Integrative Analysis of the Genes Induced by the Intestine Microbiota of Infant Born to Term and Breastfed

Bioinformatics and Biology Insights
Volume 14: 1–14

DOI: 10.1177/1177932220906168

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ABSTRACT: Nowadays, the integration of biological data is a major challenge for bioinformatics. Many studies have examined gene expression in the epithelial tissue in the intestines of infants born to term and breastfed, generating a large amount of data. The integration of these data is important to understand the biological processes involved during bacterial colonization of the newborns intestine, particularly through breast milk. This work aims to exploit the bioinformatics approaches, to provide a new representation and interpretation of the interactions between differentially expressed genes in the host intestine induced by the microbiota.

KEYWORDS: Gene expression, network, intestinal microbiota, newborn, breastfed

RECEIVED: October 15, 2019. **ACCEPTED:** January 20, 2020.

TYPE: Original Research

FUNDING: The author(s) received no financial support for the research, authorship, and/or publication of this article.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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A total of 61 differentially expressed genes (DEGs) in the intestine of newborns extracted from several bibliographic works and databases were annotated for functional analysis using the String software (http://string-db.org/), the Cystoscape software (http://www.cytoscape.org/) and the BiNGO plugin. The latter provided an evaluation of the signaling and metabolic pathways, molecular networks and biological processes for all the used genes.

The analysis revealed that *RELA, INS, IRS1, IL1B*, and *NFKBIA* are the central genes in the interaction networks produced. These networks show that the cellular differentiation of the intestinal epithelium and the development of mucosal immunity, are the most affected processes in newborns. Therefore, the global patterns of interactions supports the relationship between breastmilk and the role of the microbiota's diversity. Ergo all results consolidate the importance of breastmilk and intestinal microbiota in homeostasis.

Introduction

Breastmilk microbiota provides the transient microbiota in the newborn.[1-3] This transient microbiota is important for the implantation of the personalized intestinal microbiota of each individual; indeed, it plays a fundamental role in the development of the newborn.[4-7] Moreover, breastfeeding has demonstrated its ability to provide a balanced intestinal microbiota to the newborn, thus positively impacting the newborn's health.[8-10] Human milk can stimulate the proliferation of *Bifidobacterium* and *Lactobacillus* strains, whose role is to create an acidic environmental rich in short-chain fatty acids (SCFAs) with a protective and nutritive role at the intestinal level.[11,12] It has been shown also, that in germ-free rats, *Streptococcus thermophilus* (transient commensal bacteria) induces the epithelial stem cell differentiation.[13] As well, *Bacteroides*, very abundant in human colostrum, may have a main role in the early stages of newborn's gut colonization.[14]

Intestinal microbiota is largely studied since 2 decades, and a big amount of data are generated with Omics approaches. However, the interaction between human milk microbiota and newborn's intestinal microbiota is not completely clear. Other studies are needed to understand the powerful relationship between the human milk microbiota and the stimulation of newborn's homeostasis. Novel approaches have been developed to study the microbiota using the directed acyclic graphs (DAG) method to facilitate an understanding of the ontological profiles provided by the interaction between the induced genes in the newborn's epithelium breastfeeding.[15]

Ontological studies are becoming essential to understand the complex mechanism placed in the intestine of newborn during breastfeeding. Thus, the biological processes and Top functions can be identified through the predicted networks. The omics technological advances associated to bioinformatics tools allowed to have a global view of the function of genes and their interactions in the cell, in any given context.[16]

This study aims to provide a new view of the hierarchical ontology while showing the whole biological processes induced in the host's epithelium during breastfeeding.

Materials and Methods

This work consists of studying, through a statistical-computing approach, the representation of biological data to make their integration more efficient in the case of DEGs in the newborns' intestine. For this purpose, we have chosen the scientific

publications[17,18] which recruited healthy, full-term infants, who were exclusively breastfed at 3 months postpartum.

For that, we have used search terms for the PubMed database (www.ncbi.nlm.nih.gov/pubmed/): *Breast Feeding, Gene Expression Profiling, Infant, Newborn, Transcriptome, Feces/cytology, Proteome*. Sixty-one DEGs that were significantly higher in term infants, were selected from scientific publications. The databases were consulted (GenBank, www.ncbi.nlm.nih.gov/genbank/; GENE, www.genecards.org/) to assign each gene to its iD and their functional annotation (Tables 1 to 6). The results were then processed by different software packages: the String software (https://string-db.org/), the Cystoscape software (http://www.cytoscape.org/) and the BiNGO plugin.

Coexpression analysis by String software

This analysis was performed by 2 functions:

1. Visualization of interactions between genes by emphasizing particular criterias such as co-occurrence, coexpression, experimental evidence, existing databases, and text mining.
2. Rich statistical analysis indicates that the terms are classified by their enriched *P* value. The *P* value is calculated by a hypergeometric test and then corrected for multiple tests using the Benjamini and Hochberg method.

Ontological analysis by Cytoscape software

The networks generated by string were imported as a preexisting unformatted array in Cytoscape software.

The network analyzer plugin function provides a network customization. The size and color of the nodes have been customized according to the values of the chosen parameters:—A number of connections of the node with other proteins;—large size and light color for the weak connectivities.

The genes annotated by Cytoscape and selected manually were analyzed by the BiNGO function for network personalization. The network personalization determines a functional profile as DAG interactions. The ontological level "biological process" was chosen as a query for the analysis with the BiNGO plugin function.

Results and Discussion

String results

Sixty-one DEGs (Tables 1 to 6) were imported and analyzed by the String software. Fifty-seven genes were annotated and other 10 genes enriched the networks generated. The results were performed in 2 formats: a network with different confidence indexes (Figure 1) and a network with the different interactions between the proteins (Figure 2). Among the 67 genes annotated on String, 51 have formed a single network and 16 genes remain outside this network. A set of 51 proteins was found to be linked either directly or indirectly through one or more interacting proteins, suggesting the existence of functional links between them (Figures 1 and 2).

The central proteins of this network (Figures 1 and 2) are as follows: IL1β, RELA, INS, IRS1, and NFKBIA.

IL-1 (interleukin-1) production (Figure 1) is mainly regulated by the inflammasome, a multimeric protein complex assembled in response to various inflammatory triggers such as danger signals, microbial toxins, and crystalline substances.[20-22] A prototypical complex of inflammasome includes many proteins, among them CASP1 (caspase-1). Cleavage of CASP1 by the inflammasome leads to its activation, which in turn cleaves IL-1β (IL-1 beta).[23,24] Interleukin-1β, a proinflammatory cytokine with a wide range of systemic and local effects[25] can modulate the function of both immune and nonimmune cells. Interleukin-1β also promotes T-cell activation and survival[26] and works with other proinflammatory cytokines such as IL-33 (Figure 1) to promote epithelial restoration, repair, and mucosal healing in the intestine.[27] Interleukin-1β can also induce the positive regulation of RELA (transcription factor p65) (Figure 1) and subsequently the activation of the canonical NF-κB pathway, which is necessary for homeostatic regulation of cell death and division in intestinal epithelia, as well as for protection against development of severe acute inflammation of intestines.[28-30] Insulin receptor (INSR) mediates the pleiotropic actions of INS (insulin) (Figure 1). Insulin-binding leads to phosphorylation of several intracellular substrates, including *IRS1* (insulin receptor substrate 1), subsequently inducing various bioactivities such as growth, differentiation, survival, increased anabolism, and decreased catabolism in many types of cells.[31-33]

On the contrary, SOCS3 (suppressor of cytokine signaling 3) is regulated by several proteins within the network (Figure 1). It has an impact on multiple signaling pathways, and it is a mediator key of mucosal homeostasis. Suppressor of cytokine signaling 3 is a tumor suppressor, limiting the proliferation of intestinal epithelial cells (angiotensin-converting enzyme [ACE]) in cases of acute inflammation and tumor growth, and plays a role in wound repair.[34,35] A group of reactive proteins (black lines) is formed of: ATP5B, ATP5A1, ATP5D, ATP5H, ATP5C1, ATP5G3, and ATP5O, (Figure 1) which have a common role in displaying energy and cells communications.[36] The interconnected proteins within this network (Figure 1) reveal the antiproliferative effect of breastfeeding on the cells of the intestinal epithelium of newborns and the positive effect on cell differentiation. These observations also suggest the involvement of these proteins in metabolism, cell survival, and mucosal homeostasis.

The biological process analysis (Table 7) revealed the presence of different processes, significantly implicated in this network (P value <0.05). The most important processes were:—positive regulation of NF-kappaB transcription factor activity (GO: 0051092);—positive regulation of cellular process (GO: 0048522);—positive regulation of lipid metabolic

Table 1. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications.

ID	ABBREVIATIONS	GENES	DESCRIPTION
337	ApoA4	Apolipoprotein A4	Secreted by the small intestine on chylomicrons in the intestinal lymph in response to the absorption of fats Numerous physiological functions have been attributed to ApoA4, including a role in chylomicron assembly and lipid metabolism, a mediator of reverse cholesterol transport, an acute satiety factor, a regulator of gastric function, and finally, a modulator of homeostasis[19]
335	ApoA1	Apolipoprotein A1	This gene encodes apolipoprotein A1, which is the major protein component of high-density lipoprotein (HDL) in plasma. Defects in this gene are associated with HDL deficiencies, including Tangier disease, and with systemic non-neuropathic amyloidosis (https://www.ncbi.nlm.nih.gov/gene/335)
427	ASAH1	N-Acylsphingosine amidohydrolase 1	This gene encodes a member of the acid ceramidase family of proteins. This lysosomal enzyme, which catalyzes the degradation of ceramide into sphingosine and free fatty acid (https://www.ncbi.nlm.nih.gov/gene/427)
29956	CERS2	Céramide synthase 2	This gene encodes a protein that has sequence similarity to yeast longevity assurance gene 1. The human protein may play a role in the regulation of cell growth. Gene ontology (GO) annotations related to this gene include sphingosine N-acyltransferase activity. An important paralog of this gene is CERS3 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CERS2, https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=29956)
4534	MTM1	Myotubularin 1	This gene encodes a dual-specificity phosphatase that acts on both phosphotyrosine and phosphoserine. It is required for muscle cell differentiation, and mutations in this gene have been identified as being responsible for X-linked myotubular myopathy (https://www.ncbi.nlm.nih.gov/gene/4534)
123	PLIN2	Perilipin 2	The protein encoded by this gene belongs to the perilipin family, members of which coat intracellular lipid storage droplets. This protein is associated with the lipid globule surface membrane material and maybe involved in development and maintenance of adipose tissue (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=123)
834	CASP1	Caspase 1	This gene encodes a protein which is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution phase of cell apoptosis (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=834)
80341	BPIL1 (BPIFB2)	BPI fold containing family B member 2	This gene encodes a member of the lipid transfer/lipopolysaccharide binding protein (LT/LBP) gene family (https://www.ncbi.nlm.nih.gov/gene/80341)
240	ALOX5	Arachidonate 5-lipoxygenase	This gene encodes a member of the lipoxygenase gene family and plays a dual role in the synthesis of leukotrienes from arachidonic acid. Leukotrienes are important mediators of a number of inflammatory and allergic conditions (https://www.ncbi.nlm.nih.gov/gene/240)
7410	VAV2	Vav guanine nucleotide exchange factor 2	VAV2 is the second member of the VAV guanine nucleotide exchange factor family of oncogenes. Unlike VAV1, which is expressed exclusively in hematopoietic cells, VAV2 transcripts were found in most tissues (https://www.ncbi.nlm.nih.gov/gene/7410)
6668	SP2	Sp2 transcription factor	This gene encodes a member of the Sp subfamily of Sp/XKLF transcription factors. Sp family proteins are sequence-specific DNA-binding proteins characterized by an amino-terminal trans-activation domain and 3 carboxy-terminal zinc finger motifs This protein contains the least conserved DNA-binding domain within the Sp subfamily of proteins, and its DNA sequence specificity differs from the other Sp proteins. It localizes primarily within subnuclear foci associated with the nuclear matrix and can activate or in some cases repress expression from different promoters (https://www.ncbi.nlm.nih.gov/gene/6668)

Table 2. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications (continued).

ID	ABBREVIATIONS	GENES	DESCRIPTION
3553	IL1B	Interleukin 1 beta	The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is produced by activated macrophages as a proprotein, which is proteolytically processed to its active form by caspase 1 (CASP1/ICE). This cytokine is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (https://www.ncbi.nlm.nih.gov/gene/3553)
3383	ICAM1	Intercellular adhesion molecule 1	This gene encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. It binds to integrins of type CD11a/CD18 or CD11b/CD18 (https://www.ncbi.nlm.nih.gov/gene/3383)
90865	IL33	Interleukin 33	The protein encoded by this gene is a cytokine that binds to the IL1RL1/ST2 receptor. The encoded protein is involved in the maturation of Th2 cells and the activation of mast cells, basophils, eosinophils, and natural killer cells (https://www.ncbi.nlm.nih.gov/gene/90865)
50506	DUOX2	Dual oxidase 2	The protein encoded by this gene is a glycoprotein and a member of the NADPH oxidase family. The synthesis of thyroid hormone is catalyzed by a protein complex located at the apical membrane of thyroid follicular cells. This complex contains an iodide transporter, thyroperoxidase, and a peroxide-generating system that includes this encoded protein and DUOX1 (https://www.ncbi.nlm.nih.gov/gene/50506)
51348	KLRF1	Killer cell lectin like receptor F1	KLRF1, an activating homodimeric C-type lectin-like receptor (CTLR), is expressed on nearly all natural killer (NK) cells and stimulates their cytoxicity and cytokine release (https://www.ncbi.nlm.nih.gov/gene/51348)
4790	NF-kB	Nuclear factor kappa B subunit 1	Nuclear factor kappa B (NFKB) is a transcription regulator that is activated by various intracallular and extracellular stimuli such as cytokines, oxidant-free radicals, ultraviolet irradiation, and bacterial or viral products. Activated NFKB translocates into the nucleus and stimulates the expression of genes involved in a wide variety of biological functions. Inappropriate activation of NFKB has been associated with a number of inflammatory diseases while persistent inhibition of NFKB leads to inappropriate immune cell development or delayed cell growth (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=4790)
5468	PPAR γ	Peroxisome-proliferator-activated receptor gamma	The protein encoded by this gene is PPAR-gamma and is a regulator of adipocyte differentiation (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=5468)
5465	PPAR α	Peroxisome-proliferator-activated receptor alpha	Peroxisome-proliferator-activated receptors (PPARs) affect the expression of target genes involved in cell proliferation, cell differentiation, and in immune and inflammation responses. This gene encodes the subtype PPAR-alpha, which is a nuclear transcription factor (https://www.ncbi.nlm.nih.gov/gene/5465)
4869	NPM1	Nucleophosmin 1	The protein encoded by this gene is involved in several cellular processes, including centrosome duplication, protein chaperoning, and cell proliferation. The encoded phosphoprotein shuttles between the nucleolus, nucleus, and cytoplasm, chaperoning ribosomal proteins and core histones from the nucleus to the cytoplasm. This protein is also known to sequester the tumor suppressor ARF in the nucleolus, protecting it from degradation until it is needed (https://www.ncbi.nlm.nih.gov/gene/4869)
3156	HMGCR	3-Hydroxy-3-methylglutaryl-CoA reductase	3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase is the rate-limiting enzyme for cholesterol synthesis and is regulated via a negative feedback mechanism mediated by sterols and nonsterol metabolites derived from mevalonate, the product of the reaction catalyzed by reductase. Normally in mammalian cells this enzyme is suppressed by cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) via the LDL receptor (https://www.ncbi.nlm.nih.gov/gene/3156)

Abbreviation: ARF, alternative reading frame.

Table 3. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications (continued).

ID	ABBREVIATIONS	GENES	DESCRIPTION
3936	LCP1	Lymphocyte cytosolic protein 1	Plastins are a family of actin-binding proteins that are conserved throughout eukaryote evolution and expressed in most tissues of higher eukaryotes. In humans, 2 ubiquitous plastin isoforms (L and T) have been identified. Plastin 1 (otherwise known as fimbrin) is a third distinct plastin isoform which is specifically expressed at high levels in the small intestine (https://www.ncbi.nlm.nih.gov/gene/3936)
4792	NFKBIA	NFKB inhibitor alpha	This gene encodes a member of the NF-kappa-B inhibitor family, which contain multiple ankrin repeat domains. The encoded protein interacts with REL dimers to inhibit NF-kappa-B/REL complexes which are involved in inflammatory responses (https://www.ncbi.nlm.nih.gov/gene/4792)
5209	PFKFB3	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	The protein encoded by this gene belongs to a family of bifunctional proteins that are involved in both the synthesis and degradation of fructose-2,6-bisphosphate, a regulatory molecule that controls glycolysis in eukaryotes. This protein is required for cell cycle progression and prevention of apoptosis. It functions as a regulator of cyclin-dependent kinase 1, linking glucose metabolism to cell proliferation and survival in tumor cells (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=5209)
6280	S100-A9	S100 calcium-binding protein A9	The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation (https://www.ncbi.nlm.nih.gov/gene/6280)
9021	SOCS3	Suppressor of cytokine signaling 3	This gene encodes a member of the STAT-induced STAT inhibitor (SSI), also known as suppressor of cytokine signaling (SOCS), family. SSI family members are cytokine-inducible negative regulators of cytokine signaling. The expression of this gene is induced by various cytokines, including IL6, IL10, and interferon (IFN)-gamma. The protein encoded by this gene can bind to JAK2 kinase, and inhibit the activity of JAK2 kinase (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=9021)
54210	TREM-1	Triggering receptor expressed on myeloid cells 1	This gene encodes a receptor belonging to the Ig superfamily that is expressed on myeloid cells. This protein amplifies neutrophil and monocyte-mediated inflammatory responses triggered by bacterial and fungal infections by stimulating release of proinflammatory chemokines and cytokines, as well as increased surface expression of cell-activation markers (https://www.ncbi.nlm.nih.gov/gene/54210)
7305	TYROBP	TYRO protein tyrosine kinase-binding protein	This gene encodes a transmembrane signaling polypeptide which contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. The encoded protein may associate with the killer-cell inhibitory receptor (KIR) family of membrane glycoproteins and may act as an activating signal-transduction element (https://www.ncbi.nlm.nih.gov/gene/7305)
1051	CEBPB	CCAAT enhancer binding protein beta	This intronless gene encodes a transcription factor that contains a basic leucine zipper (bZIP) domain. Activity of this protein is important in the regulation of genes involved in immune and inflammatory responses, among other processes (https://www.ncbi.nlm.nih.gov/gene/1051)
8837	CFLAR	CASP8 and FADD like apoptosis regulator	The protein encoded by this gene is a regulator of apoptosis and is structurally similar to caspase-8. However, the encoded protein lacks caspase activity and appears to be itself cleaved into 2 peptides by caspase-8 (https://www.ncbi.nlm.nih.gov/gene/8837)
9750	FAM65B	RHO family interacting cell polarization regulator 2	This gene encodes an atypical inhibitor of the small G protein RhoA. Inhibition of RhoA activity by the encoded protein mediates myoblast fusion and polarization of T-cells and neutrophils. The encoded protein is a component of hair cell stereocilia that is essential for hearing. A splice site mutation in this gene results in hearing loss in human patients (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=9750)

Table 4. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications (continued).

ID	ABBREVIATIONS	GENES	DESCRIPTION
5265	SERPINA1	Serpin family A member 1	The protein encoded by this gene is secreted and is a serine protease inhibitor whose targets include elastase, plasmin, thrombin, trypsin, chymotrypsin, and plasminogen activator (https://www.ncbi.nlm.nih.gov/gene/5265)
57126	CD177	CD177 molecule	This gene encodes a glycosyl-phosphatidylinositol (GPI)-linked cell surface glycoprotein that plays a role in neutrophil activation. The protein can bind platelet endothelial cell adhesion molecule-1 and function in neutrophil transmigration (https://www.ncbi.nlm.nih.gov/gene/57126)
3429	IFI27	Interferon alpha inducible protein 27	Among its related pathways are cytokine signaling in immune system and innate immune system. Gene ontology (GO) annotations related to this gene include RNA polymerase II activating transcription factor binding and lamin binding (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IFI27)
10379	IRF9	Interferon regulatory factor 9	Among its related pathways are immune response IFN-gamma-signaling pathway and cytokine signaling in immune system. Gene ontology (GO) annotations related to this gene include DNA-binding transcription factor activity (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IRF9)
3937	LCP2	Lymphocyte cytosolic protein 2	This gene encodes an adapter protein that acts as a substrate of the T-cell antigen receptor (TCR)-activated protein tyrosine kinase pathway. The encoded protein associates with growth factor receptor bound protein 2 and is thought to play a role in TCR-mediated intracellular signal transduction. A similar protein in mouse plays a role in normal T-cell development and activation (https://www.ncbi.nlm.nih.gov/gene/3937)
10057	ABCC5	ATP-binding cassette subfamily C member 5	The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extracellular and intracellular membranes. ABC genes are divided into 7 distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, and white). This protein is a member of the MRP subfamily which is involved in multidrug resistance. This protein functions in the cellular export of its substrate, cyclic nucleotides (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=10057)
506	ATP5B	ATP synthase F1 subunit beta	This gene encodes a subunit of mitochondrial ATP synthase. Mitochondrial ATP synthase catalyzes ATP synthesis, utilizing an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation (https://www.ncbi.nlm.nih.gov/gene/506)
663	BNIP2	BCL2-interacting protein 2	This gene is a member of the BCL2/adenovirus E1B 19 kd-interacting protein (BNIP) family. It interacts with the E1B 19 kDa protein, which protects cells from virally induced cell death. The encoded protein also interacts with E1B 19 kDa-like sequences of BCL2, another apoptotic protector (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=663)
1030	CDKN2B	Cyclin-dependent kinase inhibitor 2B	This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6 and prevents the activation of the CDK kinases, thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by transforming growth factor (TGF) beta, which suggested its role in the TGF-beta-induced growth inhibition (https://www.ncbi.nlm.nih.gov/gene/1030)
8655	DYNLL1	Dynein light chain LC8-type 1	The complex is involved in intracellular transport and motility. The protein described in this record is a light chain and exists as part of this complex but also physically interacts with and inhibits the activity of neuronal nitric oxide synthase. Binding of this protein destabilizes the neuronal nitric oxide synthase dimer, a conformation necessary for activity, and it may regulate numerous biologic processes through its effects on nitric oxide synthase activity (https://www.ncbi.nlm.nih.gov/gene/8655)

Table 5. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications (continued).

ID	ABBREVIATIONS	GENES	DESCRIPTION
100463482	MT-RNR2-L6	MT-RNR2-Like 6	It is unclear if this is a transcribed protein-coding gene, or if it is a nuclear pseudogene of the mitochondrial MT-RNR2 gene (https://www.ncbi.nlm.nih.gov/gene/100463482)
5170	PDPK1	3-Phosphoinositide-dependent protein kinase 1	Among its related pathways are constitutive signaling by AKT1 E17K in cancer and NFAT and cardiac hypertrophy. Gene ontology (GO) annotations related to this gene include transferase activity, transferring phosphorus-containing groups, and protein tyrosine kinase activity (http://www.genecards.org/cgi-bin/carddisp.pl?gene=PDPK1)
5911	RAP2A	RAP2A, member of RAS oncogene family	Among its related pathways are ADP signaling through P2Y purinoceptor 12 and ERK signaling. Gene ontology (GO) annotations related to this gene include GTP binding and GTPase activity. An important paralog of this gene is RAP2C (http://www.genecards.org/cgi-bin/carddisp.pl?gene=RAP2A)
6337	SCNN1A	Sodium channel epithelial 1 alpha subunit	Nonvoltage-gated, amiloride-sensitive, sodium channels control fluid, and electrolyte transport across epithelia in many organs. This gene encodes the alpha subunit, and mutations in this gene have been associated with pseudohypoaldosteronism type 1 (PHA1), a rare salt-wasting disease resulting from target organ unresponsiveness to mineralocorticoids (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SCNN1A)
6670	SP3	Sp3 transcription factor	This gene belongs to a family of Sp1-related genes that encode transcription factors that regulate transcription by binding to consensus GC- and GT-box regulatory elements in target genes. This protein contains a zinc finger DNA-binding domain and several transactivation domains and has been reported to function as a bifunctional transcription factor that either stimulates or represses the transcription of numerous genes (https://www.ncbi.nlm.nih.gov/gene/6670)
55521	TRIM36	Tripartite motif containing 36	TRIM36 (tripartite motif containing 36) is a protein-coding gene. Diseases associated with TRIM36 include anencephaly and endometriosis of ovary. Among its related pathways are innate immune system and Class I MHC-mediated antigen processing and presentation. Gene ontology (GO) annotations related to this gene include ligase activity and ubiquitin-protein transferase activity (http://www.genecards.org/cgi-bin/carddisp.pl?gene=TRIM36)
3552	IL1A	Interleukin 1 alpha	The protein encoded by this gene is a member of the interleukin 1 cytokine family. This cytokine is a pleiotropic cytokine involved in various immune responses, inflammatory processes, and hematopoiesis. This cytokine is produced by monocytes and macrophages as a proprotein, which is proteolytically processed and released in response to cell injury, and thus induces apoptosis (https://www.ncbi.nlm.nih.gov/gene/3552)
6869	TACR1	Tachykinin receptor 1	This gene belongs to a gene family of tachykinin receptors. These tachykinin receptors are characterized by interactions with G proteins and contain 7 hydrophobic transmembrane regions. This gene encodes the receptor for the tachykinin substance P, also referred to as neurokinin 1 (https://www.ncbi.nlm.nih.gov/gene/6869)
5966	REL	REL proto-oncogene, NF-kB subunit	This gene encodes a protein that belongs to the Rel homology domain/immunoglobulin-like fold, plexin, transcription factor (RHD/IPT) family. Members of this family regulate genes involved in apoptosis, inflammation, the immune response, and oncogenic processes. This proto-oncogene plays a role in the survival and proliferation of B lymphocytes. Mutation or amplification of this gene is associated with B-cell lymphomas, including Hodgkin's lymphoma (https://www.ncbi.nlm.nih.gov/gene/5966)
3340	NDST1	N-deacetylase and N-sulfotransferase 1	This gene encodes a member of the heparan sulfate/heparin GlcNAc N-deacetylase/N-sulfotransferase family. The encoded enzyme is a type-II transmembrane protein that resides in the Golgi apparatus. The encoded protein catalyzes the transfer of sulfate from 3′-phosphoadenosine-5′-phosphosulfate to nitrogen of glucosamine in heparan sulfate (https://www.ncbi.nlm.nih.gov/gene/3340)

Table 6. Differentially expressed genes (DEGs) in the gut of neonates born to term and breastfed, selected from various databases and scientific publications (continued).

ID	ABBREVIATIONS	GENES	DESCRIPTION
8880	FUBP1	Far upstream element-binding protein 1	The protein encoded by this gene is a single stranded DNA-binding protein that binds to multiple DNA elements, including the far upstream element (FUSE) located upstream of c-myc. This protein is also thought to bind RNA and contains 3′-5′ helicase activity with in vitro activity on both DNA-DNA and RNA-RNA duplexes. Aberrant expression of this gene has been found in malignant tissues, and this gene is important to neural system and lung development (https://www.ncbi.nlm.nih.gov/gene?cmd=Retrieve&dopt=full_report&list_uids=8880)
50486	G0S2	G0/G1 switch 2	G0S2 (G0/G1 Switch 2) is a protein-coding gene. Among its related pathways are metabolism and regulation of lipid metabolism by peroxisome proliferator-activated receptor alpha (PPAR-alpha) (http://www.genecards.org/cgi-bin/carddisp.pl?gene=G0S2)
10765	KDM5B	Lysine demethylase 5B	This gene encodes a lysine-specific histone demethylase that belongs to the jumonji/ARID domain-containing family of histone demethylases. This protein plays a role in the transcriptional repression or certain tumor suppressor genes and is upregulated in certain cancer cells. This protein may also play a role in genome stability and DNA repair (https://www.ncbi.nlm.nih.gov/gene/10765)
4312	MMP1	Matrix metallopeptidase 1	This gene encodes a member of the peptidase M10 family of matrix metalloproteinases (MMPs). Proteins in this family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. The encoded preproprotein is proteolytically processed to generate the mature protease. This secreted protease breaks down the interstitial collagens, including types I, II, and III (https://www.ncbi.nlm.nih.gov/gene/4312)
94059	LENG9	Leukocyte receptor cluster member 9	
2101	ESRRA	Estrogen-related receptor alpha	The protein encoded by this gene is a nuclear receptor that is closely related to the estrogen receptor. This protein acts as a site-specific transcription regulator and has been also shown to interact with estrogen and the transcripton factor TFIIB by direct protein-protein contact. The binding and regulatory activities of this protein have been demonstrated in the regulation of a variety of genes including lactoferrin, osteopontin, medium-chain acyl coenzyme A dehydrogenase (MCAD) and thyroid hormone receptor genes (https://www.ncbi.nlm.nih.gov/gene/2101)
79412	KREMEN2	Kringle-containing transmembrane protein 2	This gene encodes a high-affinity dickkopf homolog 1 (DKK1) transmembrane receptor. A similar protein in mouse functions interacts with with DKK1 to block wingless (WNT)/beta-catenin signaling. The encoded protein forms a ternary membrane complex with DKK1 and the WNT receptor lipoprotein receptor-related protein 6 (LRP6) and induces rapid endocytosis and removal of LRP6 from the plasma membrane (https://www.ncbi.nlm.nih.gov/gene/79412)
4301	MLLT4 (AFDN)	Afadin, adherens junction formation factor	This gene encodes a multidomain protein involved in signaling and organization of cell junctions during embryogenesis (https://www.ncbi.nlm.nih.gov/gene/4301)
3643	INSR	Insulin receptor	This gene encodes a member of the receptor tyrosine kinase family of proteins. Binding of insulin or other ligands to this receptor activates the insulin signaling pathway, which regulates glucose uptake and release, as well as the synthesis and storage of carbohydrates, lipids, and protein (https://www.ncbi.nlm.nih.gov/gene/3643)
8639	AOC3	Amine oxidase, copper containing 3	This gene encodes a member of the semicarbazide-sensitive amine oxidase family. Copper amine oxidases catalyze the oxidative conversion of amines to aldehydes in the presence of copper and quinone cofactor. The encoded protein is localized to the cell surface, has adhesive properties as well as monoamine oxidase activity and may be involved in leukocyte trafficking (https://www.ncbi.nlm.nih.gov/gene/8639)

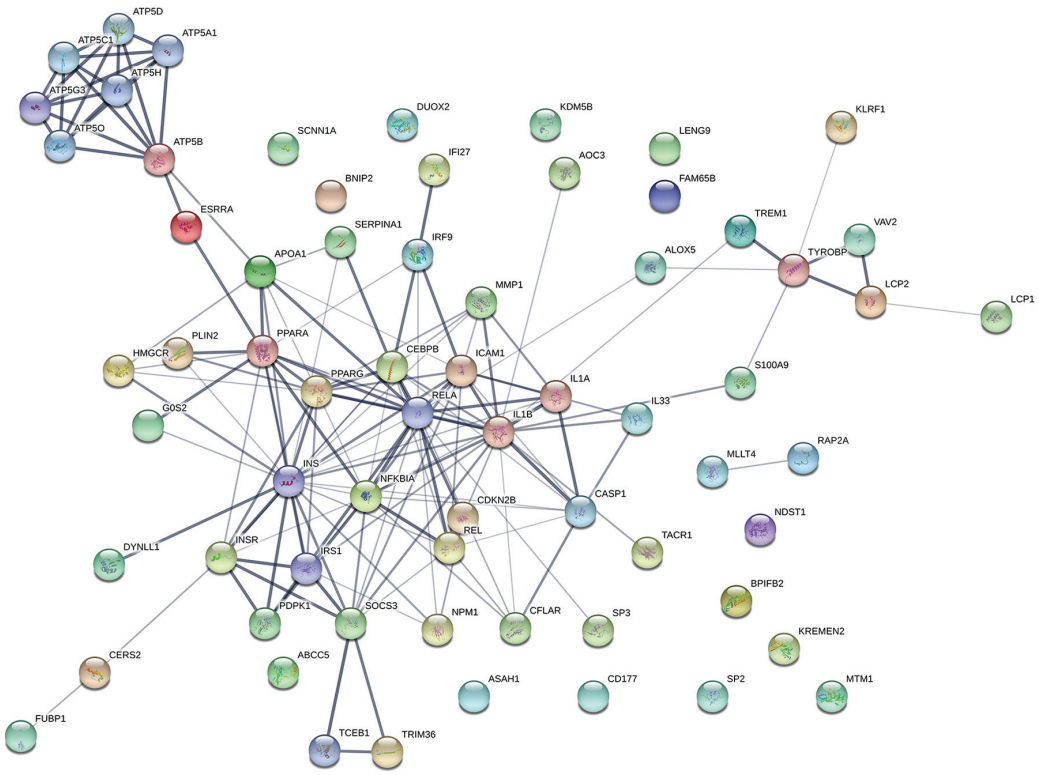

Figure 1. "Confidence view" of the protein-protein interaction network on String.
The thickness of the blue line between 2 nodes indicates the level of confidence of association between these 2. The greater the thickness, the higher the level of confidence. The confidence score for each interaction indicates the prediction of a link between 2 nodes in the same metabolic pathway at the level of the KEGG database. The size differs between proteins of which the 3-dimensional structure (large sized) is known. Nodes whose 3-dimensional structure is not yet available are small sized.

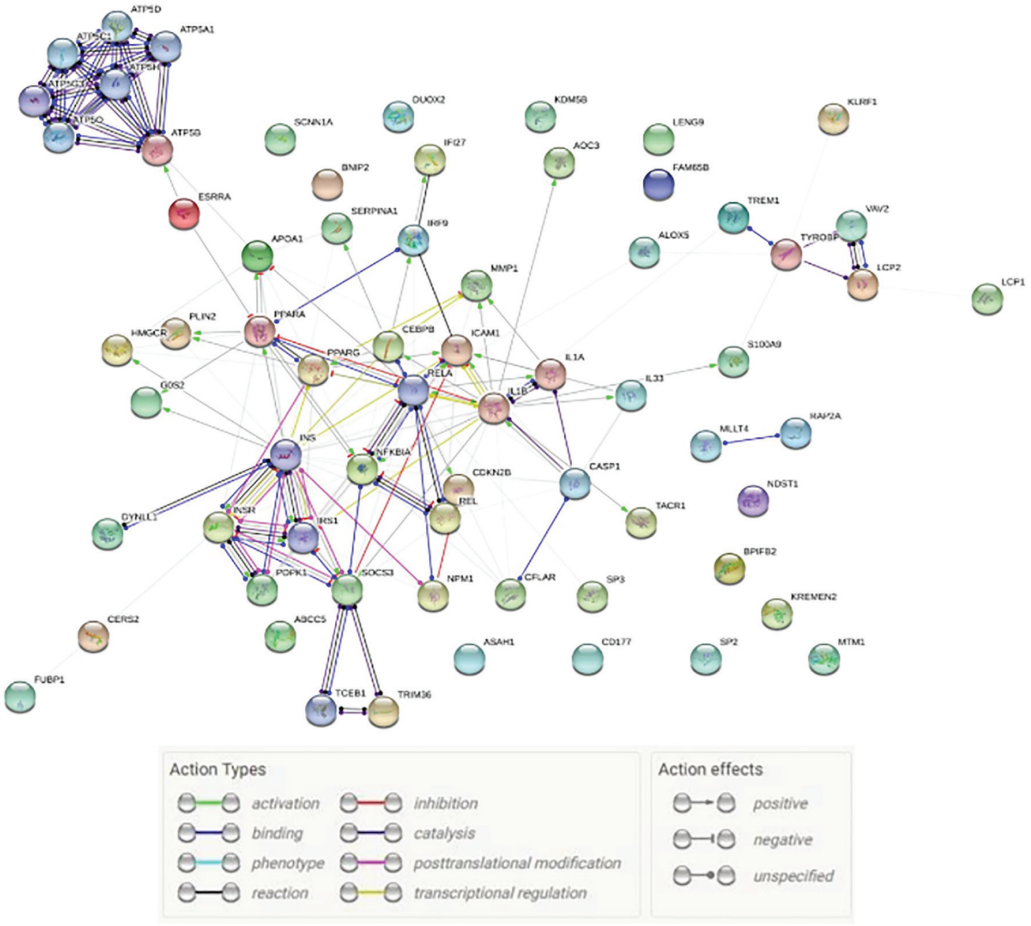

Figure 2. Types of interactions between the network proteins on String.

Table 7. GO biological processes on String.

BIOLOGICAL PROCESS (GO)			
PATHWAY ID	PATHWAY DESCRIPTION	COUNT IN GENE SET	FALSE-DISCOVERY RATE
G0:0042776	Mitochondrial ATP synthesis coupled proton transport	7	3.03e−09
G0:0002526	Acute inflammatory response	8	1.5e−07
G0:0010941	Regulation of cell death	21	1.27e−06
G0:0007165	Signal transduction	36	1.43e−06
G0:0031325	Positive regulation of cellular metabolic process	28	1.52e−06
G0:0045087	Innate immune response	17	1.52e−06
G0:0006952	Defense response	20	2.2e−06
G0:0050896	Response to stimulus	43	4.08e−06
G0:0051092	Positive regulation of NF kappaB transcription factor activity	8	4.08e−06
G0:0044700	Single organism signaling	36	4.92e−06
G0:0007166	Cell surface receptor signaling pathway	23	6.42e−06
G0:0042981	Regulation of apoptotic process	19	7.02e−06
G0:0051247	Positive regulation of protein metabolic process	19	7.57e−06
G0:0045893	Positive regulation of transcription, DNA-templated	19	8.49e−06
G0:0048522	Positive regulation of cellular process	33	9.52e−06

Abbreviation: GO, gene ontology.

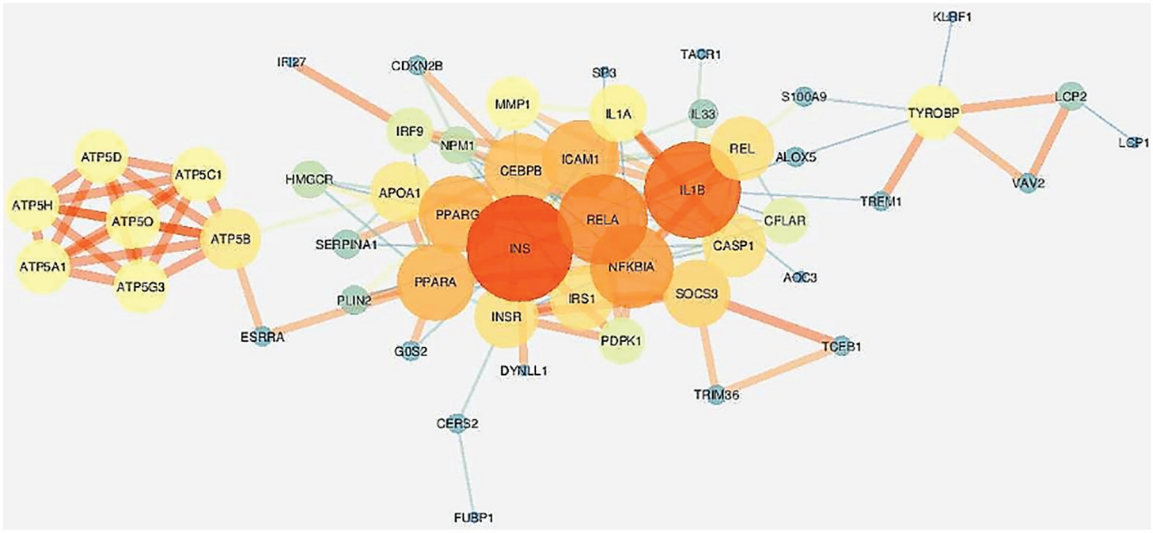

Figure 3. Gene network customized by the network analyzer plugin on Cytoscape.
The nodes of the network represent the proteins: each node represents all the proteins produced by a single gene locus encoding a protein. The edges represent protein-protein associations: the associations must be specific and significant, simply put, the proteins contribute together to a shared function; it does not necessarily mean that they physically bond to each other.

process (GO: 0045834);—response to organic substance (GO: 0010033).

Cytoscape results

The result network analyzer plugin for network visualization is shown in (Figure 3). The central genes for this network according to the chosen parameters are as follows: *INS*, *IL1B*, *NFKBIA*, and *RELA*, congruent with the results obtained by the String software.

The GO terms found by BiNGO pluging are displayed as a table of GO terms (Table 8). The functions are grouped into biological processes and the significant ones are as follows: *positive regulation of lipid metabolic process, the multiorganism process, positive regulation of the cellular process*, and *response to an organic substance*.

Positive regulation of lipid metabolic process. Of the 51 genes interacting in the network (Figure 3), 8 genes are annotated in this biological process according to the Table 8. This biological

Table 8. Table of GO terms found by the BiNGO plugin.

GO-ID	DESCRIPTION	*P* VALUE	CORRECTED *P* VALUE	CLUSTER FREQUENCY	TOTAL FREQUENCY	GENES
42776	Mitochondrial ATP synthesis coupled proton transport	3.9283e–12	5.2406e–9	6/50 12.0%	14/14 306 0.0%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5H ATP50
45834	Positive regulation of lipid metabolic process	7.0343e–12	5.2406e–9	8/50 16.0%	51/14 306 0.3%	IL1A IRS1 IL1B APOA1 PPARG PPARA VAV2 INS
14070	Response to organic cyclic substance	7.9703e–11	3.0 l37e–8	10/50 20.0%	149/14 306	IL1A SOCS3 CDKN2B SERPINA1 IL1B CASP1 PLIN2 PPARG TACR1 RELA
15985	Energy coupled proton transport, down electrochemical gradient	1.0113e–10	3.0137e–8	7/50 14.0%	42/14 306 0.2%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5G3 ATP5H ATP5O
15986	ATP synthesis coupled proton transport	1.0113e–10	3.0137e–8	7/50 14.0%	42/14 306 0.2%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5G3 ATP5H ATP5O
34220	Ion transmembrane transport	5.681e–10	1.3939e–7	7/50 14.0%	53/14 306 0.3%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5G3 ATP5H ATP5O
15992	Proton transport	1.2228e–9	2.6028e–7	7/50 14.0%	59/14 306 0.4%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5G3 ATP5H ATP5O
6818	Hydrogen transport	1.5555e–9	2.8971e–7	7/50 14.0%	61/14 306 0.4%	ATP5B ATP5A1 ATP5D ATP5C1 ATP5G3 ATP5H ATP5O
51704	Multiorganism process	4.9312e–9	8.1639e–7	16/50 32.0%	785/14 306	NPM1 SERPINA1 MMP1 INSR TACR1 CFLAR RELA ICAM1 NFKBIA IL 1A SOCS3 IL1B SP3 CAS . . .
46626	Regulation of insulin receptor signaling pathway	8.2847e–9	1.1741e–6	5/50 10.0%	21/14 306 0.1%	SOCS3 IRS1 IL1B RELA INS
48522	Positive regulation of cellular process	8.6679e–9	1.1741e–6	24/50 48.0%	2004/14 306	ESRRA CEBPB NPM1 CDKN2B IRS1 PDPK1 INSR APOA1 TACR1 CFLAR DYNLL1 RELA ICAM1 . . .
48518	Positive regulation of biological process	1.1027e–8	1.3692e–6	25/50 50.0%	2208/14 306	CEBPB IRS1 RELA ICAM1 INS SOCS3 ALOX5 CASP1 ESRRA NPM1 CDKN2B PDPK1 INSR APO . . .
19216	Regulation of lipid metabolic process	1.7458e–8	2.0010e–6	8/50 16.0%	133/14 306	IL1A IRS1 IL1B APOA1 PPARG PPARA VAV2 INS
48583	Regulation of response to stimulus	1.8859e–8	2.0071e–6	13/50 26.0%	524/14 306	NPM1 IRS1 APOA1 RELA ICAM1 INS NFKBIA IL1A SOCS3 IL1B CASP PPARG PPARA
10033	Response to organic substance	2.0898e–8	2.0759e–6	16/50 32.0%	869/14 306	CDKN2B SERPINA1 IRS1 PDPK1 INSR ATP5G3 TACR1 RELA NFKBIA IL1A SOCS3 IL1B . . .

The table has displayed the most overrepresented GO terms, sorted by their *P* value (ascending order from top to bottom). On the board is a list of GO terms (with their names and GO-IDs) for the uncorrected *P* value and the corrected *P* value. In addition, the total frequency values and a list of corresponding proteins are listed for each term and listed under the "genes" heading. Abbreviation: GO, gene ontology.

process has a significant *P* value of 5.2406e–9. The positive regulation of the lipid metabolic process (Figure 4) is related with other term children, the most significant are as follows: *the positive regulation of the metabolic process of fatty acids, the positive regulation of the catabolic process of lipids*, and *the positive regulation of the lipid kinase activity.*

The multiorganism process. Sixteen genes are annotated in this biological process according to Table 8. This biological process has a very significant *P* value of 4.9312e–9. The multiorganism process (Figure 5) is related with other term children, the most significant are as follows: *the response to another organism* and *the interspecific interaction between organisms*.

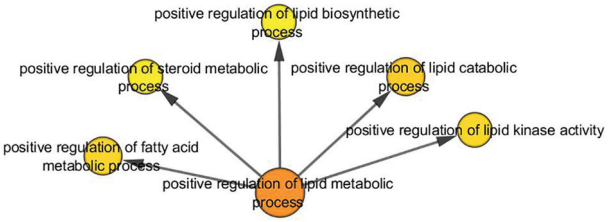

Figure 4. Acyclic-oriented graph of GO terms overrepresented for "Positive regulation of the metabolic process of lipids." GO indicates gene ontology.

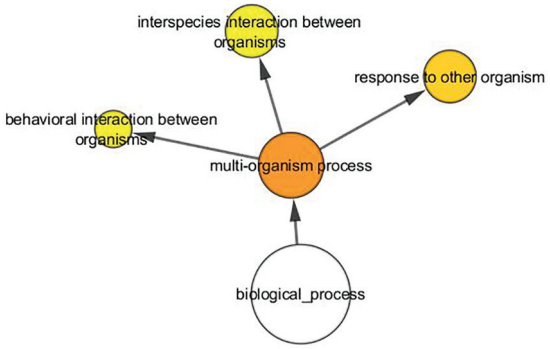

Figure 5. Acyclic-oriented graph of GO terms overrepresented for "The multiorganism process." GO indicates gene ontology.

Positive regulation of the cellular process. This biological process has a significant *P* value of 8.6679e–9, with a ratio of 24 genes annotated (Table 8). The most significant term children from the positive regulation of the cellular process (Figure 6) are as follows: *the positive regulation of the cellular communication, the positive regulation of the cellular metabolic process*, and *positive regulation of organelle organization.*

The response to an organic substance. Sixteen genes are annotated in the response to an organic substance according to the Table 8. This biological process has a significant *P* value of 2.0898e–8. The most significant term children in this biological process are (Figure 7): The response to *the cyclic organic substance, the response to molecules of bacterial origin*, and *response to the hormonal stimulus*.

The BiNGO pluging ontological analysis revealed the involvement of functional network genes in biological processes related to metabolism, communication, and survival of epithelial cells of the gut of newborns. These results are in accordance with the results obtained by String software, showing the positive impact of active foods on the homeostasis of the newborns' intestines.

Conclusion

The results of coexpression and ontological studies provide insights into global patterns of gene expression in epithelial cells of term infants. The 5 central proteins in the networks (IL1β, RELA, INS, IRS1, and NFKBIA) are the major regulators of 4 significant biological processes. These biological processes induced in the first few months of a newborns' life have concerned intestinal development, effect of nutrition,

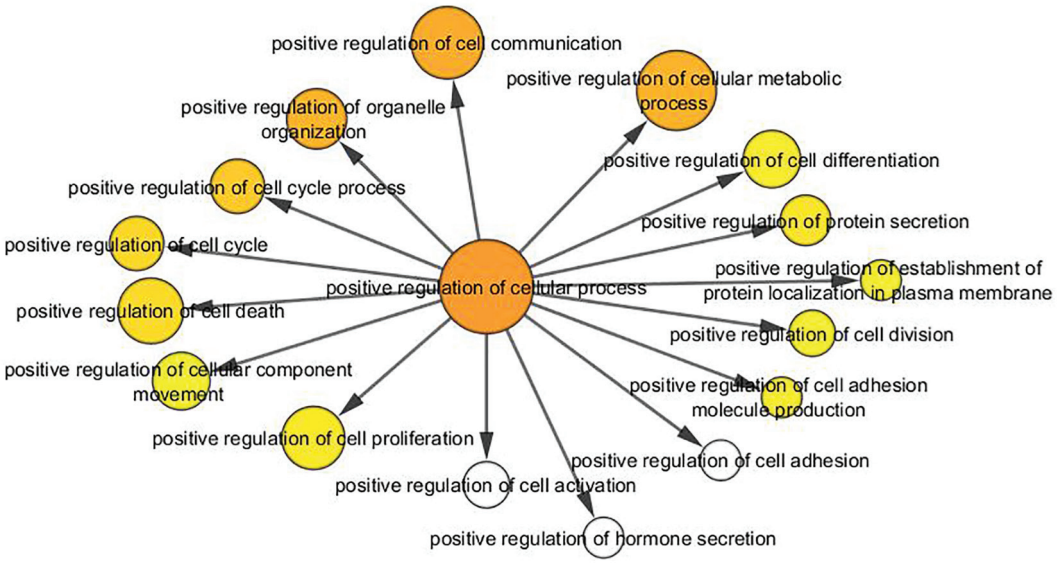

Figure 6. Acyclic-oriented graph of GO terms overrepresented for "Positive regulation of the cellular process." GO indicates gene ontology.

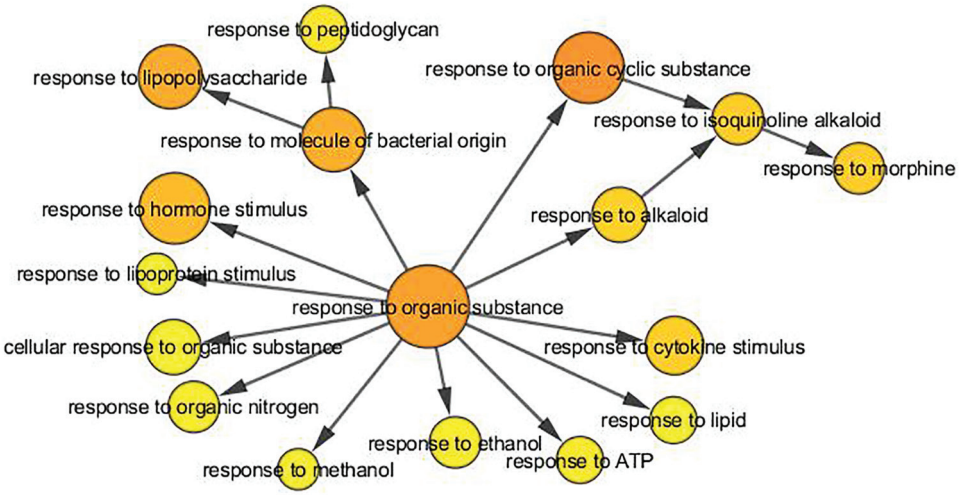

Figure 7. Acyclic-oriented graph of GO terms overrepresented for "The response to an organic substance." GO indicates gene ontology.

and impact of other environmental exposures on the intestinal microbiota colonization. Thus, this study offers a new depiction of the results to allow a better understanding of several interactions and their importance in health homeostasis.

Author Contributions

F.C., F.B., M.M., Y.S., and B.N. contributed conception and design of the study; B.N. and Y.S. collected data from different databases and scientific publications; B.N. performed the bioinformatics analysis and wrote the first draft of the manuscript; F.C., F.B., M.M., and Y.S. wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Contribution to the Field

Breastfeeding is a strategy favored by evolution, to help our descendants survive and project our genes to succeeding generations. Thus, breast milk is a vector of bacteria in the days and months after birth. Gut microbiota established during these first months of life is vital for infant health and subsequent adults. This work aims to exploit the bioinformatics approaches, to provide a new representation and interpretation of the interactions between differentially expressed genes (DEGs) in the host intestine induced by the microbiota. The results of coexpression and ontological studies provide insights into global patterns of gene expression in epithelial cells of term infants. The significant biological processes induced by the central proteins in the networks have concerned intestinal development, effect of nutrition and impact of other environmental exposures on the intestinal microbiota colonization. So, this study can contribute to a new representation of complex interactions between microorganisms genes and host genome during the development of the intestine allowing a better understanding of several interactions and their importance in health homeostasis.

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