no. 4, pp. 499–506, 2018.

[67] Z. Zhu, J. Yin, D. Li, and Z. Mao, "Role of microRNAs in the treatment of type 2 diabetes mellitus with roux-en-y gastric bypass," *Brazilian J. Med. Biol. Res.*, vol. 50, no. 3, p. e5817, 2017, doi: 10.1590/1414-431x20175817.

[68] W. Tao, X. Dong, G. Kong, P. Fang, X. Huang, and P. Bo, "Elevated circulating hsa-miR-106b, hsa-miR-26a, and hsa-miR-29b in type 2 diabetes mellitus with diarrhea-predominant irritable bowel syndrome," *Gastroenterology Res. Pract.*, vol. 2016, Apr. 2016, Art. no. 9256209.

[69] J. V. Esteves, C. Y. Yonamine, D. C. Pinto-Junior, F. Gerlinger-Romero, F. J. Enguita, and U. F. Machado, "Diabetes modulates micrornas 29b-3p, 29c-3p, 199a-5p and 532-3p expression in muscle: Possible role in GLUT4 and HK2 repression," *Frontiers Endocrinology*, vol. 9, p. 536, Sep. 2018.

MOHAMMAD ALI MONI received the Ph.D. degree in artificial intelligence and machine learning from the University of Cambridge, U.K., in 2014. From 2015 to 2017, he was a Postdoctoral Research Fellow with the Garvan Institute of Medical Research, Sydney, and he worked as an Associate Lecturer at the University of New South Wales, Australia. He is currently working as a Fellow at the University of Sydney. His research interests include artificial intelligence, machine learning, data science, and clinical bioinformatics. He received the USyd Fellowship, in 2017.

MD ABDUL AWAL received the B.Sc. degree in ECE from Khulna University, Khulna, Bangladesh, the M.Sc. degree in biomedical engineering from Khulna University of Engineering and Technology, Bangladesh, in 2011, and the Ph.D. degree in biomedical engineering from The University of Queensland, Australia. He is currently serving as an Associate Professor with the ECE Discipline, Khulna University, Khulna. His research interests include signal processing, especially, biomedical signal processing, big data analysis, image processing, time–frequency analysis, machine learning algorithms, deep learning, optimization, and computational intelligence in biomedical engineering.

M. BABUL ISLAM received the B.Sc. and M.Sc. degrees in applied physics and electronic engineering (currently electrical and electronic engineering) from the University of Rajshahi, Bangladesh, in 1992 and 1993, respectively, and the Ph.D. degree in signal processing from Shinshu University, Nagano, Japan, in 2007. From 2007 to 2010, he was working as an Associate Professor at the Department of Computer Science and Engineering, Islamic University, Kushtia, Bangladesh. He is currently a Professor of electrical and electronic engineering with the University of Rajshahi. His research interests include bioinformatics, signal processing, speech recognition, and image processing. He is a member of the IEEE SPS.

SHEIKH MOHAMMED SHARIFUL ISLAM is currently a Physician Scientist with the Institute for Physical Activity and Nutrition, Deakin University. He is also an Honorary Senior Lecturer with the Sydney Medical School, University of Sydney, and an Honorary Senior Fellow with the George Institute for Global Health, UNSW. Previously, he led the Diabetes Research Program at the Center for Control of Chronic Diseases, ICDDR,B and has experience working with United Nations Development Programme, World Health Organization, German Technical Cooperation, and as a Resident Cardiologist in Bangladesh. His research is focused on secondary prevention of diabetes and cardiovascular diseases using innovative information technologies. He is interested in wearable devices for continuous blood pressure monitoring and home blood pressure monitoring and using smart home technologies for improving cardiovascular and metabolic diseases.

MD REZANUR RAHMAN received the B.Sc. (Hons.) and M.S. degrees in biotechnology and genetic engineering from Islamic University (IU), Kushtia, Bangladesh. He is currently a Lecturer with the Department of Biochemistry and Biotechnology, School of Biomedical Science, Khwaja Yunus Ali University, Bangladesh. His research interests include systems biology and bioinformatics to study the multiomics data to decipher novel biomarkers of complex diseases.

MD. NURUL HAQUE MOLLAH received the B.Sc. and M.Sc. degrees in statistics from the University of Rajshahi, and the Ph.D. degree from ISM, Tokyo.

Then, he held a postdoctoral research position at ISM, Tokyo. He is currently a Professor of statistics with the University of Rajshahi. His research interests include robust statistical inference, multivariate statistics, data mining, big data analysis, biostatistics, bioinformatics, and computational biology.

JULIAN M. W. QUINN received the Ph.D. degree from the University of Oxford, U.K., in 1992. Then, he moved to Australia for doing his postdoctoral research at the Bone, Joint, and Cancer Group, St Vincent's Institute of Medical Research, and worked as a Senior Research Fellow, in 2014. Then, he moved to the Bone Biology Divisions, Garvan Institute of Medical Research, as a Senior Research Fellow. Over 25 years of his postdoctoral experience, he is running laboratory-based bone biology research, leading teams focused on determining drug and hormone effect on bone health, but also giving lectures and seminars, teaching and mentoring students and team members. He has long experience in scientific and technical writing and critical evaluation of manuscripts and grant applications. He also has significant skills in statistical data analysis, including large dataset studies.

MD RASHED-AL-MAHFUZ received the B.Sc. and M.Sc. degrees in computer science and engineering from the University of Rajshahi, Rajshahi, Bangladesh. In January 2011, he joined the Pabna University of Science and Technology, as a Lecturer of computer science and engineering. Then, he moved to the University of Rajshahi, as a Lecturer of computer science and engineering, where he is currently an Assistant Professor. His research interests include machine learning, big data analysis, signal and image processing, and bioinformatics.

• • •