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Digital gene expression profiling analysis of duodenum transcriptomes in SD rats administered ferrous sulfate or ferrous glycine chelate by gavage

Zhao Zhuo, Shenglin Fang, Qiaoling Hu, Danping Huang & Jie Feng

The absorption of different iron sources is a trending research topic. Many studies have revealed that organic iron exhibits better bioavailability than inorganic iron, but the concrete underlying mechanism is still unclear. In the present study, we examined the differences in bioavailability of ferrous sulfate and ferrous glycinate in the intestines of SD rats using Illumina sequencing technology. Digital gene expression analysis resulted in the generation of almost 128 million clean reads, with expression data for 17,089 unigenes. A total of 123 differentially expressed genes with a |log2(fold change)| >1 and q-value < 0.05 were identified between the $FeSO_4$ and Fe-Gly groups. Gene Ontology functional analysis revealed that these genes were involved in oxidoreductase activity, iron ion binding, and heme binding. Kyoto Encyclopedia of Genes and Genomes pathway analysis also showed relevant important pathways. In addition, the expression patterns of 9 randomly selected genes were further validated by qRT-PCR, which confirmed the digital gene expression results. Our study showed that the two iron sources might share the same absorption mechanism, and that differences in bioavailability between $FeSO_4$ and Fe-Gly were not only in the absorption process but also during the transport and utilization process.

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Age in Weeks	Body Weight	
	$FeSO_4$	Fe-Gly
4	81.5 ± 5.82	79.7 ± 7.76
6	191.7 ± 4.04	193.2 ± 10.34

Table 1. The body weights of the SD rats at 4 and 6 weeks of age. ± (n = 12).

Parameter	Unit	$FeSO_4$	Fe-Gly
BC	10 9/L	4.5 ± 0.95	4.7 ± 1.34
BC	10 12/L	5.8 ± 0.72	5.9 ± 0.39
H	/L	120.0 ± 15.72	124.7 ± 7.12
HC	%	35.9 ± 4.93	37.1 ± 2.05
C	1	61.7 ± 1.16	63.3 ± 1.75
CH		20.7 ± 0.58	21.3 ± 0.82
CHC	/L	335.0 ± 7.00	336.0 ± 11.78

Table 2. Hematological parameters in the $FeSO_4$ and Fe-Gly group rats. B D . B C C (BC), B C C (BC), H C (H), H (HC), C (C), C H (CH), C H C (CHC). ± (n = 12).

Parameter	Unit	$FeSO_4$	Fe-Gly
IBC	μ /L	103.6 ± 14.23	99.1 ± 14.66
I	μ /L	45.7 ± 5.12	66.7 ± 12.72*
A	%	45 ± 11.3	67 ± 9.0*

Table 3. Serum iron-related parameters in the SD rats. I B C (IBC), I (I), (A). ± (n = 12). *I < 0.05.

DGE . B , , . DGE F 4 F -G D .

Results

Iron status of SD rats. A D F 4 F -G (1). I , (2). (IBC) , (I) (< 0.05, 3). F -G F 4 ; , (A) (< 0.05). - (F . 1). F -G (50μ 25μ) , - . (< 0.05).

Analysis of DGE libraries. F 4 F -G A- I . , C1, C2, C3 1, 2, 3, 4. C1, C2, C3, 1, 2, 3 20,446,968, 20,983,958, 23,325,694, 21,951,812, 21,586,433, 22,458,258 , A - , - , 19,720,103, 20,414,770, 22,897,985, 21,550,142, 21,172,114, 22,056,148 . 96%.

Mapping reads to the transcriptome. F , *Rattus norvegicus* , H 2.0.9. 95% (5). I , 17,935,148 (90.95%), 18,282,777 (89.56%), 20,841,830 (91.02%), 19,627,268 (91.08%), 19,087,487 (90.15%), 19,997,125 (90.66%) C1, C2, C3, 1, 2, 3 , .

Analysis of differential gene expression. F [illegible] K . [illegible] , K [illegible] (. 1). [illegible] , 2( ) >1 - < 0.05 . A [illegible] (F . 2), [illegible] F . 3. 123 [illegible] , 83 - 40 - (. 2).

Functional analysis of differentially expressed genes. [illegible] A G (G) , 123 [illegible] : , (. 3). G [illegible] I [illegible] 6.1(69 .5( )0.5( ).4( )-29(.5( )0.5( )8.5(4)0.5( )13.5( )00.6( ))23(( )8.5( )117.)7.5(4)8.5( )0..5(

Item	C1	C2	C3	T1	T2	T3
	20,446,968 (100%)	20,983,958 (100%)	23,325,694 (100%)	21,951,812 (100%)	21,586,433 (100%)	22,458,258 (100%)
	385,382 (1.88%)	167,806 (0.80%)	22,261 (0.10%)	16,393 (0.07%)	19,553 (0.09%)	6,456 (0.03%)
	797 (<0.01%)	873 (<0.01%)	1,000 (<0.01%)	939 (<0.01%)	880 (<0.01%)	997 (<0.01%)
-	340,686 (1.67%)	400,509 (1.91%)	404,448 (1.73%)	384,338 (1.75%)	393,886 (1.82%)	394,657 (1.76%)
C	19,720,103 (96.45%)	20,414,770 (97.29%)	22,897,985 (98.17%)	21,550,142 (98.17%)	21,172,114 (98.08%)	22,056,148 (98.21%)
20	98.95%	98.93%	98.96%	98.95%	98.92%	98.95%
30	95.53%	95.42%	95.55%	95.53%	95.38%	95.52%

Table 4. Summary of sequencing analysis. C1, C2, C3: , ; 1, 2, 3: , . 20: >20; 30: >30.

Mapping Statistics	C1	C2	C3	T1	T2	T3
E	19,720,103 (100%)	20,414,770 (100%)	22,897,985 (100%)	21,550,142 (100%)	21,172,114 (100%)	22,056,148 (100%)
	18,873,056 (95.70%)	19,518,820 (95.61%)	21,935,403 (95.80%)	20,668,869 (95.91%)	20,209,758 (95.45%)	21,191,619 (96.08%)
	937,908 (4.76%)	1,236,043 (6.05%)	1,093,573 (4.78%)	1,041,601 (4.83%)	1,122,271 (5.30%)	1,194,494 (5.42%)
	17,935,148 (90.95%)	18,282,777 (89.56%)	20,841,830 (91.02%)	19,627,268 (91.08%)	19,087,487 (90.15%)	19,997,125 (90.66%)
+	9,326,071 (47.29%)	9,611,103 (47.08%)	10,844,189 (47.36%)	10,227,148 (47.46%)	9,958,528 (47.04%)	10,452,084 (47.39%)
–	9,546,985 (48.41%)	9,907,717 (48.53%)	11,091,214 (48.44%)	10,441,721 (48.45%)	10,251,230 (48.42%)	10,739,535 (48.69%)

Table 5. The data for the sequencing reads that mapped to the reference genome. C1, C2, C3: , ; 1, 2, 3: , . + , – .

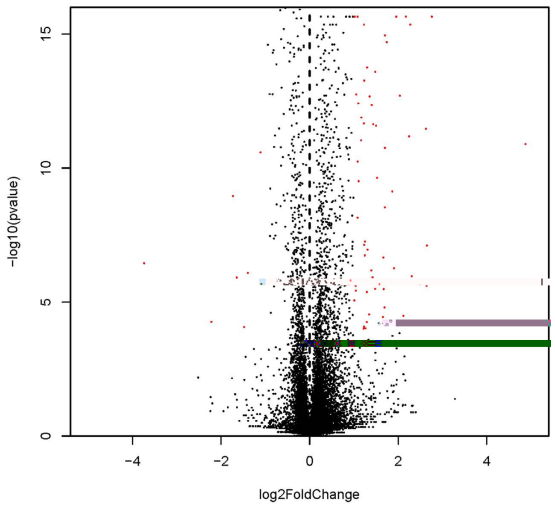

Figure 2. Volcano plot of differentially expressed genes. , ( )/ ( );
.

, A . H , H . [26]. [27], *in vivo*[28]. , , . D . I - . A- In , 128 . A 17,089

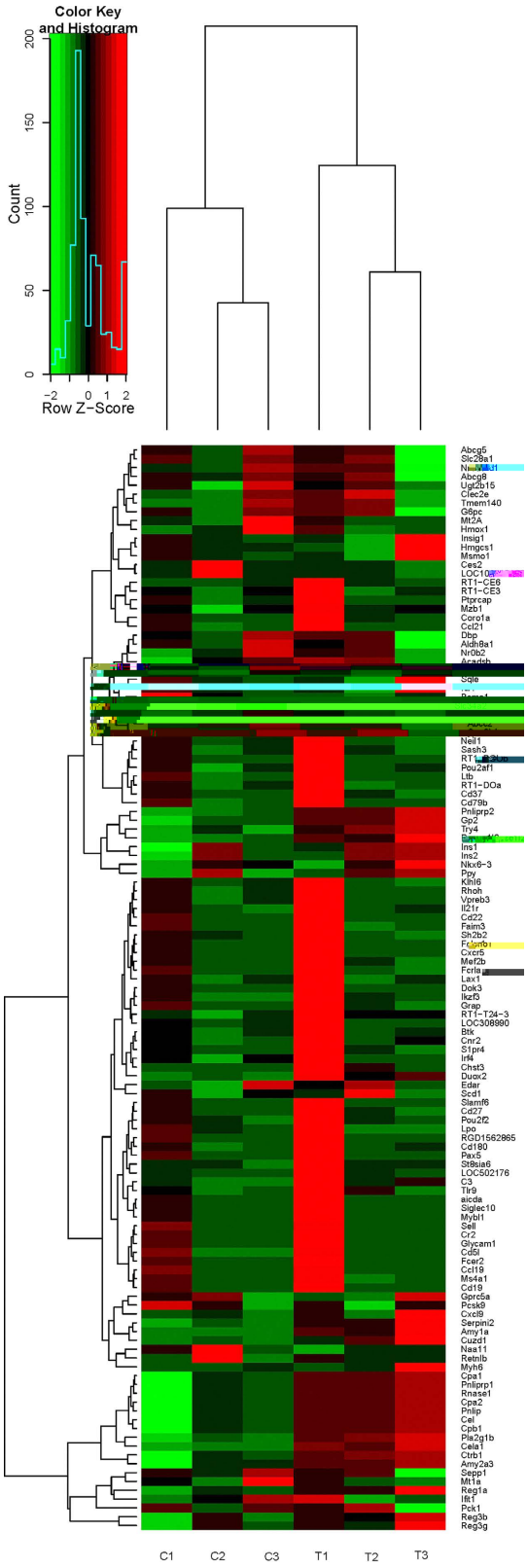

Figure 3. Heatmap of differentially expressed genes. C1, C2, C3: , ; F4 ; 1, 2, 3: , F-G .

, 100% (1). A 123 2( ) >1 <0.05 F4 F-G .

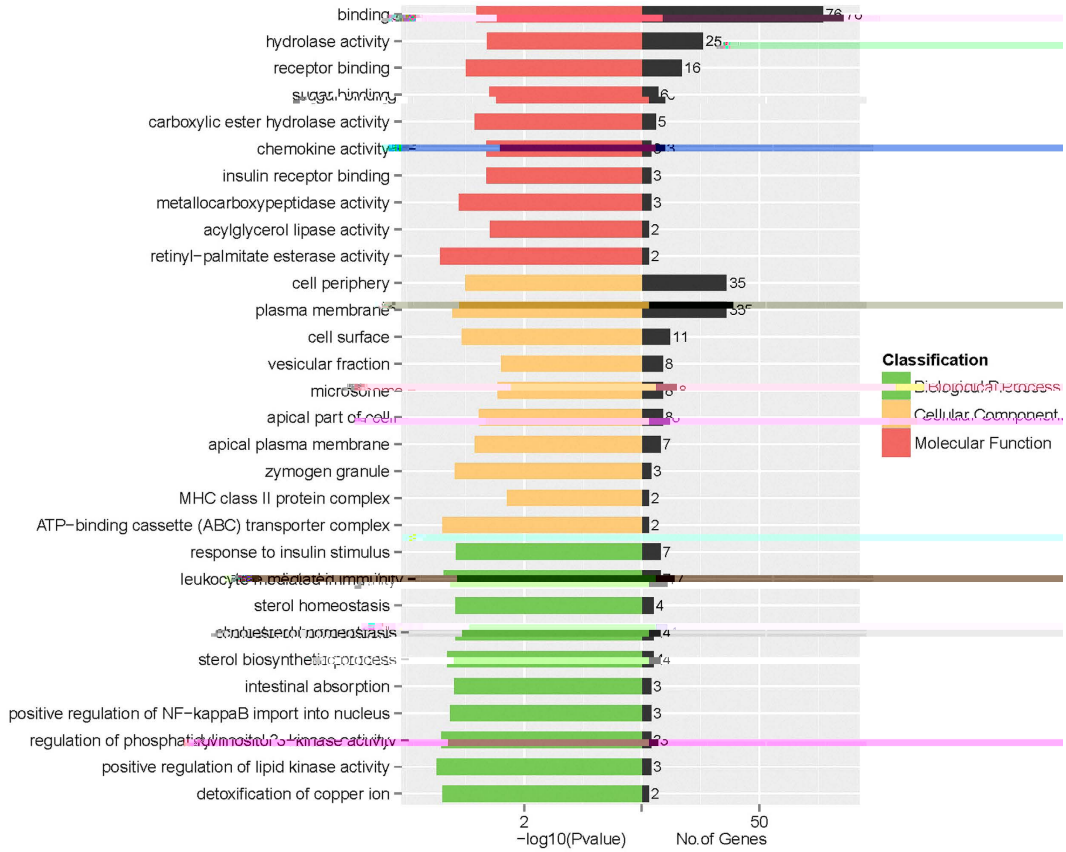

Figure 4. GO functional enrichment analysis. F 4 F - G G ; , . 3.

A- , 9 - C . C - . A G , , C 2 1, H 1, D 2, 1 G . I , KEGG 123 , , - . HIF-1 ABC . (. ., C , , K, , F , C , ,). , . . I . I - F -G , H 1, 1 2A. H 1 (H 1) [29]. , ' H 1 [30,31]. B [32], H 1 F -G F -G F [4]. (6 10 D), - (33%) . A [2+], - [33]. C 1 2A F -G , . D *Npt 2b* (34 2) [34], I , - 34 2 F -G . A - HIF-1 *Caenorhabditis elegans* *Homo sapiens*, ( ) , ,

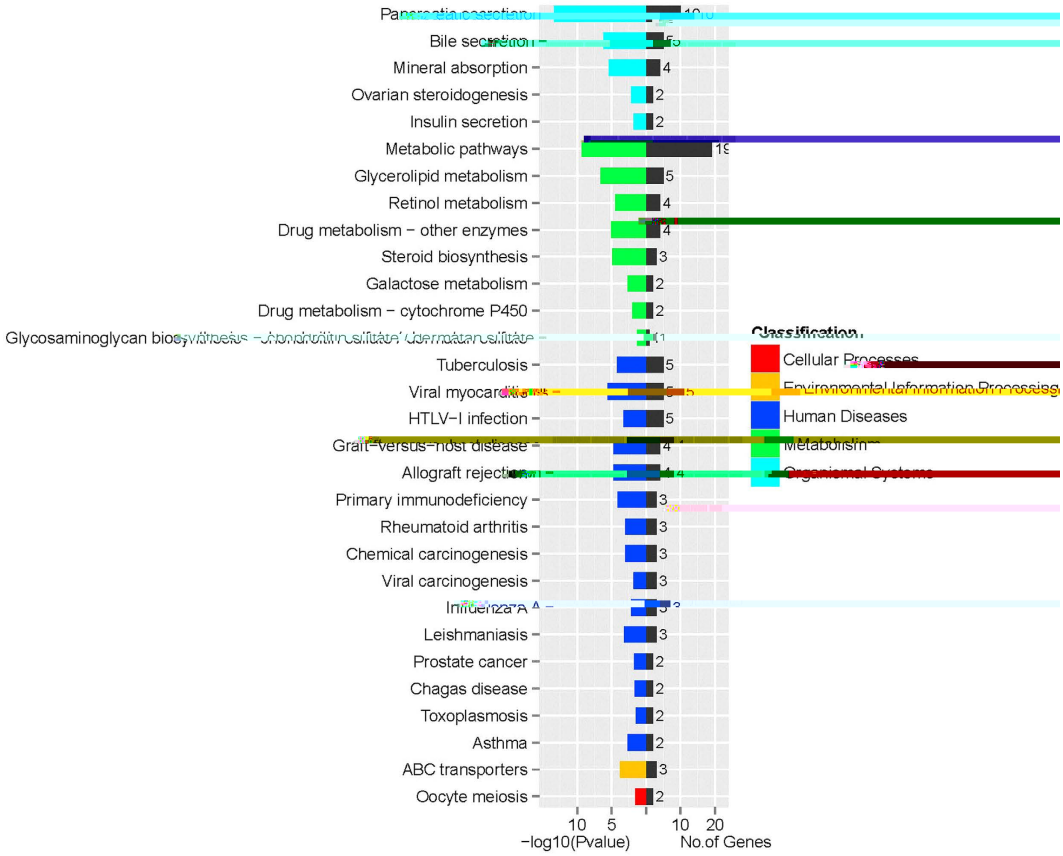

Figure 5. KEGG pathway analysis. KEGG ; , . 4.

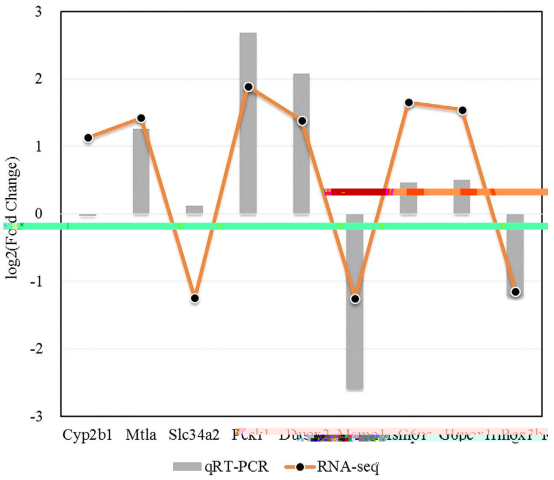

Figure 6. Gene expression determined by RNA-seq and qRT-PCR. - C F 4 F -G . GA DH, F -G F 4.

, , , , [35]. I 1 (I 1) 2 (I 2), HIF-1 - F -G . B [36], D F -G F 4 . A - (ABC) , , [37]. A

Conclusion

Methods

Animals and experimental design.

Sample collection and analysis.

Immunohistochemical staining.

Metabolic process	Gene	Function	Illumina mRNA-seq (log2 fold change)
		, , C F	0.09
		F -	−0.79
	2	; -	0.21
	3	E	−0.08
	11 2	E ;	0.15
/	C 1	I	0.21
	39 4	-	0.38
	2	H-	0.28
	5	L-	0.18
	FL C 2	H	0.70
	46 1	I	0.37
	H 1	H	1.55*
	H 2	H	0.10
	L 2	F -	0.21
	F 1	I ;	0.11
	F	I	0.05
	F	I	−0.12
	A 2	H	1.22
	A 10	I F -1;	−0.12
	A 7	I F ; I C	−0.01
	A 6	I	0.24
	25 37		−0.08
	B 2		0.62
/	A 2	; I	0.36
	40 1		0.01
	A 1	A I E;	0.09
	I 2	A I E	−0.12

Table 6. The main genes related to iron metabolism and their expression differences between the two groups. *I < 0.05. F = F -G ( )/F 4 ( );

L 1% . F , 3 ×200 . I - 6.0 (I D). , (I D/A EA).

RNA extraction and qualification. A , . A - (I), 1% . A D-1000 (, A). A260:A280 A260:A230 A 1.8 2.0, A A A 2200 (A , A) I 7.0.

Library preparation for DGE sequencing. 50. A 3μ A . B A A 200 . A - D A , A L /H K (I , A). A 2200 2.0 (L , A). 10 *in situ* H 2500 - , (2 × 100) H 2500 . A CBI 075016.

Quality control and mapping analyses. ( ) FA - . I ,

- , 20, 30 GC
. A - .
B 2.0.6, -
H 2.0.9[51]. H
-
H 0.5.4 3 [52]. , K
. K (
), ,
[53].
( )
DE [54]. DE
[55]. B
H ' [56]. C . G
2( ) >1 - <0.05 DE .

Functional analysis of differentially expressed genes. G G [57]. G
- <0.05 .
KEGG -
(://. . / /). K BA
KEGG [58].

Quantitative real-time PCR validation. A
DGE . - C (- C) -
. CBI (://. .
. / /- /) In (F I ., , C).
D A - L (H-) (K) .
- C B G C (A B)
- C (B - , A). E -
(GA DH).
95 C 1 , 95 C 15 , 63 C 25 .
$(2^{-\Delta\Delta C})$ [59].

Statistical analysis. A ± (D).)3-0.03 4 -0.0151 -0.

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J F . , F , H , D H x . F H . A .

Additional Information

Supplementary information

Competing financial interests:

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