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1 Transcriptomics of enterotoxigenic *E. coli* infection. Individual variation in intestinal
2 gene expression correlates with intestinal function.

3

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15

16 Keywords: enterotoxigenic *E. coli*; host-pathogen interaction; gene expression; micro
17 array; PAP.

18

19 Running title: Transcriptomics of ETEC infection.

20

21 Abbreviations: CYP3A29: cytochrome P450 3A29, ETEC: enterotoxigenic *E. coli*,
22 MMP1: matrix metalloproteinase-1, PAP: pancreatitis associated protein, STAT3: signal
23 transducer and activator of transcription 3, THOC4: THO complex 4.

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32 Abstract

33

34 Acute secretory diarrhea is a major cause of morbidity and mortality in young animals
35 and humans. Deaths result from excessive fluid and electrolyte losses. The disease is
36 caused by non-invasive bacteria such as *Vibrio cholerae* and *E. coli* which produce
37 enterotoxins, however, much less is known about the role of individual host responses.
38 Here we report the response of intact porcine small intestinal mucosa to infection with
39 enterotoxigenic *E. coli* (ETEC). Jejunal segments in four piglets were infused with or
40 without ETEC, and perfused for eight hours, and net absorption measured. Micro array
41 analysis at 8h post-infection showed significant differential regulation of on average
42 fifteen transcripts in mucosa, with considerable individual variation. Differential net
43 absorption varied between animals, and correlated negatively with the number of up
44 regulated genes, and with one individual gene (THO complex 4). This shows that
45 quantitative differences in gene regulation can be functionally linked to the physiological
46 response in these four animals.

47

48 Introduction

49

50 Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of secretory diarrhoea in
51 man and animals. Upon colonization ETEC can produce several toxins, the most
52 important of which is heat labile toxin (LT). LT is very similar to cholera toxin (CT).
53 ETEC strains are responsible for morbidity and mortality in neonates and infants in man.
54 Although oral rehydration therapy has reduced mortality significantly during the past
55 decades, agents which could directly inhibit the intestinal secretory machinery would be a
56 welcome addition (Farthing, 2006). However, our understanding of the exact mechanisms
57 involved is still relatively poor. A variety of mechanisms have been implied in the
58 pathogenesis of secretory diarrhea. Enterotoxins play an important role, but also the
59 enteric nervous system, and inflammatory cells such as polymorphonucleocytes are
60 involved. Furthermore, intestinal cells respond to the infection by switching on innate
61 defense mechanisms (Flach et al., 2007), which will eventually determine the outcome of
62 the disease.

63 Individual variation exists in susceptibility to the disease. This is, among others
64 associated with the presence of adhesion factors for the bacteria and receptors for
65 bacterial toxins (Harris et al., 2005). Furthermore, individual variation can be caused by
66 environmental, genetic and epigenetic factors determining the immunological response
67 (Radich et al., 2004; Shai, 2006).
68 ETEC strains cause a disease in piglets very similar to that in man. In a previous study,
69 we have described the preliminary results of a micro array analysis of an ETEC infection
70 in a pig model (Niewold et al., 2005). Here we give a more detailed analysis of the gene
71 expression, with particular attention to individual variation. Furthermore, we relate ETEC
72 associated individual gene expression differences with intestinal function.

73

74 Material and Methods

75

76 The material used derived from a previous study in which we have described some
77 preliminary results of an comparative micro array analysis of an ETEC infection in a pig
78 model (Niewold et al., 2005). We compared mucosal cDNA from normal uninfected with
79 ETEC-infected perfused small intestinal segments of four F4 receptor positive pigs (6–7-
80 week-old) under anesthesia (Niewold et al., 2005). Briefly, of a pair of segments located
81 around 25% of the length of the small intestine (anterior jejunum), before perfusion, one
82 was mock-infected with PBS only, the other was infected with 5 ml of 10^9 CFU/ml PBS
83 of ETEC (CVI-1000; typed as *E. coli* O149:K91, F4 (K88ac), LT, STb). Segments were
84 perfused for 8 h with in total 64 ml of perfusion fluid. After the experiment, the surface
85 area of each segment was measured. Net absorption was calculated as the difference
86 between inflow and outflow in $\mu\text{l}/\text{cm}^2$ intestinal wall, and the difference with the
87 absorption of the control segment given as differential absorption. Mucosal scrapings
88 were taken from the same segments, and frozen in liquid nitrogen, and stored at -70°C
89 for later transcript profiling. The animal study was approved by the local Animal Ethics
90 Commission in accordance with the Dutch Law on Animal Experimentation.

91

92 Microarray analysis

93

94 Microarray analysis was performed as described before (Niewold et al., 2005), using
95 slides spotted with the same collection of cDNA fragments (3486 clones in quadruplicate
96 from a home made porcine jejunal EST library. For a complete description of the ESTs
97 spotted on the Porcine intestinal cDNA array, see supplementary material to Hulst et al.,
98 2008). Hybridising probes were selected for further characterization when their mean
99 value of M ($n \geq 5$ or ≤ 8) was > 1.58 or < -1.58 (corresponding with a ratio greater than
100 three-fold), and with a q-value of $< 2\%$. Unknown probes were sequenced and compared
101 with the NCBI mRNA reference sequence database (refseq_rna) and/or the non redundant
102 (nr) database using the blastn option. Validation of differential expression of selected
103 genes was performed using Northern blot analysis (NB).

104

105 Northern blot analysis.

106

107 In addition to PAP and IFABP already analyzed in our earlier publication (Niewold et al.,
108 2005), the expression of a further 3 genes was analyzed using the same technique.

109 Plasmid DNA was isolated from the EST library clones homologue to human mRNA
110 coding for Fyn-related kinase (FRK, gi:31657133), poly (ADP-ribose) polymerase
111 family, member 15 (PARP 15, gi:22749258), and signal transducer and activator of
112 transcription 3 (STAT3, gi:47080105). Their inserts were sequenced to verify their
113 authenticity. Blots were hybridized and scanned as described before (Niewold et al.,
114 2005). The significance of the relationship between the values of the microarray and NB
115 was calculated by linear regression using GraphPad Prism Version 5.00 software.

116

117 Gene expression vs differential absorption.

118

119 Differential net (negative) absorption in $\mu\text{l}/\text{cm}^2$ was plotted against the differential
120 expression of separate genes, the number of genes regulated, and the number of genes up
121 and down regulated. The significance of the relationship was calculated by linear
122 regression using GraphPad Prism Version 5.00 software.

123

124 Results

125

126 Microarray analysis

127

128 The results of microarray comparisons of gene expression in segments perfused with
129 ETEC for 8h vs. control are shown in Table 1. Animals showed variation in the number
130 of genes significantly regulated ranging from 4 in animal 5 to 24 in animal 8. Also, the
131 magnitude of gene expression differences varied between animals, being smallest in
132 animal 5, and largest in animal 8. Only MMP1 and STAT3 were found to be similarly
133 (up) regulated in all four animals. Animal 5 appears to be different from the other 3
134 animals. In the latter, common regulation was seen for PAP, MMP1, STAT3, GCNT3,
135 GCLM, RIF1, THOC4, and PARP13. Other genes were found to be expressed in one or
136 two animals only.

137

138 Northern Blot analysis

139

140 Linear regression analysis of the values of the microarray and NB for PAP, IFABP
141 (Niewold et al., 2005), PARP, FRK and STAT3 showed a significant relationship ($p <$
142 0.0031 , $r^2 0.39$). Only animal 8 showed striking differences between the values obtained
143 in both techniques. Without animal 8, a highly significant correlation was found ($p <$
144 0.0001 , $r^2 0.91$).

145

146 Gene expression vs differential net absorption.

147

148 The relationship between the level of gene expression of all individual genes, the number
149 of regulated genes, and differential net absorption of individual animals was determined
150 by linear regression. No significant correlation was found for separate genes except for
151 THOC4 ($p < 0.03$, $r^2 0.95$). A near significant correlation was found for the total number
152 of genes regulated ($p > 0.07$, $r^2 0.85$), and a significant relationship with the number of up
153 regulated genes ($p < 0.03$, $r^2 0.95$) (Fig. 1).

154

155 Discussion

156

157 Multilevel etiological factors underlie almost all diseases (Shai, 2006), including
158 intestinal disease like secretory diarrhea. The intestines are a complex and dynamic
159 ecosystem (Xu & Gordon, 2003), with epithelial cells influenced by intestinal content
160 (food and microflora) in terms of differentiation and functionality. Furthermore, inborn
161 (epi-) genetic factors and the immune system (and the other components of the intestinal
162 mucosa) are essential in the defense against pathogens (Diekgraefe et al., 2000, Hooper &
163 Gordon, 2001). Individuals do vary in the reaction to pathogens, and thus vary in the
164 physiological outcome. Individual variation is on the one hand cumbersome because it
165 makes interpretation statistically difficult, on the other hand, if results obtained can be
166 linked to functional read out, it can yield valuable information (Shai, 2006, Oleksiak et
167 al., 2005). Furthermore, genes commonly active in all or most individuals, are most likely
168 to be essential ones.

169 Variation between animals could possibly be caused by loss of epithelial cells, but the
170 control for epithelial content I-FABP (Niewold et al., 2005, 2007) did not show
171 differential expression.

172 In the present experiment, three out of four animals (nr. 6, 7, and 8) reacted in a similar
173 way, whereas one was clearly different in response to a small intestinal ETEC infection.
174 Animal 5 had only 4 regulated genes, whereas all others had more than 10. Furthermore,
175 the same animal (5) was aberrant (very low) in net fluid loss. On the other extreme is
176 animal 8, showing 24 differentially regulated genes with the largest expression
177 differences and the highest net fluid loss. Although the number of animals is limited for
178 statistical analysis, it is interesting to observe that differential net absorption as the
179 parameter for intestinal function was correlated with the number of differentially
180 expressed genes, but most significantly with the number of up regulated genes. Apart
181 from animal 5, animals were very similar in the number of up regulated genes (9, 10 and
182 11), and had 80% of those in common. As described before (Niewold et al., 2005), the
183 most prominent among those is PAP. PAP has been described as an antibacterial protein,
184 and specific for gram positive bacteria in a mouse monoassociated system (Cash et al.,
185 2006). Such a system is of course very different from the one used here with normal
186 intestinal microbiota, and in contrast to Cash et al. (2006) we find no PAP expression

187 previous to infection, except in animal 5. We speculate that pre-ETEC PAP expression in
188 the latter could reflect a pre-existing infection with another agent, since we have never
189 been able to demonstrate PAP expression in the jejunum of normal, non-infected pigs
190 (data not shown). In pigs PAP is induced by gram positive organisms such as
191 *Lactobacillus plantarum* (Gross et al., 2008) as well as by the gram negative ETEC (this
192 paper), and by *Salmonella* (Niewold et al., 2007). This could suggest an antibacterial
193 spectrum not limited to gram positives as suggested by Cash et al., also because the pre-
194 existing PAP in animal 5 appears to attenuate the diarrhea.

195 The largest variation in gene expression was found in the down regulated genes. Only 3
196 constructs in 3 out of 4 animals were in common, PARP a transcription repressor, and
197 two cytochrome P450 (CYP) species. Mucosal up regulation of CYP3A29 can reflect
198 influx of polymorphonucleocytes (PMN) rather than *in situ* up regulation in *Salmonella*
199 infection (Veldhuizen et al., 2007, Niewold et al., 2007). Apparent down regulation of
200 CYP could suggest depletion of PMN, consistent with the described transmigration of
201 PMN to the intestinal lumen upon luminal infections (Hofman et al., 2000), causing loss
202 of epithelial integrity and enhanced electrogenic chloride secretion (Parkos, 1997). The
203 absence of a relationship between fluid excretion and loss of PMN as measured by
204 CYP3A29 here, suggests that fluid loss is primarily caused by LT.

205 Common regulation was seen (in animals 6, 7, 8) for PAP, MMP1, STAT3, GCNT3,
206 GCLM, RIF1, THOC4, and PARP13. This suggests a major role for these genes in
207 ETEC infection. PAP, MMP1, STAT3, and THOC4 are also present in *Salmonella*
208 infection (Niewold et al., 2007, and see above), and are clearly involved in the innate
209 defense. The same can be assumed for the other genes, although it is as yet unknown in
210 what way, with the possible exception of GCNT3. This gene is abundantly expressed by
211 goblet cells and/or differentiating immature enterocytes, and involved in forming and
212 maintenance of the epithelial mucus layer. GCNT3 up regulation was also found in
213 rotavirus infection (Hulst et al., 2008).

214

215 We conclude that the mucosal gene expression response to ETEC varies considerably
216 between animals. Animals may differ genetically or in immunological background.
217 Quantitative differences in gene regulation can be functionally linked to the physiological

218 response in these four animals. Furthermore, it is clear that the common expression found
219 for a limited number of genes (such as PAP, MMP-1, and STAT3) reflects their
220 importance in the innate defense against enteral infections. It suggests them to be
221 promising targets for intervention. Finally, these genes could also be very useful as
222 parameters for the severity of intestinal diseases, especially PAP is interesting in that
223 respect since it is known that the protein can be detected in serum (Gironella et al., 2005).

224

225 Conflict of interest

226

227 The authors declare no conflict of interest.

228

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230

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233 technique, Pim Kuurman is thanked for the statistical analyses of microarray data.

234

235 References

236

237 Cash, H.L., Whitham, C.V., Behrendt, C.L., Hooper, L.V., 2006. Symbiotic bacteria
238 direct expression of an intestinal bactericidal lectin. *Science* 313, 1126-1130.

239

240 Diekgraefe, B.K., Stenson, W.F., Korzenik, J.R., Swanson, P.E., Harrington, C.A., 2000.
241 Analysis of mucosal gene expression in inflammatory bowel disease by parallel
242 oligonucleotide arrays. *Physiol Genomics* 4, 1-11.

243

244 Farthing, M.J., 2006. Antisecretory drugs for diarrheal disease. *Dig Dis* 24, 47-58.

245

246 Flach, C.F., Qadri, F., Bhuiyan, T.R., Alam, N.H., Jennische, E., Lonroth, I., Holmgren,
247 J., 2007. Broad up-regulation of innate defense factors during acute cholera. *Infect*
248 *Immun* 75, 2343-2350.

249

250 Gironella, M., Iovanna, J.L., Sans, M., Gil, F., Peñalva, M., Closa, D., Miquel, R., Piqué,
251 J.M., Panés, J., 2005. Anti-inflammatory effects of pancreatitis associated protein in
252 inflammatory bowel disease. *Gut* 54, 1244-1253

253

254 Gross, G., van der Meulen, J., Snel, J., van der Meer, R., Kleerebezem, M., Niewold,
255 T.A., Hulst, M.M., Smits, M.A., 2008. Mannose-specific interaction of *Lactobacillus*
256 *plantarum* with porcine jejunal epithelium. *FEMS Immunol Med Microbiol* 54, 215-223.

257

258 Harris, J.B., Khan, A.I., LaRocque, R.C., Dorer, D.J., Chowdhury, F., Faruque, A.S.,
259 Sack, D.A., Ryan, E.T., Qadri, F., Calderwood, S.B., 2005. Blood group, immunity, and
260 risk of infection with *Vibrio cholerae* in an area of endemicity. *Infect Immun* 73, 7422-
261 7427.

262

263 Hofman, P., Piche, M., Far, D.F., Le Negrate, G., Selva, E., Landraud, L., Alliana-
264 Schmid, A., Boquet, P., Rossi, B., 2000. Increased *Escherichia coli* phagocytosis in
265 neutrophils that have transmigrated across a cultured intestinal epithelium. *Infect Immun*
266 68, 449-455.

267

268 Hooper, L.V., Gordon, J.I., 2001. Commensal host-bacterial relationships in the gut.
269 *Science* 292, 1115–1118.

270

271 Hulst, M., Kerstens, H., de Wit, A., Smits, M., van der Meulen, J., Niewold, T., 2008.
272 Early transcriptional response in the jejunum of germ-free piglets after oral infection with
273 virulent rotavirus. *Arch Virol* 153, 1311-1322.

274

275 Niewold, T.A., Kerstens, H.H.D., van der Meulen, J., Smits, M.A., Hulst, M.M., 2005.
276 Development of a porcine small intestinal cDNA microarray: characterization, and
277 functional analysis of the response to enterotoxic *E. coli*. *Vet Immunol Immunopathol*
278 105, 317-329.

279

280 Niewold, T.A., Veldhuizen, E.J.A., van der Meulen, J., Haagsman, H.P., Hulst, M.M.,
281 2007. The early transcriptional response of pig small intestinal mucosa to invasion by
282 *Salmonella enterica* serovar Typhimurium DT104 analyzed by cDNA microarray. Mol
283 Immunol 44, 1316-1322.
284

285 Oleksiak, M.F., Roach, J.L., Crawford, D.L., 2005. Natural variation in cardiac
286 metabolism and gene expression in *Fundulus heteroclitus*. Nat Genet 37, 67-72.
287

288 Parkos, C.A., 1997. Cell adhesion and migration. I. Neutrophil adhesive interactions with
289 intestinal epithelium. Am J Physiol 273, G763-768.
290

291 Radich, J.P., Mao, M., Stepaniants, S., Biery, M., Castle, J., Ward, T., Schimmack, G.,
292 Kobayashi, S., Carleton, M., Lampe, J., Linsley, P.S., 2004. Individual-specific variation
293 of gene expression in peripheral blood leukocytes. Genomics 83, 980-988.
294

295 Shai, R.M., 2006 Microarray tools for deciphering complex diseases. Front Biosci 11,
296 1414-1424.
297

298 Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied
299 to the ionizing radiation response. Proc Natl Acad Sci USA 98, 5116-5121.
300

301 Veldhuizen, E.J., van Dijk, A., Tersteeg, M.H., Kalkhove, S.I., van der Meulen, J.,
302 Niewold, T.A., Haagsman, H.P., 2007. Expression of beta-defensins pBD-1 and pBD-2
303 along the small intestinal tract of the pig: lack of upregulation in vivo upon *Salmonella*
304 *typhimurium* infection. Mol Immunol 44, 276-283.
305

306 Xu, J., Gordon, J.I., 2003. Honor thy symbionts. Proc Natl Acad Sci USA 100, 10452-
307 10459.
308

309 Yang, Y.H., Dudoit, S., Luu, P., Lin, D.M., Peng, J., Ngai, J., Speed, T.P., 2002.
310 Normalization for cDNA microarray data: a robust composite method addressing single
311 and multiple slide systematic variation. *Nucleic Acids Res* 30, e15.

312

313

314

315 Legends

316

317 Table 1. Differential expression (FC, fold change) between ETEC infected and mock-
318 infected SISF loops for segment pairs collected from pig 5, 6, 7 and 8. Upper panel,
319 mRNAs expressed higher in ETEC infected loops (ETEC/Mock). Lower panel, mRNAs
320 expressed lower in ETEC infected loops (Mock/ETEC). Significant differential
321 expression (FC >3.0) are in bold. The accession (acc.) number of the mRNA sequence
322 that scored the highest degree of homology (lowest E-value) is listed. The number (n) of
323 additional clones aligning to an identical mRNA species is given behind the ID of the
324 clone that scored the lowest E-value. The q-value is SAM's (Tusher et al., 2001) median
325 false discovery rate (%).

326

327 Figure 1. Relationship between differential net fluid absorption and the number of
328 regulated genes (total nr: open symbols), and the number of up regulated genes (closed
329 symbols).

330

331

332

Pig nr.	5	6	7	8	Gene name	acc. number	E-value	Tentative function
q-value; %	0.48	0.75	0.90	0.18				
ETEC/Mock								
1 (n=5)	1.5	43.2	16.4	216	<i>B. taurus</i> pancreatitis associated protein (PAP)	gi:45430002	1.0E-118	Innate defense
2	4.5	3.0	3.5	21.1	<i>S. scrofa</i> matrix metalloproteinase 1 (MMP1)	gi:2016	6.0E-31	Tissue remodelling
3	3.0	3.0	4.1	10.4	<i>H. sapiens</i> signal transducer and activator of transcription 3 (STAT3)	gi:47080105	1.0E-115	Innate defense, Transcription
4 (n=2)	1.7	3.3	3.5	8.1	<i>B. taurus</i> mucus-type core 2 beta-1,6-N-acetylglucosaminyltransferase (GCNT3)	gi:45430040	8.0E-175	Innate defense
5	1.7	3.2	3.0	10.6	<i>D. rerio</i> glutamate-cysteine ligase, modifier subunit (GCLM)	gi:41054138	3.0E-32	Glutathion biosynthesis
6	1.9	3.8	3.6	10.6	<i>D. rerio</i> RAP1 interacting factor homolog (yeast) (RIF1)	gi:124248506	1.0E-07	Cell cycle DNA damage
7	1.2	3.8	2.5	9.0	<i>B. taurus</i> interferon-induced guanylate-binding protein 1 (GBP1)	gi:194665634	3.0E-123	Innate defense
8	1.8	4.6	4.7	7.3	<i>H. sapiens</i> THO complex 4 (THOC4)	gi:55770863	1.0E-40	Transcription
9	1.5	5.0	4.0	0.7	<i>S. scrofa</i> interleukin 8 (IL8)	gi:47523123	1.0E-75	Innate defense
10 (n=2)	1.5	3.7	3.6	0.8	<i>S. scrofa</i> hypothetical protein (5'; clone 4B8); similar to <i>H. sapiens</i> membrane-spanning 4-domains, subfamily A, member 12 (gi:8923205) (MS4A12)	gi:4186144	0	Unknown
11	1.7	1.9	2.9	5.2	<i>B. taurus</i> ribosomal protein L23 (RPL23)	gi:78042491	3.0E-154	Translation/Cell cycle regulation
12	1.2	0.9	2.4	4.3	<i>H. sapiens</i> clone DNA59613 phospholipase inhibitor (UNQ511)	gi:37182060	4.0E-05	Unknown
13	1.6	1.0	2.5	4.2	<i>B. taurus</i> similar to complement factor I (CFI)	gi:84000164	8.0E-70	Innate defense
Mock/ETEC								
14 (n=3)	1.6	3.1	3.9	7.6	<i>H. sapiens</i> poly (ADP-ribose) polymerase family, member 15 (PARP15)	gi:22749258	4.0E-63	Transcription repressor
15 (n=5)	3.1	2.2	7.0	7.3	<i>S. scrofa</i> cytochrome P450 3A29 (CYP3A29)	gi:47523899	0	Metabolism
16 (n=2)	3.3	1.4	5.4	3.5	<i>S. scrofa</i> cytochrome P450 2C49 (CYP2C49)	gi:47523893	0	Metabolism
17	1.7	1.4	3.3	8.4	<i>S. scrofa</i> glutathione S-transferase (GSTA2)	gi:47523831	0	Metabolism
18 (n=9)	1.3	2.5	3.5	5.9	<i>H. sapiens</i> apolipoprotein B (APOB)	gi:105990531	5.0E-161	Metabolism
19	1.8	1.0	3.6	5.0	<i>H. sapiens</i> eukaryotic translation initiation factor 3, subunit J (EIF3J)	gi:83281437	7.0E-108	Translation
20	2.9	1.7	4.5	4.9	<i>H. sapiens</i> fyn-related kinase (FRK)	gi:31657133	0	Signal transduction
21	1.9	2.3	3.4	4.7	<i>H. sapiens</i> aldolase B, fructose-bisphosphate (ALDOB)	gi:218505812	1.0E-149	Metabolism
22	1.1	1.1	0.5	7.4	<i>C. familiaris</i> topoisomerase-related function protein 4-2 (TRF4-2)	gi:73950407	0	DNA repair
23	1.6	1.4	0.6	6.6	<i>B. taurus</i> thioredoxin (TXN)	gi:73853748	0	Transcription, oxidative stress
24	2.4	1.4	1.0	5.1	<i>S. scrofa</i> alanyl (membrane) aminopeptidase (aminopeptidase N) (ANPEP)	gi:47523627	0	Digestion
25	1.2	1.4	1.2	4.3	<i>B. taurus</i> similar to sucrase-isomaltase, intestinal (SI)	gi:166795312	0	Digestion
26	2.0	0.3	0.3	3.5	<i>B. taurus</i> similar to membrane metallo endopeptidase (MME)	gi:194663643	3.0E-156	Digestion
Control	1.2	2.1	0.6	0.7	<i>S. scrofa</i> intestinal fatty acid binding protein (IFABP)	gi:72535171	0	Metabolism

Table 1.

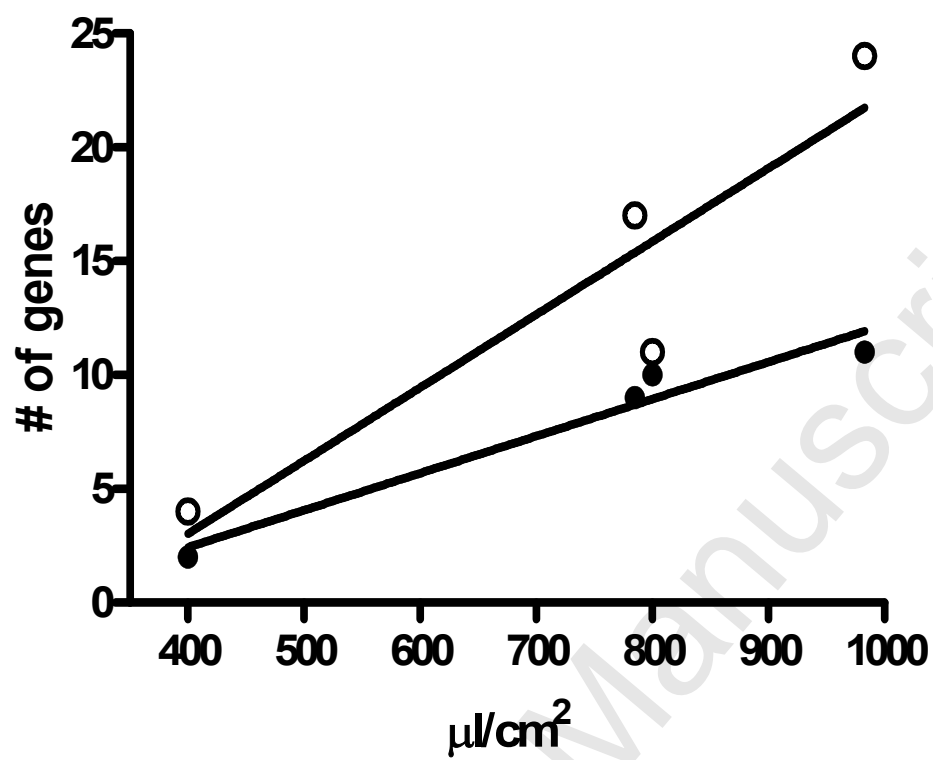


Fig 2