

# Receptor for the F4 fimbriae of enterotoxigenic *Escherichia coli* (ETEC)

Pengpeng Xia<sup>1,2</sup> · Yajie Zou<sup>1,2</sup> · Yiting Wang<sup>1,2</sup> · Yujie Song<sup>1,2</sup> · Wei Liu<sup>1,2</sup> · David H. Francis<sup>3</sup> · Guoqiang Zhu<sup>1,2</sup>

Received: 15 March 2015 / Revised: 22 April 2015 / Accepted: 24 April 2015 / Published online: 13 May 2015  
© Springer-Verlag Berlin Heidelberg 2015

**Abstract** Infection with F4<sup>+</sup> enterotoxigenic *Escherichia coli* (ETEC) responsible for diarrhea in neonatal and post-weaned piglets leads to great economic losses in the swine industry. These pathogenic bacteria express either of three fimbrial variants F4ab, F4ac, and F4ad, which have long been known for their importance in host infection and initiating protective immune responses. The initial step in infection for the bacterium is to adhere to host enterocytes through fimbriae-mediated recognition of receptors on the host cell surface. A number of receptors for ETEC F4 have now been described and characterized, but their functions are still poorly understood. The current review summarizes the latest research addressing the characteristics of F4 fimbriae receptors and the interactions of F4 fimbriae and their receptors on host cells. These include observations that as follows: (1) FaeG mediates the binding activities of F4 and is an essential component of the F4 fimbriae, (2) the F4 fimbrial receptor gene is located in a region of chromosome 13, (3) the biochemical properties of F4 fimbrial receptors that form the binding site of the bacterium are now recognized, and (4) specific receptors confer susceptibility/resistance to ETEC F4 infection in pigs. Characterizing the host–pathogen interaction will be crucial to understand the pathogenicity of the bacteria, provide insights into receptor

activation of the innate immune system, and develop therapeutic strategies to prevent this illness.

**Keywords** Fimbriae-mediated receptor · Enterotoxigenic *Escherichia coli* (ETEC) F4<sup>+</sup> · Infection and immunity

## Introduction

Fimbriae are long proteinaceous and filamentous surface structures of bacteria that play key roles in infections (Westerlund-Wikstrom and Korhonen 2005). Although the types of fimbriae vary with different kinds of bacteria, all allow bacteria to adhere tightly to cells, colonize and/or invade host cells, and subsequently survive and persist in the localized host environment to cause disease. As their principal role in disease pathogenesis is adhesion to host cells, fimbriae are frequently referred to as adhesins (Krogfelt 1991). Attachment is through highly specific interactions between fimbriae and their receptors on host cells. The fimbriae of most studied Gram-positive bacteria are known to contain specific components of extracellular matrix that allow them to recognize and adhere to receptors, such as fibronectin, collagen, and fibrinogen (Buckley et al. 2006; Wang et al. 2003). In contrast, the fimbriae of Gram-negative bacteria adhere to receptors that are glycoproteins or glycolipids, and the specific binding site is a saccharide residue (Sung et al. 2001; Van den Broeck et al. 2000; Van Gerven et al. 2008).

Enterotoxigenic *Escherichia coli* (ETEC) is a common type of Gram-negative bacteria that causes diseases by the expression of both enterotoxins and fimbriae. Various strains of these pathogens may produce a variety of fimbrial adhesions, including the colonization factor antigens F2 (CFA/I), F3 (CFA/II), and CFA/III found on pathogenic strains of human origin and F4 (K88), F5 (K99), F6 (987P), F17, F18,

✉ Guoqiang Zhu  
yzqzhu@yzu.edu.cn

<sup>1</sup> College of Veterinary Medicine, Yangzhou University, 12 East Wenhui Road, Yangzhou 225009, China

<sup>2</sup> Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou 225009, China

<sup>3</sup> Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, SD 57007, USA

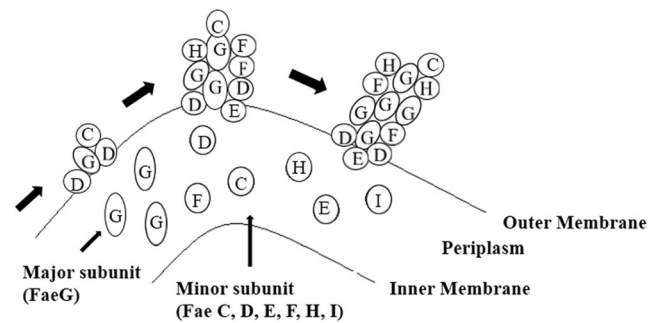
F41, F42, and F165 antigens associated with the strains of various animal origin (Evans et al. 1986; Van den Broeck et al. 2000; Zhou et al. 2012). This review focuses on the porcine pathogenic strains that express the F4 fimbriae, characteristic of F4 fimbriae, and characteristic of its receptors.

## The fimbriae of F4<sup>+</sup> ETEC

*E. coli* F4<sup>+</sup> is a major cause of severe diarrhea in neonatal and post-weaned piglets resulting in a high mortality rate in unprotected animals. The bacteria target host cells in the porcine small intestine and are able to withstand the intestinal peristalsis and flushing through their ability to adhere to the F4<sup>+</sup>-specific receptors of intestinal epithelial cells. This crucial and initial function is mediated by host-specific fimbriae on the surface of the bacteria which allows for their colonization of the small intestine and the onset of the disease (Rasschaert et al. 2010; Zhou et al. 2012).

Subsequent to the discovery of *E. coli* F4<sup>+</sup>, three serological variants of the fimbriae were found, namely, F4ab, F4ac, and F4ad. The fimbriae of these three variants share similarities in their structures including the major subunit, FaeG, and several minor subunits (FaeF, FaeH, FaeC, probably FaeI, and FaeJ), all of which are controlled by a single gene cluster (from *faeA* to *faeJ*) (Fig. 1) (Westerlund-Wikstrom and Korhonen 2005). The functions of the various subunits are highly specific: FaeA acts as a repressor of fimbrial synthesis, FaeB functions as a regulatory protein, FaeC initiates the start of fimbrial synthesis, FaeD acts as an outer membrane usher, FaeE is a periplasmic chaperone, while FaeH and FaeF are interspersed throughout the fimbrial structure acting as scaffolding for the newly forming fimbriae (Fig. 2) (Van Molle et al. 2007, 2009; Westerlund-Wikstrom and Korhonen 2005; Zhou et al. 2012).

In comparison to the minor subunits, the major subunit FaeG has a more essential role. It was reported that the FaeG subunit functions as the adhesin and facilitates the pathogen's attachment to host cells (Bakker et al. 1992). Comparative analysis of the cluster of genes in the three variants revealed that the only difference occurs in the *faeG* gene, which differs between variants in amino acid composition; different localizations of a, b, c, and d epitopes; and different specificities in attachment to cells (Van den Broeck et al. 2000). F4ab and F4ac fimbriae interacted with

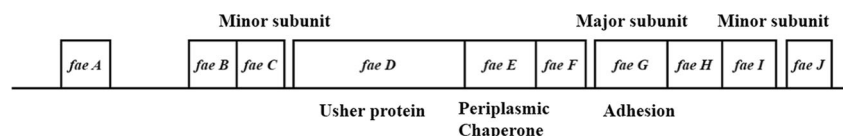


**Fig. 2** Biosynthesis structure of the F4 fimbriae: the major subunit FaeG and some other minor subunits, which are represented by C, D, E, F, G, H, and I, respectively

both sulfatide and galactosylceramide, whereas F4ad bound to gangliosylceramide and gangliosylceramide (Coddens et al. 2011). Zhang et al. (2009) proved that the amino acids 125 to 163 of the FaeG subunit were the effective site for the F4 fimbrial binding specificity. The regions of 140–145 and 151–156 amino acid residues were identified as the functional sites for the F4ab fimbriae, while the other two regions, 148–150 and 156–158 amino acids, were reported to inhibit F4ab adherence with host cells. For F4ac, the amino acids from 147 to 160 are the determinant epitopes controlling fimbrial binding capacity (Bakker et al. 1992; Van Molle et al. 2007, 2009). Unlike the ab and ac variants, the F4ad FaeG subunit interacts with a minimal galactose binding epitope via its D'-D''- $\alpha$ 1- $\alpha$ 2 binding domain, resulting in different structural and adhesive properties. Moonens et al. (2015) found that two short amino acid stretches (Phe150–Glu152 and Val166–Glu170) are the key residues providing affinity and specificity in the galactose–FaeG interaction. The crucial D'- $\alpha$ 1 loop significantly differs among the FaeG variants.

Aside from its role in fimbriae–receptor adhesion, orally administered ETEC F4 fimbriae or FaeG adhesins induced a protective mucosal immune response, especially within piglets that express the F4-specific receptor on their intestinal epithelium (Van den Broeck et al. 2002, 1999; Verdonck et al. 2004). In our own studies, we found that the *faeG*-deleted mutants had significant down-regulation of adherent ratio with host cells in comparison to that of the parent bacteria. This adherent ratio was restored when we used the corresponding complemented strain  $\Delta$ *faeG*/*pfaeG* (Zhou et al. 2013a, b; Xia et al. 2015). When taken as a whole, the data support the proposition that FaeG mediates the binding activities of the three variants and acts as an essential component of the F4 fimbriae.

**Fig. 1** The F4 gene cluster and the function of different subunits



## F4 fimbriae-mediated receptor assessed at the genetic level

The oral administration of F4 fimbriae induced a significant immune response in receptor-positive piglets but not so in receptor-negative piglets (Verdonck et al. 2004). This result is consistent with the pathogenic principle that the prerequisite for F4<sup>+</sup> *E. coli* infection is the recognition and binding of the fimbriae to specific receptors on the host cell surface. The above observation demonstrates the importance and necessity of the F4-specific receptors in the successful infection of the host and indicates their potential role in generating immunity against *E. coli* F4<sup>+</sup>.

After Sellwood et al. (1975) first reported that piglets lacking the appropriate receptors in the intestinal mucosa are resistant to the F4ac *E. coli* infection, Gibbons et al. (1977) found that the F4 receptor was inherited as a dominant Mendelian trait, where the dominant gene *S* represents susceptibility for clinical infection, while the recessive gene represents resistance. Subsequently, Edfors-Lilja et al. (1995) located the receptor gene for F4ac *E. coli* on the pig chromosome 13 in the q41 region, which is only 7.4 cm in distance from the *Tf* locus and closely linking with the receptor gene of F4ab *E. coli* ( $\theta=0.01$ ,  $Z=41.06$ ). However, the gene encoding the receptor has not yet been elucidated. Using the same family of piglets as Edfors-Lilja et al. (1995) did, Jørgensen et al. (2003) marked the receptor gene in the *SW207*, *S0075*, and *Sw225* regions of SSC13q41 by microsatellite markers and subsequently confirmed that the *Xba* I restriction enzyme site of *MUC4* intron 7 could be used for single-point polymorphic classification of piglets on the genetic level, which divided them into three categories, namely, susceptible homozygous *SS*, heterozygous *SR*, and resistant homozygous *RR* (Nguyen et al. 2012; Peng et al. 2007; Rampoldi et al. 2011). Following this classification, three groups of researchers worked together to map the F4ac receptor gene to the *Sw207–S0075* region of SSC13q41. They further hypothesized that the location of the receptor gene for F4ac is in the *Sw207–MUC4* region (Joller et al. 2009). Rampoldi et al. (2014) recently reported a new mode of inheritance of the F4ad receptor. They found that the fully adhesive receptor (F4adR<sup>FA</sup>) is presumably controlled by two genes which have an epistatic component, whereas the partially adhesive receptor (F4adR<sup>PA</sup>) is inherited as a monogenic dominant trait.

Aside from the *MUC4* gene, there are numerous other genes on pig chromosome 13 that have attracted scientific interest, including *MUC13*, *MUC20*, and *TFRC*. For example, two genes, *MUC13* and *MUC20*, controlling the expression of highly glycosylated membrane-bound proteins, are similar to the *MUC4* protein in structure and are expressed in the gastrointestinal tract in high levels (Schroyen et al. 2012b). But, the relationship between the two genes and the receptor of F4 fimbriae has not yet been elucidated. Schroyen et al. (2012a)

recently reported that the gene expression of *MUC13* and *MUC20* is not related to ETEC F4ac susceptibility in piglets. They used intestinal fatty acid binding protein 2 (IFABP2, a standard for epithelial content and reflecting the state of damage) and regenerating islet-derived 3 alpha (REG3A, a measure for inflammation in the small intestine) as controls to detect the expression level of *MUC13* and *MUC20* and found that the expression of these genes did not vary between diarrheal and non-diarrheal animals. However, Zhou et al. (2013a) reported that porcine integrin beta-5 (*ITGB5*) and *MUC13* genes play key roles in protecting the intestinal mucosa against pathogenic ETEC infection. Ren et al. (2012) found that susceptibility/resistance toward ETEC F4ac is conferred by the *MUC13* gene in pigs, including both *MUC13A* and *MUC13B* transcripts. They reported that *MUC13B* has a unique *O*-glycosylation region that forms the binding site of the bacterium, and all susceptible animals carried at least one *MUC13B* allele.

## Biochemical properties of the F4 fimbriae-mediated receptor

Several glycoproteins or glycolipids isolated from porcine intestinal epithelial cells, intestinal mucosa, or mucus could possibly act as the receptor for F4<sup>+</sup>, as they vary between F4<sup>+</sup>-susceptible and F4<sup>+</sup>-resistant piglets (Blomberg et al. 1993; Erickson et al. 1992). Considering the different serotypes of F4<sup>+</sup> *E. coli*, Erickson et al. (1997) proposed a receptor model: receptor bc composed of the 210 and 240 kDa glycoproteins which adhere to K88ab and K88ac; receptor b constituted by the 74 kDa glycoprotein which only adheres to K88ab (Grange and Mouricout 1996); receptor d, a glycolipid which only adheres to K88ad (Willemsen and de Graaf 1992); and receptor bcd (yet to be identified) which appears to bind to all three serotypes of F4<sup>+</sup> *E. coli* (Erickson et al. 1997) (Table 1). Bijlsma et al. (1982) proposed that candidates as potential F4Rs must conform to the following conditions: (1) only adhere to the specific F4<sup>+</sup> adhesin, (2) be found only in the brush borders of the small intestine, and (3) exhibit the same adhesion properties in different animals. Therefore, only three identified substances meet the conditions mentioned above, i.e., the 74-kDa receptor protein GP74 (TF) that binds to F4ab, the receptor IGLad (intestinal neutral glycosphingolipid) that binds F4ad, and the 210 and 240-kDa F4ab and F4ac receptor proteins also known as intestinal mucin-type glycoprotein (IMTGP).

In other investigations, Edfors et al. (1986) found that the density of receptors corresponds with selected sections of the intestine: the highest concentration of receptors is located in the central portion of the small intestine and the smallest concentration of receptors occurs in the cecum. This pattern was confirmed when samples were obtained from the same

**Table 1** Characteristics of putative F4-specific intestinal brush border receptors

Adhesiveness	Receptor	Characterization	Molecular mass (kDa)	Reference
ab, ac	bc	Glycoproteins	210, 240	Erickson et al. (1997)
ab	b	Glycoproteins	74	Grange and Mouricout (1996)
ad	d	Glycosphingolipid	?	Willemsen and de Graaf (1992)
ab, ac, ad	bcd	Glycoproteins	45–70	Erickson et al. (1997)

receptor-positive piglet. It is worth noting that the activities of receptors in the proximal, middle, and distal sections of the jejunum are different as well. Francis et al. (1998) found that the concentrations of bacteria in the jejunum and ileum of pigs expressing IMTGP were higher than in pigs that did not express IMTGP. Willemsen and de Graaf (1992) reported that the presence of receptors in the brush border fraction of intestinal epithelial cells is independent of age but that it is hard to isolate the same receptors in the mucus of 6-month-old pigs, while these receptors are easy to detect in 1-week-old and 35-day-old post-weaning piglets.

### Characterization of F4 fimbriae-specific receptors

Nguyen et al. (2012) found different F4 receptor profiles in pigs based on their *mucin 4* polymorphism; they proved that these glycoproteins (>250 kDa) are F4 receptor candidates and highly associated with the MUC4-susceptible genotype. In order to verify the relationship of the *MUC4* gene and the F4<sup>+</sup> adhesion phenotypes, there has been much focusing on MUC4. MUC4 protein is a high-molecular-weight glycoprotein which is involved in the development of carcinoma, tumor migration, and inflammatory bowel disease (Linden et al. 2008; Moniaux et al. 2004). The MUC4 protein stimulates the immune system of the healthy body to defend itself against pathogens. This protein protects and lubricates the epithelial mucosa, while allowing transport of small soluble substrates; it aids in the capture of some particulate materials and prevents bacteria and other particulates from making contact with the epithelium. However, if the protein is over-expressed, it may enhance the tumor cell proliferation rate and promote the migration of tumor cells by inhibition of apoptosis or by changing binding activities between cells. Crohn's disease and ulcerative colitis are reported to be associated with the over-expression of MUC4 protein (Dorofeyev et al. 2013; Srivastava et al. 2011). Moreover, the protein also has involvement with endometriosis and infertility or cervical dysplasia disease (Chang et al. 2011). Unfortunately, research on the mucin 4 proteins and F4<sup>+</sup> receptors has yet to make progress in terms of a solution for the ETEC F4<sup>+</sup> infection.

Most intriguingly, it is reported that porcine aminopeptidase N (APN), a Zn<sup>2+</sup> membrane-bound exopeptidase belonging to the family of zinc-dependent metalloproteinase M1, is highly expressed in the intestinal mucosa in conjunction with

F4 fimbriae, induces mucosal immunity in host cells, and is associated with the MUC4 susceptible genotype (Sjostrom et al. 2000; Nguyen et al. 2012; Snoeck et al. 2008). Erickson et al. (1994) and Melkebeek et al. (2012) each found that the adherent ratio of F4 fimbriae is significantly reduced when the intestinal brush border receptor  $\alpha$  2–3,6,8 sialic acid binding site was changed. Further experiments have shown that the interaction between the sialic acid binding lectin of fimbriae and sialic acid residues of the APN protein results in the binding of APN and F4 fimbriae. *N*-glycosidase F removes the N-terminal sugar chain of the brush border receptors and reduces the binding between the receptor and F4 fimbriae (Erickson et al. 1994; Kramer et al. 2005; Melkebeek et al. 2012). Snoeck et al. (2008) proved that APN can promote endocytosis of F4ac fimbriae within intestinal epithelial cells. Melkebeek et al. (2012) found that APN has the ability to induce a strong immune response even in the absence of an adjuvant or an auxiliary substance, producing IgA, IgG, and IgM antibodies. Furthermore, an undetectable level of APN will facilitate phagocytosis of intestinal epithelial cells and induce a chain of immune responses, which suggest that APN could be used as a potential target for a vaccine antigen to cross the epithelial barrier (Rasschaert et al. 2010). In our study, we found that APN has an important influence on the adherence between ETEC F4<sup>+</sup> and host cells in vitro and that the adherence ratio of F4<sup>+</sup> is APN dose dependent (unpublished paper). However, the functional binding domain of the APN protein for ETEC F4<sup>+</sup> and the interaction between APN and F4 still remain to be elucidated.

### Summary of the research of the F4 fimbrial antigen and its receptors

The swine industry is often plagued by newborn and weaning diarrhea, which is mostly caused by ETEC F4<sup>+</sup>. The important virulence factor of this pathogen is the F4 fimbriae, which is a macromolecular component that interacts with the host receptors in a complementary and specific fashion, and is used by the host for antigen presenting and mucosal immunity stimulation. Because the mucosa is the first barrier to the external environment, and efficient mucosal immunity could be induced by direct antigen presenting, it is important to obtain an active and effective immune protection of the mucosa after the passive protection from the colostrum has been depleted.

For years, antibiotics, vaccines, or some other routine preventive measures were used to reduce morbidity and mortality in the pig industry, but the use of antibiotics and vaccines also results in various issues, including the increase of production cost, the residue of antibiotics or other drugs, and vaccine failure.

Despite the current knowledge on ETEC F4 receptors, there remain problems unresolved: It is difficult to locate the exact region of receptor gene on chromosome 13 and choose the appropriate candidate genes to study; it is yet inconclusive as to whether the F4 receptors for domestic and foreign breeds are of the same origin; it is hard to determine which key factors affect the number and relevance of F4 receptors. The lack of convincing evidence relevance of and methods to identify F4 receptors and their function motivates further research.

In order to find the mechanism of interaction between F4<sup>+</sup> *E. coli* and the host, since 1975, the researchers have been focusing on the F4-specific receptors and their numerous characteristics. In the literature, the F4 receptor gene was inherited as a dominant Mendelian trait and located on pig chromosome 13, the *Sw207–S0075* region. Depending on these, the piglets could be divided into three categories of *SS*, *SR*, and *RR* (Jørgensen et al. 2003; Joller et al. 2009; Peng et al. 2007). Subsequently, Nguyen et al. (2012) proved that the polymorphism of *MUC4* intron 7 could be used for the differentiation of the F4 receptor on the genetic level. Moreover, the glycoproteins or glycolipids isolated from porcine intestinal epithelial cells, intestinal mucosa, or mucus were found to be varied between F4<sup>+</sup>-susceptible and F4<sup>+</sup>-resistant piglets (Bijlsma et al. 1982). Considering that these glycoproteins or glycolipids have different biochemical properties and different correlation with piglet susceptibility to F4<sup>+</sup> *E. coli* infections, it seems likely that these may act as a biologically relevant receptor for F4<sup>+</sup> *E. coli* or at least relative to the expression of such a receptor (Bijlsma et al. 1982; Francis et al. 1998). In addition, GP74 (TF), IGLad, IMTGP, and some other candidate receptors were found to be related with different variants of F4<sup>+</sup> *E. coli* (Bijlsma et al. 1982; Van den Broeck et al. 2000).

Comparative analysis of three variants, the only difference occurs in their major FaeG subunit of F4 fimbriae, which results in different specificities in attachment to cells (Bakker et al. 1992). In our studies, we also observed that FaeG directly mediates the binding activities of the three variants (Xia et al. 2015). There is also some pieces of evidence that oral administration of F4 fimbriae to induce effective mucosal immune response is F4 receptor (F4R) dependent, even after the booster immunization, F4R-negative pigs have no F4<sup>+</sup>-specific antibody-secreting cells (ASC) being induced (Rasschaert et al. 2010; Snoeck et al. 2008; Van den Broeck et al. 2002, 1999). Thus, the presence of F4 receptor on the brush borders of villous enterocytes is a prerequisite for inducing the immune response, which can potentially be conducted for the further study about the difference between F4R positive and

negative pigs and for genetically breeding the pigs against the F4<sup>+</sup> infection.

## The new receptor APN and future studies

The newly found receptor of F4<sup>+</sup> *E. coli*, the APN protein, proved to be efficient in promoting endocytosis of F4 fimbriae into intestinal epithelial cells and in inducing a strong immune response, even in the absence of an adjuvant or an auxiliary substance (Snoeck et al. 2008). The  $\alpha$  2–3,6,8 sialic acid binding site of APN was found to be involved in binding F4 fimbriae (Melkebeek et al. 2012). In our study, we found that the adherence ratio of F4<sup>+</sup> is APN dose dependent, and APN has an important influence on the adherence between three variants and host cells in vitro. These results suggest that APN could be used as a potential target for the binding of a vaccine antigen.

Moreover, considering that the ligand–receptor interactions are critical for disease pathogenesis, interference with the binding of an adhesin to its specific host receptor is considered to be a promising anti-infective strategy. For example, Paton et al. (2010) constructed a bacterium expressing a mimic of lacto-*N*-neotetraose (LNT; Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc) and neutralized approximately 94 % of the LT activity in ETEC culture lysates by expressing the *Neisseria*  $\beta$ 1-4galactosyltransferase gene *lgtA*, *lgtB*, and *lgtE* in CWG308. Watts et al. (2012) used a recombinant *E. coli* 83972 strain with a surface-located oligosaccharide P fimbriae receptor mimic to prevent urinary tract infections (UTI), which was efficient in inhibiting any of the three PapG adhesin variants of P fimbriae. It is reported that the biological activity of APN was positively correlated with the degradation of L-leucine-*p*-nitroanilide and could be inhibited by a higher concentration of ZnCl<sub>2</sub> or some other inhibitor (Chen et al. 2013; Demaegdt et al. 2004; Ma et al. 2013). The results showed that the APN protein activities were ion dependent, and this protein could be used as the target to decrease the binding of F4<sup>+</sup> fimbriae and the host cells. Exploitation of this finding could be the constructing of a recombinant *E. coli* strain with a surface-located F4 fimbriae receptor mimic to prevent the F4<sup>+</sup> infection or the genetic selection of piglets to resist F4<sup>+</sup> *E. coli* infection. In conclusion, the findings mentioned above might open up novel ideas for breeding and production to improve the pig industry as a whole.

**Acknowledgments** This study was supported by grants from the Chinese National Science Foundation Grant (No. 30571374, 30771603, 31072136, and 31270171) and the Genetically Modified Organisms Technology Major Project of China (2014ZX08006-001B), a project funded by the Priority Academic Program of Development Jiangsu High Education Institution, Program for Changjiang Scholars and Innovative Research Team In University “PCSIRT” (IRT0978), and program granted for Scientific Innovation Research of College Graduate in Jiangsu province (KYLX\_1359).

**Conflict of interest** The authors declared no conflict of interest.

## References

- Bakker D, Willemsen PT, Simons LH, van Zijderveld FG, de Graaf FK (1992) Characterization of the antigenic and adhesive properties of FaeG, the major subunit of K88 fimbriae. *Mol Microbiol* 6(2):247–55. doi:10.1111/j.1365-2958.1992.tb02006.x
- Bijlsma I, de Nijs A, van der Meer C, Frik J (1982) Different pig phenotypes affect adherence of *Escherichia coli* to jejunal brush borders by K88ab, K88ac, or K88ad antigen. *Infect Immun* 37(3):891–4
- Blomberg L, Krivan HC, Cohen PS, Conway PL (1993) Piglet ileal mucus contains protein and glycolipid (galactosylceramide) receptors specific for *Escherichia coli* K88 fimbriae. *Infect Immun* 61(6):2526–31
- Buckley JM, Wang JH, Redmond HP (2006) Cellular reprogramming by gram-positive bacterial components: a review. *J Leukocyt Biol* 80(4):731–41. doi:10.1189/jlb.0506312
- Chang CY, Chang HW, Chen CM, Lin CY, Chen CP, Lai CH, Lin WY, Liu HP, Sheu JJ, Tsai FJ (2011) *MUC4* gene polymorphisms associate with endometriosis development and endometriosis-related infertility. *BMC Med* 9:19. doi:10.1186/1741-7015-9-19
- Chen LZ, Du LP, Li MY (2013) The first inhibitor-based fluorescent imaging probe for aminopeptidase N. *Drug Discov Ther* 7(3):124–5. doi:10.5582/ddt.2013.v7.3.124
- Coddens A, Valis E, Benktander J, Angstrom J, Breimer ME, Cox E, Teneberg S (2011) Erythrocyte and porcine intestinal glycosphingolipids recognized by F4 fimbriae of enterotoxigenic *Escherichia coli*. *PLoS One* 6(9), e23309. doi:10.1371/journal.pone.0023309
- Demaegdts H, Laeremans H, De Backer JP, Mosselmans S, Le MT, Kersemans V, Michotte Y, Vauquelin G, Vanderheyden PM (2004) Synergistic modulation of cystinyl aminopeptidase by divalent cation chelators. *Biochem Pharmacol* 68(5):893–900. doi:10.1016/j.bcp.2004.05.046
- Dorofeyev AE, Vasilenko IV, Rassokhina OA, Kondratiuk RB (2013) Mucosal barrier in ulcerative colitis and Crohn's disease. *Gastroenterol Res Pract* 2013:431231. doi:10.1155/2013/431231
- Edfors-Lilja I, Gustafsson U, Duval-Iflah Y, Ellergren H, Johansson M, Juneja RK, Marklund L, Andersson L (1995) The porcine intestinal receptor for *Escherichia coli* K88ab, K88ac: regional localization on chromosome 13 and influence of IgG response to the K88 antigen. *Anim Genet* 26(4):237–42
- Edfors-Lilja I, Petersson H, Gahne B (1986) Performance of pigs with or without the intestinal receptor for *Escherichia coli* K88. *Anim Sci* 42(03):381–387. doi:10.1017/S000335610001816X
- Erickson A, Baker D, Bosworth B, Casey T, Benfield D, Francis D (1994) Characterization of porcine intestinal receptors for the K88ac fimbrial adhesin of *Escherichia coli* as mucin-type sialoglycoproteins. *Infect Immun* 62(12):5404–10
- Erickson AK, Billey LO, Srinivas G, Baker DR, Francis DH (1997) A three-receptor model for the interaction of the K88 fimbrial adhesin variants of *Escherichia coli* with porcine intestinal epithelial cells. *Adv Exp Med Biol* 412:167–73
- Erickson AK, Willgohs JA, McFarland SY, Benfield DA, Francis DH (1992) Identification of two porcine brush border glycoproteins that bind the K88ac adhesin of *Escherichia coli* and correlation of these glycoproteins with the adhesive phenotype. *Infect Immun* 60(3):983–8
- Evans MG, Waxler GL, Newman JP (1986) Prevalence of K88, K99, and 987P pili of *Escherichia coli* in neonatal pigs with enteric colibacillosis. *Am J Vet Res* 47(11):2431–4
- Francis DH, Grange PA, Zeman DH, Baker DR, Sun R, Erickson AK (1998) Expression of mucin-type glycoprotein K88 receptors strongly correlates with piglet susceptibility to K88(+) enterotoxigenic *Escherichia coli*, but adherence of this bacterium to brush borders does not. *Infect Immun* 66(9):4050–5
- Gibbons RA, Sellwood R, Burrows M, Hunter PA (1977) Inheritance of resistance to neonatal *E. coli* diarrhoea in the pig: examination of the genetic system. *Theor Appl Genet* 51(2):65–70. doi:10.1007/BF00299479
- Grange PA, Mouricout MA (1996) Transferrin associated with the porcine intestinal mucosa is a receptor specific for K88ab fimbriae of *Escherichia coli*. *Infect Immun* 64(2):606–10
- Jørgensen CB, Cirera S, Anderson SI, Archibald AL, Raudsepp T, Chowdhary B, Edfors-Lilja I, Andersson L, Fredholm M (2003) Linkage and comparative mapping of the locus controlling susceptibility towards *E. coli* F4ab/ac diarrhoea in pigs. *Cytogenet Genome Res* 102(1–4):157–62. doi:10.1159/000075742
- Joller D, Jørgensen CB, Bertschinger HU, Python P, Edfors I, Cirera S, Archibald AL, Burgi E, Karlsson-Mortensen P, Andersson L, Fredholm M, Vogeli P (2009) Refined localization of the *Escherichia coli* F4ab/F4ac receptor locus on pig chromosome 13. *Anim Genet* 40(5):749–52. doi:10.1111/j.1365-2052.2009.01881.x
- Kramer W, Girbig F, Corsiero D, Pfenninger A, Frick W, Jahne G, Rhein M, Wendler W, Lottspeich F, Hochleitner EO, Orso E, Schmitz G (2005) Aminopeptidase N (CD13) is a molecular target of the cholesterol absorption inhibitor ezetimibe in the enterocyte brush border membrane. *J Biol Chem* 280(2):1306–20. doi:10.1074/jbc.M406309200
- Krogfelt KA (1991) Bacterial adhesion: genetics, biogenesis, and role in pathogenesis of fimbrial adhesins of *Escherichia coli*. *Rev Infect Dis* 13(4):721–35
- Linden SK, Florin TH, McGuckin MA (2008) Mucin dynamics in intestinal bacterial infection. *PLoS One* 3(12), e3952. doi:10.1371/journal.pone.0003952
- Ma C, Li X, Liang X, Jin K, Cao J, Xu W (2013) Novel beta-dicarbonyl derivatives as inhibitors of aminopeptidase N (APN). *Bioorg Med Chem Lett* 23(17):4948–52. doi:10.1016/j.bmcl.2013.06.058
- Melkebeek V, Rasschaert K, Bellot P, Tilleman K, Favoreel H, Deforce D, de Geest BG, Goddeeris BM, Cox E (2012) Targeting aminopeptidase N, a newly identified receptor for F4ac fimbriae, enhances the intestinal mucosal immune response. *Mucosal Immunol* 5(6):635–45. doi:10.1038/mi.2012.37
- Moniaux N, Varshney GC, Chauhan SC, Copin MC, Jain M, Wittel UA, Andrianifahanana M, Aubert JP, Batra SK (2004) Generation and characterization of anti-MUC4 monoclonal antibodies reactive with normal and cancer cells in humans. *J Histochem Cytochem* 52(2):253–61. doi:10.1177/002215540405200213
- Moonen K, van den Broeck I, de Kerpel M, Deboeck F, Raymaekers H, Remaut H, de Greve H (2015) Structural and functional insight in the carbohydrate receptor binding of F4 fimbriae producing enterotoxigenic *Escherichia coli*. *J Biol Chem* 290(13):8409–19. doi:10.1074/jbc.M114.618595
- Nguyen VU, Goetstouwers T, Coddens A, van Poucke M, Peelman L, Deforce D, Melkebeek V, Cox E (2012) Differentiation of F4 receptor profiles in pigs based on their mucin 4 polymorphism, responsiveness to oral F4 immunization and in vitro binding of F4 to villi. *Vet Immunol Immunopathol* 152(1–2):93–100. doi:10.1016/j.vetimm.2012.09.015
- Paton AW, Morona R, Paton JC (2010) Bioengineered bugs expressing oligosaccharide receptor mimics: toxin-binding probiotics for treatment and prevention of enteric infections. *Bioeng Bugs* 1(3):172–7. doi:10.4161/bbug.1.3.10665
- Peng QL, Ren J, Yan XM, Huang X, Tang H, Wang YZ, Zhang B, Huang LS (2007) The *g.243A>G* mutation in intron 17 of *MUC4* is significantly associated with susceptibility/resistance to ETEC F4ab/ac

- infection in pigs. *Anim Genet* 38(4):397–400. doi:10.1111/j.1365-2052.2007.01608.x
- Rampoldi A, Jacobsen MJ, Bertschinger HU, Joller D, Burgi E, Vogeli P, Andersson L, Archibald AL, Fredholm M, Jorgensen CB, Neuenschwander S (2011) The receptor locus for *Escherichia coli* F4ab/F4ac in the pig maps distal to the *MUC4-LMLN* region. *Mamm Genome* 22(1–2):122–9. doi:10.1007/s00335-010-9305-3
- Rampoldi A, Bertschinger HU, Burgi E, Dolf G, Sidler X, Bratus A, Vogeli P, Neuenschwander S (2014) Inheritance of porcine receptors for enterotoxigenic *Escherichia coli* with fimbriae F4ad and their relation to other F4 receptors. *Animal* 8(6):859–66. doi:10.1017/s1751731114000779
- Rasschaert K, Devriendt B, Favoreel H, Goddeeris BM, Cox E (2010) Clathrin-mediated endocytosis and transcytosis of enterotoxigenic *Escherichia coli* F4 fimbriae in porcine intestinal epithelial cells. *Vet Immunol Immunopathol* 137(3–4):243–50. doi:10.1016/j.vetimm.2010.05.016
- Ren J, Yan X, Ai H, Zhang Z, Huang X, Ouyang J, Yang M, Yang H, Han P, Zeng W, Chen Y, Guo Y, Xiao S, Ding N, Huang L (2012) Susceptibility towards enterotoxigenic *Escherichia coli* F4ac diarrhea is governed by the *MUC13* gene in pigs. *PLoS One* 7(9), e44573. doi:10.1371/journal.pone.0044573
- Schroyen M, Stinckens A, Verhelst R, Geens M, Cox E, Niewold T, Buys N (2012a) Susceptibility of piglets to enterotoxigenic *Escherichia coli* is not related to the expression of MUC13 and MUC20. *Anim Genet* 43(3):324–7. doi:10.1111/j.1365-2052.2011.02241.x
- Schroyen M, Stinckens A, Verhelst R, Niewold T, Buys N (2012b) The search for the gene mutations underlying enterotoxigenic *Escherichia coli* F4ab/ac susceptibility in pigs: a review. *Vet Res* 43(1):70. doi:10.1186/1297-9716-43-70
- Sellwood R, Gibbons RA, Jones GW, Rutter JM (1975) Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. *J Med Microbiol* 18(3):405–11. doi:10.1099/00222615-8-3-405
- Sjostrom H, Noren O, Olsen J (2000) Structure and function of aminopeptidase N. *Adv Exp Med Biol* 477:25–34. doi:10.1007/0-306-46826-3\_2
- Snoeck V, van den Broeck W, de Colvenaer V, Verdonck F, Goddeeris B, Cox E (2008) Transcytosis of F4 fimbriae by villous and dome epithelia in F4-receptor positive pigs supports importance of receptor-dependent endocytosis in oral immunization strategies. *Vet Immunol Immunopathol* 124(1–2):29–40. doi:10.1016/j.vetimm.2006.10.014
- Srivastava SK, Bhardwaj A, Singh S, Arora S, Wang B, Grizzle WE, Singh AP (2011) MicroRNA-150 directly targets MUC4 and suppresses growth and malignant behavior of pancreatic cancer cells. *Carcinogenesis* 32(12):1832–9. doi:10.1093/carcin/bgr223
- Sung MA, Fleming K, Chen HA, Matthews S (2001) The solution structure of PapGII from uropathogenic *Escherichia coli* and its recognition of glycolipid receptors. *EMBO Rep* 2(7):621–7. doi:10.1093/embo-reports/kve133
- van den Broeck W, Bouchaut H, Cox E, Goddeeris BM (2002) F4 receptor-independent priming of the systemic immune system of pigs by low oral doses of F4 fimbriae. *Vet Immunol Immunopathol* 85(3–4):171–8. doi:10.1016/S0165-2427(01)00429-9
- van den Broeck W, Cox E, Goddeeris BM (1999) Receptor-dependent immune responses in pigs after oral immunization with F4 fimbriae. *Infect Immun* 67(2):520–6
- van den Broeck W, Cox E, Oudega B, Goddeeris BM (2000) The F4 fimbrial antigen of *Escherichia coli* and its receptors. *Vet Microbiol* 71(3–4):223–44
- van Gerven N, de Greve H, Hernalsteens JP (2008) Inactivated Salmonella expressing the receptor-binding domain of bacterial adhesins elicit antibodies inhibiting hemagglutination. *Vet Microbiol* 131(3–4):369–75. doi:10.1016/j.vetmic.2008.04.001
- van Molle I, Joensuu JJ, Buts L, Panjikar S, Kotiaho M, Bouckaert J, Wyns L, Niklander-Teeri V, de Greve H (2007) Chloroplasts assemble the major subunit FaeG of *Escherichia coli* F4 (K88) fimbriae to strand-swapped dimers. *J Mol Biol* 368(3):791–9. doi:10.1016/j.jmb.2007.02.051
- van Molle I, Moonens K, Garcia-Pino A, Buts L, de Kerpel M, Wyns L, Bouckaert J, de Greve H (2009) Structural and thermodynamic characterization of pre- and postpolymerization states in the F4 fimbrial subunit FaeG. *J Mol Biol* 394(5):957–67. doi:10.1016/j.jmb.2009.09.059
- Verdonck F, Cox E, van der Stede Y, Goddeeris BM (2004) Oral immunization of piglets with recombinant F4 fimbrial adhesin FaeG monomers induces a mucosal and systemic F4-specific immune response. *Vaccine* 22(31–32):4291–9. doi:10.1016/j.vaccine.2004.04.016
- Wang JE, Dahle MK, McDonald M, Foster SJ, Aasen AO, Thiemermann C (2003) Peptidoglycan and lipoteichoic acid in gram-positive bacterial sepsis: receptors, signal transduction, biological effects, and synergism. *Shock* 20(5):402–14. doi:10.1097/01.shk.0000092268.01859.0d
- Watts RE, Tan CK, Ulett GC, Carey AJ, Totsika M, Idris A, Paton AW, Morona R, Paton JC, Schembri MA (2012) *Escherichia coli* 83972 expressing a P fimbriae oligosaccharide receptor mimic impairs adhesion of uropathogenic *E. coli*. *J Infect Dis* 206(8):1242–9. doi:10.1093/infdis/jis493
- Westerlund-Wikstrom B, Korhonen TK (2005) Molecular structure of adhesin domains in *Escherichia coli* fimbriae. *Int J Med Microbiol: IJMM* 295(6–7):479–86. doi:10.1016/j.ijmm.2005.06.010
- Willemsen PT, de Graaf FK (1992) Age and serotype dependent binding of K88 fimbriae to porcine intestinal receptors. *Microbiol Pathog* 12(5):367–75
- Zhang W, Fang Y, Francis DH (2009) Characterization of the binding specificity of K88ac and K88ad fimbriae of enterotoxigenic *Escherichia coli* by constructing K88ac/K88ad chimeric FaeG major subunits. *Infect Immun* 77(2):699–706. doi:10.1128/IAI.01165-08
- Zhou C, Liu Z, Liu Y, Fu W, Ding X, Liu J, Yu Y, Zhang Q (2013a) Gene Silencing of Porcine *MUC13* and *ITGB5*: candidate genes towards *Escherichia coli* F4ac adhesion. *PLoS One* 8(7), e70303. doi:10.1371/journal.pone.0070303
- Zhou H, Zhu J, Zhu G (2012) Fimbriae of animal-originated enterotoxigenic *Escherichia coli*—a review. *Acta Microbiol Sin* 52(6):679–86
- Zhou M, Duan Q, Zhu X, Guo Z, Li Y, Hardwidge PR, Zhu G (2013b) Both flagella and F4 fimbriae from F4ac<sup>+</sup> enterotoxigenic *Escherichia coli* contribute to attachment to IPEC-J2 cells in vitro. *Vet Res* 44:30. doi:10.1186/1297-9716-44-30
- Xia P, Song Y, Zou Y, Yang Y, Zhu G (2015) F4 enterotoxigenic *Escherichia coli* (ETEC) adhesion mediated by the major fimbrial subunit FaeG. *J Basic Microbiol*. doi:10.1002/jobm.201400901