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DISSERTAÇÃO DE MESTRADO

COMPARAÇÃO DOS TRANSCRIPTOMAS DE SUÍNOS AFETADOS COM HÉRNIA ESCROTAL E HÉRNIA UMBILICAL

ARIENE FERNANDA GRANDO RODRIGUES

CHAPECÓ, 2020.

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COMPARAÇÃO DOS TRANSCRIPTOMAS DE SUÍNOS AFETADOS COM HÉRNIA UMBILICAL E HÉRNIA ESCROTAL

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Zootecnia, Área de Concentração Ciência e Produção Animal, da Universidade do Estado de Santa Catarina (UDESC), como requisito parcial para obtenção de grau de **Mestre em Zootecnia**

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Elaborada por
ARIENE FERNANDA GRANDO RODRIGUES

como requisito parcial para obtenção do grau de
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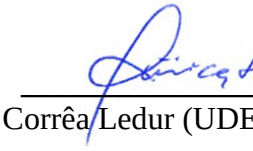

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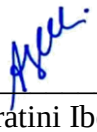

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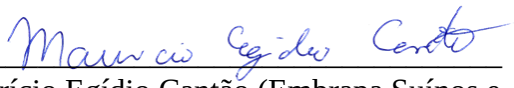

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RESUMO
Dissertação de Mestrado
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COMPARAÇÃO DOS TRANSCRIPTOMAS DE SUÍNOS AFETADOS COM HÉRNIA ESCROTAL E HÉRNIA UMBILICAL

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Chapecó, 27 de fevereiro de 2020

A hérnia é considerada um dos defeitos congênitos mais comuns encontrados em suínos. As mais observadas são escrotais (HE), inguinais (HI) e umbilicais (HU). As hérnias causam prejuízo para a suinocultura mundial devido a redução da eficiência produtiva, além de afetar negativamente o bem-estar do animal. Vários estudos genômicos já foram conduzidos, porém, ainda não foi possível identificar os genes responsáveis pela formação das hérnias em suínos, dificultando a seleção contra essas características. Dessa forma, buscou-se comparar o perfil de expressão dos transcriptomas relacionados às hérnias escrotal e umbilical e identificar genes candidatos aos dois tipos de hérnia, utilizando análises de RNA-Seq. Coletaram-se amostras biológicas de anel inguinal e umbilical de suínos com HE, HU e livres destes defeitos, as quais passaram pela extração do RNA. Após, foram preparadas bibliotecas de cDNA e estas sequenciadas na plataforma Illumina. As sequências passaram por análise de controle de qualidade e foram alinhadas e mapeadas contra o genoma de referência do suíno (*Sus scrofa*, v11.1). Posteriormente, foram identificados os genes expressos nos tecidos e os genes diferencialmente expressos (DE) quando comparados os grupos controle e afetados. O perfil de expressão dos transcriptomas relacionados à HE e HU foi comparado para identificar genes DE nos dois tipos de hérnia. Realizaram-se análises para a descoberta de polimorfismos nesses genes com posterior anotação daqueles encontrados para as duas hérnias estudadas. Em cada grupo, compararam-se os genes DE e foi verificado se estes estavam em regiões de QTL (*Quantitative trait loci*) já relatadas para suínos. Após comparação dos dois transcriptomas (HE e HU), observou-se que 94,91% dos genes encontrados estavam contidos em ambos os grupos. Quando comparadas amostras de animais afetados com aquelas de seus respectivos grupos controle, identificaram-se 627 genes DE para HE e 199 para HU, dos quais 35 genes estavam DE em ambos os grupos. Estes genes participam de 108 processos biológicos que envolvem desde o sistema imunológico até a organização celular. Dos genes DE em ambos os grupos, dois (*ACAN* e *BCHE*) estão em regiões de QTL já relatadas para hérnia escrotal. Considerou-se os genes *MAP1LC3C*, *VIT*, *ACAN*, *ACER2*, *KCNMA1* e *SYNPO2* candidatos ao surgimento dos dois tipos de defeito por apresentarem expressão equivalente em ambas as hérnias e participarem nos processos de adesão celular, organização do citoesqueleto, produção de colágeno, relaxamento muscular e autofagia. Identificaram-se 67 polimorfismos no tecido do anel inguinal e 76 no anel umbilical dos quais 11 e 14 são novos, respectivamente. Além disso, foi observada uma variante com função deletéria localizada no gene *ITGAM*, que participa do processo biológico de diferenciação celular ectodérmica. Considera-se que o perfil da expressão desses genes possa interferir no desenvolvimento normal do tecido, causar enfraquecimento e diminuir a resistência do local, podendo levar a formação de ambas as hérnias em suínos. Assim, avançou-se no conhecimento dos genes relacionados ao surgimento da HE e HU, contribuindo para a compreensão do mecanismo genético relacionado aos dois tipos de hérnia em suínos.
Palavras-chave: RNA-Seq, expressão gênica, melhoramento animal, sequenciamento.

ABSTRACT
Master's Dissertation
Programa de Pós-Graduação em Zootecnia
Universidade do Estado de Santa Catarina

TRANSCRIPTOME COMPARISON OF PIGS AFFECTED WITH SCROTAL HERNIA AND UMBILICAL HERNIA

AUTHOR: Ariene Fernanda Grando Rodrigues
ADVISER: Mônica Corrêa Ledur
Chapecó, February 27, 2020

Hernia is considered one of the main birth defects found in pigs. The most common are scrotal (SH), inguinal (IH) and umbilical (UH). Hernias cause losses in pig production worldwide due to reduced production efficiency, also negatively affecting the animal's welfare. Several genomic studies have already been conducted; however, it has not yet been possible to identify the genes responsible for the formation of hernias in pigs, hindering the selection against these characteristics. Thus, we aimed to compare the expression profile of transcriptomes related to scrotal and umbilical hernias and to identify candidate genes for both types of hernia, using RNA-Seq analyses. Biological samples of inguinal and umbilical rings from pigs with HE, HU and without any of the defects were collected and submitted to RNA extraction. The cDNA libraries were prepared and sequencing was performed using Illumina platform. After the reads quality control, they were aligned and mapped against the swine reference genome (*Sus scrofa*, v11.1). Subsequently, the genes expressed in each tissue were identified as well as the DE genes between the control and affected groups. The expression profile of SH and UH-related transcriptomes was then compared in order to identify genes that were DE in both types of hernia. Analyses were carried out to discover polymorphisms in these genes with further annotation of those found in both hernias studied. The DE genes of each group were compared and verified if they were located in QTL regions (Quantitative trait loci) already reported for pigs. After comparing the two transcriptomes (HE and HU), 94.91% of the genes found were contained in both groups. When samples of affected animals were compared with those of their respective control groups, 627 DE genes were identified for SH and 199 for UH, of which 35 genes were DE in both groups. These genes participate in 108 biological processes (BP) that involve since the immune system to cellular organization. From the DE genes in both groups, two genes (*ACAN* and *BCHE*) were in QTL regions already reported for scrotal hernia. Furthermore, the *MAP1LC3C, VIT, ACAN, ACER2, KCNMA1* and *SYNPO2* genes were considered candidates to the appearance of both types of defect for having equivalent expression in the two types of hernia and participating in cell adhesion, cytoskeleton organization, collagen production, muscle relaxation and autophagy BP. A total of 67 polymorphisms were identified in the inguinal ring and 76 in the umbilical ring tissues, of which 11 and 14 were new, respectively. Also, a variant with deleterious function was found in the *ITGAM* gene, which participates in the BP of ectodermal cell differentiation. The expression profile of these genes possibly interferes with the normal development of the tissue, causing weakness and decreasing the resistance of the site, which can lead to the formation of both hernias in pigs. Therefore, progress has been made in the knowledge of genes related to the emergence of SH and UH contributing to a better understanding of the genetic mechanism related to both types of hernia in pigs.
Keywords: RNA-Seq, gene expression, animal breeding, sequencing.

SUMÁRIO

1 REVISÃO DE LITERATURA

1.1 SUINOCULTURA

A carne suína é a mais consumida no mundo, representando 42,1% do consumo global de carnes, de acordo com o Departamento de Agricultura dos Estados Unidos (USDA, 2019). Segundo o USDA, de 2017 para 2018 houve um crescimento de 1,8% no consumo mundial de carne suína. No Brasil, o consumo desta proteína vem crescendo nos últimos anos, passando de 13 kg/hab no ano de 2007 para 15,90 kg/hab em 2018, conforme dados apresentados pela Associação Brasileira de Proteína Animal (ABPA, 2019). Para tanto, no ano de 2019, o Brasil produziu 42,5 milhões de cabeças de suínos, o que resultou em 3,975 milhões de toneladas de carne (USDA, 2019).

De acordo com o USDA, em 2019 o Brasil consumiu 3,1 milhões de toneladas de carne suína e exportou 875.000 de toneladas do produto. Segundo o Ministério da Economia, Indústria, Comércio Exterior e Serviços (MDIC, 2019), os principais destinos de exportação no período de janeiro a novembro de 2019 foram China, Hong Kong, Rússia, Chile, Uruguai, Singapura e Argentina. Essa grande produção só é possível graças a criação em larga escala destes animais e, para um maior ganho, se faz necessário eliminar ou minimizar características que influenciam negativamente na vida dos suínos ou que resultem em perdas produtivas (FERREIRA et al., 2014).

A evolução produtiva na cadeia suinícola acontece em virtude das tecnologias e pesquisas implantadas em nutrição, manejo, genética, ambiência, sanidade, bem-estar, dentre outras (HECK, 2009). O conjunto de ações positivas ligadas à genética e ambiente resultam em melhores níveis de produção e produtividade, mas se faz necessário lembrar que as mudanças realizadas em caráter ambiental são transitórias e as de âmbito genético são permanentes e estáveis, ou seja, transferidas de geração em geração (PEREIRA, 2008).

Em setembro de 2003, o sequenciamento de genoma suíno foi iniciado (SCHOOK et al., 2005) e ao longo dos anos foi sendo complementado. Em 2012, foi publicada a sequência genômica de referência do *Sus scrofa* (GROENEN et al., 2012). A partir desses avanços, por volta de 350 genomas completos de suínos estão disponíveis (GROENEN, 2016). A montagem do genoma facilitou a identificação de genes e polimorfismos relacionados a características de interesse, principalmente comercial (ERNST; STEIBEL, 2013). Grande parte destas características é influenciada por múltiplos genes ou *loci* que controlam características quantitativas (QTL; do inglês, *Quantitative trait loci*) (ERNST; STEIBEL,

2013) e a busca por essas regiões tem sido realizada deste a década de 90 (ANDERSSON et al., 1994).

Com o auxílio da genética molecular, novos genes vêm sendo revelados como candidatos associados a características importantes para a produção, como a qualidade da carcaça e da carne (DUARTE et al., 2018), permitindo também uma melhor compreensão da expressão de genes como, por exemplo, em tecido adiposo (MIAO et al., 2018). A evolução dessas tecnologias traz para a suinocultura a possibilidade de melhorar índices produtivos e reprodutivos, além de diminuir o aparecimento de anomalias anatômicas, como os diversos tipos de hérnia, que resultam em prejuízos para toda a cadeia.

1.2 HÉRNIAS

Dentre os defeitos congênitos mais comuns em suínos, pode-se citar a hérnia escrotal (HE), a inguinal (HI) e a hérnia umbilical (HU) (MATTSSON, 2011). Essas anomalias prejudicam o bem-estar animal, causam problemas de saúde secundários, além de comprometer o crescimento normal dos animais (GRINDFLEK et al., 2006; MILLER et al., 1995) e aumentar a mortalidade na granja (STRAW; BATES; MAY, 2009). A morte dos animais, nesses casos, acontece em consequência ao estrangulamento das alças intestinais ou por fatores secundários ao aparecimento desses defeitos (PERROTT, 2004).

De maneira geral, hérnias são saliências formadas pela passagem anormal de algum conteúdo através de músculos ou tecidos (CLARNETTE; HUTSON, 1997). Na suinocultura, as hérnias causam prejuízos aos produtores, aos frigoríficos, além de prejudicar o bem-estar dos animais em função de dor e estresse. Por isso, a recomendação é que animais herniados sejam abatidos precocemente ou que seja realizado reparo cirúrgico no local afetado (SOBESTIANSKY; CARVALHO; BARCELLOS, 2012).

O abate de animais herniados requer um cuidado especial, pois ao manejar o suíno, o intestino pode ser perfurado, o que contamina a carcaça e gera condenação. Como a variação de velocidade de abate é constante e adaptada para animais sem quaisquer defeitos, ao se deparar com a hérnia, a contaminação é quase inevitável (STRAW; BATES; MAY, 2009). Em alguns casos, animais que apresentam hérnias em idade de abate são comercializados por um preço abaixo do mercado (SEARCY-BERNAL; GARDNER; HIRD, 1994; PERROTT, 2004), o que gera perdas econômicas (YUN et al., 2017), além de apresentarem menores taxas de crescimento e maiores taxas de mortalidade quando comparados aos suínos saudáveis (STRAW; BATES; MAY, 2009). É importante destacar que esses animais que morrem antes

do abate ocupam espaço, consomem alimento e, muitas vezes, sofrem com dores abdominais, então a eutanásia é uma opção sensata ao identificar um animal com hérnia (STRAW; BATES; MAY, 2009).

A incidência da HE/HI em criações suinícolas varia de 0,34% a 0,42% (SEVILLANO et al., 2015) e da HU de 0,55% e 1,5% (SEARCY-BERNAL; GARDNER; HIRD, 1994) e pode variar dependendo da raça dos animais e do manejo utilizados (SOBESTIANSKY; CARVALHO, 2007). Esta variação se altera também dependendo do sexo e da linhagem genética dos animais (VOGT; ELLERSIECK,1990 e RUTTEN-RAMOS; DEEN, 2006). Normalmente, a HE pode surgir já nos primeiros dias de vida e a HU um pouco mais tardiamente (MANALAYSAY et al., 2017; SEARCY-BERNAL; GARDNER; HIRD, 1994).

Straw, Bates e May (2009) realizaram um experimento em uma granja comercial de terminação na Carolina do Norte, EUA, no qual as anormalidades anatômicas (HE e HU) foram avaliadas em um grupo de suínos para verificar taxas de crescimento e mortalidade. De acordo com suas conclusões, a prevalência e as taxas de mortalidade não diferiram por gênero quando avaliada HU. A mortalidade foi maior no grupo dos afetados para os dois tipos de hérnia em comparação aos animais saudáveis, mas esse aumento não foi significativo quando avaliada entre os diferentes tamanhos de HU. As lesões comuns encontradas na necropsia dos suínos foram caracterizadas por peritonite com intestino estrangulado. Nas fêmeas não foi encontrada HI (STRAW; BATES; MAY, 2009). Pommerehn et al. (2014) evidenciam que o sexo dos animais pode ser um fator predisponente ao surgimento de HI e HU.

Relatos têm sido descritos que a idade em que a hérnia dos suínos comumente aparece é de 9 a 14 semanas (SEARCY-BERNAL; GARDNER; HIRD, 1994). Possivelmente, um distúrbio do tecido conjuntivo resulta em hérnias primárias, e as secundárias, como as incisionais por exemplo, são ocasionadas por falhas técnicas, que induzem uma ferida crônica (FRANZ, 2008). A função da matriz extracelular é apresentada pela medicina como um importante fator na formação de hérnia. As evidências demonstram que vários distúrbios relacionados ao colágeno predispõem à esta formação em humanos (CONNER; PEACOCK, 1973; FRANZ, 2008). Zheng et al. (2002) observaram que a quantidade de colágeno tipo III imaturo estava aumentada em duas vezes nos fibroblastos da pele de pacientes com HI, quando comparada à de pessoas sem o defeito. Contudo, para a síntese e reticulação de colágeno, as vitaminas C, A e B6 são de fundamental importância (BENATI; BERTONE, 2013).

A persistência da doença nos rebanhos, as perdas geradas por estes defeitos e a falta de clareza quanto aos genes que estão envolvidos no desenvolvimento da anomalia evidenciam a

importância da realização de estudos relacionados à hérnia, que contribuam para o entendimento de suas causas principais e para o melhor conhecimento de sua etiologia (SOUZA et al., 2020).

Como alternativa para minimizar os prejuízos que estes defeitos trazem ao plantel, os produtores e empresas realizam um processo de seleção que visa descartar reprodutores que possam estar transmitindo genes associados às anomalias (SOBESTIANSKY; CARVALHO; BARCELLOS, 2012; TAYLOR, 1995). Porém, como os estudos relacionados à genética envolvida com estes defeitos ainda são escassos, os resultados dessa seleção nem sempre são satisfatórios.

1.2.1 Hérnia escrotal / inguinal

A HE (Figura 1) é caracterizada pela passagem do conteúdo abdominal através do canal inguinal malformado, chegando até o escroto (GRINDFLEK et al., 2006). Existem alguns processos associados ao surgimento desse defeito em diferentes espécies como, por exemplo, uma falha na obliteração do processo vaginal (CLARNETTE; LAM; HUTSON, 1998) ou por uma involução falha do anel inguinal interno (CLARNETTE; HUTSON, 1997).

Figura 1 – Hérnia escrotal em leitão de creche

Fonte: Renato Irineu Pangoni (2019)

Conforme citado por Pommerehn et al. (2014), a HE é um defeito que atinge apenas machos. Já a HI ocorre principalmente em machos, mas seu aparecimento não é restrito a este sexo (TIRANTI et al., 2002). Gong et al. (1994) indicaram que a frequente transmissão

vertical e alta taxa de segregação sugere herança autossômica dominante com penetrância incompleta e influência sexual para HI indireta congênita. Foi observada a transmissão paterna preferencial da anomalia, o que pode sugerir o papel do *imprinting* genômico na etiologia dessa condição (GONG et al., 1994). Entre as raças *Duroc*, *Landrace* e *Yorkshire*, a prevalência de HE foi de 0,6%, 1,0% e 1,5%, respectivamente (VOGT; ELLERSIECK, 1990). Já em estudo realizado com as raças *Landrace* e *Large White*, a incidência de HE/HI em suínos foi de 0,34% e 0,42%, respectivamente (SEVILLANO et al., 2015).

Como em humanos o fechamento do anel inguinal ocorre primeiro no lado esquerdo, a presença da hérnia pode ser unilateral (BRANDT, 2008). Segundo o mesmo autor, parede abdominal e região inguinal com estruturas enfraquecidas, em conjunto com a descida testicular e uma obliteração incompleta do processo vaginal, podem provocar HI. De acordo com Beuermann et al. (2009), a HE pode surgir como consequência de uma baixa resistência na região inguinal. Essa falha está atrelada a ocorrência de distúrbios no metabolismo e hidrólise de componentes da matriz extracelular como o colágeno e as estruturas das fibras musculares (BENDAVID, 2004), que por sua vez comprometem a reparação do tecido conjuntivo realizada pelos fibroblastos (FRANZ, 2008).

É provável que a manifestação da hérnia seja determinada por uma falha na involução do processo vaginal, já que os defeitos relacionados à estrutura da parede abdominal não explicam a ocorrência de HE na totalidade (LORENZETTI, 2018). O comprometimento de inervações que agem no processo vaginal, como consequência de uma atrofia muscular que gera danos musculares progressivos da região, auxilia no entendimento relacionado à protrusão da hérnia, pois a atrofia muscular leva à redução da espessura da parede por perda da força contrátil e perda de massa muscular, enfraquecendo a região inguinal. (AMATO et al., 2012).

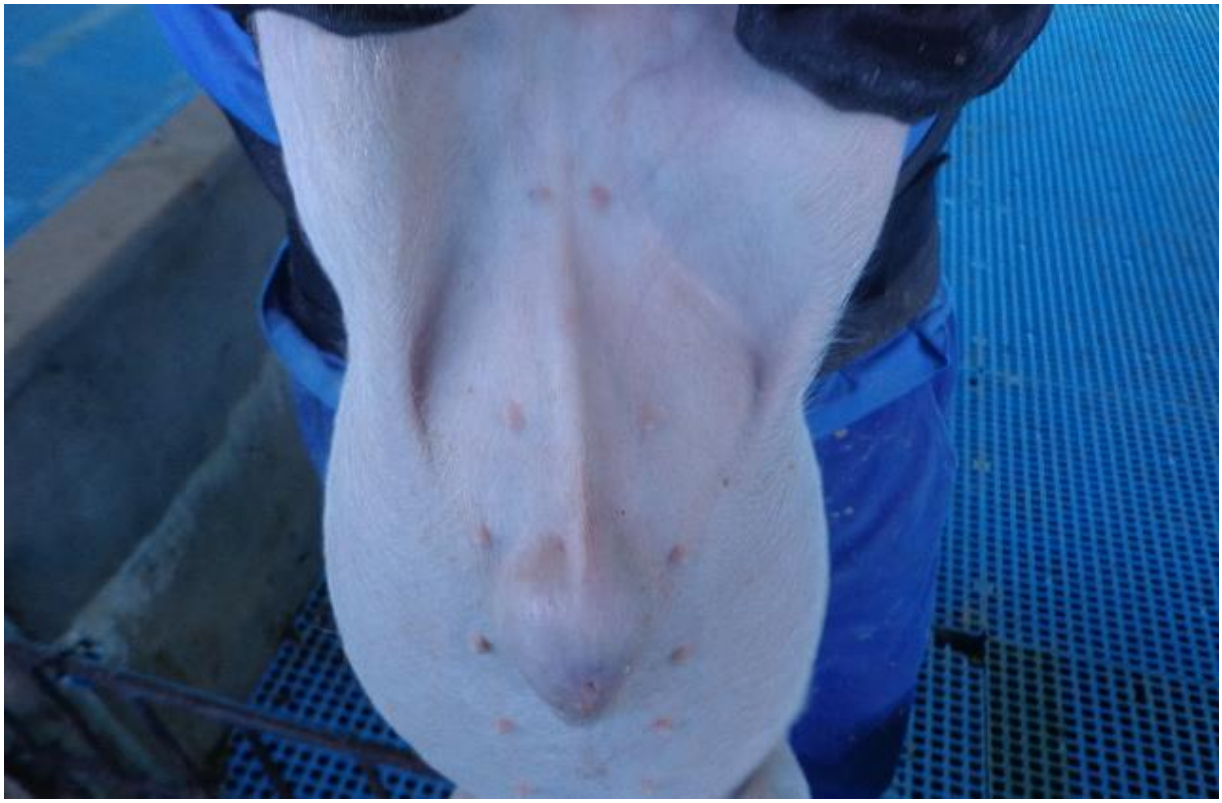
Em estudo realizado com tecidos de leitões criptorquidas ou herniados, foi observado que os processos de sinalização celular, apoptose e o encerramento da invaginação podem sofrer interferência quando observados níveis baixos de cálcio (BEUERMANN et al., 2009).

A protrusão do conteúdo herniário no escroto ou no canal inguinal é o que define a HI e HE. Se, na formação da hérnia, o intestino entrar em contato com a pele, esta é classificada como direta. Caso não haja esse contato, ou seja, se o intestino estiver revestido pelo peritônio ou pela túnica vaginal, é chamada indireta (GRINDFLEK et al., 2006). Esta classificação está diretamente relacionada com o nível de gravidade do defeito, sendo que as diretas desenvolvem um estímulo à formação de aderências e, consequentemente, podem causar obstrução intestinal, baixo desempenho, além de afetar o bem-estar (KEENLISIDE, 2006).

1.2.2 Hérnia umbilical

A HU (Figura 2) é definida como um deslocamento de conteúdo abdominal para a região umbilical (MILLER et al., 1995) e pode estar associada a uma falha do processo natural de fechamento do anel umbilical (SEARCY-BERNAL; GARDNER; HIRD, 1994). Essa falha pode estar relacionada a fatores genéticos e não genéticos, como infecções de umbigo, lesões no local, obesidade, corte do cordão realizado incorretamente, entre outras (PETERSEN et al., 2008; WARREN; ATKESON, 1931). Além disso, existem estudos que propõem que o aparecimento de hérnia pode ser induzido por um defeito muscular (MILLER et al., 1995) ou por uma anormalidade na síntese metabólica do colágeno (BENDAVID, 2004).

Figura 2 – Hérnia umbilical em leitão de creche



Fonte: Alyne Evangelista Barbosa (2019)

Conforme o manejo, a linhagem e o lote de produção, a incidência de HU pode atingir valores diferentes (SOBESTIANSKY; CARVALHO, 2007). Grindflek et al. (2018) realizaram pesquisa a partir de suínos da raça *Landrace* da Noruega e obtiveram como resultado uma incidência de 0,55%. Já em suínos dos Estados Unidos, em período anterior,

Searcy-Bernal, Gardner e Hird (1994) observaram uma incidência de 1,5% do mesmo defeito em um rebanho comercial, obtido a partir de fêmeas da raça Yorshire e machos de diversas raças. A HU pode causar desconforto e dor, que se agravam quando fatores secundários se associam ao defeito, e o bem-estar dos suínos fica comprometido (MILLER et al., 1995).

Em sua grande maioria, o saco herniário que se forma é composto de partes do intestino delgado, omento e gordura (SUTRADHAR et al., 2009). Seu aparecimento nem sempre pode ser observado ao nascimento, pois em alguns casos aparece quando os suínos já estão no período de crescimento (SUTRADHAR et al., 2009). Isso explica a dificuldade que a cadeia suinícola possui para eliminar tal defeito de seus rebanhos.

De acordo com Searcy-Bernal, Gardner e Hird (1994) e Young e Angus (1972), o surgimento de HU em suínos é causada pelo fechamento incompleto do cordão (coto) umbilical após o nascimento, e isto ocorre em função de uma variabilidade genética na musculatura do cordão umbilical (músculos de suporte ao redor do coto enfraquecidos), de uma infecção do coto umbilical, ou até mesmo pelo conjunto dos dois acontecimentos, permitindo assim a projeção dos intestinos através da parede abdominal. Ainda, os mesmos autores sugerem uma causa hereditária e descrevem que existe diferença entre as linhagens quando avaliada a chance do surgimento de HU.

A melhor forma para se diagnosticar corretamente uma HU é através da palpação local, pois esta anomalia, quando observada somente, pode ser confundida com outras patologias como abscessos (SOBESTIANSKY; CARVALHO; BARCELLOS, 2012). Ao realizar a avaliação clínica, normalmente é observado um conteúdo solto dentro do saco herniário, que corresponde principalmente às alças intestinais (SOBESTIANSKY; CARVALHO; BARCELLOS, 2012).

Em humanos, ainda no ciclo gestacional, a HU pode se desenvolver de duas maneiras diferentes: uma na fase embrionária (anteriormente a oitava semana de gestação) e uma na fase fetal (posteriormente a oitava semana de gestação) (MUSCHAWECK, 2003). Na fase embrionária, a hérnia é causada por uma falha no fechamento da parede abdominal que leva o cordão umbilical a unir-se ao abdômen e deslocar o intestino e até mesmo o fígado (MUSCHAWECK, 2003). Na fase fetal, caso o intestino não realize seu movimento normal de retorno à cavidade, ocorre um defeito na parede, que leva a herniação (MUSCHAWECK, 2003).

A principal causa do surgimento tardio de HU em humanos, justificada por Muschaweck (2003), é a cedência da cicatriz umbilical, que ocorre de forma gradual. Esse fato ocorre por consequência de uma pressão intra-abdominal elevada, que pode ser resultante

de problemas como obesidade, ascite, tumores no local, entre outros (MUSCHAWECK, 2003), o que acontece de maneira bastante semelhante em suínos (PETERSEN et al., 2008; WARREN; ATKESON, 1931).

1.3 GENÉTICA ENVOLVIDA COM AS HÉRNIAS

Com o intuito de trazer melhorias para a produção de suínos, como resistência às doenças e defeitos congênitos, a atenção nessa cadeia tem se voltado para o melhoramento genético. Com essa ferramenta, é possível se obter melhorias na qualidade da carne e nas características de produção (ABCS, 2017). Entre os defeitos congênitos mais frequentes observados nas criações comerciais de suínos pode-se citar o criptorquidismo e hérnias escrotais e umbilicais (MATTSSON, 2011) os quais implicam negativamente no desempenho zootécnico dos animais, além de causar dor e estresse (GRINDFLEK et al., 2006). De acordo com Sobestiansky, Carvalho e Barcellos (2012) as hérnias são transmitidas de geração para geração, ou seja, são hereditárias. Segundo Magee (1951), Rutten-Ramos e Deen (2006), Ding et al. (2009) e Du et al. (2009), fatores hereditários influenciam na maioria dos casos de HI, porém Rutten-Ramos e Deen (2006) lembram que as HU não são resultado de herança simples. Além disso, Elansary et al. (2015), Vogt e Ellersieck (1990), Taylor (1995) e Thaller, Dempfle e Hoeschele (1996) expõem a possibilidade de que o modo de herança para a susceptibilidade a HE/HI seja poligênico, o que foi recentemente confirmado por Sevillano et al. (2015).

Estudos que estimaram a herdabilidade para HE/HI e HU demonstraram, respectivamente, os valores de 0,31 (SEVILLANO et al., 2015) e 0,25 (THALLER; DEMPFLE; HOESCHELE, 1996), o que evidencia a influência genética no surgimento destes defeitos. O conhecimento dos mecanismos genéticos associados à formação da HE/HI e da HU é de extrema importância para o auxílio da compreensão das causas fundamentais da anomalia.

A musculatura do umbigo de suínos pode ser afetada pela variabilidade genética, e esse fator somado a um ambiente desfavorável pode tornar os animais mais suscetíveis ao aparecimento da hérnia (STRAW; BATES; MAY, 2009). Vogt e Ellersieck (1990) realizaram um estudo durante 8 anos e identificaram diferenças raciais na prevalência de HE, e que a prevalência é maior entre irmãos completos do sexo masculino, quando comparada com a de irmãos do sexo masculino na população geral. Concluíram então, que a suscetibilidade a esse defeito é herdada por meio de múltiplos *loci*. Grindflek et al. (2006) identificaram regiões

genômicas associadas à suscetibilidade a HE e HI em suínos, usando uma varredura do genoma para marcadores associados a estas hérnias.

Quando estudados casos de hérnia da parede abdominal em pacientes que também apresentavam aneurismas da aorta abdominal, foi observado um defeito comum da matriz extracelular no metabolismo do colágeno em ambos os tecidos (parede vascular e parede abdominal), o que sugere uma predisposição genética à formação das hérnias na parede abdominal (FRIEDMAN et al., 1993; HALL et al., 1995).

Estudos já demonstraram que existem fatores genéticos envolvidos no desenvolvimento de hérnias (COOK; HASTHORPE; HUTSON, 2000; KOSKIMIES et al., 2003) e, a partir desse conhecimento, genes que participam do processo natural de descida do testículo, obliteração do processo vaginal e fechamento do anel inguinal, são estudados para que se possa avaliar se existe alguma associação relacionada ao surgimento das hérnias (KUBOTA et al., 2002; HUTSON et al., 2000).

Em suínos, Ding et al. (2009) relacionaram regiões nos cromossomos SSC 2, 4, 8 (lócus SW 933), 13 e 16 como suscetíveis à ocorrência de HE. Grindflek et al. (2006) detectaram regiões de *loci* que controlam características quantitativas (*Quantitative trait loci* - QTLs) sugestivos para HE e HI em sete cromossomos (SSC1, 2, 5, 6, 15, 17 e X) quando comparados animais saudáveis e com hérnia, além de identificarem genes (*INLS3*, *MIS* e *CGRP*) envolvidos significativamente com o surgimento de HE. Foram descobertos por Sevillano et al. (2015), em suínos *Landrace*, 22 polimorfismos de base única (SNPs) presentes nos cromossomos SSC 1, 2, 4, 10 e 13, assim como 10 SNPs em cromossomos de suínos *Large White* (SSC 3, 5, 7, 8 e 13), que estão localizados em regiões de QTLs, e que podem interferir na incidência da HE/HI destas populações. No momento existe o registro de 48 QTLs associados ao surgimento de HE no banco de dados QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/SS/traitmap?trait_ID=358 - 03/01/2020).

Como a HE tem se mostrado uma anomalia hereditária, é considerado que a ocorrência e sua regulação podem ser controladas por diversos genes (VOGT; ELLERSIECK, 1990; ELANSARY et al., 2015; TAYLOR, 1995). Alguns genes como *ELF5* (*E74 like ETS transcription fator 5*), *COL23A1* (*Collagen type XXIII alpha 1 chain*) (DU et al., 2009), *MMP2* (*Matrix metallopeptidase 2*), *HOXA10* (*Homeobox A10*), *COL2α1* (*Collagen type II α1*), *ZFPM2* (*Zinc finger protein multitype 2*) (ZHAO et al., 2009), *GUSB* (*β-glucuronidase*) (BECK et al., 2006) e *INSL3* (*Leydin insulin-like hormone*) (KNORR et al., 2004) têm sido investigados, pois poderiam estar envolvidos na manifestação da HE.

Já para HU, existem 54 QTLs relacionados à sua manifestação inseridos no banco de dados QTLdb (https://www.animalgenome.org/cgi-bin/QTLdb/SS/traitmap?trait_ID=596 – 19/01/2020), dos quais, alguns são constituídos de regiões menores e mais específicas do genoma, e outros de regiões mais amplas (SOUZA et al., 2020). Li et al. (2019) detectaram um SNP no gene *CAPN9* (*Calpain 9*) do cromossomo 14 do suíno significativamente associado à HU, além de observarem a existência de uma mutação no exon 10 do mesmo gene em animais afetados com o defeito, a qual não se apresentava no gene dos animais saudáveis. Em suínos de linhagem comercial, quatro genes candidatos localizados em regiões de QTL associadas à HU foram identificados, sendo eles: *TBX15* (*T-Box 15*) e *WARS2* (*Tryptophanyl TRNA Synthetase 2, Mitochondrial*) no *SSC4*, e *LIP1* (*Lipase 1*) e *RBM11* (*RNA Binding Motif Protein 11*) no *SSC13* (FERNANDES et al., 2018). Um QTL no cromossomo 14 do suíno da raça *Landrace* foi altamente correlacionado com HU, e os genes *LIF* (*Leukemia inhibitory fator*) e *OSM* (*Oncostatin M*), localizados nesta região de QTL, foram identificados como genes candidatos a este defeito (GRINDFLEK et al., 2018).

1.4 ANÁLISE DE EXPRESSÃO GÊNICA E TRANSCRIPTOMA

O objetivo principal de uma pesquisa de genômica funcional é estudar a função de genes através de seus níveis de expressão, possibilitando a indicação dos mecanismos envolvidos em processos biológicos (BUTTE, 2002). A expressão gênica é o processo pelo qual as informações codificadas em um gene são usadas para direcionar a montagem de uma molécula (ZAHA, 2014).

O transcriptoma é o conjunto completo de transcritos em uma linhagem celular ou tecido e sua quantidade, para uma condição fisiológica ou estágio de desenvolvimento específico. Sua compreensão é essencial para interpretar os elementos funcionais do genoma, revelar os constituintes moleculares das células e tecidos, e entender o desenvolvimento de uma anomalia ou doença (WANG; GERSTEIN; SNYDER, 2009; QIU et al., 2013).

Diferentes técnicas podem ser utilizadas para a obtenção de dados de expressão gênica. A PCR é uma técnica considerada simples, pela qual moléculas de DNA ou DNA complementar (cDNA) são amplificadas milhares ou milhões de vezes de uma forma bastante rápida, além de ser bastante sensível, o que possibilita a amplificação de DNA a partir de uma quantidade mínima de amostra (ZAHA, 2014b). No caso do cDNA, a amplificação é realizada por PCR com transcrição reversa (RT-PCR), em que o DNA complementar ao RNA é sintetizado, utilizando a transcriptase reversa, e depois amplificado por PCR, e pode ser

utilizada tanto para conseguir uma sonda específica, como para analisar a expressão do gene em um determinado tipo de célula ou tecido (ZAHA, 2014b). A PCR quantitativa em tempo real (qPCR) é um método de detecção e quantificação reprodutível dos produtos gerados durante cada ciclo de amplificação, os quais são proporcionais à quantidade de molde disponível no início do processo de PCR e no caso da PCR quantitativa com transcrição reversa em tempo real (qRT-PCR), deve ocorrer a transcrição reversa de moléculas de RNA em cDNA de uma amostra pela enzima transcriptase reversa e, posteriormente, a PCR em tempo real (ZAHA, 2014b). A qRT-PCR é aplicada para quantificar os níveis de expressão de mRNA, determinar o número de cópias de um fragmento de DNA, analisar o número de cópias e expressão de um transgene, entre outras aplicações (ZAHA, 2014b).

Existem técnicas que se baseiam em hibridização como *microarrays* (BUTTE, 2002; ALLISON et al., 2006), que envolvem a extração de RNA de amostras biológicas, o qual é copiado, incorporando nucleotídeos fluorescentes ou um marcador que é manchado com fluorescência (BUTTE, 2002). O RNA marcado é hibridizado com um *microarray* por um período, após o qual o excesso é lavado e o *microarray* é escaneado sob luz laser (BUTTE, 2002). Com os *microarrays* de cDNA, para os quais cada sonda tem sua própria característica de hibridização, cada *microarray* mede duas amostras e fornece um nível de medição relativo para cada molécula de RNA (BUTTE, 2002).

Além destas, pode-se citar as técnicas baseadas em sequenciamento, como o RNA-Seq (WANG; GERSTEIN; SNYDER, 2009; SENGUPTA et al., 2011), que foi utilizado no experimento deste trabalho. Nesta técnica, uma população de RNA é convertida em uma biblioteca de fragmentos de cDNA com adaptadores conectados a uma ou ambas as extremidades. Cada molécula é sequenciada, e as sequências resultantes são alinhadas a um genoma ou transcriptoma de referência ou montadas *de novo* (quando não são utilizadas referências) para produzir um mapa de transcrição em escala de genoma que consiste na estrutura transcricional e/ou nível de expressão de cada gene (WANG; GERSTEIN; SNYDER, 2009; MORTAZAVI et al., 2008). A partir disto, a expressão dos genes relacionados a condições específicas é quantificada (NAGALAKSHMI; WAERN; SNYDER, 2010; MARIONI et al., 2008). O RNA-Seq permite, então, detectar a expressão diferencial em diferentes tratamentos ou condições (QIAN et al., 2014). Ele apresenta vantagens quando comparado a outros métodos transcriptômicos, como não depender de conhecimento prévio dos genes em estudo (RAZ et al., 2011), ou seja, não se limitar à detecção de transcritos que correspondam à sequência genômica existente, além de utilizar menos amostra de RNA (WANG; GERNTEIN; SNYDER, 2009).

Com relação ao sequenciamento, informações importantes como o número de replicatas biológicas a serem usadas e a escolha da profundidade do sequenciamento são necessárias (ROBLES et al., 2012). Uma característica distinta das moléculas de RNA é que elas ocorrem em uma ampla gama de tamanhos, então RNAs muito pequenos (<100pb), como microRNAs (miRNA), devem ser capturados e sequenciados por uma estratégia independente, pois as estratégias de seleção de tamanho acabam os excluindo da análise geral (GRIFFITH et al., 2015).

Pesquisas recentes vêm confirmando a colaboração que análises de RNA-Seq fornecem em nível molecular (LIU et al., 2018; PAN et al., 2019; MIAO et al., 2018). Usando amostras de fígado suíno, Horodyska et al. (2019) identificaram 922 genes diferencialmente expressos (DE) entre os animais de alta e baixa eficiência alimentar, e com isso, buscou-se explanar sobre mudanças no metabolismo, crescimento hepático e resposta imune a partir de diferentes eficiências alimentares. Du et al. (2014) identificaram 5.516 novos transcritos na placenta e 9.061 nos testículos, dos quais 159 demonstraram-se específicos para placenta e 252 para testículos.

Estudos já identificaram, utilizando RNA-Seq, genes candidatos à ocorrência de hérnia escrotal (ROMANO et al., 2020) e de hérnia umbilical (SOUZA et al., 2020) em suínos, porém, ainda não existe estudo que, utilizando amostras do anel inguinal e umbilical, compare o perfil de expressão destes transcriptomas. O estudo desses dados, em conjunto, proporciona o progresso para a identificação de genes que estejam envolvidos na manifestação dos dois tipos de hérnia (escrotal e umbilical), além de contribuir para o entendimento dos mecanismos genéticos associados à ocorrência destes defeitos em suínos.

1.5 OBJETIVOS

1.5.1 Objetivo geral

Comparar o perfil de expressão dos transcriptomas relacionados às hérnias escrotal e umbilical e identificar genes diferencialmente expressos nos dois tipos de hérnia.

1.5.2 Objetivos específicos

- Verificar se os genes que influem sobre a formação de hérnia escrotal são os mesmos que controlam a formação de hérnia umbilical.
- Elucidar vias biológicas e redes de interação gênica relacionadas à manifestação das hérnias escrotais e umbilicais.
- Verificar a presença de polimorfismos entre sequências de mRNA nos transcriptomas relacionados às hérnias escrotal e umbilical.
- Gerar conhecimento sobre os mecanismos moleculares envolvidos com o processo herniário em suínos.

2 MANUSCRITO

Os resultados desta dissertação são apresentados na forma de um manuscrito, com sua formatação de acordo com as orientações da revista Journal of Animal Science and Biotechnology.

2.1 MANUSCRITO I

Common genes involved with scrotal and umbilical hernia in pigs

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Common genes involved with scrotal and umbilical hernia in pigs

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Abstract

Background: Hernia is one of the most common defects in pigs. The most prevalent are the scrotal (SH), inguinal (IH) and umbilical (UH) hernias, which cause pain, discomfort and economic losses. Some genomic regions associated with SH and UH have already been identified in pigs. However, no comparative study involving RNA-Seq has been performed with umbilical and inguinal ring tissues. Therefore, we compared the inguinal ring transcriptome of normal and SH-affected pigs with the umbilical ring transcriptome of normal and UH-affected pigs to discover genes and pathways involved with the development of both types of hernia.

Results: A total of 13,307 transcripts was expressed in the inguinal and 13,302 in the umbilical ring tissues with 94.91% of them present in both tissues. From those, 627 genes were differentially expressed (DE) between normal and SH-affected pigs and 199 between normal and UH-affected pigs. From those, 35 genes were DE in both groups, which participate in 108 biological processes (BP) ranging from the immune system to the cellular organization. Two of the DE genes in both types of hernia, *ACAN* and *BCHE*, were mapped in QTL regions already reported for SH. A total of 67 polymorphisms was identified in the DE genes in the inguinal ring and 76 in the umbilical ring tissue, of which 11 and 14 were novel, respectively. A SNP with deleterious function was identified in the *ITGAM* gene, which participates of the ectodermic cellular differentiation BP. The *MAP1LC3C, VIT, ACAN, ACER2, KCNMA1* and *SYNPO2* genes can be highlighted as candidates to trigger both types of hernia, because they have similar expression profiles in both hernia groups and participate

in cell adhesion, cytoskeleton organization, production of collagen, muscle relaxation and autophagy BP.

Conclusion: We generated the first comparative study of the pig umbilical and inguinal ring transcriptomes, which allowed improving our knowledge on the genes related to the onset of SH and UH. These findings contribute to the understanding of the genetic mechanism involved with these two types of hernia in pigs and probably in other mammals.

Keywords: gene expression, congenital defects, RNA-Seq, transcriptomics, swine.

Background

Swine production is one of the most important livestock activities in the world. Evolution and growth of this activity are mainly due to the development of technologies that combine genetics, management, nutrition and well-being [1], which increases productivity and brings the final product closer to what the consumer idealizes. Meat quality and feed efficiency are traits that have been prioritized by genetic breeding programs [2, 3, 4]. However, in recent years, studies have also been carried out to improve our knowledge on diseases that persist in production, which bring losses to the entire chain [5 – 9]. Among these, scrotal (SH) / inguinal (IH) and umbilical hernias (UH) are birth defects often found in pigs [10], causing pain and discomfort to the animals and, consequently, economic losses related to meat [11, 12] and increased risk of death [13].

Scrotal hernia is mainly characterized by the displacement of intestinal loops to the scrotal sac, resulting from an abnormality in the inguinal ring [11]. Failure to obliterate the vaginal process [14], impairment of the innervations that act on the site [15] or the failed

involution of the internal inguinal ring [16] are processes associated to the manifestation of this defect. Moreover, SH can arise as a result of low resistance of the inguinal region [17] caused by disturbances in the metabolism and hydrolysis of the extracellular matrix components, such as collagen and muscle fiber structures [18], compromising the repair of connective tissue [19]. The incidence of SH in pigs is influenced by genetics and environmental factors. Sevillano et al. [20] analyzed the incidence of SH/IH in Landrace and Large White pigs and the results were 0.34% and 0.42%, respectively.

Umbilical hernia is characterized by the passage of abdominal contents (mainly intestine) to the hernial sac, in the umbilical region [12]. The discomfort and pain can be aggravated when secondary factors are associated with the defect, and the pig welfare is compromised [12]. UH can be related to genetic and non-genetic factors, such as navel infections, lesions at the site, obesity and incorrect cord cutting [21, 22]. This defect has been associated with a failure in the process of umbilical ring closure [23, 24]. The incidence of UH varies according to the management, the breed line and the production lot [25], and values between 0.55% [26] and 1.5% [23] have already been observed. Generally, the UH is not observed at birth, and this defect appears when the pigs are already in the growing period [27]. This demonstrates the difficulty that pig farmers have to eliminate such defect from their herds.

The heritability for SH/IH and UH were estimated in 0.31 [20] and 0.25 [28], respectively, which shows moderate genetic influence in the appearance of these defects. The knowledge of the genetic mechanisms associated with the formation of these anomalies is important to understand their underlying causes, regardless of the type of hernia studied. In pigs, quantitative trait loci (QTL) were detected for the occurrence of SH in pig chromosomes (SSC) 2, 4, 8 (locus SW 933), 13 and 16 [29]. Also, suggestive QTL for IH and SH were found in seven chromosomes (SSC1, 2, 5, 6, 15, 17 and X) when comparing healthy and

herniated pigs [11]. In addition, genes significantly involved with the manifestation of SH (*INLS3*, *MIS* and *CGRP*) were identified [11]. Twenty-two single nucleotide polymorphisms (SNPs) located on chromosomes 1, 2, 4, 10 and 13 in Landrace pigs, and 10 SNPs on SSC 3, 5, 7, 8 and 13 of Large White pigs, were mapped to QTL regions associated with the incidence of SH/IH in these populations [20]. To date, there is a record of 48 QTL associated with the appearance of HE/HI in pigs in the QTLdb database [30].

Regarding UH, there are 54 QTL related to their manifestation in pigs curated in the QTLdb database [30]. A SNP in the *CAPN9* gene (Calpain 9) on SSC14 significantly associated with UH ($P = 1.97 \times 10^{-10}$) has already been detected [31]. In commercial pigs, four candidate genes were identified in QTL regions associated with HU, namely *TBX15* (T-Box 15) and *WARS2* (Tryptophanyl TRNA Synthetase 2, Mitochondrial) in chromosome 4, and *LIPI* (Lipase I) and *RBM11* (RNA Binding Motif Protein 11) in chromosome 13 [32]. A QTL on chromosome 14 of the Landrace breed was highly correlated with UH, where the *LIF* (Leukemia inhibitory factor) and *OSM* (Oncostatin M) genes were identified as candidates for this defect [26].

Although several genetic studies have been performed, no comparison between large-scale gene expression profile of pigs affected with SH from those affected with UH was reported to date. Therefore, the objective of this study was to investigate the mechanisms and genes common to these two types of hernia by comparing the SH and UH transcriptomes.

Methods

This study was performed with the approval of the Embrapa Swine and Poultry National Research Center Ethical Committee of Animal Use (CEUA) under the protocol number 011/2014.

Animals and sample collection

Eighteen pigs were selected from a Landrace female line from the same Nucleus farm, located in Santa Catarina State, SC, Brazil. From those, five were females affected (case) with UH, five normal (control) females for UH, four males with SH and four control males for SH. Control animals were normal pigs, without any type of hernia, came from hernia-free litter and were contemporary to the cases. The animals were selected at approximately 60 days of age for SH and 90 days of age for UH. At the Embrapa Swine and Poultry National Research Center, located in Concórdia, SC, Brazil, the pigs were euthanized by electrocution stunning for ten seconds, followed by bleeding, in accordance with the practices recommended by the Ethics Committee. The necropsy was performed for general evaluation and to confirm the phenotypes. In the pathological analysis, the two groups of animals were confirmed: hernia-affected or without hernias (Fig. 1). Tissue samples from the inguinal and umbilical rings were collected for investigating the scrotal and umbilical hernias, respectively (Fig. 1). Samples were immediately placed in liquid nitrogen and stored in ultra-freezer (-80 °C) for RNA extraction. Samples from those tissues were also collected and placed in 4% paraformaldehyde for histopathological analysis.

Figure 1 Pathological analysis. Legend: A) Swine affected with scrotal hernia. B) Region affected with scrotal hernia (inguinal ring). C) Swine affected with umbilical hernia. D) Region affected with umbilical hernia (umbilical ring).

Histopathological analysis of the inguinal and umbilical ring tissues

The samples previously collected were routinely processed for histopathology, dehydrated in a series of increasing concentrations of ethanol, diaphanized with xylol and embedded in paraffin. Tissue sections with 2 to 5 μm thickness were cut with an automatic microtome, stained using the hematoxylin and eosin (HE) method and analyzed by optical microscopy. The cell types were evaluated in a 10x eyepiece with 5x to 100x objectives.

RNA Extraction

Total RNA extraction was performed using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. The tissues were macerated in liquid nitrogen with a mortar and pistil, properly treated for use with RNA. For each 100 mg of tissue, 1 mL of the Trizol reagent was added, which was mixed using the vortex, and then incubated for five minutes at room temperature (RT, 25ºC). Then, 200 μL of chloroform were added, shaking vigorously with the hands for 15 seconds and incubating at RT for another five minutes. A 11,000 xg refrigerated centrifugation for 15 minutes was performed, 600 μL of the aqueous phase was transferred to a new tube and 600 μL of 70% ethanol were added and homogenized by inversion. This volume was then added to a mini RNeasy silica column (Qiagen) and centrifuged for 15 seconds at 8000 xg. Then, the eluate was discarded and 700 μL of the RW1 buffer was added, followed by centrifugation for 15 seconds at 8000 xg. Then, two washes were performed with 500 μL of RPE buffer and, finally, the RNAs were eluted in 50 μL of RNAse-free water. The quantity and quality of total RNA were assessed by quantification in a Biodrop spectrophotometer (Biodrop, UK), in a 1% agarose gel and in the Bioanalyzer

Agilent 2100 (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA Integrity Number (RIN) > 8.0 were used to prepare the RNA-Seq libraries.

RNA-Seq libraries preparation and sequencing

The RNA of four normal and four SH-inguinal ring and five normal and five UH-umbilical ring tissues were submitted to library preparation with the TruSeq mRNA Stranded Sample Preparation kit (Illumina, Inc., San Diego CA). For this, 2 μg of total RNA was purified using magnetic microspheres for poly-A selection. The libraries were quantified by qPCR and evaluated in Bioanalyzer (Agilent), prior to sequencing. Libraries passing the quality control were sent for RNA sequencing in HiSeq 2500 equipment (Illumina) at the Functional Genomics Center of ESALQ/USP, in a paired-end protocol (2 x 100 bp). All samples of each hernia and their respective controls were placed in the same lane.

Quality control and differentially expressed genes

The quality control analysis and mapping were performed using BAQCOM pipeline available in the Github repository [33]. BAQCOM pipeline uses Trimmomatic tool [34] to remove short reads (<70 bp), reads with low quality (QPhred <24) and adapter reads. The sequences were mapped against the swine reference genome (*Sus scrofa*, assembly 11.1) available on the Ensembl version 95 [35] using the STAR 2.7.0 program [36]. To verify the consistency of the expression pattern between the sample groups, a multidimensional scaling (MDS) plot was performed in RStudio 1.1.463 [37] with the EdgeR package [38] from R 3.5.3 (R Core Team, 2019). After, differentially expressed (DE) genes in the analyzed tissues (case and control for each hernia) were selected based on the level of False Discovery Rate (FDR < 0.05) after the Benjamini-Hochberg (BH) method for multiple correction tests [40].

188

189 **Transcriptomes characterization of scrotal and umbilical hernia**

190 Initially, SH and UH-related transcriptomes were characterized as the total number of
191 transcripts, number of protein-encoding genes, miRNAs, lncRNAs and non-characterized
192 genes in the swine genome using the Biomart tool available in Ensembl 95 [35]. A manual
193 annotation of non-characterized genes in the porcine genome was performed using DAVID
194 6.8 database [40]. The comparison between both transcriptomes was carried out to identify
195 genes expressed in both types of hernia, SH and UH.

196

197 **Comparison of differentially expressed genes and in silico functional analysis**

198 The classification of DE genes was performed according to the database available in Ensembl
199 95 and further enrichment in DAVID 6.8 [40]. A comparison of DE genes between both
200 conditions was performed to verify if the genes were involved in the manifestation of the two
201 types of hernia, and whether there was agreement or not between gene expression profiles in
202 both conditions. To indicate possible similarity of the proteins not characterized in the DE
203 genes, the UniProt database [41] was used to collect the amino acid sequence from each
204 protein, and these sequences were then inserted in the BLASTp tool from the NCBI [42], in
205 which the result with the lowest e-value was considered [43]. A gene interaction network was
206 built with the DE genes common to both types of hernia using the STRING database [44, 45].
207 The gene ontology (GO) was evaluated in the Panther [46] and DAVID 6.8 [40] databases,
208 followed by clusterization in the REVIGO tool [47]. Furthermore, it was verified if the DE
209 genes were located in QTL regions previously reported in the Pig QTL Database [30].

210

Identification of polymorphisms

For the polymorphisms discovery between the transcriptome of animals affected with each type of hernia, the Genome Analysis Tool Kit (GATK) [48] was used with the Picard 2.5 toolkit [49]. The search for single nucleotide polymorphisms (SNPs) and InDels (insertions and deletions) was carried out following the filtering parameters and sequence quality suggested by the best practices protocol [50, 51]. The polymorphisms annotation for the two hernias studied was performed using the VEP (Variant Effect Predictor) tool [52] with standard parameters available in the Ensembl 95 database [35] and using the KEGG Pathway Database [53]. Therefore, this annotation allowed the discovery of new polymorphisms, as well as to verify their location and possible function in the genome. Using the Biomart data mining tool [35], miRNAs were observed, and a manual comparison was made between them to identify common miRNAs between SH and UH. Subsequently, a search in the miRBase database version 22 [54, 55] was performed to obtain individual information for each miRNA.

Results

Histopathological analysis of the inguinal and umbilical ring tissues

In the microscopic evaluation, the group affected with SH (Fig. 2A and 2B) presented greater number of connective tissue fibers compared to the control group and, in the UH-affected group (Fig. 2C and 2D), the connective tissue was denser than in the control group.

Figure 2 Histopathological slide stained with hematoxylin and eosin (HE). Legend: A) Sample from the SH-control group and B) Sample from the SH-affected group. A larger number of connective fibers is observed in the sample of the SH-affected group than in the

sample from the SH-control group. C) Sample from the UH-control group and D) Sample from the UH-affected group. Connective tissue interspersed with adipose tissue is observed in the sample of the UH-control group, while in the sample from the UH-affected group, only proliferated connective tissue is observed.

Sequencing and mapping

The RNA sequencing of all samples (n = 18) generated approximately 465 million paired-end reads and, after the quality control analyses, 13.84% of these were removed, resulting in approximately 400 million reads (86.16%) (Additional file 1: Table S1). The MDS plot shows a clear separation between the samples from the two evaluated types of hernia (Additional file 2: Figure 1). Around 93.50% of reads were mapped against the swine reference genome (*Sus scrofa* 11.1) [35], with individual samples ranging from 87.73% to 96.05%, distributed between the groups of healthy and affected by SH or UH. From those, 78.77% of all reads were mapped in genes. From the 31,907 annotated genes in the swine reference genome (*Sus scrofa* 11.1) [35], 13,307 (41.71%) genes were expressed in the inguinal ring and 13,302 (41.69%) in the umbilical ring.

Characterization of the scrotal and umbilical hernia transcriptomes

In the global inguinal ring transcriptome, 13,307 genes were identified and 13,302 genes were expressed in the umbilical ring tissue, with the majority of the genes appearing in both types of hernia (Fig. 3). From these data, the transcripts were classified according to the Ensembl 95 database [35] (Table 1).

Figure 3 Distribution of transcripts identified in the pig inguinal and umbilical ring tissue samples. Legend: SH – Scrotal hernia group; UH – Umbilical hernia group. For the

SH, the inguinal ring tissue was evaluated, and for the UH, the umbilical ring tissue was analyzed.

Table 1. Characterization of the transcripts identified in samples of the inguinal and umbilical ring.

Annotated transcripts	**SH**		**UH**	
LncRNA	68	0.53%	77	0.60%
MiRNA	5	0.04%	4	0.03%
Mt rRNA	2	0.02%	2	0.02%
Mt tRNA	1	0.01%	1	0.01%
Processed pseudogene	1	0.01%	0	0%
Protein-coding	12,601	98.55%	12,598	98.50%
Pseudogene	90	0.70%	91	0.71%
Ribozime	1	0.01%	1	0.01%
ScaRNA	1	0.01%	1	0.01%
SnoRNA	13	0.10%	14	0.11%
SnRNA	1	0.01%	0	0%
Y RNA	2	0.02%	1	0.01%
Total annotated transcripts	12,786		12,790	

After comparing the two transcriptomes (SH and UH), 94.91% of the genes were identified in both groups (Fig. 3). The Venn diagram also presents the number of transcripts expressed exclusively in each type of tissue.

Differently expressed genes

In the pig inguinal ring transcriptome, 627 genes were differentially expressed (FDR <0.05) between the control and the SH-affected group. Out of those, 435 genes (69.38%) were downregulated and 192 (30.62%) were upregulated in the SH-affected pigs compared to the normal animals. Regarding the genes expressed in the umbilical ring, 199 were DE between normal and UH-affected pigs. From those, 129 were downregulated (64.82%) and 70 (35.18%) upregulated in the UH-affected pigs when compared to the normal ones. In the samples from the SH group, 98.09% of the DE genes were characterized as protein coding

278 genes, 0.64% as lncRNA, 0.32% as pseudogenes, 0.32% as C immunoglobulins, 0.32% as
279 miscRNA, 0.16% as encoding immunoglobulins V and 0.16% as ribozyme. In the UH
280 transcriptome, 92.46% were protein coding genes, 3.52% immunoglobulin C coding genes,
281 1.51% pseudogenes, 1.01% miscRNAs, 1.01% mitochondrial ribosomal RNA and 0.50%
282 lncRNA.

283

284 **Differentially expressed genes common to both SH and UH transcriptomes**

285 After comparing the DE genes in the analysis of the samples referring to SH and UH, 35 DE
286 genes were present in both transcriptomes (Table 2).

287

288 Table 2. Differentially expressed genes identified in both scrotal (SH) and umbilical hernia
289 (UH) groups.

ENSEMBL ID	Gene symbol	Chromosome	Gene name	SH-logFC	SH-FDR	UH-logFC	UH-FDR
ENSSSCG00000001832	*ACAN*	7	Aggrecan	2.913	0.001	2.788	0.040
ENSSSCG00000034213	*ACER2*	1	Alkaline ceramidase 2	-3.066	0.001	-2.373	0.004
ENSSSCG00000036223	*ACKR1*	4	Atypical chemokine receptor 1 (Duffy blood group)	-1.119	0.030	-1.023	0.034
ENSSSCG00000010370	*ANXA8*	14	Annexin A8	2.026	0.000	1.744	0.004
ENSSSCG00000032709	*ARL4A*	9	ADP ribosylation factor like GTPase 4[a]	-1.308	0.001	-1.199	0.031
ENSSSCG00000033350	*BCHE*	13	Butyrylcholinesterase	1.011	0.028	1.557	0.009
ENSSSCG00000028567	*BTNL9*	2	Butyrophilin like 9	-1.356	0.010	-2.268	0.016
ENSSSCG00000002662	*C16orf74*	6	Chromosome 16 open reading frame 74	1.818	0.025	1.661	0.008
ENSSSCG00000006736	*CD2*	4	CD2 molecule	2.275	0.028	-1.990	0.002
ENSSSCG00000009138	*CFI*	8	Complement factor I	2.254	0.000	1.904	0.029
ENSSSCG00000011524	*CHL1*	13	Cell adhesion molecule L1 like	-1.776	0.004	-1.321	0.009
ENSSSCG00000021588	*DAPK2*	1	Death associated protein kinase 2	-1.347	0.026	-1.473	0.013
ENSSSCG00000012126	*GPM6B*	X	Glycoprotein M6B	-1.047	0.009	-1.244	0.022
ENSSSCG00000002847	*GPT2*	6	Glutamic--pyruvic transaminase 2	-3.752	0.002	1.571	0.029
ENSSSCG00000036438	*GPX3*	16	Glutathione peroxidase 3	-2.184	0.000	-2.762	0.006
ENSSSCG00000017010	*INSYN2B*	16	Inhibitory synaptic factor family	3.436	0.000	1.667	0.020

			member 2B				
ENSSSCG00000002245	*KATNBL1*	7	Katanin regulatory subunit B1 like 1	0.877	0.040	1.147	0.002
ENSSSCG00000010325	*KCNMA1*	14	Potassium calcium-activated channel subfamily M alpha 1	0.982	0.042	1.652	0.001
ENSSSCG00000034838	*MAP1LC3C*	10	Microtubule associated protein 1 light chain 3 gamma	6.715	0.000	3.819	0.002
ENSSSCG00000004191	*MOXD1*	1	Monooxygenase DBH like 1	-2.980	0.010	2.570	0.033
ENSSSCG00000011133	*PFKFB3*	10	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	-1.135	0.008	-1.450	0.010
ENSSSCG00000007528	*PHACTR3*	17	Phosphatase and actin regulator 3	1.727	0.012	1.986	0.028
ENSSSCG00000007470	*RIPOR3*	17	RIPOR family member 3	-1.119	0.049	-1.377	0.042
ENSSSCG00000009111	*SYNPO2*	8	Synaptopodin 2	-1.293	0.006	-1.194	0.045
ENSSSCG00000014834	*UCP3*	9	Uncoupling protein 3	-2.693	0.024	-3.262	0.016
ENSSSCG00000008501	*VIT*	3	Vitrin	-2.317	0.004	-3.709	0.029
ENSSSCG00000015766	*WDR17*	15	WD repeat domain 17	-1.761	0.000	-1.773	0.019
ENSSSCG00000013714		2		2.087	0.048	1.813	0.019
ENSSSC00000037142		AEMK02000452.1	Cysteine-rich protein 1	1.222	0.000	-4.322	0.010
ENSSSCG00000031037		14		3.813	0.000	-4.021	0.029
ENSSSCG00000032582		14		3.873	0.000	-3.822	0.012
ENSSSCG00000036224		3		3.369	0.001	-3.010	0.045
ENSSSCG00000036983		AEMK02000452.1		4.568	0.001	-5.951	0.001
ENSSSCG00000037009		AEMK02000452.1		3.679	0.024	-3.785	0.031
ENSSSCG00000039111		AEMK02000452.1		4.596	0.000	1.180	0.041

From the 35 DE genes found in both tissues (inguinal and umbilical ring), 34 were protein coding and one was an immunoglobulin C coding gene. Moreover, eight transcripts (22.86%) were uncharacterized proteins (Table 3), of which six were similar to the amino acid sequences of the pig immunoglobulin and other was similar to another predicted protein in pigs (Table 3). When the relative expression of the 35 common DE genes from each group that represents a type of hernia was compared based on the log2 fold-change (logFC), 26 of these genes had a similar expression profile in the two types of hernia (Fig. 4A), and nine had opposite expression profiles considering both types of hernia (Fig. 4B).

Table 3. Differentially expressed genes in the inguinal and umbilical ring annotated as uncharacterized protein.

Gene ID	Description	e-value	Query cover (%)	Identity (%)	Accession (RefSeq)
ENSSSCG00000013714	Mucin-16 [*Sus scrofa*]	8e-157	100	93.1	XP_020940777.1
ENSSSCG00000036224	Ig kappa chain V-C region (PLC18) [*Sus scrofa domesticus*]	5e-89	82	85	PT0219
ENSSSCG00000031037	Immunoglobulin lambda-like polypeptide 5 precursor [*Sus scrofa*]	3e-71	99	99.09	NP_001230248.1
ENSSSCG00000032582	Immunoglobulin lambda-like polypeptide 5 precursor [*Sus scrofa*]	7e-68	94	92.04	NP_001230248.1
ENSSSCG00000036983	IgG heavy chain precursor [*Sus scrofa*]	0.0	74	79.78	BAM75547.1
ENSSSCG00000037009	IgG heavy chain precursor [*Sus scrofa*]	0.0	100	100	BAM75542.1
ENSSSCG00000039111	IgG heavy chain constant region [*Sus scrofa*]	1e-74	100	100	BAM66306.1
ENSSSCG00000037142	Cysteine-rich protein 1 [*Camelus dromedarius*]	3e-41	36	94.37	KAB1277051.1

Figure 4 Common differentially expressed genes for scrotal and umbilical hernias and their respective control groups. Legend: A) Genes with similar expression profile and B) with opposite expression profile in the two types of hernia based on the LogFC.

Of the 35 genes DE in both types of hernia, a network with 27 of them was built and the *MAP1LC3C* and *MUC16* genes grouped the two largest clusters of the network (Fig. 5). One cluster was related to macroautophagy including the *MAP1LC3C*, *ATG3*, *ATG5* and *ATG12* genes (Fig. 5) and the other cluster was composed by the mucin gene family (*MUC4*, *MUC6*, *MUC16* and *MUC20*) (Fig. 5), which plays an important role protecting against environmental stress. A third group was related to the complement and coagulation cascade composed of genes *C3*, *CFH* and *CFI* (Fig. 5).

Figure 5 Gene interaction network with differentially expressed genes common to both scrotal and umbilical hernias. Legend: Gene network built with 27 of the 35 differentially expressed genes common to both types of hernia obtained with the STRING database using information from *Sus scrofa* proteins.

The enrichment analysis in the PANTHER database [46] using the 35 genes DE for the two hernias studied highlighted four metabolic pathways, namely: Huntington's disease (P00029) (*ARL4A*); Muscarinic receptor signaling pathway 1 and 3 of acetylcholine (P00042) (*BCHE*); Acetylcholine muscarinic receptor 2 and 4 signaling pathway (P00043) (*BCHE*) and Acetylcholine receptor nicotinic signaling pathway (P00044) (*BCHE*). The enrichment of this set of 35 DE genes using the DAVID 6.8 database [40] indicated that those genes participate in 108 biological processes (BP) (Additional file 1: Table S2). The *KCNMA1* gene (Potassium calcium-activated channel subfamily M alpha 1) was the most enriched in BP, appearing in 18 of them (Additional file 1: Table S2). These BP were clustered in nine macro biological processes (superclusters) using the REVIGO tool [47] (Table 4).

The 26 DE genes with similar expression profile enriched 99 BP (Additional file 1: Table S3). Considering the molecular function, the set of 35 genes was present in 57 different molecular functions mainly comprising binding, catalytic activity, molecular function regulator, structural molecule activity and transport activity. Using the QTL database [30], two DE genes in both groups of hernias studied here were located in QTL regions already identified as being associated to SH hernia in pigs: the *ACAN* and *BCHE* genes were mapped, respectively, in the QTLs 55892 (SSC7) and 8794 (SSC13).

Table 4. Macro biological processes (superclusters) enriched with the 35 differentially expressed genes common to both types of hernia.

Superclusters	Genes		
	Upregulated in both groups	**Downregulated in both groups**	**Opposite expression profile**
Cell adhesion (GO: 0022610)		*CHL1*	*CD2*
Biological regulation (GO: 0065007)	*ANXA8*	*ACKR1*, *SYNPO2*	*MOXD1*, ENSSSCG00000036224, ENSSSCG00000039111
Cellular process (GO: 0009987)	*PHACTR3*, *ANXA8*, *MAP1LC3C*	*VIT*, *SYNPO2*, *BTNL9*	*GPT2*, *MOXD1*, *CD2*, ENSSSCG00000039111, ENSSSCG00000036224
Development (GO: 0032502)	*PHACTR3*	*VIT*	
Immune system process (GO: 0002376)		*ACER2*, *BTNL9*	*CD2*, ENSSSCG00000032582, ENSSSCG00000039111, ENSSSCG00000031037, ENSSSCG00000036224
Location (GO: 0051179)	*ANXA8*, *MAP1LC3C*	*ARL4A*, *UCP3*	ENSSSCG00000036224, ENSSSCG00000039111
Metabolic process (GO: 0008152)		*UCP3*, *PFKFB3*	*GPT2*, *MOXD1*, ENSSSCG00000036224, ENSSSCG00000039111
Multicellular organismal process (GO: 0032501)	*ACAN*, *PHACTR3*	*ACKR1*, *CHL1*, *GPM6B*, *VIT*	
Response to stimulus (GO: 0050896)		*ACKR1*, *GPX3*, *UCP3*	ENSSSCG00000037009, ENSSSCG00000036983, ENSSSCG00000036224, ENSSSCG00000039111

Identification of polymorphisms

Using the GATK program, 67 polymorphisms were identified in the inguinal ring tissue samples (Additional file 1: Table S4) and 76 in the umbilical ring tissue samples (Additional file 1: Table S5). Comparing the transcriptomes of pigs affected with each type of hernia, the polymorphisms were then classified (Table 5). From the 67 polymorphisms related to scrotal hernia, 56 (83.58%) have already been described in VEP tool and 11 (16.42%) are considered new (Additional file 1: Table S4). Of the 76 polymorphisms referring to umbilical hernia, 62 (81.58%) have been previously described in VEP tool and 14 (18.42%) are new (Additional file 1: Table S5).

Table 5. Classification of polymorphisms found in samples from the inguinal and umbilical ring tissues from normal, and scrotal and umbilical hernia-affected pigs, respectively.

	Scrotal hernia		Umbilical hernia	
Polymorphism type	**N°**	**(%)**	**N°**	**(%)**
Insertion	10	14.93	6	7.90
Deletion	4	5.97	3	5.26
SNP	53	79.10	67	86.84
Total	**67**	**100**	**76**	**100**

Considering the whole transcriptome of the two tissues, the variants detected for SH and UH were classified according to the functional region indicating their possible consequences in gene regulation (Table 6). Most of the SNPs in the SH group (37.74%) were classified as synonymous variants (Additional file 1: Table S4), and in the UH group, most were of the UTR3' type (44.78%) (Additional file 1: Table S5). In the SH group, two observed variants had calculated SIFT (Sorting Intolerant From Tolerant) score classified as tolerant (SIFT score> 0.05) (Table 7). One of them has already been described in the dbSNP database [35] and the other was classified as new. From the variants belonging to the UH group, six had the calculated tolerance prediction score (SIFT) detected, one of them being deleterious (SIFT $\leq$ 0.05) and five tolerant (SIFT> 0.05) (Table 7), all of which were already present in the dbSNP database [35]. These six variants belong to six genes, two of which were enriched for metabolic pathways in the KEGG Pathway Database [53] (Table 8). The frameshift type variants were located in two genes (*NCOA7* and *SEC62*), of which one was enriched for a metabolic pathway in the same database [53] (Table 8). The SNPs of the SH group were observed in 17 different genes, which enriched nine BP (Table 9) in the DAVID 6.8 database [40]. The SNPs found in the UH group were mapped in 24 genes, which enriched six biological processes (Table 10).

Table 6. Variants annotated in different functional classes in samples from inguinal and umbilical ring tissues.

Variant type	Scrotal Hernia (%)	Umbilical Hernia (%)
Intronic	23.88	5.26
Synonym	29.85	23.68
Missense	2.99	7.89
Splicing	1.49	-
UTR5'	5.97	6.58
UTR3'	34.33	47.37
Downstream	1.49	6.58
Frameshift	-	2.63

Table 7. Missense variants observed in groups with SIFT score calculated in the dbSNP database (Ensembl).

Group	Variant	Location	Impact	Gene	SIFT
Scrotal hernia	rs325370594	16:20418972-20418972	Moderate	*RAI14*	Tolerant (1)
	-	7:64303141-64303141	Moderate	*RALGAPA1*	Tolerant (0.63)
Umbilical hernia	rs325089032	6:81571496-81571496	Moderate	*ELOA*	Tolerant (0.1)
	rs327289001	3:17254444-17254444	Moderate	*ITGAM*	Deleterious (0.01)
	rs789266896	3:17628688-17628688	Moderate	*RNF40*	Tolerant (0.6)
	rs330957838	3:17468302-17468302	Moderate	*SETD1A*	Tolerant low confidence (0.34)
	rs337670844	3:17399477-17399477	Moderate	*ZNF646*	Tolerant (0.08)
	rs323115420	3:16964045-16964045	Moderate	*ZNF713*	Tolerant (0.65)

Table 8. Genes with elevated impact variants enriched in metabolic pathways with the KEGG Pathway Database.

Variant	Gene	Pathway (ssc[1])
New (Frameshift)	*SEC62*	Protein exports (ssc03060); Protein processing in the endoplasmic reticulum (ssc04141).
rs327289001 (Missense)	*ITGAM*	Rap1 signaling path (ssc04015); Phagosome (ssc04145); Cell adhesion molecules (CAMs) (ssc04514); Hematopoietic cell line (ssc04640); Transendothelial migration of leukocytes (ssc04670);

		Regulation of the actin cytoskeleton (ssc04810); Whooping cough (ssc05133); Legionellosis (ssc05134); Leishmaniasis (ssc05140); Amebiasis (ssc05146); Infection by Staphylococcus aureus (ssc05150); Tuberculosis (ssc05152); Incorrect regulation of transcription in cancer (ssc05202).
rs330957838 (Missense)	*SETD1A*	Lysine degradation (ssc00310).

[1]Metabolic pathway identifying code described for *Sus scrofa* by the KEGG Pathway.

Table 9. Biological processes enriched with genes harboring SNPs in the scrotal hernia group.

David Term	Biological process	Enriched genes
GO:0010604	Positive regulation of the metabolic process of macromolecules	*TBX3*, ***MYRF***, ***MYLIP, PARP3***
GO:0055088	Lipid homeostasis	*ACACA*, ***MYYLIP***
GO:0009893	Positive regulation of the metabolic process	*TBX3*, ***MYRF***, ***MYLIP, PARP3***
GO:0043170	Metabolic process of the macromolecule	*TBX3*, ***MYRF***, ***MYLIP***, ***PARP3***, *ACACA*, ***DDB2***
GO:0065008	Regulation of biological quality	*TBX3, ACACA*, ***MYLIP, PARP3***
GO:0045935	Positive regulation of the compound metabolic process containing nucleobase	*TBX3*, ***MYRF, PARP3***
GO:0051173	Positive regulation of the metabolic process of nitrogen compounds	*TBX3*, ***MYRF, PARP3***
GO:0042592	Homeostatic process	*ACACA*, ***MYLIP, PARP3***
GO:0033554	Cellular stress response	*TBX3*, ***DDB2, PARP3***
GO:0060249	Anatomical structure homeostasis	*ACACA*, ***PARP3***

The genes in bold were upregulated in the scrotal hernia-affected group.

Table 10. Biological processes enriched with genes harboring SNPs in the umbilical hernia group.

GO Term	Biological process	Enriched genes
GO:0010468	Regulation of gene expression.	*RPRD1A, RNF40, ELOA*, ***VIM***, *ZNF629*, ***ZNF713***
GO:0010977	Negative regulation of the development of neuron projection	***EPHB2, VIM***
GO:0060255	Regulation of the metabolic process of macromolecules	***ITGB2***, *RPRD1A, RNF40, ELOA*, ***VIM***, *ZNF629*, ***ZNF713***
GO:0031345	Negative regulation of cellular projection organization	***EPHB2 e VIM***
GO:0019222	Regulation of the metabolic process	***ITGB2***, *RPRD1A, RNF40, ELOA*, ***VIM***, *ZNF629*, ***ZNF713***
GO:0045665	Negative regulation of neuron differentiation	***EPHB2, VIM***

The genes in bold were upregulated in the umbilical hernia-affected group.

In the SH group, the genes corresponding to exonic regions, in which the variants were observed, were not enriched by the KEGG Pathway Database [53]. Considering the type of impact caused by the variants, the results were distributed as shown in Figures 6A and 6B for the scrotal and umbilical hernias, respectively. These figures show that more than 60% of the variants represent variations of modifying impact for both types of hernia.

Figure 6 Impact caused by variants and its frequency. Legend: A) Samples from the scrotal hernia group. B) Samples from the umbilical hernia group.

Among the transcripts present in the analyzed samples, five miRNAs were identified in the SH transcriptome and four in the UH transcriptome (Table 11). Of these, three were expressed in both types of hernia. No DE miRNAs belonging to the evaluated samples were identified.

Table 11. MiRNAs identified in the transcriptomes of the pig inguinal and umbilical ring tissues.

ENSEMBL ID	Name/ Symbol	miRBase	Location	Group
ENSSSCG00000018513	ssc-mir-145 (MIR145)	MI0002417	2: 150.580.126 – 150.580.211	SH
ENSSSCG00000018758	ssc-mir-214 (MIR214)	MI0002441	9: 114.527.990 - 114.528.101	SH/UH
ENSSSCG00000019065	ssc-mir-186 (MIR186)	MI0002456	6: 141.943.328 - 141.943.409	SH
ENSSSCG00000034634	ssc-mir-6782	MI0031620	AEMK02000489.1: 40.305 - 40.379	UH
ENSSSCG00000035742	-	-	12: 14.578.144 - 14.578.210	SH/UH
ENSSSCG00000037094	ssc-mir-9810	MI0031577	4: 83.070.363 – 83.070.457	SH/UH

SH stands for scrotal hernia and UH for umbilical hernia.

Discussion

Some studies investigating genes involved with the occurrence of hernias have been performed using candidate genes and GWAS approaches [11, 26, 29, 31, 32, 56 – 59]. More recently, functional candidate genes were prospected for scrotal [6] and umbilical [7] hernias by our group using RNA-Seq approach. Nevertheless, since the molecular mechanisms involved with these traits are not yet completely understood, a comparison between the transcriptome of umbilical and scrotal hernias was performed here, allowing the identification of several common genes differentially expressed in both conditions.

Transcriptome characterization

Gene expression studies obtained from samples of the inguinal and the umbilical ring tissue are quite recent. Lorenzetti [5] and Romano et al. [6] performed gene expression analyses from the pig inguinal ring and Souza et al. [7] performed analyses with umbilical ring samples to investigate scrotal and umbilical hernias, respectively. Information of gene expression in those tissues are scarce and there are still many gaps to be elucidated in this field.

From the transcripts characterization of the two tissues (Table 1), a great similarity between the groups of both types of hernia was observed when comparing the number of each class of transcripts, implying that the appearance of both hernias may be related to the same set of genes or family of genes. This large number of transcripts that are expressed in both groups can also be seen in the Venn diagram (Fig. 3). With the exception of processed pseudogenes and snRNA, which were not identified in the UH group, the percentage of each type of transcript was similar. Thus, the expression profile of the genes in the inguinal ring

tissue was very similar to the profile found in the umbilical ring, being compatible with the histopathological composition of these two tissues (Fig. 2).

Common differentially expressed genes in scrotal and umbilical hernias

From the DE genes observed in each type of hernia, 35 were common to both groups. Among these, nine genes (*CD2*, *GPT2*, *MOXD1*, *ENSSSCG00000031037*, *ENSSSCG00000032582*, *ENSSSCG00000036224*, *ENSSSCG00000036983*, *ENSSSCG00000037009* and *ENSSSCG00000039111*) had different expression profiles when comparing both types of hernia. This behavior may have occurred due to the expression be tissue specific (inguinal ring and umbilical ring) for those genes. Other reasons could be the differences in sex and age between the groups evaluated for the two types of hernia. The other 26 DE genes have shown similar expression in both types of hernia, of which 14 genes were downregulated and 12 were upregulated in pigs affected by both types of hernia.

From the gene interaction network (Figure 5), three DE genes were enriched in both types of hernia. The *MAP1LC3C* (Microtubule associated protein 1 light chain 3 gamma) interacted in the group of the macroautophagy BP (GO: 0016236) [60]. Macroautophagy is the main path involved in inducing general renewal of cytoplasmic constituents in eukaryotic cells and is essential for cell survival, development, differentiation and homeostasis [62 - 65]. The Gene Ontology (GO) annotations related to this gene include the assembly and maturation of the autophagosome (Additional file 1: Table S4). Marcelino et al. [66] indicated the *MAP1LC3C* gene as a candidate for the formation of UH in pigs since this gene was upregulated in the affected compared to the normal pigs. In our research, the *MAP1LC3C* gene exhibited the same behavior, being upregulated in affected animals of both types of hernia when compared to the control groups (Fig. 4A). Moreover, this expression profile can

be one of the causes of the hernia onset, since the high expression of this gene can cause excessive autophagy and interfere with normal tissue development [67].

The *CFI* gene (Complement factor I) was grouped in the cluster of the coagulation cascade metabolic pathway and complement system (Figure 5). The coagulation cascade is a sequence of interconnected reactions in order to clot the local blood when a blood vessel injury occurs [68]. The complement system is a proteolytic cascade in blood plasma and a mediator of innate immunity [69]. The GO annotations related to this gene include endocytosis (content absorption through membrane invagination process) and proteolysis (protein degradation process) (Additional file 1: Table S2). The *CFI* gene, like the *MAP1LC3C*, was upregulated in animals with hernia when compared to the control group (Fig. 4A). The *CFI* encodes the trypsin-like protein Serine-protease [41], which plays an essential role in regulating the immune response, controlling all the complement pathways [70]. The participation of the *CFI* in these pathways and processes, taken together with its expression profile, suggests that this gene could be involved with as a consequence of the disorders.

The *MUC16* (Mucin-16) gene encodes a protein of the mucin family, which are O-glycosylated proteins found in the apical surfaces of the epithelium and play an important role in the formation of a protective mucous barrier [71]. This gene was enriched in the gene network (Figure 5) as a participant in processing O-glycan BP (GO: 16266) [60]. This process is related to the gradual addition of carbohydrate residues or carbohydrate derivatives to form the O-glycan structure [72]. The *MUC16* gene was also enriched as an integral cell membrane component BP (GO: 16021) [60]. According to Blalock et al. [73], the *MUC16* build a protective barrier to the epithelial cell surface, where binding proteins are associated with its tail, linking it to the actin cytoskeleton. This gene was upregulated in the affected group of

485 both types of hernia compared to their respective control groups (Fig. 4A), thus configuring a
486 defense system that might have arisen as a consequence of the hernias formation.

487

488 **Enriched biological processes**

489 When the 99 BP enriched by the 26 DE genes with an equivalent profile in both types of
490 hernia (Additional file 1: Table S3) were evaluated, the BP of cell adhesion, apoptosis,
491 organization of the actin cytoskeleton and organization of collagen fibrils can be highlighted,
492 because they are generally linked to the formation of hernias. The enriched genes for cell
493 adhesion, *VIT* (Vitrin), *ACER2* (Alkaline Ceramidase 2) and *CHL1* (Cell Adhesion Molecule
494 L1 Like) were downregulated in the affected animals compared to the control groups and
495 *ACAN* (Aggrecan) was upregulated in the affected animals. The cell adhesion BP allows the
496 interaction among cells, and between cells and the extracellular matrix [74, 75]. This BP has
497 already been related to tissue maintenance and cell differentiation [76, 77]. The *CHL1* gene
498 was enriched with the process of homophilic cell adhesion via plasma membrane adhesion
499 molecules. *ACER2* participates in the specific BP of negative regulation of cell adhesion
500 mediated by integrin and negative regulation of cell matrix adhesion. The *VIT* gene enriched
501 the process of positive regulation of cell substrate adhesion. Thus, the reduced expression of
502 these genes that actively participate in cell adhesion interferes with the integrity of tissues,
503 which can be determinant for the appearance of both types of hernia.

504 The *KCNMA1* gene (Potassium Calcium-Activated Channel Subfamily M Alpha 1),
505 which was upregulated in animals affected with hernia, and *ACER2* (downregulated) were
506 enriched in the apoptosis BP. This process is related to the regulation of programmed cell
507 death, which is extremely important for the maintenance of the development of living beings
508 [78]. The overexpression of this gene can compromise the tissue as a result of an

accumulation of immature cells in the region, which can influence the appearance of hernias, especially when associated with unfavorable environmental factors. *ACER2* was enriched with the specific process of activating cysteine-type endopeptidase activity, involved in the apoptotic process, and the *KCNMA1* was enriched for positive regulation of the apoptotic process. This last gene was also enriched for the relaxation process of the vascular smooth muscle that is related to the negative regulation of the contraction of this muscle. The relaxation is mediated by a decrease in the phosphorylation state of the myosin light chain [79]. As the expression of this gene was higher in herniated than in normal pigs, the *KCNMA1* can be pointed out as a candidate gene for the formation of umbilical and scrotal hernia, since the lack of local muscle contraction facilitates the passage of the abdominal content through the rings.

Biological processes that regulate the activities of collagen and its structures have been indicated in the enrichment of the *ACAN* and *VIT* genes. The first gene was related to the condensation of mesenchymal cells that differentiate into chondrocytes, organization of collagen fibrils and the development of chondrocytes [60]. The *VIT* gene, on the other hand, was related to the morphogenesis of chondrocytes in the cartilage of the growth plate, in which the structures of a chondrocyte are generated and organized [80]. The *ACAN* gene was upregulated in animals affected with hernia, which is in accordance with the histopathological analyses that evidenced a larger amount of collagen compared to normal pigs. Moreover, *ACAN* upregulation in animals affected with hernia can generate an exaggerated collagen production, which has already been related to hernia by Harrison, Sanniec and Janis [81].

Regarding the organization of the cytoskeleton, especially those processes related to actin, two genes were enriched, *SYNPO2* (Synaptopodin 2) and *ENSSSCG00000037142*. *SYNPO2* has been enriched specifically for the process of positive regulation of the actin filament bundles set. The organization of the actin cytoskeleton is carried out at the cellular

level and results in the assembly, disposition of the constituent parts or disassembly of the structures, including the filaments and their associated proteins [60]. In our study, this gene was downregulated in animals with hernia. This negative regulation can be a predisposing factor to hernia, since the non-assembly and organization of the structures that make up the tissue can make it less resistant.

DE genes located in QTL regions for hernias and polymorphisms characterization

Several studies have been carried out to identify QTL regions related to umbilical and scrotal hernia [11, 20, 29, 82, 83]. Among the DE genes in the two types of hernia, *ACAN* and *BCHE* (Butyrylcholinesterase) are highlight since they have already been located in QTL regions associated to scrotal/inguinal hernia [20, 29]. Even with scientific reports relating these two genes only with QTL regions for scrotal hernia, in our study, the expression profile of these two genes was equivalent in both types of hernia, being upregulated in the affected animals. Souza [7] indicated some genes as strong candidates to trigger umbilical hernias in pigs, and *ACAN* is among them.

According to the results, variations in the transcripts may be related to the manifestation of the different types of hernia. In both groups, most of the polymorphisms detected were SNPs, followed by insertions and deletions (Additional file 1: Table S4 and S5). In the SH group, a new SNP was identified on chromosome 13 (13: 34083960-34083960), which is located within a QTL region (QTL ID 55898) associated with scrotal hernia [11, 20, 29]. This SNP was mapped in the *PARP3* gene (Member of the poly ADP-ribose 3 polymerase family), which acts in the repair pathways by base excision, apoptosis and necroptosis, participating in biological processes of DNA repair [42]. Moreover, Piórkowska et al. [84] carried out research with Polish Landrace and Pulawska pigs and

pointed out the participation of the *PARP3* gene in the regulation of the actin cytoskeleton BP. The muscle tissues belonging to the regions where the hernias occur are classified as skeletal striatum, which are formed by myofibrils composed by actin and myosin [85]. As mentioned by Bendavid [18], disturbances in the structures of muscle fibers cause low resistance in the inguinal region, which can lead to scrotal hernia.

The 53 SNPs observed in the SH group were located in 17 genes (Additional file 1: Table S4), which have been enriched in nine biological processes (Table 9). Most of these BP were related to homeostasis, which are processes that maintain the stability of the structure of the analyzed tissue (GO:0042592) [60]. The *ACACA* gene, enriched in these BP, participates in processes that maintain the stability of anatomical structures of the site [42]. *ACACA* was downregulated in the SH-affected animals (Table 9) indicating the development of an unstable structure of the inguinal ring, which can influence the development of hernia. In humans, this gene participates in the fatty acid synthesis BP [42], which reinforces the histopathological findings that showed greater amount of adipose tissue in normal than in SH-affected pigs (Fig. 2 A and B). From the SNPs found in the SH group, all variants were tolerated (Table 7). According to the SIFT score, two had a moderate impact classification, so they can alter the effectiveness of the encoded protein. This means that the function of the proteins resulting from these sites has not been altered, since the SIFT score is a tool that predicts whether the variant affects the function of the protein or not [35]. These SNPs were located in two genes, *RAI14* and *RALGAPA1*; the first has already been annotated and the second has no identification in the VEP tool. No high impact polymorphisms were identified in the SH group.

The 67 SNPs found in the UH group were mapped in 24 genes (Additional file 1: Table S5). These genes were enriched in six biological processes (Table 10) [40], all of which were related to some type of regulation, mainly metabolic. The *EPHB2* and *VIM* genes were

583 enriched in BP that interrupts the processes of cellular projections formation (GO: 0031345)
584 [60]. These two genes were upregulated in animals affected with HU when compared to the
585 control group (Table 10). The *VIM* gene encodes an intermediate filament protein that is part
586 of the cytoskeleton [42]. Lazarides [86] reported that high amount of this filament is observed
587 in the early stages of myogenesis in humans, and is hardly identified in adult muscles. Thus,
588 the levels of this protein indicate functionality feature. The upregulation of *VIM* in the
589 umbilical ring tissue of the UH-affected animals suggests that this gene may be involved with
590 a consequence of UH since Miller et al. [12] reported that the appearance of hernia can be a
591 consequence of a muscular defect.

592 Polymorphisms that had a high impact rating in the UH group (Additional file 1: Table
593 S5) were identified in two genes (*NCOA7* and *SEC62*). These variants still do not have
594 identification in the tool used, but they were classified as insertions of the Frameshift type.
595 Therefore, they can cause an interruption in the translation reading frame, because the number
596 of inserted nucleotides is not multiple of three [35]. The *NCOA7* (Nuclear receptor coactivator
597 7) is involved in the biological process of RNA polymerase II transcription and negative
598 regulation of the cellular response to oxidative stress. The *SEC62* (Preprotein translocation
599 factor) is related to the regulation of post-translational protein transport to the membrane BP
600 and was mapped in a QTL region for stillborn pigs [87]. The detection of these
601 polymorphisms is important because they can alter not only processes related to hernias, but
602 all important processes for biological maintenance, possibly resulting in transcription failures
603 or disruption in the transport of translated proteins by lack of regulation.

604 The SNPs classified as having moderate impact for the UH group were found in six
605 genes (Additional file 1: Table S5), with the SNP rs327289001 being highlighted due to its
606 deleterious SIFT score. This SNP is located in the *ITGAM* gene that participates in the
607 biological process of ectodermal cell differentiation [35]. This process is related to the

608 specialization of previously non-specialized cells, which acquire structure and functioning of
609 ectodermic cells. This differentiation integrates the processes involved in the commitment of a
610 cell to its specific purpose (GO: 0010668) [60]. In the embryonic gastrulation phase, the
611 formation of germ layers (ectoderm, mesoderm and endoderm) occurs, which will give rise to
612 specific tissues and organs [88]. The ectoderm is the external layer of a developing embryo
613 and gives rise to epidermis, hair, nails, cutaneous and mammary glands, tooth enamel, inner
614 ear, lens, and the anterior part of the pituitary gland, besides others related to the neural tube
615 and neural crest [88]. A SNP with deleterious SIFT score indicates that the function of the
616 protein can be altered due to the polymorphism, which in this case can result in non-
617 differentiated cells, compromising the formation of resistant tissues, which, when associated
618 with environmental factors such as obesity, can lead to hernia. SNPs located in QTL regions
619 associated with UH were not found in the current study.

620 The SNP rs339972872 from the SH group and the SNPs rs324236192 and
621 rs340781986 from the UH group were located in the same gene (*ACACA*) (Additional file 1:
622 Tables S4 and S5). These are synonym SNPs and were classified as low impact. According to
623 Stachowiak et al. [89], the *ACACA* gene is involved with performance traits in pigs.

624 From the expressed miRNAs, three were identified in the groups of both hernias. One
625 of them, *ssc-mir-214*, plays an important role in the regulation of ovarian function and in the
626 induction of granular ovarian cells to induce follicular development [90]. The *ssc-mir-145*,
627 which was identified only in samples from the SH group, is involved in the development of
628 adipose tissue [91].

629 We conducted the first comparative study of the pig inguinal and umbilical ring tissue
630 transcriptomes. The results demonstrated similarities related to the expression profile of the
631 whole transcriptome and DE genes in both types of hernia. The *ACAN* gene, which had
632 already been associated to the appearance of scrotal hernia, showed similar behavior in the

633 data obtained from the umbilical hernia group. Moreover, the *MAP1LC3C*, *VIT*, *ACER2*,
634 *KCNMA1* and *SYNPO2* genes were highlighted as candidates for the formation of the two
635 types of hernias evaluated in our studied for presenting equivalent expression in both hernias
636 and for being involved in biological processes such as cell adhesion, cytoskeleton
637 organization, collagen production, muscle relaxation and autophagy. However, further studies
638 are needed to identify the expression profile of these same genes in younger animals to
639 improve our interpretation of the gene regulation mechanisms triggering the formation of
640 hernias. The knowledge of the genetic factors that control the manifestation of both scrotal
641 and umbilical hernia brings possibilities to the pig production chain to develop actions to
642 reduce the appearance of these defects in their herds, aiming to reduce economical losses and
643 favoring the animal welfare.

644

645

646 **Conclusion**

647 The expression profile of the inguinal and umbilical ring transcriptomes showed great
648 similarity. Thirty-five differentially expressed genes between normal and affected samples
649 were common to both types of hernia. The *MAP1LC3C*, *ACAN*, *VIT*, *ACER2*, *KCNMA1* and
650 *SYNPO2* genes are indicated as strong candidates for the appearance of both defects. A total
651 of 11 and 14 new SNPs were identified in the samples related to the scrotal hernia and
652 umbilical hernia, respectively. Moreover, a SNP with predicted deleterious function was
653 identified in the *ITGAM* gene, which might be related to the appearance of umbilical hernia in
654 pigs. Finally, the expression profile of these genes possibly interferes with the normal
655 development of the tissues, causing weakness and decreasing the resistance of the site, which
656 can lead to the formation of both types hernia in pigs.

657

658 **List of abbreviations**

659 **BP**: Biological process.

660 **DE**: Differentially expressed.

661 **GATK**: Genome Analysis Tool Kit.

662 **GO**: Gene ontology.

663 **HE**: Hematoxylin and eosin.

664 **IH**: Inguinal hernia.

665 **MDS**: Multidimensional scaling.

666 **QTL**: Quantitative trait loci.

667 **SH**: Scrotal hernia.

668 **SIFT:** Sorting intolerant from tolerant.

669 **SNP**: Single nucleotide polymorphism.

670 **UH**: Umbilical hernia.

671 **VEP**: Variant Effect Predictor.

672

673 **Declarations**

674 **Ethics approval**: All animal procedures were performed in accordance with the Ethics
675 Committee on Animal Utilization of the Embrapa Swine and Poultry National Research
676 Center, under protocol number 011/2014.

Consent for publication: Not applicable.

Availability of data and material: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions: JOP, MCL and AMGI conceived and designed the experiment. JOP, AMGI, LODC, MAZM, IRS and MCL were responsible for the data collection. AFGR, AMGI, JOP, MAZM and MCL performed the experiment. AFGR, AMGI, HCO and MEC performed the data analysis. AFGR, AMGI, JOP, IRS, MRS, MEC and MCL interpreted the results and wrote the manuscript. All authors reviewed, edited and approved the final manuscript.

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Figure 1 Pathological analysis. Legend: A) Swine affected with scrotal hernia. B) Region affected with scrotal hernia (inguinal ring). C) Swine affected with umbilical hernia. D) Region affected with umbilical hernia (umbilical ring).

Figure 2 Histopathological slide stained with hematoxylin and eosin (HE). Legend: A) Sample from the SH-control group and B) Sample from the SH-affected group. A larger number of connective fibers is observed in the sample of the SH-affected group than in the sample from the SH-control group. C) Sample from the UH-control group and D) Sample from the UH-affected group. Connective tissue interspersed with adipose tissue is observed in the sample of the UH-control group, while in the sample from the UH-affected group, only proliferated connective tissue is observed.

Figure 3 Distribution of transcripts identified in the pig inguinal and umbilical ring tissue samples. Legend: SH – Scrotal hernia group; UH – Umbilical hernia group. For the SH, the inguinal ring tissue was evaluated, and for the UH, the umbilical ring tissue was analyzed.

Figure 4 Common differentially expressed genes for scrotal and umbilical hernias and their respective control groups. Legend: A) Genes with similar expression profile and B) with opposite expression profile in the two types of hernia based on LogFC.

Figure 5 Gene interaction network with differentially expressed genes common to both scrotal and umbilical hernias. Legend: Gene network built with 27 of the 35 differentially expressed genes common to both types of hernia obtained with the STRING database using information from *Sus scrofa* proteins.

Figure 6 Impact caused by variants and its frequency. Legend: A) Samples from the scrotal hernia group. B) Samples from the umbilical hernia group.

Additional file 1:

.docx

Table S1 Average of reads sequenced, removed in the quality control analysis and mapped in each group of samples. Legend: SH – N: Samples from the scrotal hernia group – normal pigs. SH – A: Samples from the scrotal hernia group – affected pigs. UH – N: Samples from the umbilical hernia group – normal pigs. UH– A: Samples from the umbilical hernia group – affected pigs.

Table S2 Biological processes of differentially expressed genes in the groups of the two types of hernia, comparing normal and affected pigs. Legend: Genes in bold were upregulated in the affected group for both types of hernia, and those underlined were downregulated for one type of hernia and e upregulated for the other type of hernia.

Table S3 Enrichment for biological process of the 26 DE genes with similar expression profile between both types of hernias.

Table S4 Polymorphisms identified in samples of the pig inguinal ring.

Table S5 Polymorphisms identified in samples of the pig umbilical ring.

Additional file 2:

.docx

Figure S1 Multidimentional scaling (MDS) plot showing the samples used to generate the transcriptome of the inguinal ring for scrotal hernia (SH) in red and the transcriptome of the umbilical ring for umbilical hernia (UH) in blue.

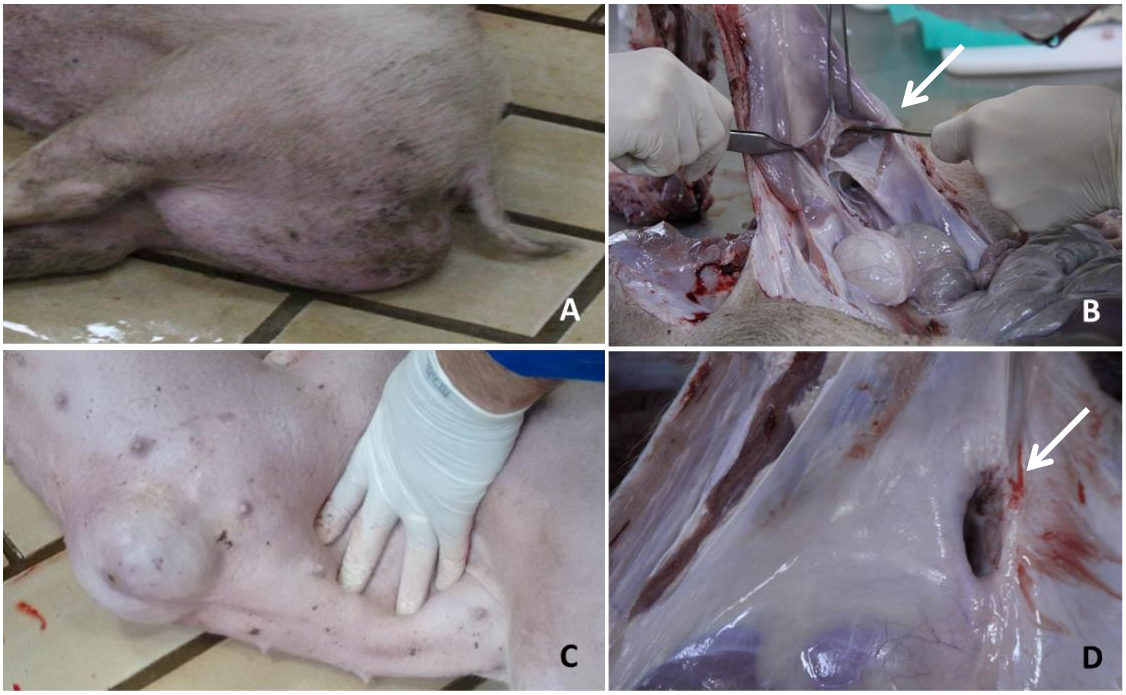


Figure 1 Pathological analysis. Legend: A) Swine affected with scrotal hernia. B) Region affected with scrotal hernia (inguinal ring). C) Swine affected with umbilical hernia. D) Region affected with umbilical hernia (umbilical ring).

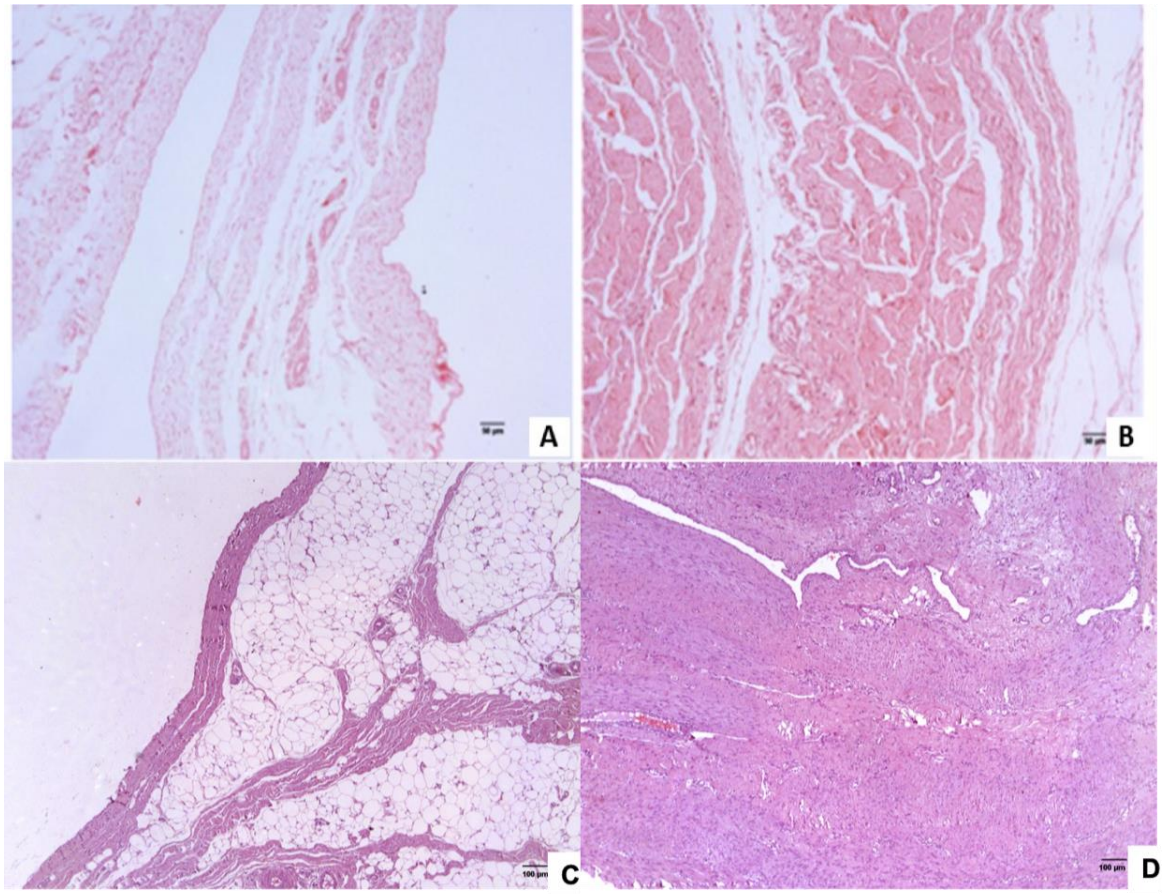


Figure 2 Histopathological slide stained with hematoxylin and eosin (HE). Legend: A) Sample from the SH-control group and B) Sample from the SH-affected group. A larger number of connective fibers is observed in the sample of the SH-affected group than in the sample from the SH-control group. C) Sample from the UH-control group and D) Sample from the UH-affected group. Connective tissue interspersed with adipose tissue is observed in the sample of the UH-control group, while in the sample from the UH-affected group, only proliferated connective tissue is observed.

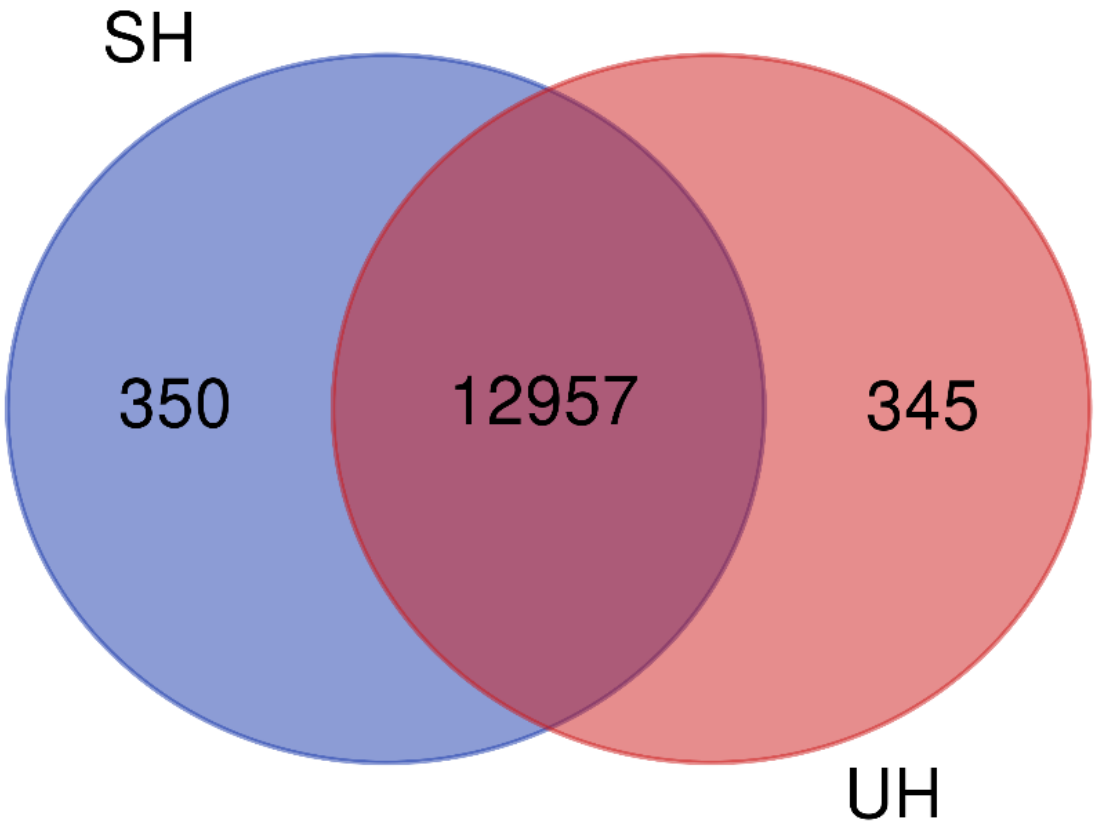
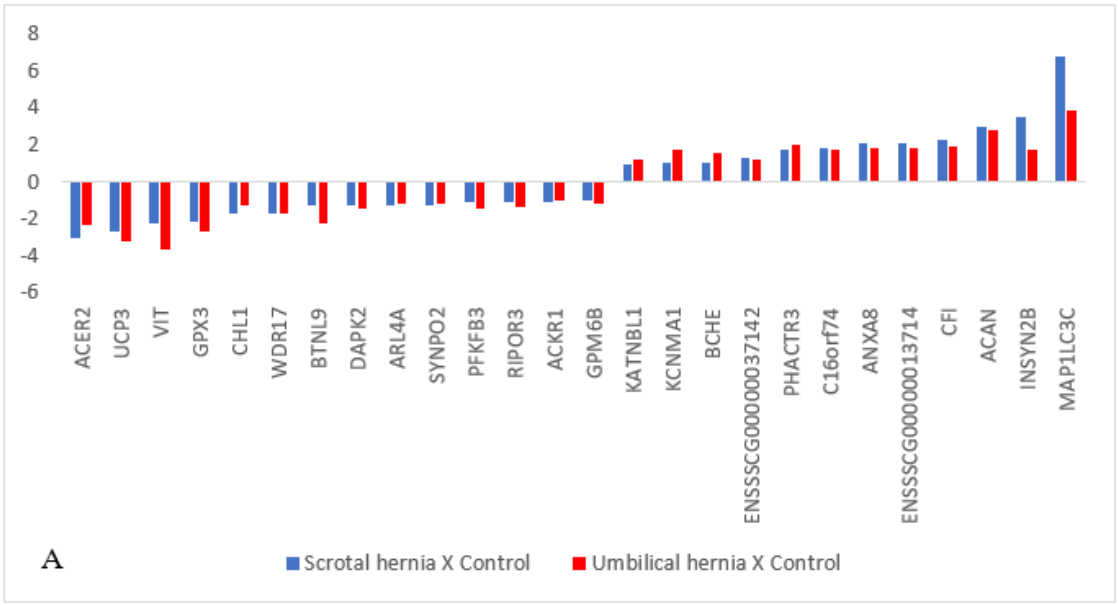


Figure 3 Distribution of transcripts identified in the pig inguinal and umbilical ring tissue samples. Legend: SH – Scrotal hernia group; UH – Umbilical hernia group. For the SH, the inguinal ring tissue was evaluated, and for the UH, the umbilical ring tissue was analyzed.



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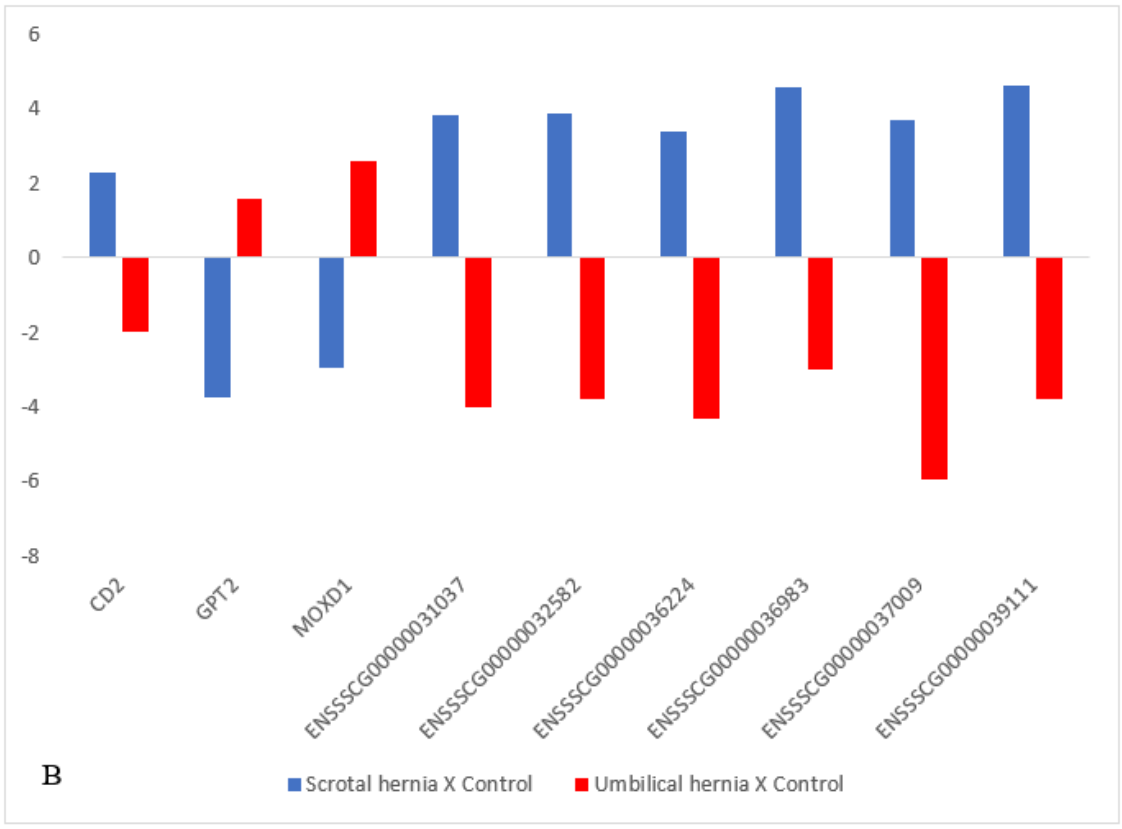

1084

1085 **Figure 4 Common differentially expressed genes for scrotal and umbilical hernias and**
1086 **their respective control groups.** Legend: A) Genes with similar expression profile and B)
1087 with opposite expression profile in the two types of hernia based on the LogFC.

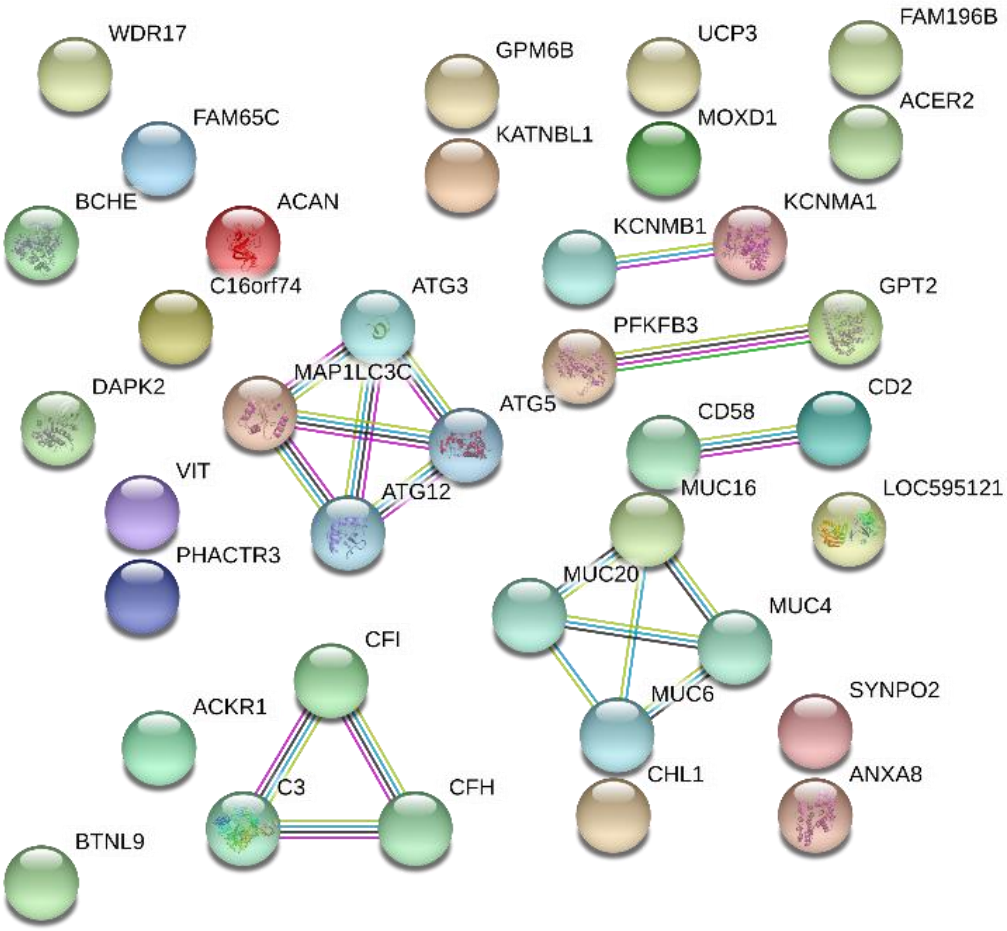

Figure 5 Gene interaction network with differentially expressed genes common to both scrotal and umbilical hernias. Legend: Gene network built with 27 of the 35 differentially expressed genes common to both types of hernia obtained with the STRING database using information from *Sus scrofa* proteins.

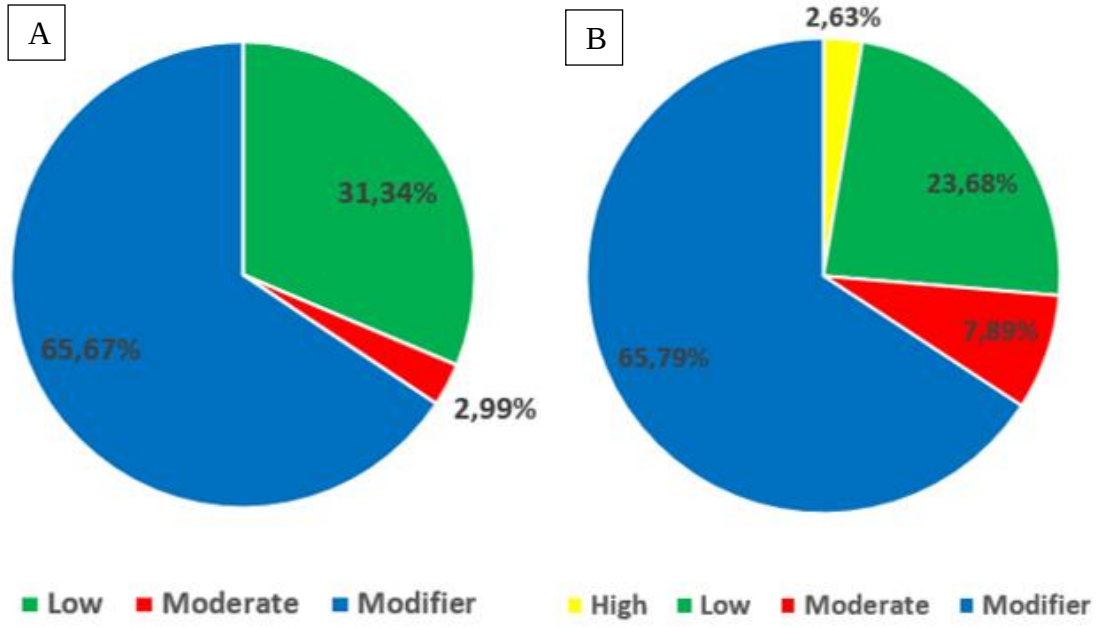

Figure 6 Impact caused by variants and its frequency. Legend: A) Samples from the scrotal hernia group. B) Samples from the umbilical hernia group.

Additional file 1

Table S1 Average of reads sequenced, removed in the quality control analysis and mapped in each group of samples.

Samples	Sequenced reads	Removed reads	Pairs reads	Mapped reads	% of mapped reads	Reads mapped in genes	% of reads mapped in genes
SH – N	28362296	5693664	22668632	21337739	94.10	17756382	83.22
SH – A	27838413	4232821	23605592	21850701	92.60	18446649	84.42
UH – N	24356034	2727661	21628373	19902246	91.99	16584959	83.33
UH – A	23738367	2214464	21523903	20502663	95.24	17607460	85.88

Legend: SH – N: Samples from the scrotal hernia group – normal pigs.
SH – A: Samples from the scrotal hernia group – affected pigs.
UH – N: Samples from the umbilical hernia group – normal pigs.
UH– A: Samples from the umbilical hernia group – affected pigs.

Table S2 Biological processes of the 35 differentially expressed genes common to both types of hernia, comparing normal and affected pigs.

Term_ID	David biological processes	Enriched genes
GO:0001502	Cartilage condensation	***ACAN***
GO:0007155	Cell adhesion	***ACAN***, *CD2*
GO:0007417	Central nervous system development	***ACAN***
GO:0002063	Chondrocyte development	***ACAN***
GO:0030199	Collagen fibril organization	***ACAN***
GO:0007507	Heart development	***ACAN***
GO:0030166	Proteoglycan biosynthetic process	***ACAN***
GO:0001501	Skeletal system development	***ACAN***
GO:0009058	Biosynthetic process	*GPT2*
GO:0042420	Dopamine catabolic process	*MOXD1*
GO:0042421	Norepinephrine biosynthetic process	*MOXD1*
GO:0006589	Octopamine biosynthetic process	*MOXD1*
GO:0055114	Oxidation-reduction process	*MOXD1*, *GPX3*
GO:0032515	Negative regulation of phosphoprotein phosphatase activity	***PHACTR3***
GO:0043666	Regulation of phosphoprotein phosphatase activity	***PHACTR3***
GO:0030198	Extracellular matrix organization	*VIT*
GO:0003429	Growth plate cartilage chondrocyte morphogenesis	*VIT*
GO:0010811	Positive regulation of cell-substrate adhesion	*VIT*
GO:0032233	Positive regulation of actin filament bundle assembly	*SYNPO2*
GO:0006897	Endocytosis	***CFI***
GO:0006508	Proteolysis	***CFI***
GO:0030007	Cellular potassium ion homeostasis	***KCNMA1***
GO:0034220	Ion transmembrane transport	***KCNMA1***
GO:0006811	Ion transport	***KCNMA1***
GO:0060073	Micturition	***KCNMA1***
GO:0045794	Negative regulation of cell volume	***KCNMA1***
GO:0043065	Positive regulation of apoptotic process	***KCNMA1***
GO:1902632	Positive regulation of membrane hyperpolarization	***KCNMA1***
GO:0071805	Potassium ion transmembrane transport	***KCNMA1***
GO:0006813	Potassium ion transport	***KCNMA1***
GO:0034765	Regulation of ion transmembrane transport	***KCNMA1***
GO:0042391	Regulation of membrane potential	***KCNMA1***
GO:0060087	Relaxation of vascular smooth muscle	***KCNMA1***
GO:0051592	Response to calcium ion	***KCNMA1***
GO:0034465	Response to carbon monoxide	***KCNMA1***
GO:0001666	Response to hypoxia	***KCNMA1***
GO:0006970	Response to osmotic stress	***KCNMA1***
GO:0060083	Smooth muscle contraction involved in micturition	***KCNMA1***
GO:0055085	Transmembrane transport	***KCNMA1***
GO:0016197	Endosomal transport	***ANXA8***

GO:0007032	Endosome organization	***ANXA8***
GO:1900138	Negative regulation of phospholipase A2 activity	***ANXA8***
GO:1900004	Negative regulation of serine-type endopeptidase activity	***ANXA8***
GO:0046835	Carbohydrate phosphorylation	*PFKFB3*
GO:0006003	Fructose 2,6-bisphosphate metabolic process	*PFKFB3*
GO:0006000	Fructose metabolic process	*PFKFB3*
GO:0016310	Phosphorylation	*PFKFB3, DAPK2*
GO:0008344	Adult locomotory behavior	*CHL1*
GO:0007411	Axon guidance	*CHL1*
GO:0050890	Cognition	*CHL1*
GO:0070593	Dendrite self-avoidance	*CHL1*
GO:0035640	Exploration behavior	*CHL1*
GO:0007156	Homophilic cell adhesion via plasma membrane adhesion molecules	*CHL1*
GO:0043524	Negative regulation of neuron apoptotic process	*CHL1*
GO:0001764	Neuron migration	*CHL1*
GO:0031175	Neuron projection development	*CHL1, GPM6B*
GO:0051612	Negative regulation of serotonin uptake	*GPM6B*
GO:1990845	Adaptive thermogenesis	*UCP3*
GO:1990542	Mitochondrial transmembrane transport	*UCP3*
GO:0006839	Mitochondrial transport	*UCP3*
GO:1902600	Proton transmembrane transport	*UCP3*
GO:0009409	Response to cold	*UCP3*
GO:0008150	Biological_process	*WDR17*
GO:0006468	Protein phosphorylation	*DAPK2*
GO:0050776	Regulation of immune response	*BTNL9*
GO:0050852	T cell receptor signaling pathway	*BTNL9*
GO:0006955	Immune response	*ENSSSCG00000031037, ENSSSCG00000036224*
GO:0002377	Immunoglobulin production	*ENSSSCG00000031037, ENSSSCG00000036224*
GO:0050873	Brown fat cell differentiation	*ARL4A*
GO:0006886	Intracellular protein transport	*ARL4A*
GO:0016192	Vesicle-mediated transport	*ARL4A*
GO:0006919	Activation of cysteine-type endopeptidase activity involved in apoptotic process	*ACER2*
GO:0006974	Cellular response to DNA damage stimulus	*ACER2*
GO:0035690	Cellular response to drug	*ACER2*
GO:0046514	Ceramide catabolic process	*ACER2*
GO:0006672	Ceramide metabolic process	*ACER2*
GO:0030330	DNA damage response, signal transduction by p53 class mediator	*ACER2*
GO:0006629	Lipid metabolic process	*ACER2*
GO:0033629	Negative regulation of cell adhesion mediated by integrin	*ACER2*
GO:0001953	Negative regulation of cell-matrix adhesion	*ACER2*
GO:0090285	Negative regulation of protein glycosylation in Golgi	*ACER2*

GO:0010942	Positive regulation of cell death	*ACER2*
GO:0008284	Positive regulation of cell population proliferation	*ACER2*
GO:0042981	Regulation of apoptotic process	*ACER2*
GO:0010506	Regulation of autophagy	*ACER2*
GO:0032526	Response to retinoic acid	*ACER2*
GO:0046512	Sphingosine biosynthetic process	*ACER2*
GO:0035973	Aggrephagy	***MAP1LC3C***
GO:0000045	Autophagosome assembly	***MAP1LC3C***
GO:0097352	Autophagosome maturation	***MAP1LC3C***
GO:0006914	Autophagy	***MAP1LC3C***
GO:0000422	Autophagy of mitochondrion	***MAP1LC3C***
GO:0006995	Cellular response to nitrogen starvation	***MAP1LC3C***
GO:0009267	Cellular response to starvation	***MAP1LC3C***
GO:0016236	Macroautophagy	***MAP1LC3C***
GO:0070098	Chemokine-mediated signaling pathway	*ACKR1*
GO:0006954	Inflammatory response	*ACKR1*
GO:0032642	Regulation of chemokine production	*ACKR1*
GO:0006958	Complement activation, classical pathway	*ENSSSCG00000036224*
GO:0042742	Defense response to bacterium	*ENSSSCG00000036224*
GO:0006911	Phagocytosis, engulfment	*ENSSSCG00000036224*
GO:0006910	Phagocytosis, recognition	*ENSSSCG00000036224*
GO:0050871	Positive regulation of B cell activation	*ENSSSCG00000036224*
GO:0098869	Cellular oxidant detoxification	*GPX3*
GO:0042744	Hydrogen peroxide catabolic process	*GPX3*
GO:0051289	Protein homotetramerization	*GPX3*
GO:0006979	Response to oxidative stress	*GPX3*
GO:0030036	Actin cytoskeleton organization	***ENSSSCG00000037142***

Legend: Genes in bold were upregulated in the affected group for both types of hernia, and those underlined were downregulated for one type of hernia and upregulated for the other type of hernia.

Table S3 Enrichment for biological process of the 26 DE genes with similar expression profile between both types of hernias.

Ensembl ID	GO Term	Biological process
ENSSSCG00000037142	GO:0030036	Actin cytoskeleton organization
ENSSSCG00000034213	GO:0006919	Activation of cysteine-type endopeptidase activity involved in the apoptotic process
ENSSSCG00000014834	GO:1990845	Adaptive thermogenesis
ENSSSCG00000011524	GO:0008344	Adult locomotive behavior
ENSSSCG00000034838	GO:0035973	Aggrephagy
ENSSSCG00000034838	GO:0000045	Autophagosome assembly
ENSSSCG00000034838	GO:0097352	Autophagosome maturation
ENSSSCG00000034838	GO:0006914	Autophagy
ENSSSCG00000034838	GO:0000422	Mitochondria autophagy
ENSSSCG00000011524	GO:0007411	Axonal orientation
ENSSSCG00000015766	GO:0008150	Biological process
ENSSSCG00000032709	GO:0050873	Differentiation of brown fat cells
ENSSSCG00000011133	GO:0046835	Carbohydrate phosphorylation
ENSSSCG00000001832	GO:0001502	Condensation of cartilage
ENSSSCG00000001832	GO:0007155	Cell adhesion
ENSSSCG00000036438	GO:0098869	Cell oxidant detoxification
ENSSSCG00000010325	GO:0030007	Cell potassium ion homeostasis
ENSSSCG00000034213	GO:0006974	Cellular response to DNA damage stimulus
ENSSSCG00000034213	GO:0035690	Cellular response to the drug
ENSSSCG00000034838	GO:0006995	Cellular response to nitrogen starvation
ENSSSCG00000034838	GO:0009267	Cellular response to starvation
ENSSSCG00000001832	GO:0007417	Central nervous system development
ENSSSCG00000034213	GO:0046514	Catabolic ceramide process
ENSSSCG00000034213	GO:0006672	Metabolic process of ceramide
ENSSSCG00000036223	GO:0070098	Chemokine-mediated signaling pathway
ENSSSCG00000001832	GO:0002063	Chondrocyte development
ENSSSCG00000011524	GO:0050890	Cognition
ENSSSCG00000001832	GO:0030199	Organization of collagen fibrils
ENSSSCG00000011524	GO:0070593	Dendrite self-avoidance
ENSSSCG00000034213	GO:0030330	Response to DNA damage, signal transduction by the mediator of class p53
ENSSSCG00000009138	GO:0006897	Endocytosis
ENSSSCG00000010370	GO:0016197	Endosomal transport
ENSSSCG00000010370	GO:0007032	Endosomal organization

ENSSSCG00000011524	GO:0035640	Exploration behavior
ENSSSCG00000008501	GO:0030198	Organization of the extracellular matrix
ENSSSCG00000011133	GO:0006003	Metabolic process of fructose 2,6-bisphosphate
ENSSSCG00000011133	GO:0006000	Fructose metabolic process
ENSSSCG00000008501	GO:0003429	Morphogenesis of chondrocytes in the growth plate cartilage
ENSSSCG00000001832	GO:0007507	Heart development
ENSSSCG00000011524	GO:0007156	Homophilic cell adhesion via plasma membrane adhesion molecules
ENSSSCG00000036438	GO:0042744	Hydrogen peroxide catabolic process
ENSSSCG00000036223	GO:0006954	Inflammatory response
ENSSSCG00000032709	GO:0006886	Intracellular protein transport
ENSSSCG00000010325	GO:0034220	Ion transmembrane transport
ENSSSCG00000010325	GO:0006811	Ion transport
ENSSSCG00000034213	GO:0006629	Lipid metabolic process
ENSSSCG00000034838	GO:0016236	Macroautophagy
ENSSSCG00000010325	GO:0060073	Urination
ENSSSCG00000014834	GO:1990542	Mitochondrial transmembrane transport
ENSSSCG00000014834	GO:0006839	Mitochondrial transport
ENSSSCG00000034213	GO:0033629	Negative regulation of cell adhesion mediated by integrin
ENSSSCG00000010325	GO:0045794	Negative regulation of cell volume
ENSSSCG00000034213	GO:0001953	Negative regulation of cell matrix adhesion
ENSSSCG00000011524	GO:0043524	Negative regulation of the neuron apoptotic process
ENSSSCG00000010370	GO:1900138	Negative regulation of phospholipase A2 activity
ENSSSCG00000007528	GO:0032515	Negative regulation of phosphoprotein phosphatase activity
ENSSSCG00000034213	GO:0090285	Negative regulation of protein glycosylation in Golgi
ENSSSCG00000010370	GO:1900004	Negative regulation of serine endopeptidase activity
ENSSSCG00000012126	GO:0051612	Negative regulation of serotonin uptake
ENSSSCG00000011524	GO:0001764	Neuron migration
ENSSSCG00000011524	GO:0031175	Development of neuron projection
ENSSSCG00000012126	GO:0031175	Development of neuron projection
ENSSSCG00000036438	GO:0055114	Oxidation reduction process
ENSSSCG00000011133	GO:0016310	Phosphorylation
ENSSSCG00000021588	GO:0016310	Phosphorylation
ENSSSCG00000009111	GO:0032233	Positive regulation of the actin filament bundle assembly
ENSSSCG00000010325	GO:0043065	Positive regulation of the apoptotic process

ENSSSCG00000034213	GO:0010942	Positive regulation of cell death
ENSSSCG00000034213	GO:0008284	Positive regulation of cell population proliferation
ENSSSCG00000008501	GO:0010811	Positive regulation of cell substrate adhesion
ENSSSCG00000010325	GO:1902632	Positive regulation of membrane hyperpolarization
ENSSSCG00000010325	GO:0071805	Transmembrane transport of potassium ions
ENSSSCG00000010325	GO:0006813	Transport of potassium ions
ENSSSCG00000036438	GO:0051289	Protein homotetramerization
ENSSSCG00000021588	GO:0006468	Protein phosphorylation
ENSSSCG00000001832	GO:0030166	Proteoglycan biosynthetic process
ENSSSCG00000009138	GO:0006508	Proteolysis
ENSSSCG00000014834	GO:1902600	Transmembrane proton transport
ENSSSCG00000034213	GO:0042981	Regulation of the apoptotic process
ENSSSCG00000034213	GO:0010506	Autophagy regulation
ENSSSCG00000036223	GO:0032642	Regulation of chemokine production
ENSSSCG00000028567	GO:0050776	Regulation of the immune response
ENSSSCG00000010325	GO:0034765	Regulation of transmembrane ion transport
ENSSSCG00000010325	GO:0042391	Regulation of the membrane potential
ENSSSCG00000007528	GO:0043666	Regulation of phosphoprotein phosphatase activity
ENSSSCG00000010325	GO:0060087	Vascular smooth muscle relaxation
ENSSSCG00000010325	GO:0051592	Response to calcium ion
ENSSSCG00000010325	GO:0034465	Response to carbon monoxide
ENSSSCG00000014834	GO:0009409	Cold response
ENSSSCG00000010325	GO:0001666	Response to hypoxia
ENSSSCG00000010325	GO:0006970	Response to osmotic stress
ENSSSCG00000036438	GO:0006979	Response to oxidative stress
ENSSSCG00000034213	GO:0032526	Response to retinoic acid
ENSSSCG00000001832	GO:0001501	Development of the skeletal system
ENSSSCG00000010325	GO:0060083	Smooth muscle contraction involved in urination
ENSSSCG00000034213	GO:0046512	Sphingosine biosynthetic process
ENSSSCG00000028567	GO:0050852	T cell receptor signaling pathway
ENSSSCG00000010325	GO:0055085	Transmembrane transport
ENSSSCG00000032709	GO:0016192	Vesicle-mediated transport

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Table S4 Polymorphisms identified in samples of the pig inguinal ring.

Variant	Polymorphism type	Location	Consequence	Impact	Symbol	Gene
New	Deletion	6:82851652-82851653	Intronic	Modifier	*RSRP1*	ENSSSCG00000034449
New	Deletion	7:104221878-104221879	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
rs1108216348	Deletion	2:134533476-134533477	UTR5'	Modifier	*P4HA2*	ENSSSCG00000020915
rs1114124998	Deletion	5:9499095-9499096	Intronic	Modifier	*DDX17*	ENSSSCG00000000104
New	Insertion	13:122703207-122703207	UTR5'	Modifier	*VPS8*	ENSSSCG00000011788
New	Insertion	14:37596310-37596310	UTR3'	Modifier	*TBX3*	ENSSSCG00000009865
New	Insertion	2:101385604-101385604	UTR3'	Modifier	*MCTP1*	ENSSSCG00000025286
New	Insertion	7:104221039-104221039	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
New	Insertion	7:104221369-104221369	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
New	Insertion	7:104223100-104223100	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
New	Insertion	8:99783036-99783036	UTR5'	Modifier	*ANKRD50*	ENSSSCG00000022173
rs698169541	Insertion	7:64368649-64368649	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs699814235	Insertion	1:45178864-45178864	UTR3'	Modifier	*PTP4A1*	ENSSSCG00000025941
rs707857689	Insertion	7:64370059-64370059	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
New	SNP	13:34083960-34083960	UTR3'	Modifier	*PARP3*	ENSSSCG00000023033
New	SNP	7:64303141-64303141	Missense	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs318677442	SNP	2:134534068-134534068	Intronic	Modifier	*P4HA2*	ENSSSCG00000020915
rs319937494	SNP	7:12137039-12137039	UTR3'	Modifier	*MYLIP*	ENSSSCG00000001063
rs320772137	SNP	2:134568695-134568695	Intronic	Modifier	*PDLIM4*	ENSSSCG00000014274
rs320812009	SNP	7:64369632-64369632	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs321437076	SNP	2:15365561-15365561	Intronic	Modifier	*DDB2*	ENSSSCG00000013243
rs321448108	SNP	7:104223338-104223338	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414

rs321482827	SNP	16:20430280-20430280	Synonym	Low	*RAI14*	ENSSSCG00000016824
rs322028481	SNP	7:64370049-64370049	Downstream	Modifier	*INSM2*	ENSSSCG00000001949
rs323170636	SNP	2:134520258-134520258	Synonym	Low	*P4HA2*	ENSSSCG00000020915
rs323684157	SNP	7:104256746-104256746	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs324657636	SNP	2:134567763-134567763	Intronic	Modifier	*P4HA2*	ENSSSCG00000020915
rs325370594	SNP	16:20418972-20418972	Missense	Modifier	*RAI14*	ENSSSCG00000016824
rs326057680	SNP	4:88876043-88876043	Intronic	Modifier	-	ENSSSCG00000006350
rs326292614	SNP	7:12136748-12136748	UTR3'	Modifier	*MYLIP*	ENSSSCG00000001063
rs327405343	SNP	2:134570264-134570264	UTR3'	Modifier	*PDLIM4*	ENSSSCG00000014274
rs328500299	SNP	2:134502458-134502458	UTR3'	Modifier	*P4HA2*	ENSSSCG00000020915
rs328617030	SNP	14:37595933-37595933	UTR3'	Modifier	*TBX3*	ENSSSCG00000009865
rs330964251	SNP	2:134569316-134569316	Splicing region Synonym	Low	*PDLIM4*	ENSSSCG00000014274
rs330986417	SNP	4:88559715-88559715	Synonym	Low	*OLFML2B*	ENSSSCG00000006345
rs331580151	SNP	2:134523104-134523104	Synonym	Low	*P4HA2*	ENSSSCG00000020915
rs331672044	SNP	7:104234431-104234431	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs331683704	SNP	7:12136979-12136979	UTR3'	Modifier	*MYLIP*	ENSSSCG00000001063
rs332730821	SNP	7:104239412-104239412	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs333120728	SNP	7:104256830-104256830	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs333503612	SNP	7:64368656-64368656	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs334410412	SNP	2:134520288-134520288	Synonym	Low	*P4HA2*	ENSSSCG00000020915
rs334902692	SNP	7:104247760-104247760	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs336367802	SNP	7:12133527-12133527	Synonym	Low	*MYLIP*	ENSSSCG00000001063
rs337177437	SNP	7:104254924-104254924	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs337356541	SNP	2:134566986-134566986	Intronic	Modifier	*PDLIM4*	ENSSSCG00000014274
rs337778431	SNP	2:134568657-134568657	Intronic	Modifier	*PDLIM4*	ENSSSCG00000014274
rs338426616	SNP	2:134570329-	UTR3'	Modifier	*PDLIM4*	ENSSSCG00000014274

		134570329				
rs339297902	SNP	7:64369109-64369109	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs339523164	SNP	7:104223402-104223402	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
rs339972872	SNP	12:38745989-38745989	Synonym	Low	*ACACA*	ENSSSCG00000017694
rs341675974	SNP	7:12136596-12136596	UTR3'	Modifier	*MYLIP*	ENSSSCG00000001063
rs341856430	SNP	7:64368731-64368731	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs342773249	SNP	7:64369687-64369687	Intronic	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs343682724	SNP	4:88846962-88846962	UTR3'	Modifier	*FCRLA*	ENSSSCG00000030246
rs343732012	SNP	7:64372117-64372117	UTR3'	Modifier	*RALGAPA1*	ENSSSCG00000032377
rs344350584	SNP	2:134505397-134505397	Synonym	Low	*P4HA2*	ENSSSCG00000020915
rs345069964	SNP	6:74482956-74482956	UTR5'	Modifier	*TMEM51*	ENSSSCG00000021026
rs345905406	SNP	7:104247817-104247817	Synonym	Low	*SEL1L*	ENSSSCG00000002414
rs346022448	SNP	2:134502393-134502393	UTR3'	Modifier	*P4HA2*	ENSSSCG00000020915
rs695824576	SNP	7:59713114-59713114	Synonym	Low	*CD276*	ENSSSCG00000001917
rs700364715	SNP	7:104221886-104221886	UTR3'	Modifier	*SEL1L*	ENSSSCG00000002414
rs712681004	SNP	2:9762085-9762085	UTR3'	Modifier	-	ENSSSCG00000013078
rs80801180	SNP	4:88559748-88559748	Synonym	Low	*OLFML2B*	ENSSSCG00000006345
rs81214013	SNP	2:134514074-134514074	Synonym	Low	*P4HA2*	ENSSSCG00000020915
rs81218171	SNP	2:134570121-134570121	Synonym	Low	*PDLIM4*	ENSSSCG00000014274
rs81365336	SNP	2:134516657-134516657	Synonym	Low	*P4HA2*	ENSSSCG00000020915

1180 **Table S5 Polymorphisms identified in samples of the pig umbilical ring.**
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Variant	Polymorphism type	Location	Consequence	Impact	Symbol	Gene
New	Deletion	3:16844063-16844064	UTR3'	Modifier	-	ENSSSCG00000020808
New	Deletion	3:17617319-17617323	UTR3'	Modifier	*ZNF629*	ENSSSCG00000007780
New	Deletion	6:80843072-80843073	UTR3'	Modifier	*EPHB2*	ENSSSCG00000003527
New	Deletion	6:80843074-80843081	UTR3'	Modifier	*EPHB2*	ENSSSCG00000003527
New	Insertion	1:37122230-37122230	Frameshift	High	*NCOA7*	ENSSSCG00000004222
New	Insertion	1:77585583-77585583	UTR3'	Modifier	*FYN*	ENSSSCG00000004421
New	Insertion	13:108676707-108676707	Frameshift	High	*SEC62*	ENSSSCG00000029608
New	Insertion	4:128903094-128903094	Intronic	Modifier	-	ENSSSCG00000047605
rs709055765	Insertion	6:82481037-82481037	UTR3'	Modifier	*CLIC4*	ENSSSCG00000038994
rs710955781	Insertion	13:50397619-50397619	UTR3'	Modifier	*EOGT*	ENSSSCG00000011504
New	SNP	12:3760896-3760896	UTR3'	Modifier	*AFMID*	ENSSSCG00000033636
New	SNP	13:207513656-207513656	UTR3'	Modifier	*ITGB2*	ENSSSCG00000025133
New	SNP	3:16078557-16078557	Synonym	Low	-	ENSSSCG00000007733
New	SNP	3:17082026-17082026	UTR3'	Modifier	*C16orf58*	ENSSSCG00000007753
New	SNP	3:17591144-17591144	Downstream	Modifier	*BCL7C*	ENSSSCG00000007776
New	SNP	3:51803551-51803551	UTR3'	Modifier	*IL18R1*	ENSSSCG00000008159
rs1107804156	SNP	3:17618533-17618533	Intronic	Modifier	*ZNF629*	ENSSSCG00000007780
rs1108762720	SNP	3:16851119-16851119	Synonym	Low	-	ENSSSCG00000020808
rs320729536	SNP	3:16382619-16382619	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs322669402	SNP	3:17459844-17459844	UTR3'	Modifier	*HSD3B7*	ENSSSCG00000032369
rs323015047	SNP	6:119763116-119763116	UTR3'	Modifier	*RPRD1A*	ENSSSCG00000027700
rs323115420	SNP	3:16964045-16964045	Missense	Modifier	*ZNF713*	ENSSSCG00000029029
rs323662654	SNP	3:16384658-16384658	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs323726488	SNP	3:16383246-16383246	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs324198007	SNP	3:17491763-17491763	Synonym	Low	*ORAI3*	ENSSSCG00000007770
rs324205762	SNP	3:16971143-16971143	UTR5'	Modifier	*ZNF713*	ENSSSCG00000029029
rs324236192	SNP	12:38624714-38624714	Synonym	Low	*ACACA*	ENSSSCG00000017694
rs324583382	SNP	3:16384043-16384043	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs325089032	SNP	6:81571496-81571496	Missense	Modifier	*ELOA*	ENSSSCG00000025440

rs325937498	SNP	3:17466691-17466691	Synonym	Low	*SETD1A*	ENSSSCG00000007782
rs326053487	SNP	3:16383454-16383454	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs326115442	SNP	6:80824416-80824416	Synonym	Low	*EPHB2*	ENSSSCG00000003527
rs326942919	SNP	3:17246305-17246305	Synonym	Low	*ITGAM*	ENSSSCG00000007754
rs327289001	SNP	3:17254444-17254444	Missense	Modifier	*ITGAM*	ENSSSCG00000007754
rs327572607	SNP	6:80837841-80837841	Synonym	Low	*EPHB2*	ENSSSCG00000003527
rs327947675	SNP	3:17399455-17399455	Synonym	Low	*ZNF646*	ENSSSCG00000026817
rs329707669	SNP	3:16383919-16383919	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs330195537	SNP	10:43520964-43520964	Intronic	Modifier	*VIM*	ENSSSCG00000011033
rs330731365	SNP	3:16844265-16844265	UTR3'	Modifier	-	ENSSSCG00000020808
rs330957838	SNP	3:17468302-17468302	Missense	Modifier	*SETD1A*	ENSSSCG00000007782
rs331463738	SNP	12:38129509-38129509	UTR3'	Modifier	*DHRS11*	ENSSSCG00000017690
rs332268785	SNP	3:16385727-16385727	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs333208968	SNP	3:16383250-16383250	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs333661817	SNP	3:16383284-16383284	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs333780109	SNP	3:16971156-16971156	UTR5'	Modifier	*ZNF713*	ENSSSCG00000029029
rs334463568	SNP	18:11660822-11660822	UTR3'	Modifier	*CREB3L2*	ENSSSCG00000016520
rs335540465	SNP	3:17613531-17613531	Synonym	Low	*ZNF629*	ENSSSCG00000007780
rs336364520	SNP	3:16383470-16383470	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs337670844	SNP	3:17399477-17399477	Missense	Modifier	*ZNF646*	ENSSSCG00000026817
rs339276563	SNP	3:17468216-17468216	Synonym	Low	*SETD1A*	ENSSSCG00000007782
rs339771716	SNP	3:17461694-17461694	Synonym	Low	*HSD3B7*	ENSSSCG00000032369
rs339771716	SNP	3:17461694-17461694	Downstream	Modifier	*STX1B*	ENSSSCG00000021238
rs340480028	SNP	3:17460060-17460060	UTR3'	Modifier	*HSD3B7*	ENSSSCG00000032369
rs340781986	SNP	12:38624687-38624687	Synonym	Low	*ACACA*	ENSSSCG00000017694
rs341960076	SNP	3:16855237-16855237	Synonym	Low	-	ENSSSCG00000020808
rs342012840	SNP	3:16971089-16971089	UTR5'	Modifier	*ZNF713*	ENSSSCG00000029029
rs342283188	SNP	6:80843098-80843098	UTR3'	Modifier	*EPHB2*	ENSSSCG00000003527
rs343615406	SNP	3:16971169-16971169	UTR5'	Modifier	*ZNF713*	ENSSSCG00000029029
rs343894209	SNP	3:17466745-17466745	Synonym	Low	*SETD1A*	ENSSSCG00000007782
rs343913735	SNP	3:16844271-16844271	UTR3'	Modifier	-	ENSSSCG00000020808
rs344858642	SNP	3:17244108-17244108	Synonym	Low	*ITGAM*	ENSSSCG00000007754
rs344892486	SNP	3:17610468-17610468	UTR5'	Modifier	*ZNF629*	ENSSSCG00000007780

rs345204099	SNP	3:16384270-16384270	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs345481021	SNP	6:80824380-80824380	Synonym	Low	*EPHB2*	ENSSSCG00000003527
rs345676220	SNP	3:17491423-17491423	UTR3'	Modifier	*ORAI3*	ENSSSCG00000007770
rs346223430	SNP	6:80843336-80843336	UTR3'	Modifier	*EPHB2*	ENSSSCG00000003527
rs694561033	SNP	3:16385959-16385959	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs697381317	SNP	3:17591079-17591079	Downstream	Modifier	*BCL7C*	ENSSSCG00000007776
rs699677308	SNP	3:17591133-17591133	Downstream	Modifier	*BCL7C*	ENSSSCG00000007776
rs701844432	SNP	3:16383956-16383956	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs706065940	SNP	3:17591138-17591138	Downstream	Modifier	*BCL7C*	ENSSSCG00000007776
rs713060696	SNP	3:16383936-16383936	UTR3'	Modifier	*KCTD7*	ENSSSCG00000040985
rs713239492	SNP	3:17612980-17612980	Synonym	Low	*ZNF629*	ENSSSCG00000007780
rs789266896	SNP	3:17628688-17628688	Missense	Modifier	*RNF40*	ENSSSCG00000007786
rs793318116	SNP	3:17460656-17460656	Synonym	Low	*HSD3B7*	ENSSSCG00000032369
rs81389091	SNP	6:80842615-80842615	UTR3'	Modifier	*EPHB2*	ENSSSCG00000003527

Additional file 2

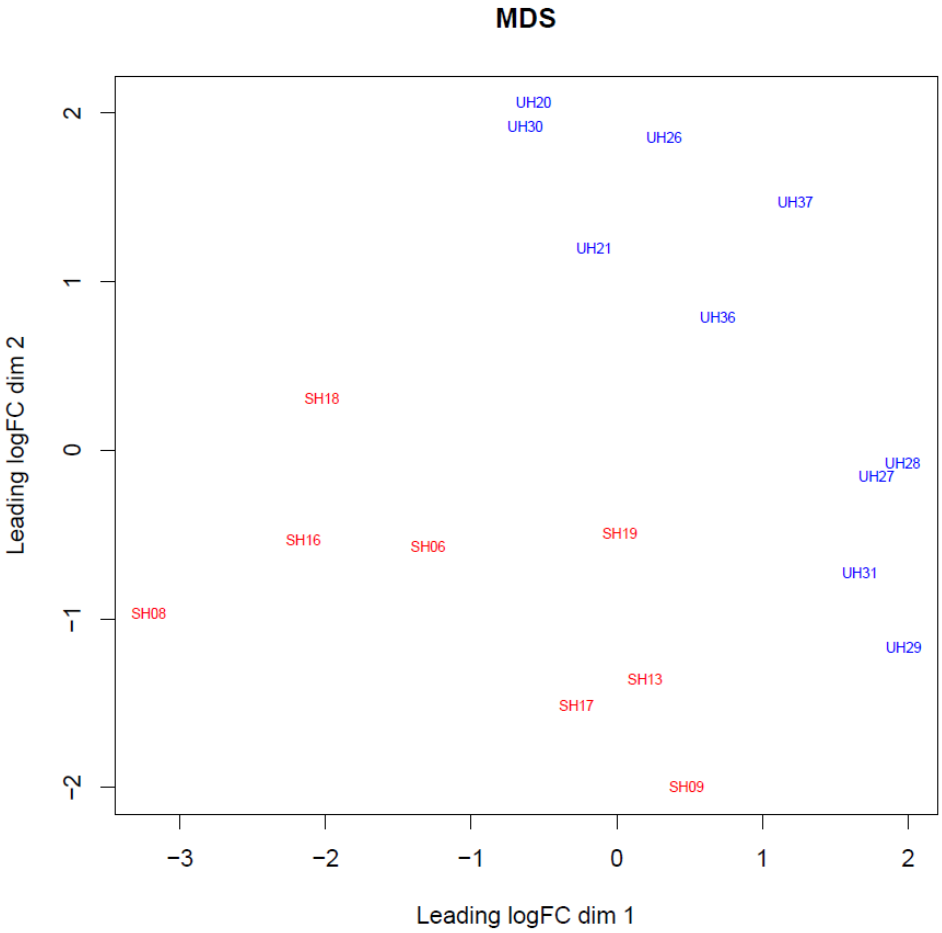

Figure S1 Multidimensional scaling (MDS) plot showing the samples used to generate the transcriptome of the inguinal ring for scrotal hernia (SH) in red, and the transcriptome of the umbilical ring for umbilical hernia (UH) in blue.

3 CONSIDERAÇÕES FINAIS

O estudo dos transcriptomas do tecido do anel inguinal e umbilical de suínos afetados com hérnia escrotal (HE) e umbilical (HU) foi realizado para comparar o perfil de expressão destes transcriptomas e identificar genes diferencialmente expressos nos dois tipos de hérnia, proporcionando avanço no conhecimento dos genes relacionados ao surgimento da HE e HU. Esses resultados contribuem para a compreensão dos mecanismos genéticos que envolvem os dois tipos de hérnia em suínos.

Para HE, 627 genes DE foram identificados entre animais normais e afetados e para HU 199 genes DE, dos quais 35 pertenciam a ambos os tipos de hérnia. Destes, 26 genes apresentaram perfil de expressão semelhante em ambos os grupos de hérnia e foram submetidos a uma base de dados para enriquecimento e análise dos processos biológicos relacionados a estes genes. Adesão celular, apoptose, organização do citoesqueleto de actina e organização das fibrilas de colágeno foram os processos biológicos destacados, pois apresentam afinidade com os fatores fisiológicos envolvidos na manifestação de hérnias.

Os genes *ACAN* e *BCHE* foram os que apresentaram informações interligadas à manifestação da hérnia. Além de serem enriquecidos em processos biológicos característicos na formação da hérnia e estarem presentes em regiões de QTL relacionadas à hérnia escrotal, também apresentaram perfil de expressão semelhante nos dois tipos desta patologia. Além destes, os genes *MAP1LC3C*, *VIT*, *ACAN*, *ACER2*, *KCNMA1* e *SYNPO2* foram considerados genes candidatos para a formação dos dois tipos de defeitos.

Neste estudo também foram investigadas variantes alélicas nos genes DE, sendo que 67 polimorfismos foram identificados nos genes DE no tecido do anel inguinal e 76 nos genes DE no tecido do anel umbilical, dos quais 11 e 14 são novos, respectivamente.

A partir da obtenção de todos os dados deste estudo, confirmou-se que a manifestação desses defeitos é devido a atuação de vários genes. No entanto, são necessários mais estudos para identificar o perfil de expressão desses mesmos genes em diferentes idades como, por exemplo, em animais mais jovens, para aprimorar nossa interpretação dos mecanismos de regulação gênica.

O conhecimento dos fatores genéticos que envolvem tanto a manifestação da hérnia escrotal quanto da umbilical são de grande importância para a cadeia produtiva de suínos, pois favorece o desenvolvimento de estratégias efetivas para reduzir o surgimento destes defeitos nos rebanhos.

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ANEXOS

Embrapa	Certificado de Conduta Ética	ETICA 1/1

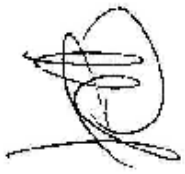
CERTIFICADO

Certificamos que o Protocolo nº**(000/AAAA): 011/2014**, sob título **"Identificação de genes e polimorfismos associados à formação de hérnias em suínos pela combinação do sequenciamento exômico total e do RNA",** sob responsabilidade de **Mônica Ledur** está de acordo com os Princípios Éticos na Experimentação Animal, adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), **TENDO SIDO CONSIDERADO APROVADO PELA** Comissão de Ética no Uso de Animais (CEUA/CNPSA) em reunião realizada em **07/ 11/ 2014**.

CERTIFICATE

We certify that the Protocol nº **(000/YYYY): 011/2014**, under the following title **"Identification of genes and polymorphisms associated with formation of hernias in swines combining RNA and whole exons sequencing."** is in agreement with the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation (COBEA) and was approved by the Embrapa Swines and Poultry Ethical Committee for Animals utilization in experimentation (CEUA/CNPSA) in **11/07/2014**.

Concórdia, **07/11/2014**.



Presidente CEUA/CNPSA

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