An efficient method for mining cross-timepoint gene regulation sequential patterns from time course gene expression datasets

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Abstract

Background: Observation of gene expression changes implying gene regulations using a repetitive experiment in time course has become more and more important. However, there is no effective method which can handle such kind of data. For instance, in a clinical/biological progression like inflammatory response or cancer formation, a great number of differentially expressed genes at different time points could be identified through a large-scale microarray approach. For each repetitive experiment with different samples, converting the microarray datasets into transactional databases with significant singleton genes at each time point would allow sequential patterns implying gene regulations to be identified. Although traditional sequential pattern mining methods have been successfully proposed and widely used in different interesting topics, like mining customer purchasing sequences from a transactional database, to our knowledge, the methods are not suitable for such biological dataset because every transaction in the converted database may contain too many items/gene.

Results: In this paper, we propose a new algorithm called CTGR-Span (Cross-Timepoint Gene Regulation Sequential pattern) to efficiently mine CTGR-SPs (Cross-Timepoint Gene Regulation Sequential Patterns) even on larger datasets where traditional algorithms are infeasible. The CTGR-Span includes several biologically designed parameters based on the characteristics of gene regulation. We perform an optimal parameter tuning process using a GO enrichment analysis to yield CTGR-SPs more meaningful biologically. The proposed method was evaluated with two publicly available human time course microarray datasets and it was shown that it outperformed the traditional methods in terms of execution efficiency. After evaluating with previous literature, the resulting patterns also strongly correlated with the experimental backgrounds of the datasets used in this study.

Conclusions: We propose an efficient CTGR-Span to mine several biologically meaningful CTGR-SPs. We postulate that the biologist can benefit from our new algorithm since the patterns implying gene regulations could provide further insights into the mechanisms of novel gene regulations during a biological or clinical progression. The Java source code, program tutorial and other related materials used in this program are available at http://websystem.csie.ncku.edu.tw/CTGR-Span.rar.

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Background

Over the past decade, a great number of studies on time course issue have become increasingly important since most clinical/biological events, such as infection-related chronic/acute inflammatory responses [1-3], drug treatment-related experiments [4], cell cycle-arrest [5] or other important issues [6], require a period of time in which aberrant alterations in gene expression would lead to different outcomes. Therefore, through performing a consecutive monitoring of massive gene expressions and discovering their regulations during clinical/biological manifestations, the hidden layer of biological mechanisms could be unveiled. However, to our knowledge, these is no effective method can handle this issue although the high-throughput microarray is a powerful tool and has been widely utilized to efficiently detect differentially expressed genes among a group of patients in a time course experiment [3,4]. These authors only focused on how to identify differentially expressed genes varied with time but actually we did not know whether these genes are associated with each other or not. Their results did not show the valuable information.

Sequential pattern mining is one of the most important topics in the field of data mining, especially for the database systems. The fundamental meaning of a sequential pattern refers to a set of singleton frequent items/differentially expressed genes that are followed by another set of items/differentially expressed genes in the time-stamp ordered transaction. Therefore, once the potential gene regulations occurred in a period of time, it could be identified by mining such sequential patterns from a dataset-converted database. Referring to previous studies, several parental algorithms using different computational designs, such as AprioriAll [7], SPADE [8] and PrefixSpan [9], have been successfully proposed and used for different databases to discover their own sequential patterns. The Apriori-like (level-wise) GSP [10] and pattern-growth-based Prefixgrowth [11] as well as DELISP [12] are evolutionarily designed incorporating with many constraints such as the size of gap among the sequence-involved singleton items, or a time interval within which items are observed as belonging to the same transaction even if they originate from different transactions. Besides, any possible subpatterns derived from each parental sequential pattern also satisfy the user-set constraint values. This property is called downward closure [7-12]. Therefore, any possible subpatterns of each sequential pattern, particularly for the longer ones, need to be generated during the decomposing process that is time-consuming and space-exhausting. Once both shorter and longer sequential patterns have the same occurrence times across all transactions in the database, i.e., closed sequential patterns, the shorter ones will be eliminated from the final resulting patterns. For this purpose, some newer algorithms like incorporating with constraints, CTSP [13], and without constraints, CloSpan [14], were then designed to tackle this problem. In addition to these traditional algorithms, an increasing number of extended methods have also been performed on some interesting topics. For example, an algorithm called WSpan [15] could be used to determine weighted sequential patterns from a transactional database, and the MAGIIC [16] was designed to discover the structure motifs from protein sequences. However, to the best of our knowledge, all of the aforementioned methods are not suitable for the widely used microarray data, as a large-scale DNA microarray-based platform normally consists over tens of thousands of probes genes, e.g., over 45,000 probes genes in rice and over 20,000 probes genes in human arrays. A set of differentially expressed genes (significant singleton gene items) on a single array could be individually considered as a single transaction. In that way, each transaction (each time point contained gene items) may contain too many significant singleton gene items after converting the numeric datasets into the format (discrete) of transactional databases [17]. This is called a long transaction issue. However, to date, there exists no method which can efficiently handle such kind of issue. Actually, a lot of items would frequently occur at most time points. They are similar to the housekeeping genes, which are very insensible to an extracellular stimulus; instead, they play critical roles as maintenance genes in the basic cellular functions [18]. Moreover, mining sequential patterns containing too many such items may increase the difficulty in interpreting the resulting gene regulations. The performance of the preceding sequential pattern mining methods would also be limited to these simultaneous items.

In this paper, we propose a new algorithm called CTGR-Span (Cross-Timepoint Gene Regulation Sequential pattern) with some biologically designed parameters to solve the issue mentioned above by mining CTGR-SPs (Cross-Timepoint Gene Regulation Sequential Patterns). The CTGR-Span ensures that all of the resulting patterns imply gene regulations, which take place across different time points during the course of biological observations. The method is an extended and improved version of our previous paper [19] presented in the 2012 IEEE International Conference on Bioinformatics and Biomedicine (BIBM). The most important changes include: first, we designed a new optimal parameter tuning procedure for the proposed algorithm to ideally determine suitable conditions in pattern mining. The procedure has a merit that there is no need to additionally compute the standard deviation of time intervals in a time course dataset. Based on this design, then we compared our method with two representative sequential pattern mining algorithms, namely GSP and PrefixSpan, in execution efficiency and effectiveness. The resulting patterns were validated using a
manual literature survey and an automatic Gene Ontology enrichment analysis [20]. Finally, more explanations for the proposed algorithm have also been added to this paper like i) providing complete examples for readily understanding both our proposed algorithm and the new parameter tuning procedure, and ii) performing more experimental results on the two publicly available human disease-related time course microarray datasets [3,4].

The rest of this paper is organized as follows. The proposed method and materials for analysis are described in Methods. In Results and Discussion, we give the experimental results of the proposed method on two time course gene expression datasets. Concluding remarks are given in Conclusions.

Methods
In this section, we introduce how to efficiently discover CTGR-SPs (Cross-Timepoint Gene Regulation Sequential Patterns) from a time course microarray dataset through 3 main parts: i) an introduction to the experimental background of 2 input microarray datasets, ii) how to convert a numeric dataset into a transactional database, and iii) the kernel of the CTGR-Span (Cross-Timepoint Gene Regulation Sequential pattern) and its required biologically designed arguments.

Input microarray datasets
We tested this paper presenting method using the same input datasets as our previous works [19]. In brief, 2 time course gene expression microarray datasets (GSE6377 [3] and GSE11342 [4]) were downloaded from the GEO database. In GSE6377, McDunn et al. attempted to detect 8,793 transcriptional changes in 11 ventilator-associated pneumonia patients’ leukocytes across 10 time points. For the other GSE11342, Taylor et al. monitored 22,283 gene expression changes in peripheral blood monocytes of 20 hepatitis C virus infected patients across the first 10 weeks right after treating with the Peg-interferon alfa-2b plus ribavirin.

Converting microarray datasets into transactional databases
The sequential patterns could be mined directly from a transactional database if the data are discrete. The microarray-involved probe/gene expression values need to be discretized into singleton items within every transaction. Here we show you an example from Table 1 to 3. Table 1 shows the probe/gene expression values of 3 genes G1 to G3 over 4 time points TP1 to TP4 with a fixed interval (1 day). The experimental design is performed in 3 patients. The first time point of this example is regarded as a baseline for deriving the significant items at each time point. All of the values are then divided by the first time point. The divided values can be presented in a fold change matrix as Table 2. The absolute fold changes exceeding a fold-change threshold are further defined as the significant genes. Suppose that the threshold is set as 1.5, only the eligible significant genes can be preserved as new items as shown in Table 3. Take patient 1 for instance, up-regulated G1, down-regulated G2 and down-regulated G3 occur at the second time point that will be presented within the same parentheses (transaction). In this example, a set of 3 time-ordered transactions for each patient is called a sequence.

However, the content of the converted transactional databases will be affected by different threshold settings. In this study, the threshold of GSE6377 is set as 1.03 and the threshold of GSE11342 is set as 1.5, based on the same criteria used for the original datasets [3,4].

CTGR-Span: cross-timepoint gene regulation sequential pattern
Since the CTGR-Span is designed based on a pattern-growth-based manner [9] for mining CTGR-SPs, we will present the kernel procedure and meanwhile show the main differences between the traditional pattern-growth-based and our methods using a readily understood example. Finally, we present several extra biologically designed parameters toward more meaningful CTGR-SPs in biology.

| Table 1 Example of time course microarray dataset |
|-----------------------------------------------|
| Patient IDs | Genes | TP1 | TP2 | TP3 | TP4 |
|-------------|-------|-----|-----|-----|-----|
| 1           | G1    | 249 | 656 | 100 | 50  |
|             | G2    | 333 | 100 | 777 | 989 |
|             | G3    | 500 | 250 | 157 | 333 |
| 2           | G1    | 123 | 950 | 135 | 354 |
|             | G2    | 222 | 987 | 592 | 80  |
|             | G3    | 300 | 222 | 246 | 735 |
| 3           | G1    | 500 | 121 | 100 | 50  |
|             | G2    | 400 | 777 | 520 | 60  |
|             | G3    | 100 | 300 | 400 | 500 |

TPn/m: gene/probe reading values at time point n.

| Table 2 Fold changes of gene/probe reading values |
|-----------------------------------------------|
| Patient IDs | Genes | TP1/1 | TP2/1 | TP3/1 | TP4/1 |
|-------------|-------|-------|-------|-------|-------|
| 1           | G1    | 1.00  | 2.63  | -2.49 | -4.98 |
|             | G2    | 1.00  | -3.33 | 2.33  | 2.97  |
|             | G3    | 1.00  | -2.00 | -3.18 | -1.50 |
| 2           | G1    | 1.00  | 7.72  | 1.10  | 2.88  |
|             | G2    | 1.00  | 4.45  | 2.67  | -2.78 |
|             | G3    | 1.00  | -1.35 | -1.22 | 2.45  |
| 3           | G1    | 1.00  | -4.13 | -5.00 | -10.00|
|             | G2    | 1.00  | 1.94  | 1.30  | -6.67 |
|             | G3    | 1.00  | 3.00  | 4.00  | 5.00  |

TPn/m: gene/probe reading values at time point n relative to m.
Kernel procedure

The main strength of the CTGR-Span is to overcome a problem that the transactions have too many items/significant genes. According to our design, there also have several advantages: i) the items within transactions do not need to be sorted in advance, ii) the mining results will not be affected by different sorting types, iii) more meaningful sequential patterns implying gene regulations in biology can be successfully discovered relative to the traditional sequential pattern mining algorithms [7-12], and iv) massive repeated redundant patterns will not be identified. The following examples guide you how to trace the mining processes to explore the patterns from a microarray dataset-converted database. A set of sequences containing 4 patients' transactions is shown in Table 4. Each transaction consists of several significant gene items $G_{p/n}$. In this example, we set a minimum support (minSupp) as 50%, which means if any one of the items occurs in at least 2 different individual sequences (each patient has its own sequence), we call these items as frequent items and further to generate CTGR-SPs through a prefix-projection-based manner [9] in the following steps:

**Step 1: Find length-1 CTGR-SPs**

After scanning the $S$, the frequent items of length-1 including $G_{1+}$, $G_{2-}$ and $G_{3+}$ can be successfully identified since they appear over one half of the sequences. Therefore, these 3 frequent items are regarded as the length-1 CTGR-SPs.

**Step 2: Divide search space**

Each item within the set of length-1 CTGR-SPs is individually considered as a prefix to find its postfixes in which they are also frequent in the $S$.

**Step 3: Find postfixes of CTGR-SPs**

For each identified prefix, the subsets of CTGR-SPs can be identified using a depth-first search-based manner in the prefixes projected databases.

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### Table 3 Converted transactional database

| Patient IDs | Sequences |
|-------------|-----------|
| 1           | $<(G_1,G_2,G_3,G_4)_1,(G_1,G_2,G_3)_2,(G_1,G_2,G_3)_3>^c$ |
| 2           | $<(G_1,G_2,G_3)_1,(G_1,G_2,G_3)_2>^c$ |
| 3           | $<(G_1,G_2,G_3,G_4)_1,(G_1,G_2,G_3)_2>^c$ |

$< >$: a sequence; $^c$: a transaction of time point t; $G_{p/n}$: significantly up- or down-regulated gene item.

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### Table 4 Example of transactional database

| Patient IDs | Sequences |
|-------------|-----------|
| 1           | $<(G_1)_1,(G_2,G_3)_2,(G_1)_3>^c$ |
| 2           | $<(G_1)_1,(G_2,G_3)_2,(G_1)_3>^c$ |
| 3           | $<(G_1)_1,(G_2,G_3)_2,(G_1)_3>^c$ |
| 4           | $<(G_1)_1,(G_2,G_3)_2,(G_1)_3>^c$ |

$< >$: a sequence; $^c$: a transaction of time point t; $G_{p/n}$: significantly up- or down-regulated gene item.
Table 5 Comparison of patterns between a traditional pattern-growth-based approach and CTGR-Span

| Prefixes | Traditional projected databases | Projected databases of CTGR-Span | Traditional sequential patterns | CTGR-SPs |
|----------|---------------------------------|---------------------------------|--------------------------------|----------|
| $G_{14}$ | <(G2, G11), (G11)>              | <(G2, G11), (G11)>              | <(G11)>                        | <(G11)>  |
|          |                                 |                                 |                                |          |
| $G_3$    |                                 |                                 |                                |          |
|          |                                 |                                 |                                |          |
| $G_{14}$ |                                 |                                 |                                |          |
|          |                                 |                                 |                                |          |
|          |                                 |                                 |                                |          |

$G_{14}$: significantly up- or down-regulated gene item; < >: a sequence; t: a transaction of time point t; _: indexed prefix; *: redundant patterns derived from traditional pattern-growth-based sequential pattern mining methods.

efficiency and useful than the traditional pattern-growth-based methods.

Biological parameter designs
As stated above, we have introduced the main differences between the traditional and our proposed method. Then we intend to describe how to enrich the patterns with more meaningful in biology. In addition to the inherent parameter minSupp for mining traditional patterns, we additionally introduce 3 parameters: minimum timepoint support (minTSupp), sliding window size (SWS) and maximum time constraint (maxTC) to the CTGR-Span to mine more meaningful sequential patterns in gene regulation based on some biological characteristics. Since the fundamental definitions of these parameters have been shown in the section II, MATERIALS AND METHODS, of our previous conference paper [19], here we briefly describe their main characteristics and followed by some concrete examples.

minTSupp (minimum timepoint support). After converting the input microarray datasets into the transactional datasets, thousands of items are contained in each transaction. The average lengths of the transactions of the two datasets are presented as two bars at the leftmost N tick shown in Figure 1. The continuously expressed genes at all-time points may not be susceptible to the cellular responses. They may have a propensity for being housekeeping or maintenance genes [18]. In this regard, some well-studied housekeeping genes (HGs) contained in each transaction will be removed. Based on the similar concept, if the items constitutively appear in most time points, these HG-like items can also be further removed from the transactions using the proposed parameter minTSupp. The average lengths of transactions in both input datasets as the functions of varying minTSupp are shown in Figure 1.

SWS (sliding window size). Mining sequential patterns implying gene regulations across fixed time points may cause the resulting patterns inadequate because the response times among a set of genes through transcription regulations are not identical. The sliding window size (SWS) parameter can flexibly allow the patterns containing items to be derived from the same/different time points. Here we show you an example extended from Table 4. Table 6 shows the projected databases of length-1 CTGR-SPs when the SWS is set as 1. Since the time intervals between the transactions contained in the length-1-projected databases and the prefixes not exceed 1 (SWS = 1), the transactions-involved items and the prefixes may actually take place at the same time point. In this case, the gene items involved in a-prime-symbol-marked transactions indicate that they occur with the
Table 6 Example of SWS = 1

| Prefixes | Projected databases | CTGR-SPs |
|----------|---------------------|----------|
| G1_1     | \(<(G_1 \cup G_2 \cup G_3)\)\> | \(<(G_1, G_2)\)\> |
|          | \(<(G_2 \cup G_3)\)
| G2_1     | \(<(G_1 \cup G_2 \cup G_3)\)\> | \(<(G_1, G_2)\)\> |
|          | \(<(G_2 \cup G_3)\)
| G3_1     | \(<(G_1 \cup G_2 \cup G_3)\)\> | \(<(G_1, G_2)\)\> |
|          | \(<(G_2 \cup G_3)\)

G_1: significantly up- or down-regulated gene item; <>: a sequence; (): a transaction of time point t; _: indexed prefix; *: redundant patterns derived from traditional pattern-growth-based sequential pattern mining methods.

prefixes at the same time point even if all of them originate from different time points.

maxTC (maximum time constraint). Normally, the cells need to react quickly to resist adverse environmental changes, massive short-term gene regulations should be more pronounced within a cellular signaling transduction. In this regard, when setting smaller values of the parameter maxTC, a pattern containing two gene items with a big time gap will not be generated. Table 7 shows the length-1-projected databases and CTGR-SPs from an extended example of Table 4 when maxTC is set as 1. The possible postfixes for generating length-2 CTGR-SPs only will be checked till the transactions marked with a prime symbol.

Results and discussion

In this section, we presented the experimental results of the proposed CTGR-Span of two time course gene expression datasets. Because performing the program with different parameter values would yield diverse results, all of the parameters used in this study will be tuned according to the biological backgrounds of the datasets. By introducing the tuned parameter values to the CTGR-Span, the resultant CTGR-SPs will then be evaluated with previous literature and a GO enrichment analysis to reveal their reliability in biology. Meanwhile, in terms of the performance, the execution efficacy between the traditional and our proposed methods will also be examined in this study.

Optimal parameter tuning

In addition to the inherent parameter minSupp of the traditional methods, we additionally introduced 3 parameters minTSupp, SWS and maxTC to the CTGR-Span. However, two questions might arise as to how to set these parameter values for most biologists and whether these parameters are useful for mining gene regulations. In this section, we performed an optimal parameter tuning process to obtain a general rule for setting the parameters without additionally calculating the standard deviations of the time intervals of a dataset in advance [19]. Based on the impact degree of each parameter setting to the numbers of the resulting CTGR-SPs, we examined the parameters in an order of minTSupp (Table 8 and Supplementary Table 1 to 3 in Additional file 1), minSupp (Table 8 and Supplementary Table 1 to 3 in Additional file 1), maxTC (Table 9 and 10) and SWS (Table 11 and 12). Several characteristics of the mined CTGR-SPs of two input datasets are presented in these tables. However, here arises a question as to how to judge which condition (combination of parameter values) is more suitable for further exploration - it is a trade-off that higher parameter values would allow fewer patterns to be mined, but lower parameter values would dramatically increase the number of marginal patterns. Both quantity and quality of the resultant patterns are necessary to be taken into account in this work. In the first dataset (GSE6377), McDunn et al. have proven that as the ventilator associated pneumonia (VAP) patients recovered from critical illness complicated by acute infection, the general trajectory (riboleukogram) converged, consistent with an immune attractor [3]. Eighty five genes involved in the inflammatory response were identified with consistent changes in abundance during seven days bracketing the diagnosis of VAP. For the other dataset (GSE11342), Taylor et al. identified 85 significantly up/down-regulated genes involved in the immune response from the blood monocytes of hepatitis C patients during the first 10 weeks of treatment with the Peg-interferon alfa-2b plus ribavirin in peripheral [4]. We used a Gene Ontology (GO) enrichment analysis [20] to test if the longest CTGR-SPs-involved at least two genes
Table 8 Characteristics of mined sequential patterns (\(\text{minSupp} = \text{variable} \) and \(\text{minTSupp} = 100\%\))

| \(\text{minTSupp}\) | GSE6377 | GSE11342 |
|----------------------|---------|----------|
| 100%                 | 95%     | 90%      | 85% | 80% | 75% | 100% | 95% | 90% | 85% | 80% | 75% | 100% |
| # of CTGR-SPs        | 417     | 426      | 4,762| 5,090| 181,295| 181,170| 6,948,828| 32   | 224 | 964 | 3,077| 11,105| 6,053| 17,412|
| # of longest CTGR-SPs| 81      | 81       | 59   | 59   | 176,552| 176,552| 208,297   | 274  | 224 | 964 | 3,077| 11,105| 6,053| 17,412|
| Maximal length of CTGR-SPs | 4 | 4 | 6 | 6 | 6 | 7 | 4 | 4 | 4 | 4 | 5 | 5 |
| # of genes in CTGR-SPs | 212     | 211      | 1,006| 996  | 2,821  | 2,826  | 5,313     | 25   | 138 | 466 | 1,132| 2,011 | 2,801| 4,142 |
| # of longest CTGR-SPs | 14      | 14       | 11   | 11   | 214    | 214    | 214       | 274  | 224 | 964 | 3,077| 11,105| 6,053| 17,412|
| # of gene pairs in longest CTGR-SPs | 81| 81| 59| 59| 176,552| 176,552| 208,297| 274| 224| 964| 3,077| 11,105| 6,053| 17,412|
| -Log(p-value)†       | 0.34    | 0.34     | 0.00 | 0.00 | 0.55   | 0.55   | 0.29†     | 0.00†| 0.12†| 0.26†| 0.91†| 0.00†| 1.58†| 4.11† |

%: \(\text{minSupp}\) value presented as percentage; †: test longest CTGR-SPs-involved genes in inflammatory response using GO enrichment analysis; ††: test longest CTGR-SPs-involved genes in immune response using GO enrichment analysis; -: no complete patterns.

Table 9 Characteristics of mined sequential patterns in GSE6377 (\(\text{maxTC} = \text{variable, minSupp} = 95\% \) and \(\text{minTSupp} = 100\%\))

| \(\text{d}\) | 2d | 3d | 4d | 5d | 6d | 7d | 8d | 9d | ≥ 10d |
|-------------|----|----|----|----|----|----|----|----|-------|
| # of CTGR-SPs | 157 | 157 | 166 | 166 | 180 | 180 | 298 | 306 | 426   |
| # of longest CTGR-SPs | 157 | 157 | 9   | 9   | 17  | 17  | 58  | 58  | 81    |
| Maximal length of CTGR-SPs | 1   | 1   | 3   | 3   | 4   | 4   | 4   | 4   | 4     |
| # of genes in CTGR-SPs | 157 | 157 | 169 | 169 | 179 | 179 | 201 | 202 | 211   |
| # of genes in longest CTGR-SPs | 0   | 0   | 7   | 7   | 10  | 10  | 12  | 12  | 14    |
| # of gene pairs in longest CTGR-SPs | 0  | 0   | 11  | 11  | 27  | 27  | 50  | 50  | 70    |
| -Log(p-value)† | - | - | - | - | - | - | - | - | 0.34 |

: \# of days of SWS; †: test longest CTGR-SPs-involved genes in inflammatory response using GO enrichment analysis; -: no p-values.

Table 10 Characteristics of mined sequential patterns in GSE11342 (\(\text{maxTC} = \text{variable, minSupp} = 95\% \) and \(\text{minTSupp} = 100\%\))

| \(\text{d}\) | 28d | 31d | 34d | 37d | 40d | 43d | 46d | 49d | 52d | 55d | 58d | 61d | 64d | ≥ 67d |
|-------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| # of CTGR-SPs | 112 | 112 | 120 | 126 | 157 | 165 | 160 | 163 | 161 | 194 | 194 | 220 | 242 |       |
| # of longest CTGR-SPs | 112 | 112 | 8   | 14  | 45  | 2   | 2   | 2   | 2   | 28  | 28  | 28  | 28  |       |
| Maximal length of CTGR-SPs | 1   | 1   | 3   | 3   | 4   | 4   | 4   | 4   | 4   | 4   | 4   | 4   | 4   |       |
| # of genes in CTGR-SPs | 112 | 112 | 123 | 132 | 132 | 132 | 132 | 132 | 132 | 136 | 136 | 136 | 140 |       |
| # of genes in longest CTGR-SPs | 0   | 0   | 11  | 11  | 42  | 4   | 4   | 4   | 4   | 21  | 21  | 21  | 21  |       |
| # of gene pairs in longest CTGR-SPs | 0  | 0   | 7   | 11  | 42  | 4   | 4   | 4   | 4   | 21  | 21  | 21  | 21  |       |
| -Log(p-value)†† | - | - | 1.02 | 0.74 | 0.40 | 0   | 0   | 0   | 1.31 | 1.31 | 1.31 | 1.31 |       |

: \# of days of SWS; ††: test longest CTGR-SPs-involved genes in immune response using GO enrichment analysis; -: no p-values.

Table 11 Characteristics of mined sequential patterns in GSE6377 (\(\text{SWS} = \text{variable, maxTC} = \infty \) days, \(\text{minSupp} = 95\% \) and \(\text{minTSupp} = 100\%\))

| \(\text{d}\) | 0d | 1d | 2d | 3d | 4d | 5d | 6d | 7d | 8d | 9d | ≥ 10d |
|-------------|----|----|----|----|----|----|----|----|----|----|-------|
| # of CTGR-SPs | 352 | 419 | 203 | 203 | 169 | 169 | 201 | 189 | 279 | 354 | 423 |
| # of longest CTGR-SPs | 81 | 81 | 46 | 46 | 3 | 3 | 201 | 189 | 279 | 354 | 423 |
| Maximal length of CTGR-SPs | 4 | 4 | 3 | 3 | 2 | 2 | 1 | 1 | 1 | 1 | 1 |
| # of genes in CTGR-SPs | 206 | 212 | 178 | 178 | 174 | 174 | 187 | 183 | 197 | 209 | 213 |
| # of genes in longest CTGR-SPs | 14 | 14 | 11 | 11 | 2 | 2 | 11 | 9 | 15 | 20 | 21 |
| # of gene pairs in longest CTGR-SPs | 70 | 70 | 33 | 33 | 5 | 5 | 0 | 0 | 0 | 0 | 0 |
| -Log(p-value)† | 0.37 | 0.37 | 0.44 | 0.44 | 0.44 | 0.44 | - | - | - | - |       |

: \# of days of SWS; †: test longest CTGR-SPs-involved genes in inflammatory response using GO enrichment analysis; -: no p-values.
under the conditions are relevant to the corresponding biological manifestations (inflammatory response in GSE6377 and immune response in GSE11342). We focused on the longest CTGR-SPs containing at least two gene items because the longer patterns not only contain more significant gene items but also carried more information in a consecutive gene regulation according to the original design of the algorithm. The testing results are presented as -log(p-value) in the tables.

First of all, if the same significant gene items occur too frequent during a time period, they may be similar to the HGs. Then, the significant patterns should occur as frequently as possible in a group of patients. For these two reasons, we tested both minTSupp and minSupp from 70% to 100% as shown in Table 8 and Supplementary Table 1 to 3 in Additional file 1. Apparently, the longest CTGR-SPs revealed no biologically significant when minTSupp was set as 70% or 80% regardless of the values of minSupp. Although the minTSupp was set as 90%, the common values of minSupp suitable for these two input datasets were 85%, 80%, 75% and 70%. Unfortunately, the number of genes involved in the CTGR-SPs was too high (over 250 patterns). It might be difficult for most biologists to work with the high number. In spite of these limitations, we could still successfully obtain a suitable common condition for the two datasets when minTSupp and minSupp were set as 100% and 95%, respectively.

Once the values of minTSupp and minSupp have been decided, we subsequently tested all possible values of maxTC in both two datasets as shown in Table 9 (GSE6377) and Table 10 (GSE11342). The maxTC was set from the beginning as largest time interval, 2 days (21-19) in GSE6377 and 28 days (70-42) in GSE11342, to the end as the values which included most transactions bracketing the maximal time interval, 10 days (21-11) in GSE6377 and 67 days (70-3) in GSE11342. For each dataset, the maxTC would be increased with the first minimum time interval, 1 day (1-0) in GSE6377 and 3 days (3-0) in GSE11342, to ensure any possible conditions would be tested. Apparently, according to the same criteria mentioned in the above paragraph, there was a suitable common condition for the two datasets when the values of maxTC were set as ∞ days.

Finally, we fixed the previous three parameter values and tested the SWS as shown in Table 11 (GSE6377) and Table 12 (GSE11342). The values of SWS in both datasets were set from the beginning as 0 to the end as the values which included most transactions bracketing the maximal time interval, 10 days in GSE3677 and 66 days in GSE11342. The values of SWS were also increased with a fixed interval. Then, we could successfully observe a suitable common condition when the value of SWS was set as 3 days. These tables also demonstrate that these suitable common conditions were neither the rule number nor rule length dependent. Incorporating with the domain knowledge of biology to the parameter designs might had a great benefit on discovering the CTGR-SPs with potential gene regulations. Therefore, these optimal parameter values could be certainly considered as the default settings to most biologists even if they have no any experiences before.

**High performance of CTGR-Span**

In this section, we compared the performance of our proposed CTGR-Span and the traditional sequential pattern mining algorithms such as the GSP and PrefixSpan in terms of execution efficiency. For achieving a fair comparison, we performed the GSP, PrefixSpan and CTGR-Span with same parameter settings on both input datasets. The resultant patterns and execution times are presented in Table 8 and Table 13 respectively. However, the traditional algorithms did not allow complete patterns (indicated with “−” in Table 8) to be identified in 2 weeks. Meanwhile, their patterns already have produced tens of millions of patterns. It might be complicated for biologist for find further usage of such massive patterns. In contrast, our proposed CTGR-Span only needed to take several hours in a worst case that the minSupp was set as 70%. (Table 13). These results clearly showed the efficiency of CTGR-Span.

**Evaluation with literature**

After performing the optimal parameter tuning process, we set the parameter SWS = 3 days, maxTC = ∞ days, minTSupp = 95% and minSupp = 100% for the further exploration of CTGR-SPs in biology. As stated in the section of optimal parameter tuning, the evaluation criteria for GO enrichment analysis were based on the experimental backgrounds of those two datasets to preliminarily test which condition with longest CTGR-SPs-involved genes is much related to the inflammatory response caused by the ventilator-associated pneumonia (GSE6377) and the immune response after drug treatments in hepatitis C patients (GSE11342). In this section, we attempted to further address whether these patterns contain potential genes/regulations which have not been reported in previous literature yet. We scrutinized and evaluated the longest CTGR-SPs derived genes from the two input datasets using a manual literature survey. Table 14 and Table 15 show the evaluation results of GSE6377 and GSE11342, respectively. If the patterns contain same items, they will be presented as a single item from left (prefix) to right. For example, in the top-4 data rows of Table 14, there are 4 CAV1+-, prefixed CTGR-SPs: <(CAV1+)(GNG7+)(EIF2D+)>), <(CAV1+)(GNG7+)(FTSJ2+), <(CAV1+)(GNG7+)(NR2E1+)>, and <(CAV1+)(GNG7+)(TMOD3+)>. The CAV1+, and GNG7+,
can be individually grouped and presented as a single item in the table.

After the evaluating process, 78% (54/69 hits) in Table 14 and 73% (29/40 hits) in Table 15 of the patterns-involved genes could be successfully referred to some literature. In other words, the remaining genes might play potential roles during the time course. As stated in the previous example, it has been proven that up-regulated caveolin-1 (CAV1) would regulate NF-kappa B activation and lung inflammatory response to sepsis induced by lipopolysaccharide [21]. The upregulation of nuclear receptor subfamily 2, group E, member 1 (NR2E1) has been revealed by a microarray analysis of mice infected with influenza virus A and Streptococcus pneumonia [22]. A relation/regulation might exist between these two genes since they were strongly related to the pneumonia [21,22]. Coincidentally, in Table 15, upregulated chemokine (C-X-C motif) ligand 10 (CXCL10) has also been reported in the original paper that CXCL10 would be transiently induced early in treatment with Peg-interferon alfa-2b plus ribavirin in peripheral blood monocytes (PBMC) of hepatitis C patients [4]. It could be successfully regarded as plasma indicator for predicting the outcome of antiviral therapy in patients with hepatitis C [23]. Therefore, via this literature evaluation, we postulated that the remaining unreported genes and their relations of the identified patterns in both datasets are highly valuable to be explored in the future.

Conclusions
In this study, our proposed CTGR-Span overcomes the flaws of the traditional sequential pattern mining methods. Although the transactional databases converted from the large-scale time course microarray gene expression datasets have too many items/significant genes within every transaction, the gene regulations over a period of time can still be efficiently identified. The CTGR-Span runs dramatically faster than the traditional methods. In addition to the improvement of execution times, we incorporated the characteristics of gene regulation in the parameter designs and further used a GO enrichment analysis to yield the CTGR-SPs more meaningful biologically. After evaluating with previous literature, the identified patterns correlate very well with the experimental backgrounds of the two input datasets. Therefore, we postulated that our approach could provide more biological insights into the underlying mechanisms of certain biological or clinical progresses, and it also could be readily applied to other research topics of interest.

Table 12 Characteristics of mined sequential patterns in GSE11342 (SWS = variable, maxTC = ∞ days, minSupp = 95% and minTSupp = 100%)

| d | 0d | 3d | 6d | 9d | 12d | 15d | 18d | 21d | 24d | 27d | 30d | 33d | 36d | 39d | 42d | 45d | 48d | 51d | 54d | 57d | 60d | 63d | 66d |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # of CTGR-SPs | 214 | 211 | 221 | 194 | 154 | 135 | 131 | 127 | 125 | 128 | 125 | 127 | 136 | 157 | 163 | 163 | 163 | 163 | 187 | 190 | 198 | 217 |
| # of longest CTGR-SPs | 28 | 25 | 25 | 82 | 37 | 17 | 17 | 14 | 10 | 13 | 13 | 7 | 10 | 157 | 163 | 163 | 163 | 163 | 187 | 190 | 198 | 217 |
| Maximal length of CTGR-SPs | 4 | 4 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| # of genes in CTGR-SPs | 136 | 134 | 136 | 134 | 127 | 124 | 123 | 121 | 120 | 121 | 119 | 121 | 125 | 132 | 132 | 132 | 132 | 132 | 132 | 136 | 136 | 136 |
| # of genes in longest CTGR-SPs | 3 | 3 | 3 | 15 | 10 | 9 | 9 | 9 | 8 | 5 | 5 | 4 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| # of gene pairs in longest CTGR-SPs | 21 | 19 | 19 | 59 | 26 | 16 | 16 | 14 | 10 | 12 | 10 | 10 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| -Log(p-value)†† | 1.26 | 1.37 | 1.37 | 0.70 | 0.00 | 0.00 | 0.00 | 0.00 | 0.86 | 0.86 | 0.65 | 0.53 | 0.40 | - | - | - | - | - | - | - | - |

Table: # of days of SWS; ††: test longest CTGR-SPs-involved genes in immune response using GO enrichment analysis; -: no p-values.

Table 13 Execution times (hr) of mined sequential patterns (minSupp = variable and minTSupp = 100%)

| GSE6377 | GSE11342 |
|---------|----------|
| 100%    | 95%      | 90%      | 85%      | 80%      | 75%      | 70%      | 100%    | 95%      | 90%      | 85%      | 80%      | 75%      | 70%      |
| GSP     | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        |
| PrefixSpan | -      | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        | -        |
| CTGR-Span | 0      | 0        | 0.03     | 0.03     | 1.65     | 1.65     | 220.88   | 0        | 0        | 0        | 0        | 0.05     | 0.23     | 0.93     |

%: minSupp value presented as percentage; -: over 2 weeks.
Table 14 Longest CTGR-SPs of GSE6377 (SWS = 3 days, max TC = ∞ days, min Supp = 95% and min TSupp = 100%)

| I1       | I2      | I3      | Supports |
|----------|---------|---------|----------|
| CAV1+ [21] | GNG7+   | EIF2D+ [24] | 100% (11/11) |
|          |         | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
| CCL20- [26] | KIF4A+ [27] | FTSJ2+ | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
| CSF3R- [28] | GNG7+   | CHST7+   | 100% (11/11) |
|          |         | EIF2D+ [24] | 100% (11/11) |
|          |         | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
| KIF4A+ [27] | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
| DGKQ+ [29] | GNG7+   | FTSJ2+   | 100% (11/11) |
| NUDT4+ [30] | CDC25A+ [31] | NR2E1- [22] | 100% (11/11) |
|          | GNG7+   | NR2E1- [22] | 100% (11/11) |
|          | KIF4A+ [27] | EIF2D+ [24] | 100% (11/11) |
|          |         | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | SOAT1- [32] | 100% (11/11) |
| TLR6- [33] | CORO1A+ [34] | 100% (11/11) |
|          | KAT2B- [35] | 100% (11/11) |
|          | NR2E1- [22] | 100% (11/11) |
|          | PLAGL1- [22] | 100% (11/11) |
| NUDT4P1+ | CDC25A+ [31] | NR2E1- [22] | 100% (11/11) |
|          | GNG7+   | NR2E1- [22] | 100% (11/11) |
|          | KIF4A+ [27] | EIF2D+ [24] | 100% (11/11) |
|          |         | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | SOAT1- [32] | 100% (11/11) |
| TLR6- [33] | CORO1A+ [34] | 100% (11/11) |
|          | KAT2B- [35] | 100% (11/11) |
|          | NR2E1- [22] | 100% (11/11) |
|          | PLAGL1- [22] | 100% (11/11) |
| STX4- [36] | CDC25A+ [31] | NR2E1- [22] | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
|          | KIF4A+ [27] | EIF2D+ [24] | 100% (11/11) |
|          |         | FTSJ2+   | 100% (11/11) |
|          |         | NR2E1- [22] | 100% (11/11) |
|          |         | TMOD3- [25] | 100% (11/11) |
| TLR6- [33] | CORO1A+ [34] | 100% (11/11) |
|          | KAT2B- [35] | 100% (11/11) |
|          | NR2E1- [22] | 100% (11/11) |
|          | PLAGL1- [22] | 100% (11/11) |

Pneumonia-associated genes reported in previous literature; Ii: the nth item in a CTGR-SP; +: expressed genes; -: repressed genes.
Additional material

**Additional file 1: Characteristics of mined sequential patterns**

(minSupp = 70–100% and minTSupp =70%–90%)

**List of abbreviations**

CTGR-Span: Cross-Timepoint Gene Regulation Sequential pattern; CTGR-SPs: Cross-Timepoint Gene Regulation Sequential Patterns; minTSupp: minimum timepoint support; minSupp: minimum support; SWS: sliding window size; maxTC: maximum time point support; GO: gene ontology.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

CPC, YCL, YLT and VST conceived and designed the entire experiments. CPC carried out the computational studies, performed the statistical analysis and drafted the manuscript. YLT participated in the data interpretations and helped to draft the manuscript. YCL obtained funding and made critical study supervision. All authors read and approved the final manuscript.

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**Declarations**

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Table 15 Longest CTGR-SPs of GSE11342 (SWS = 3 days, maxTC = ∞ days, minSupp = 95% and minTSupp = 100%)

| L₁    | L₂    | L₃    | L₄     | Supports    |
|-------|-------|-------|--------|-------------|
| CXCL10+ [23] | IFIT2+ [38] | ZNF710- | FECH+ [39] | 95% (19/20) |
|        |       |       | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| HBZ+  |       |       | FECH+ [39] | 95% (19/20) |
|        |       |       | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 100% (20/20) |
|        |       |       | TRIM46+ | 95% (19/20) |
| SELENBP1+ [42] |       |       | HBZ+ | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| PPP4R4+ |       |       | FECH+ [39] | 95% (19/20) |
|        | IFIT2+ [38] | IFIT2+ [38] | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| HBZ+  |       |       | FECH+ [39] | 95% (19/20) |
|        |       |       | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 100% (20/20) |
|        |       |       | TRIM46+ | 95% (19/20) |
| SELENBP1+ [42] |       |       | HBZ+ | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| PPP4R4+ |       |       | FECH+ [39] | 95% (19/20) |
|        | IFIT2+ [38] | IFIT2+ [38] | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| HBZ+  |       |       | FECH+ [39] | 95% (19/20) |
|        |       |       | BPGM+ [40] | 95% (19/20) |
|        |       |       | SNCA+ [41] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 100% (20/20) |
|        |       |       | TRIM46+ | 95% (19/20) |
| SELENBP1+ [42] |       |       | HBZ+ | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
|        |       |       | SELENBP1+ [42] | 95% (19/20) |
| PPP4R4+ |       |       | TNFSF10+ [43] | 95% (19/20) |
|        | IFIT2+ [38] | HBZ+ | SELENBP1+ [42] | 95% (19/20) |

[1]: hepatitis C-associated genes reported in previous literature; Lₙ: the nth item in a CTGR-SP; +: expressed genes; -: repressed genes.
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