Chromosomal neighbourhoods allow identification of organ specific changes in gene expression

Rishi Das Roy[1*], Outi Hallikas[1], Mona M. Christensen[1], Elodie Renvoisé[1,2], Jukka Jernvall[1,3*]

[1]Institute of Biotechnology, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland.
[2]5 Rue de Portland, 72100 Le Mans, France.
[3]Department of Geosciences and Geography, University of Helsinki, P.O. Box 64, FI-00014 Helsinki, Finland.
[*]To whom correspondence should be addressed. Email: rishi.dasroy@helsinki.fi and jernvall@fastmail.fm

ABSTRACT

Although most genes share their chromosomal neighbourhood with other genes, distribution of genes has not been explored in the context of individual organ development; the common focus of developmental biology studies. Because developmental processes are often associated with initially subtle changes in gene expression, here we explored whether neighbouring genes are informative in the identification of differentially expressed genes. First, we quantified the chromosomal neighbourhood patterns of genes having related functional roles in the mammalian genome. Although the majority of protein coding genes have at least five neighbours within 1 Mb window around each gene, very few of these neighbours regulate development of the same organ. Analyses of transcriptomes of developing mouse molar teeth revealed that whereas expression of genes regulating tooth development changes, their neighbouring genes show no marked changes, irrespective of their level of expression. Finally, we test whether inclusion of gene neighbourhood in the analyses of differential expression could provide additional benefits. For the analyses, we developed an algorithm, called DELocal that identifies differentially expressed genes by comparing their expression changes to changes in adjacent genes in their chromosomal regions. Our results show that DELocal provides distinct benefits in the identification of even subtle changes in gene expression. Future studies, including the detection of differential expression, may benefit from, and further characterize the significance of gene-gene neighbour relationships.

INTRODUCTION

One key aspect of development and differentiation is regulation of gene expression. During development, genes are expressed in a highly dynamic manner, and perturbations of the expression dynamics underlie many diseases and developmental defects. Regulation of gene expression through developmental time can be examined by quantifying gene expression levels at two or more time points. However, because development is typically a gradual process, detecting differential gene expression can be challenging due to the changes being initially subtle. This challenge underscores the continuing need for different strategies to identify biologically meaningful changes in gene expression.

One topic that has been intensively studied in the connection to gene expression is the genome organization. A fundamental aspect discovered is the difference in gene distribution between eukaryotes and prokaryotes. In many compact prokaryotic genomes the co-functioning genes are physically linked and expressed under a single promoter in a shared operon [1]. In eukaryotes, genes have been thought to be scattered throughout the genome without order related to expression or function [2]. However, even though there does not appear to be any simple logic in the distribution of eukaryotic genes, it is not truly random as most genes cannot be moved around in the genome without seriously disturbing their functionality [3]. Non-random distribution is found both at the small scale, relating to a limited number of genes, and at the large scale, concerning large chromosomal regions [3, 4]. Thus, clustering has been detected among co-expressed genes, such as the housekeeping genes, as also within functional groups, such as interacting pairs of proteins or biological processes containing thousands of genes [5, 6]. Overall, factors contributing to the genome organization include at least evolutionary history and current evolutionary forces, mechanisms of rearrangement, mechanisms of regulation of gene expression and control of chromatin composition. The main source of non-random gene order in mammalian genomes has been suggested to be tandem duplications [7].

Although the previous studies have uncovered several factors driving genome organization, distribution of genes has not been explored in the context of individual organ development; the common focus of developmental biology and regeneration studies. In this study, we first review the spatial distribution of protein coding genes in the mammalian genome and quantify genes involved in development of the same organ. Our specific organ system for in-depth analyses of gene neighbourhoods and gene expression is the mammalian tooth. Especially developing mouse molar teeth provide many well characterized genes to examine links between gene neighbourhood and expression [[8] and references therein]. We focus on 1 Mb chromosomal neighbourhoods around each organ specific gene as these encompass the majority of both regulatory

regions and constrained architecture seen in the conservation of large or very orderly gene clusters. These include Hox genes and immunoglobulin genes, whose spatial organization on the chromosome is crucial to their regulation and function. Still, the Hox-gene type strict conserved spatial co-localization of co-regulated genes is not the general pattern in mammalian genomes [7] and the patterns of gene clustering related to gene function are delicate and complex.

For detection of differentially expressed genes in organ development, we developed a new method, called DELocal. This method does not depend solely on the read count from transcriptomes, but it includes in the analysis the expression data of the neighbouring genes. This allows DELocal to identify more subtle gene expression changes during development. Using embryonic mouse dental transcriptomes obtained with both microarray and RNAseq, we show how DELocal compares favourably to other methods to identify differentially expressed (DE) genes [9-14].

MATERIALS AND METHODS

Ethics statement

All mouse studies were approved and carried out in accordance with the guidelines of the Finnish national animal experimentation board under licenses KEK16-021, ESAVI/2984/04.10.07/2014 and ESAV/2363/04.10.07/2017.

Gene sets and gene neighbourhood analyses

The start position, chromosome name and gene biotype (protein coding, lncRNA, ncRNA, pseudogene) of every gene of mouse and human genomes were downloaded from Ensembl database using R package biomaRT [15] in February, 2020. Further analysis was limited to protein coding genes. 302 genes linked to tooth development were marked as tooth developmental genes (Supplementary Table S1 and refs [8, 16]). Throughout the study, the gene start coordinates are used as the position of the gene in the chromosome. The same coordinates are used to measure the distances between genes. A 1 Mb neighbourhood around each gene was defined around the gene start position and the genes within this window were marked as neighbours of the corresponding gene. The GO slim terms were acquired from AmiGO [17] and they are listed in Supplementary Table S2. For each GO term, the corresponding genes were downloaded again from Ensembl using biomaRT. The artificial GO terms of different sizes for simulation were created by randomly sampling protein coding genes from genome using custom R scripts. For every group size, 10,000 simulations were performed and for each simulation density of genes from the same artificial GO term in 1Mb neighbourhood was calculated.

RNAseq library preparation

Developing mouse molar teeth from embryonic days 13.5 (E13) and 14.5 (E14) were dissected from wild type C57BL/Ola embryos. For RNAseq, five biological replicates were used. The samples were stored in RNAlater (Qiagen GmbH, Hilden, Germany) in -75°C. RNA was extracted first twice with guanidinium thiocyanate-phenol-chloroform extraction and then further purified using RNeasy Plus micro kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. RNA quality of representative samples was assessed with 2100 Bioanalyzer (Agilent, Santa Clara, CA) and the RIN values were 9 or higher. The RNA concentrations were determined by Qubit RNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA). The cDNA libraries were prepared with Ovation RNAseq System V2 (Nugene, Irvine, CA), and sequenced with NextSeq500 (Illumina, San Diego, CA).

Microarray library preparation

Mouse E13 and E14 teeth were dissected from wild type NMRI embryos. Five biological replicates were used. The amount of RNA available in each sample was measured with 2100 Bioanalyzer (Agilent, Santa Clara, CA). Only the samples showing a RIN value above 9 were used for the microarray analysis.

Gene Expression analysis

Gene expression was measured both in microarray (platform: GPL6096, Affymetrix Mouse Exon Array 1.0) and RNAseq (platforms GPL19057, Illumina NextSeq 500). The microarray gene signals were normalized with aroma.affymetrix [18] package using Brainarray custom CDF (Version 19, released on Nov 13, 2014) [19]. The RNAseq reads (84 bp) were evaluated and bad reads were filtered out using FastQC [20], AfterQC [21] and Trimmomatic [22]. This resulted in on average 63 million reads per sample. Then good reads were aligned with STAR [23] to Mus_musculus.GRCm38.dna.primary_assembly.fa genome and counts for each gene was performed by HTSeq [24] tool using Mus_musculus.GRCm38.90.gtf annotation. On average 89% reads were uniquely mapped to Mus musculus genome. Additionally, RNAseq count values were normalized using DESeq2 [14]. All the transcriptomic data are available in GEO under the accession number GSE142201.

DELocal

In DELocal, it is hypothesized that differentially expressed genes have different expression dynamics compared to their neighbouring genes. We used a similar logic to ESLiM [25], an algorithm which detects changes in exon usage. In an analogy, a

neighbourhood could be assumed as a single gene and genes in the neighbourhood are equivalent to exons of the gene. Then identifying a DE gene is comparable to detecting alternative splicing. Note that in principle one could choose the neighbouring gene or genes randomly across the genome, but this would result in different result in each randomization.

In this algorithm, gene's expression is modelled as a linear relationship with median expression of neighbourhood genes, such as,

$$\hat{g}_{ij} = s_i \times N\widetilde{g}w_{ij} + b_i \quad \text{……. (i)},$$

where $\hat{g}_{ij}$ is expected expression of *i*-th gene in *j*-th sample, $N\widehat{gw}_{ij}$ is median expression of *N* nearest neighbouring genes within 1 Mb window of the *i*-th gene from *j*-th sample and b_i is base line expression level of gene g_i. The slope s_i of every gene g_i depends on its neighbouring genes. Therefore, the difference between expected and observed values or residual,

$$r_{ij} = g_{ij} - \hat{g}_{ij} \quad \text{…… (ii)}$$

where g_{ij} is observed value. For DE genes, these residual values will be significantly different in different biological conditions.

Furthermore, with the aid of the residuals r_{ij} observed g_{ij} could be formulated as follow,

$$g_{ij} = s_i \times N\widetilde{g}w_{ij} + b_i + r_{ij} \quad \text{…. (iii)}$$

Noticeably, this relationship Eq. (iii) is independent of experimental condition and only dependent on neighbouring gene. Therefore, similar to ESLiM, DE genes are detected through significantly deviated residual values between the desired contrasts using Empirical Bayes statistics, available from Limma package [12]. We tested the performance of DELocal using from 1 to 14 neighbouring genes (*N* in Eq. (iii)). The log-normalized and normalized count values were used in DELocal respectively for microarray and RNAseq data. There are 334 protein coding genes in mouse genome which do not have any other protein coding gene in their 1Mb neighbourhood. Therefore, we also used available non-protein coding genes from the neighbourhood in DELocal analysis. However, after the inclusion of non-protein coding genes there are still 17 protein coding genes without any neighbours within 1 Mb.

Performance measures

DELocal was compared with different publicly available tools applicable both for

microarray or RNAseq : RankProd[9], SAM[10], DEMI[11], Limma[12], edgeR[13] and DESeq2 [14]. All these programs were executed with default parameters. Genes reported with p-value <= 0.05 by these tools were marked as differentially expressed gene and used to evaluate the performance of each tool using the following metrices and receiver operating characteristic (ROC) curves.

- Sensitivity (Recall) - TPR, true positive rate TPR = TP / (TP + FN)
- Specificity - SPC, true negative rate SPC = TN / (TN + FP)
- Precision - PPV, positive predictive value PPV = TP / (TP + FP)
- Accuracy - ACC = (TP + TN) / (TP + FP + FN + TN)
- F1 score F1 = 2TP / (2TP + FP + FN)
- Mathews correlation coefficient (MCC) $= \frac{TP \times TN - FP \times FN}{\sqrt{[(TP+FP)(TP+FN)(TN+FP)(TN+FN)]}}$

where TP, true positive; FP, false positive; TN, true negative; FN, false negative

Tooth developmental genes were used to find the true and false positive rate for the analyses (Supplementary Table S1 and refs [8, 16]). The areas under the ROC curves were calculated with ROCR [26].

RESULTS

Majority of protein coding genes have neighbouring genes within 1 Mb window

As our focus of interest is developmental regulation, we first obtained an overall pattern of distribution of developmental genes by tabulating how closely genes are located in the genome. For example, the human genome (GRCh38.p13) is 4.5*10-9 base pairs long and has 20,449 protein coding genes, which means, on average, one gene for every 220,060 bases (Ensembl release 99). Similarly, for the mouse genome, this number is 154,290 (GRCm38.p6). Consequently, on average, 5 to 6 genes should reside in each 1 Mb window in the mouse genome. To express these statistics as a neighbourhood, we can state that each gene has 4 to 5 neighbouring genes within a 1Mb window. This observation is obviously a broad generalization, but it does indicate that genes tend to have some neighbours within 1 Mb. To examine the neighbourhood patterns in more detail, we examined the 1Mb neighbourhoods of protein coding genes of the mouse genome. For every gene, number of neighbouring genes within 1 Mb window was counted (Figure 1A, Supplementary Figure S1). This simple calculation shows that, at the level of all protein coding genes, majority of genes in mouse genome have more than 4 neighbours, the median number of neighbours being 15 (Supplementary Figure S2). This

tabulation indicates that there is some level of clustering of genes in the eukaryotic genome, a pattern well established in the literature [27].

Majority of protein coding genes lack neighbours regulating the same organ

To contrast the genome-wide pattern of gene neighbourhood with that of a single organ system, we first tabulated gene neighbourhoods associated with the development of the mouse tooth. This specific organ focus basically illustrates whether genes participating in the regulation of the same organ are also located close to each other. Here mouse molar development provides a good example because its gene regulation is relatively well understood, and because tooth development itself is relatively autonomous process [8, 28]. We analysed the 1 Mb neighbourhoods of the tooth developmental genes (TDG, Supplementary Table S1) and the number of TDGs that are sharing the same neighbourhood. The results show that TDGs are mostly located far from each other, suggesting that genes regulating this specific developmental process are not co-localized (Figure 1B). Taken together, although majority of protein coding genes are to some extent clustered in the mouse genome, TDGs tend to be located far from each other (Figure 1).

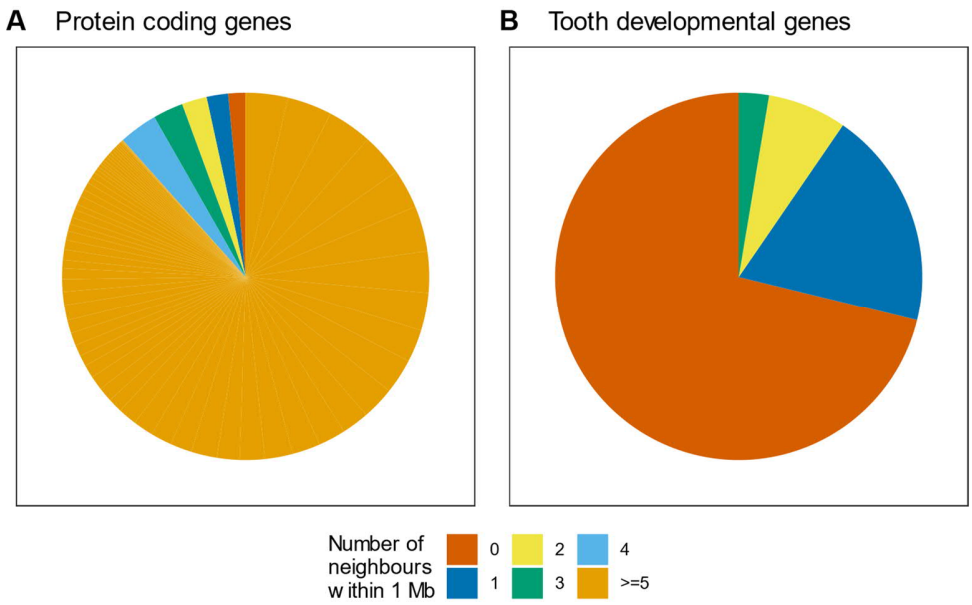

Figure 1. Although protein-coding genes typically have neighbours, tooth genes lack other tooth gene neighbours within 1 Mb windows around each gene. (**A**) The number of neighbouring genes within 1 Mb window around each gene tabulated from the mouse genome for all protein coding genes and (**B**) genes involved in tooth

development. Majority of 21,971 protein coding genes have at least five neighbours whereas most of 302 tooth developmental genes lack tooth genes as neighbours. This pattern suggests that genes with specific functions are sparsely distributed.

Next we examined how representative the developing mouse tooth (Figure 1B) is of other developmental systems. We used the gene ontology (GO) terms to provide an approximation of the adjacency of genes involved in the same developmental process. Here we focused in the genes belonging to mouse GO slims (a concise list of terms) of "biological process" [29, 30]. For every gene belonging to these terms, its 1 Mb neighbourhood was investigated to tabulate genes belonging to the same term. The tabulations show that most genes belonging to a certain GO term are sparsely distributed in the chromosomes (Figure 2) which is in accordance with previous studies [3, 31]. There are only few genes from broader, or high level, GO terms that are densely located (more than 3 genes from the same GO term within 1 Mb neighbourhood). However, these few GO terms represent very broad descriptions of biological functions. With more precise GO terms (Figure 2, bottom row), genes tend to have no neighbours belonging to the same GO term. For generality, we examined these patterns in human genome and they remained largely the same (Supplementary Figure S3).

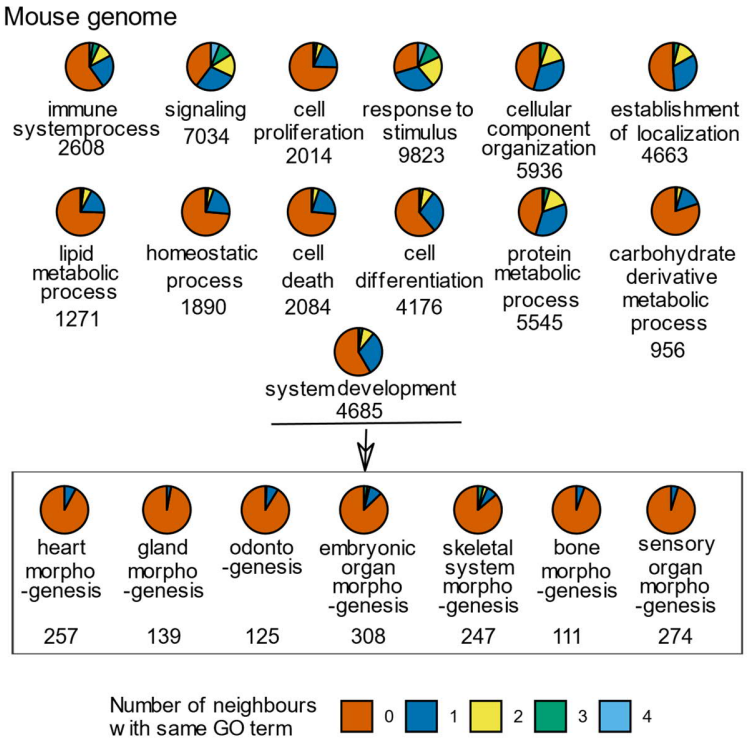

Figure 2. The more specific the GO term is the fewer neighbours its genes have

from the same GO term within 1 Mb. Each pie represents the genes of one GO term under the root GO term 'biological processes'. The top three rows represent GO slim terms. The GO terms in the box are children of the GO term 'system development'. The color-coding indicates the number of neighbours that a gene has from the same GO term. The number of genes is indicated under the GO term. Analysis was done for mouse genome. See Supplementary Table S2 for GO IDs.

The scarcity of neighbouring genes in the precise GO term categories is perhaps not surprising considering the limited number of genes in each category. To test if the patterns are a simple function of group size, we performed simulations where genes were assigned randomly to artificial GO terms containing different numbers of genes. For every group size, 10,000 simulations were performed and for each simulation density of genes from the same artificial GO term in 1Mb neighbourhood was calculated. A plot showing the percentage of genes lacking neighbours shows the expected decrease as the number of genes increases in the artificial GO terms (Figure 3). The empirical patterns largely follow the randomizations, although some real GO terms with small number of genes (300 and less) show slightly lower percentages, indicating that there is a slight tendency for higher spatial clustering of genes belonging to the same GO terms than in the simulations. This may partly be due to GO terms having paralogous genes that are sometimes located near each other in the genome. Nevertheless, up to GO term categories containing 1000 genes, 80 % of genes have no neighbours belonging to the same GO terms. These patterns indicate that the general expectation for the genes sharing the same genomic neighbourhood (Figure 1A) is that they participate in the regulation of different systems and organs. To the extent that this is the general rule of genomic organization, it provides opportunities for new computational approaches. Below we develop a method to detect differential expression using additional information from the neighbouring genes and test it using our data on the developing mouse tooth.

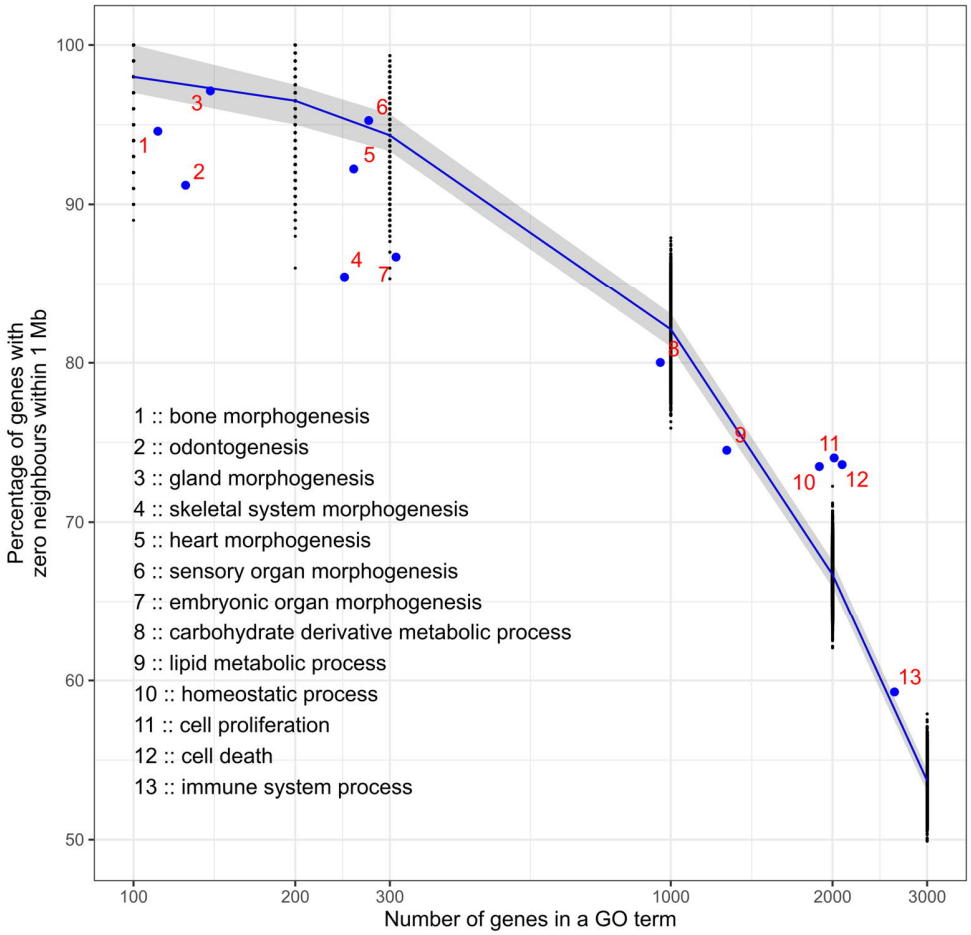

Figure 3. Real GO terms with lower gene numbers are slightly more clustered in the genome than artificial GO terms with the same gene numbers. Artificial GO terms with different numbers of genes were made with randomly selected genes and their distribution across the genome was measured. For each group size, 10,000 simulations were executed and for each simulation the percentage of genes with zero within-1Mb-neighbours with the same artificial GO term were counted (black dots). Real GO terms are marked with blue dots. See Supplementary Table S2 for GO IDs. The blue line is median, and the shadow shows the observations between 1st and 3rd quartile.

Neighbouring genes do not show organ-specific changes in expression

Because genes linked to tooth development do not appear to be co-localized in the genome (Figure 1B), we checked whether, in a developing tooth, these genes differ in their expression dynamics compared to the other genes in their neighbourhood. We examined differential expression of genes at the onset of tooth crown formation, between mouse embryonic day 13.5 (E13) and 14.5 (E14) when many of the TDGs are known to be upregulated [8, 32]. For example, in visual inspection *Ctnna1*, *Shh*, *Foxi3* and

Sostdc1, all genes required for normal tooth developmental [33], show upregulation between E13 and E14 (Figure 4). In a strong contrast, the other genes in their neighbourhoods show no marked changes between E13 and E14, irrespective of their expression levels (Figure 4). Therefore, the distribution of genes involved in the regulation of the tooth (Figure 1 and 4) is manifested also at the level of differential expression. Building from this observation, we developed a new algorithm, DELocal, to identify DE genes based on their neighbours' expression dynamics (Methods). To evaluate its potential performance, we used gene expression data from embryonic mouse dental tissues generated by both microarray and RNAseq.

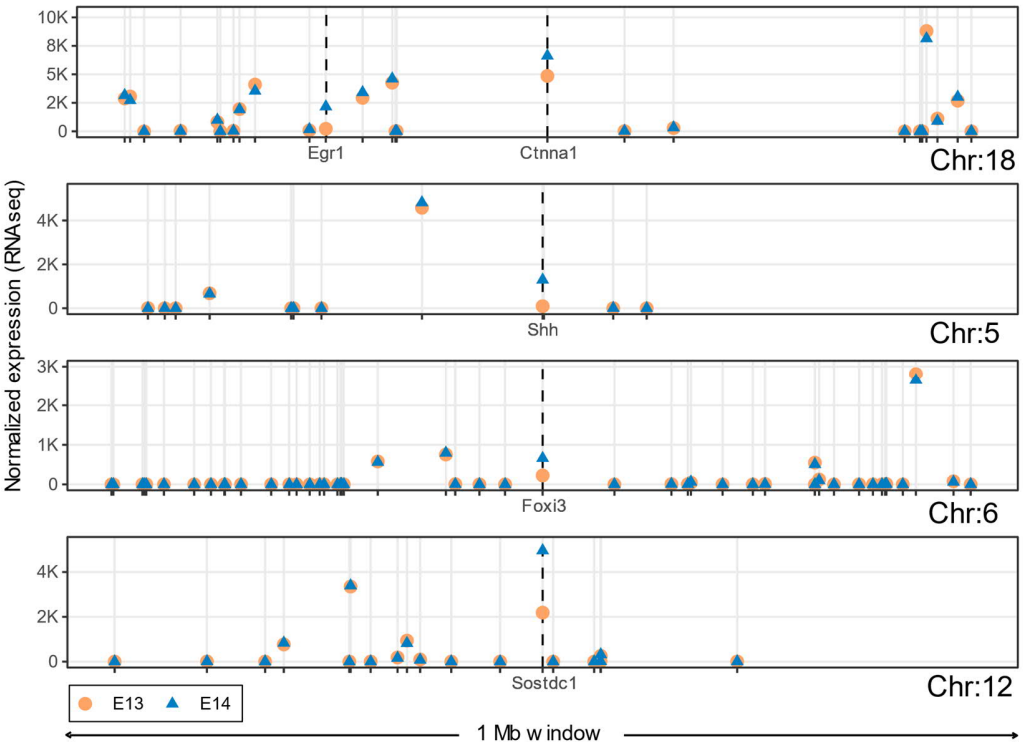

Figure 4. Only tooth developmental genes are differentially expressed within 1 Mb windows in developing mouse molar. Median expression levels of the tooth developmental genes *Ctnna1*, *Shh, Foxi3* and *Sostdc1* and their neighbouring genes at developmental stages E13 and E14. Regardless of the expression level, the surrounding genes show little change between the two stages, compared to the tooth developmental genes. *Egr1* in the 1 Mb window of *Ctnna1* is also a tooth developmental gene.

The number of neighbours is not critical for detecting differential expression

Our hypothesis of neighbouring genes being informative in the detection of differential expression is dependent on the definition of 'neighbourhood'. Therefore, it is important to

determine the right number of neighbours to include in the analysis by the DELocal algorithm. To define the optimal number of neighbours we tested different numbers (1-14) of closest genes within a fixed window (1 Mb) surrounding the gene of interest. We evaluated the performance of DELocal with different numbers of closest neighbours in identifying the genes involved in tooth development (TDGs). Again we contrasted the expression levels between E13 to E14 molar teeth (for 302 tooth genes), or so-called bud stage to cap stage transition, when many TDGs are known to be active [8]. The Matthews correlation coefficient (MCC) scores were measured for different numbers of neighbours to examine the strength of DELocal to identify TDGs (true positive; TP) as well as non-TDGs (true negatives; TN). The MCC score was chosen to optimize the model due to very few TPs, or imbalanced dataset. The results show that DELocal produces similar and stable MCC scores on both microarray and RNAseq datasets, even though RNAseq data produces slightly higher MCC scores than microarray (Figure 5). We note that only one nearest gene is enough to obtain close to the highest MCC score. However, for RNAseq the best MCC score corresponds to 5 nearest neighbours. Because there are fewer genes available in microarray analyses compared to RNAseq, in the rest of the analyses we used DELocal with 5 neighbours both for the RNAseq and microarray data.

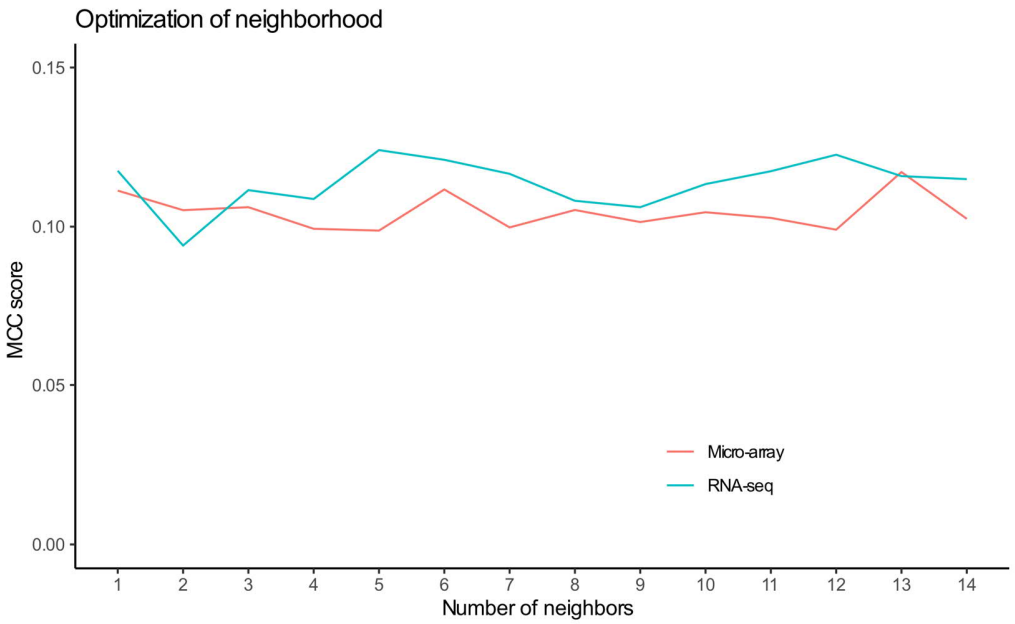

Figure 5. DELocal performance is not strongly dependent on the number of gene neighbours used in the analysis. Every gene is evaluated in relation to its neighbouring genes. In the absence of any “gold standard” for the number of neighbours, different numbers of nearest genes (within 1 Mb window) were used to identify the DE genes. The overall performances were measured using MCC. The performance of DELocal using RNAseq data was slightly better than with microarray data.

DELocal shows high precision compared to other methods

Microarray is one of the earliest successful high throughput technologies to measure a large number of gene expressions, and consequently there are a good number of statistical methods to identify DE genes from datasets generated by this platform. Hence, the performance of DELocal can be evaluated by comparisons to these methods using a microarray dataset. However, microarray is limited to only the genes which have been targeted by microarray probes. Therefore, the expression of all the genes cannot be accessed, resulting in fewer neighbouring genes being sampled. To obtain a more comprehensive readout of DE genes, RNAseq was also used to evaluate DELocal performance. The performance of all these methods was measured by their ability to identify differentially expressed TDGs.

For analysis of performance, we used the receiver operating curve (ROC) [26] depicting the true positive rate against the false positive rate of DE genes. The analyses show that DELocal outperforms other methods in identifying TDGs using both microarray and RNAseq (Figure 6). The performance is most similar to Limma/DEMI (microarray) and Limma/DESeq (RNAseq). We used also other metrices like specificity, recall (sensitivity), precision and MCC to evaluate and compare the different methods. DELocal shows high specificity and accuracy compared to other methods for microarray data (Supplementary Figure S4).

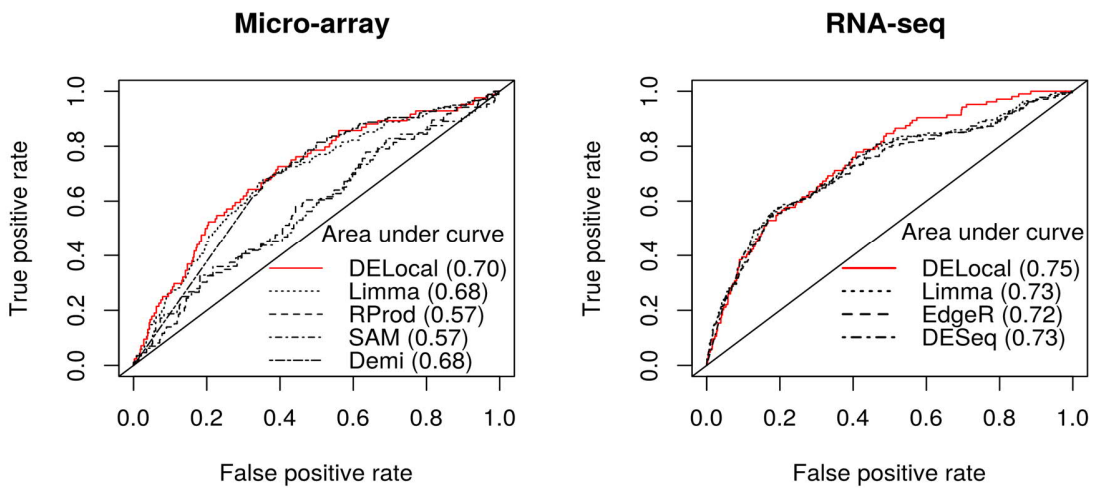

Figure 6. Compared to other methods, DELocal is powerful in detecting differentially expressed genes. Receiver operating characteristic (ROC) curves and areas under the curves (within the parenthesis) show that DELocal outperforms other methods on both microarray and RNAseq data.

In RNAseq data, DELocal outperforms other methods except in recall (sensitivity) (Figure 7). The MCC scores remain equivalent to each other. The TDG dataset is imbalanced due to the large number of non-TDGs (true negatives), which hinders the evaluation of accuracy, but does not affect F1 or MCC. Considering that the objective of many experiments is to find true positives, the F1 score, which is a compound-term of precision and recall, is an important metrics. The F1 score ranges from zero (bad) to one (good), and the F1 scores of all of the methods remain suboptimal. Nevertheless, DELocal using RNAseq clearly outperforms other methods also in F1 score. Below we discuss the results from RNAseq only.

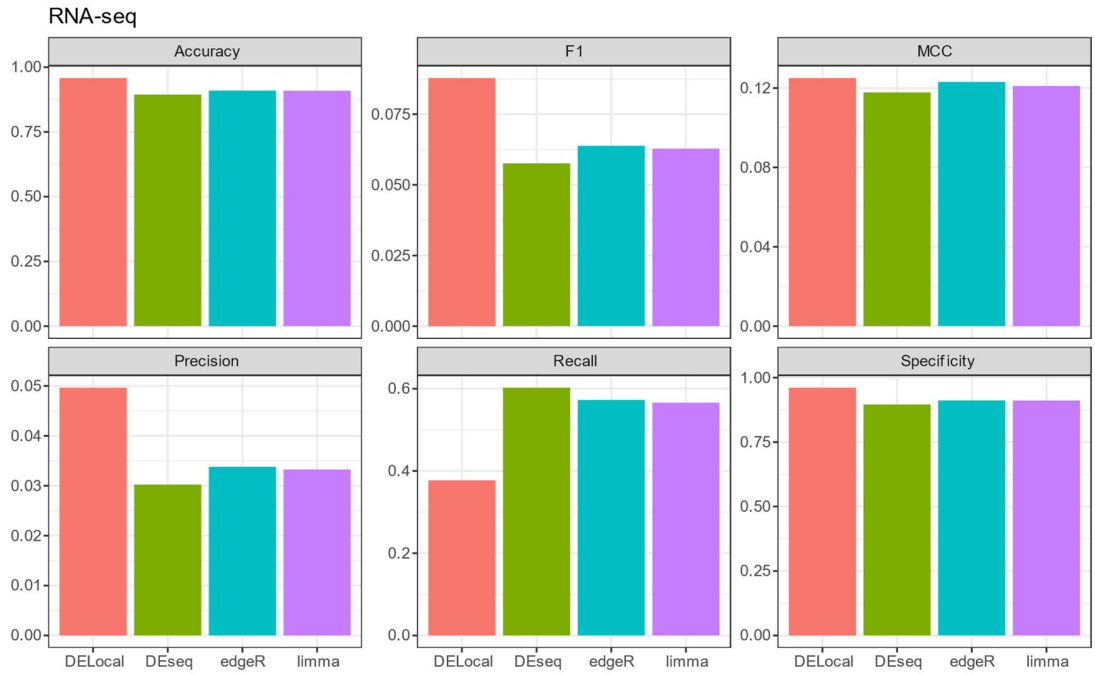

Figure 7. Comparison of DELocal with earlier methods of identifying differential expression. Evaluation matrices show that, except for recall (sensitivity), DELocal outperforms earlier methods in every matrix. However due to large number of true negatives, the significance scores of precision, F1 and MCC remained negligible. The evaluation matrices are explained in materials and method section. The analysis was done using RNAseq data. For microarray data see supplementary figure S4.

Differentially expressed genes missed by DELocal tend to have local paralogues

The DELocal algorithm appears to efficiently identify the DE genes (having high precision) as well as to filter out non TDGs (having high specificity). Still, DELocal missed 60 differentially expressed TDGs which are identified by all the other methods in RNAseq

dataset. DELocal is built on the hypothesis that every true DE gene should have neighbours and none of them should be differentially expressed. Consequently, DELocal may fail to identify those DE genes whose neighbours are also differentially expressed. For instance, 2 out of 5 neighbours of *Bmp7* were DE genes which could be the reason of failure of DELocal to detect *Bmp7* (Figure 8). Additionally, presence of paralogous genes in the neighbourhood may contradict with our hypothesis, like *Dlx1* and *Dlx2* in chromosome 11 (Figure 8), *Dlx5* and *Dlx6* in chromosomes 6, and *Dlx1* and *Dlx2* in chromosome 2, for which it is known that the pairs of these genes are co-regulated and can compensate for the deletion of one another [34-37]. Additionally, the other neighbouring paralogs in our set of TDGs are *Cyp26c1* with *Cyp26a1*, and *Cdh1* with *Cdh3* [38]. Therefore, at least some of the genes missed by DELocal should be possible to detect by incorporating information about gene paralogues into the algorithm.

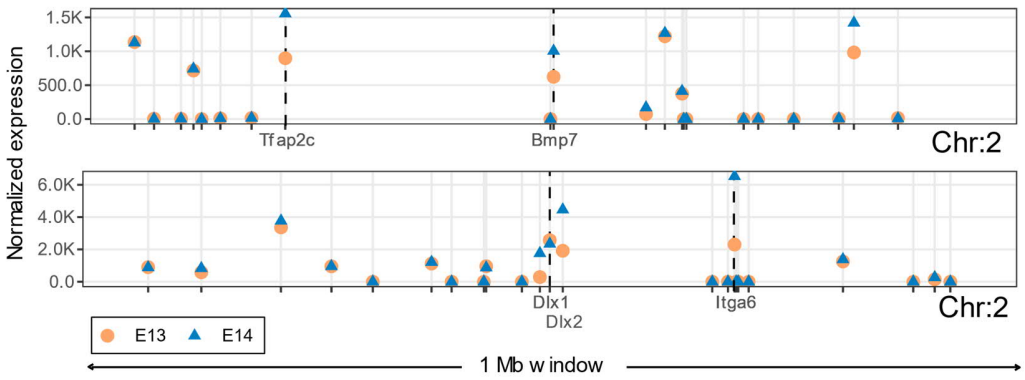

Figure 8. Differentially expressed genes in the same neighbourhood interfere with the detection of differentially expressed genes by DELocal. DELocal failed to identify *Bmp7* due to differential expression patterns of its neighbouring genes (top)**.** *Dlx1* and *Dlx2 are p*aralogous TDGs which are in the same neighbourhood (bottom). Only tooth developmental genes are labelled here.

DISCUSSION

Only seven protein-coding genes in Ensembl mouse annotation lack neighbours altogether within their 1 Mb windows (Figure 1A). Hence, the overwhelming majority of mouse genes have neighbours, and this pattern of organization is likely to apply to all mammals, if not beyond. In the genomic scale of thousands of genes, neighbouring genes can be co-expressed in eukaryotes [39]. Our analyses, focused on individual organ development, suggest that the neighbouring genes are very rarely involved in the regulation of the same organ or tissue (Figure 2), and that this is also reflected in the lack of co-expression of neighbours (Figure 4). The lack of co-expressed neighbours is partly explained by the limited number of genes that are associated with individual organ

regulation (Figures 2 and 3). In contrast, more general processes, such as cell proliferation or cell death, are linked to many thousands of genes (Figure 2), increasing the likelihood of neighbourhood co-expression. Indeed, an analysis using high-level GO terms containing thousands of genes found that functionally related genes are often located close to each other [6]. It is plausible that evolution of organs such as teeth may have co-opted genes from multiple high-level clusters, perhaps further explaining the rarity of co-expressed neighbours in organ regulation.

A distinct advantage of the rarity of co-expressed neighbours in organ development is that this information can be used to detect even subtle changes in gene expression. To this end, here we developed the DELocal algorithm to identify differentially expressed genes by incorporating expression information from chromosomal neighbourhood. DELocal provided highly precise detection of differentially expressed genes in mouse tooth development (Figures 6 and 7), suggesting that this method can provide additional benefits in the analysis of developmental systems.

More technically, the developed DELocal algorithm is based on linear models that have been successfully implemented and used in Limma, DeSeq2 and many other methods to identify DE genes [12, 14]. With linear models, gene expression can be modelled in two or more biological conditions and thereafter DE genes of different contrasts of interest can be found. Linear models are advantageous compared to other methods in that they can model complex experimental conditions with multiple factors. Here we used DELocal to determine the genes that are differentially expressed between bud and cap stage in the developing mouse tooth. The extensive list of genes active in tooth development (7, 27) allowed us to optimize and evaluate the performance of DELocal. Considering that the in vivo bud and cap stage differences in gene expression are relatively subtle, the high specificity and accuracy of DELocal is promising. Obviously, the optimization requires a list of genes of interest. However, DELocal can also be run without any prior knowledge of genes that are active in a particular developmental process. Figure 3 shows that in relation to the number of neighbours, the performance of DELocal is very stable and even only one nearest neighbour could be sufficient to build the models. The implication of this is that DELocal could be used with only a single neighbour contrast in the absence of any reference/training gene set.

It remains to be explored whether the rarity of neighbouring genes regulating the same process is related, for example, to the requirements of spacing required for folding of the DNA, or whether it is the result of historical processes of genome rearrangements or co-option of genes in the evolution of organs. Regardless, our results on the developing mouse tooth suggest that the lack of organ specific neighbours is also manifested at the level of differential expression. We further show that it is possible to develop computational approaches to detect even subtle changes in gene expression

using the additional information from the neighbouring genes.

FUNDING

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Conflict of interest statement. None declared.

DATA AVAILABILITY

The DELocal algorithm is freely available here https://github.com/dasroy/DELocal as an R package. The microarray and RNAseq data are deposited in the NCBI Gene Expression Omnibus with accession codes GSE141907 and GSE142199.

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