

## CONCISE COMMUNICATION

# An integrative multi-omic analysis reveals a major metabolic rewiring between baby foreskin keratinocytes and adult female abdominal keratinocytes

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**Abstract**

Even though its development starts early in utero, neonatal skin is still immature at birth relative to adult and undergoes a maturation process extending to the first years of life. It is now established that the stratum corneum is thinner and dryer and that skin contains less natural moisturizing factors and lipids in newborns compared to children and adults. Moreover, it has been shown that skin surface area expansion is not linear throughout life and is peaking perinatally, suggesting that baby skin has a higher epidermal cellular turnover. Despite growing resources showing differences between adult and infant skin physiology, molecular and metabolic specificities of baby skin are still poorly understood. To address this critical knowledge gap, we performed an integrative transcriptomic and metabolomic study comparing human primary foreskin and abdominal keratinocytes from male babies and female adults, respectively. Based on state-of-the-art integrative frameworks, our analyses revealed a major shift in the global energetic metabolism in baby foreskin keratinocytes compared to adult abdominal keratinocytes, highlighting increased amino acid metabolism and mitochondrial oxidative phosphorylation in baby cells to fuel the citric acid cycle, while showing glycolysis as the major cell energy source in adult cells.

**KEYWORDS**

cell energy, metabolomic, skin barrier, skin development, transcriptomic

## 1 | BACKGROUND

At birth, the newborn shifts from a constant temperature, wet and sheltered environment to a dry highly variable surrounding potentiating water loss, mechanical trauma and infections. Even though its development starts early in utero, neonatal skin is still immature relative to adult and undergoes a maturation process extending to the first years of life. It is now known that baby skin is different from adult skin in terms of structure, composition and function. Indeed, the infant

epidermis and the stratum corneum are thinner and are composed of smaller cells. Babies also experience higher water loss through the skin, cell renewal is twice as fast, and baby skin contains less natural moisturizing factors.<sup>1</sup> The epidermis is morphologically composed of distinct and stratified layers reflecting the differentiation of keratinocytes that migrate from the basal layer to the superficial layer. Epidermal renewal relies on the balance between proliferation and differentiation of epidermal stem cells and on the removal of superficial dead cells (corneocytes) by desquamation to maintain skin

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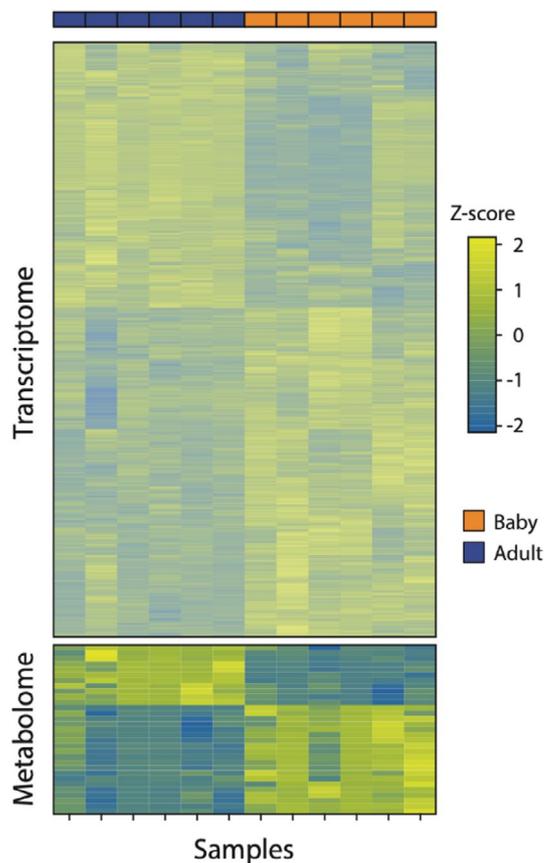
homeostasis or to restore epidermal structure and functions.<sup>2</sup> Skin uses energy metabolism through a dynamic and adaptive process to support its development and to maintain homeostasis.<sup>3,4</sup> Throughout the cell life cycle, energy is required for cell growth and maintenance. Cells produce this energy mainly in the form of adenosine triphosphate (ATP) by several metabolic pathways depending on different conditions. The citric acid (TCA) cycle is the main source of cell energy. It is fuelled by glycolysis, fatty acid oxidation and amino acid pathways that produce acetyl coenzyme A (acetyl CoA). Thereby, one glucose molecule is degraded into two pyruvate molecules by glycolysis in the cytosol. On one hand, pyruvate molecules generated by glycolysis are transported to the mitochondrial matrix to be converted into acetyl CoA.<sup>5</sup> On the other hand, deaminated amino acids can produce pyruvate, acetyl CoA or other components contributing to the TCA cycle into mitochondria. For example, lysine is degraded to acetyl CoA through acetoacetate, phenylalanine feeds the TCA cycle by fumarate and glycine, and serine and threonine are converted to pyruvate, the precursor of acetyl CoA.<sup>5</sup>

## 2 | QUESTIONS ADDRESSED

In this study, we explored potential differences in cell metabolism between infant and adult skin using an integrative transcriptomic and metabolomic analysis of cultured foreskin and abdominal keratinocytes from infant and adult donors, respectively.

## 3 | EXPERIMENTAL DESIGN

Human primary keratinocytes were provided by Sterlab (Sophia Antipolis, France) from 6 baby (male) to 6 adult (female) volunteers. Briefly, keratinocytes were isolated from foreskin for babies and from abdominal skin for adults by Sterlab. After thawing, keratinocytes were seeded at 500 000 cells in a 150 cm<sup>2</sup> flask and cultured in KGM (keratinocyte growth medium)-Gold basal medium supplemented with KGM-Gold Keratinocyte SingleQuots Kit (Lonza) and in a 90% humidified incubator with 5% CO<sub>2</sub> at 37°C until reaching 80–90% of confluence. They were passaged once to the third until reaching 80–90% of confluence. Then, RNAs from 5 million cell pellets were extracted using RNeasy 96 QIAcube HT Kit (Qiagen) and supernatants were collected and frozen at –80°C. Samples were finally sent to Genex (Longjumeau, France) for transcriptomic analysis and Metabolon (Morrisville, USA) for metabolomic profiling (procedural details for the transcriptomic and metabolomic analyses are provided in the Supplementary Information section—Supplementary Methods). For both transcriptomic and metabolomic data, a background correction followed by a quantile normalization (QN) was applied using the RMA (robust multi-array) framework as implemented in the *limma* R package.<sup>6</sup> Quality check was performed to properly evaluate the impact of the QN and filtering using extensive data visualization before and after normalization. The differential analyses were conducted using *limma* to identify genes and metabolites



**FIGURE 1** Heatmap depicting the Z-score expression and relative quantity of probes (top) and metabolites (bottom) differentially expressed between baby foreskin keratinocytes and adult abdominal keratinocytes. Row and columns were ordered using hierarchical clustering based on Pearson's correlation and Ward's D2 aggregation criterion

differentially expressed between baby and adult volunteers considering thresholds at 10% for the FDR (false discovery rate)-corrected p-values and 20% change in expression level. Gene Set Enrichment Analysis (GSEA) was then performed using the *fgsea* R package<sup>7</sup> to identify sets of genes and metabolites that are over-represented among genes and metabolites up- or down-regulated using the Molecular Signatures Database (<https://www.gsea-msigdb.org/gsea/msigdb>) curated gene sets. Metabolomic and transcriptomic data were finally integrated using the *MetaboAnalystR* R framework considering the KEGG database.<sup>8</sup> Since baby foreskin keratinocytes were sampled from males and adult abdominal keratinocytes from females, all probes matching genes contained in allosomes were excluded before performing the analyses (*i.e.* 2143 probes, corresponding to 1,505 genes).

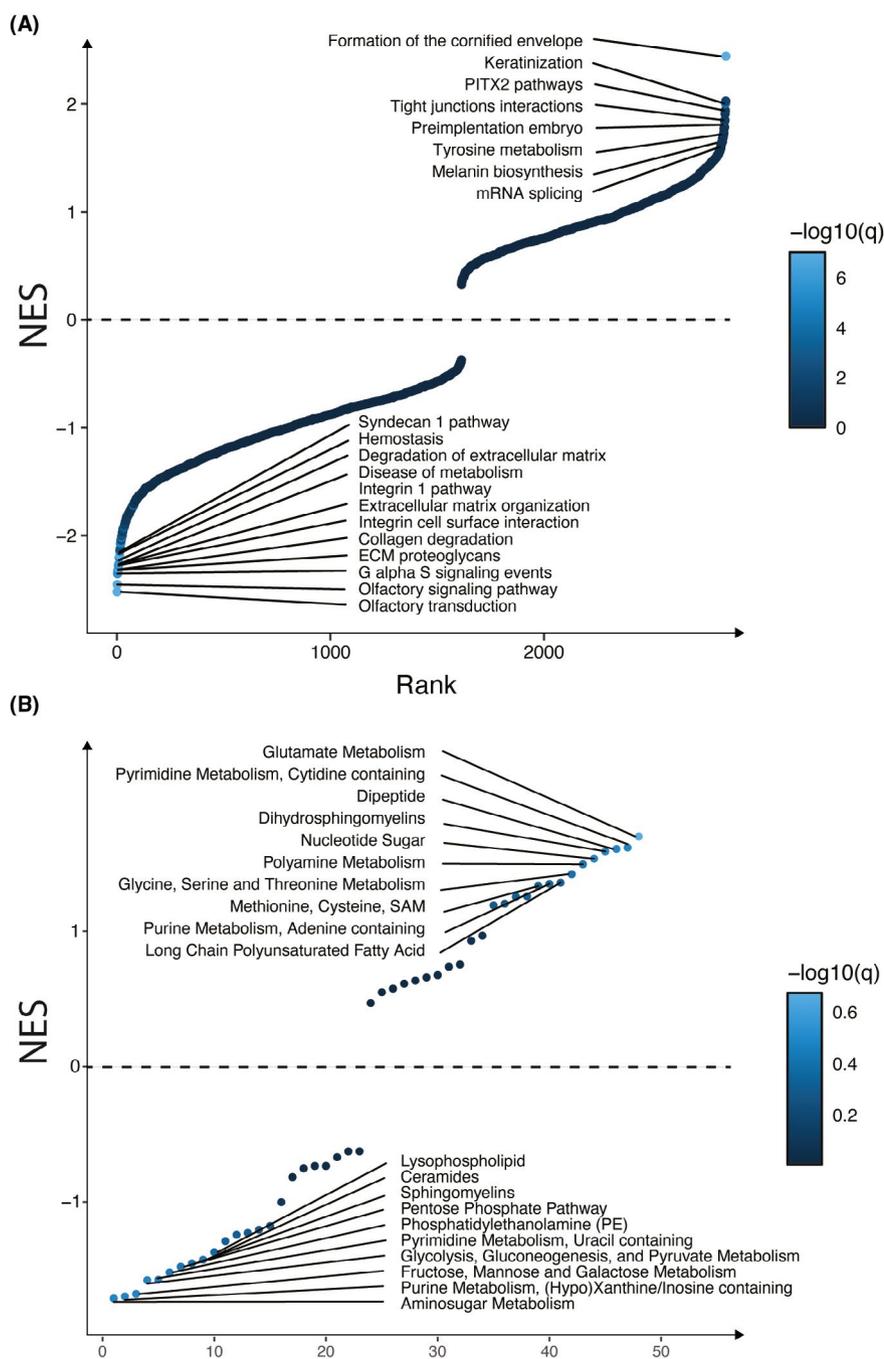
## 4 | RESULTS

The differential transcriptomic analysis revealed a total of 3,189 dysregulated probes (1,769 up-regulated and 1,420 down-regulated in baby foreskin keratinocytes (BFK) vs. adult abdominal keratinocytes

(AAK), Figure 1 top panel and Figure S1A). The results of the differential expression analysis are provided in Table S1. The raw data are publicly available on Gene Expression Omnibus (accession number GSE183891). The differential metabolomic analysis revealed 65 dysregulated metabolites (37 up-regulated and 28 down-regulated in BFK vs. AAK, Figure 1 bottom panel and Figure S1B). The results of the differential analysis are provided in Table S2.

From the transcriptome perspective, the GSEA revealed an up-regulation of molecular pathways involved in skin maturation in BFK (formation of the cornified envelope, keratinization, *PITX2* pathways and melanin synthesis, Figure 2A) and a down-regulation of pathways connected to cell turnover and replication (G alpha S signalling events,

extracellular matrix (ECM) proteoglycan, collagen degradation and ECM organization, Figure 2A). These major differences were also reflected in the results of the GSEA performed on the metabolome, pinpointing an increased production of amino acid-related metabolites (dipeptide, glycine metabolism and methionine metabolism, Figure 2B) and a decreased production of ceramides (ceramides and sphingomyelins) and sugars (glycolysis, gluconeogenesis, pyruvate metabolism, fructose, mannose and galactose, Figure 2B) in BFK supernatants. Considering gene expression level as a readout for enzymatic activities, the integrative analysis finally confirmed the increased activity of metabolic pathways involved in amino acid synthesis and degradation in BFK (lysine degradation, glycine, serine, threonine metabolism, cysteine and



**FIGURE 2** (A and B) Ranking plots showing the Nested Enrichment Score (NES) computed with a Gene Set Enrichment Analysis on the Reactome database for transcriptomic data (A) and the KEGG database for metabolomic data (B). The name of relevant top hit pathways is given

methionine metabolism and phenylalanine metabolism, Figure 3 right) and the decreased activity of metabolic pathways related to sugar metabolism (glycolysis, gluconeogenesis, starch and sucrose metabolism, Figure 3 left). Interestingly, this analysis also revealed a decreased activity of nucleotide-related pathways (purine, pyrimidine and nitrogen metabolism) in BFK (Figure 3 left).

This study shows functional differences between baby foreskin and adult abdominal skin cells when placed in similar conditions (monolayered primary keratinocytes at subconfluence) suggesting similar differences in the skin. Of interest, recent studies have highlighted the possibility of running such transcriptomic analysis on non-cultured skin samples through single-cell transcriptomic analysis.<sup>9,10</sup> This type of analysis would be of great interest to further confirm the present observations.

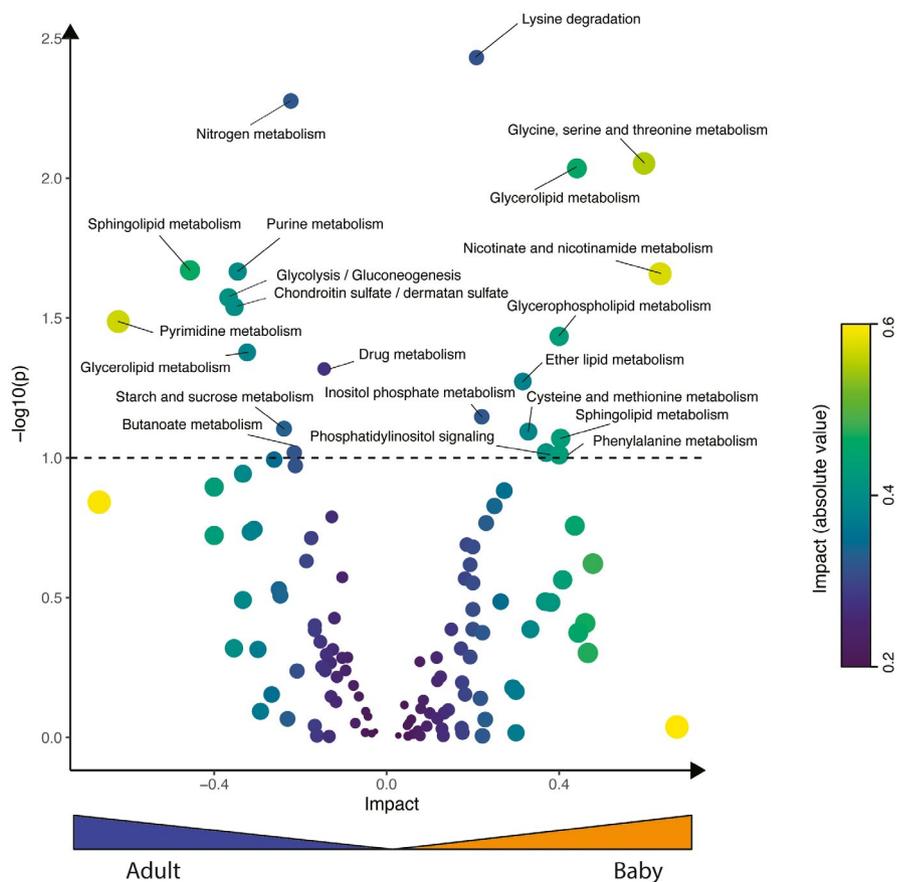
Since BFK were sampled from male donors and AAK from female donors, a portion of the observed differential expression might be attributed to a gender bias as previously identified by Naqvi et al.<sup>11</sup> However, a comparative analysis of the genes affected by a sex bias in the study of Naqvi et al. and the present data showed that only 1.9% of the differentially expressed genes in that study might be affected by a sex bias when considering the list of sex-biased genes in skin<sup>11</sup> (Table 1) and up to 9.6% if considering genes affected by sex bias in at least one tissue. This comparative analysis showed that the effect of age is predominant as compared to a potential sex bias.

Body location might also contribute to some extent to the observed dysregulation. Indeed, it is well known that some physiological

parameters, such as epidermal thickness, vary among body sites.<sup>12,13</sup> However, to our knowledge, no major shift in cellular energy/metabolism has been reported in keratinocytes from different anatomical sites.

The switch of metabolic pathways between baby foreskin keratinocytes and adult abdominal keratinocytes highlighted in the integrative analysis of the transcriptomic and metabolomic data shows that baby foreskin cells mainly use mitochondrial oxidative phosphorylation through up-regulation of (a) nicotinate and nicotinamide metabolism and (b) amino acid metabolism, as a source of energy (Figure 3). Indeed, we observed the up-regulation of lysine degradation in BFK associated with the production of acetyl CoA (Figure S1E) and the increased metabolism of glycine, threonine and serine (glucogenic amino acids, Figure 3) implicated in pyruvate production, which might fuel the TCA cycle in mitochondria, thus generating energy for baby cells. This mitochondrial activity generates reactive oxygen species (ROS), which has been shown to promote epidermal differentiation. Indeed, mitochondrial ROS generation is involved in activation of Notch and beta-catenin pathways, both implicated in keratinocyte differentiation.<sup>14,15</sup> The activation of Notch signalling in BFK is also highlighted in our study (Figure S2).

Conversely, the glycolysis pathway is preferred in adult abdominal keratinocytes (Figure 3). This phenomenon was already observed in skin ageing involving a decline of mitochondrial function associated with a shift to a non-mitochondrial pathway increasing glycolysis, glucose uptake and lactate production in old fibroblasts.<sup>16</sup>



**FIGURE 3** Volcano plot summarizing the result of the integrative analysis and showing the concomitant impact of metabolomic and transcriptomic differences between baby foreskin keratinocytes and adult abdominal keratinocytes on KEGG pathway activities

TABLE 1 Comparative analysis of genes affected by a sex bias (Naqvi et al. 2019) and the present data

	Current study	
	3189	
Number of genes differentially expressed in BFK vs AAK	Considering genes affected by sex bias in skin <sup>a</sup>	Considering genes affected by sex bias in at least one tissue <sup>a</sup>
Number of genes affected by sex bias (Naqvi et al. 2019)	269	1568
Number of overlapping genes between current study and Naqvi et al. study	61	307
% of differentially expressed genes potentially affected by a sex bias in current study	1,9%	9,6%

<sup>a</sup>In Naqvi et al. study.

Increased glycolysis in AAK could be correlated with the up-regulation of purine and pyrimidine metabolism in AAK (Figure 3). Indeed, glycolysis may also serve to feed the pentose phosphate pathway (PPP). The PPP generates nicotinamide adenine dinucleotide phosphate (NADPH), a reducing equivalent used in anabolic reactions, such as lipid and nucleic acid synthesis and in the regeneration of glutathione.<sup>17</sup> Therefore, up-regulation of the PPP (Figure 2b) could be as a first-line response to oxidative stress or to nucleotide synthesis, essential for proliferation.

Although glycolysis generates less ATP than oxidative phosphorylation in mitochondria, proliferative cells preferentially use glycolysis when glucose availability is sufficient.<sup>18</sup> The ECM also plays a major role in this switch through focal adhesion, which mediates the activation of phosphatidylinositol 3-kinase (Pi3K) pathway, increasing glycolysis to maintain cell proliferation.<sup>19</sup> This is in line with the up-regulation of ECM organization (Figure 2A), focal adhesion, Pi3K pathway (Figure S3) and glycolysis that we observed in adult abdominal keratinocytes.

In this study, ceramides and sphingomyelins are down-regulated in baby foreskin keratinocytes as compared to adults (Figure 2B), which might contribute to the weaker barrier often observed in baby skin. Indeed, ceramides and sphingomyelins are constituents of the extracellular lipids of the stratum corneum and stratum granulosum, which provide barrier integrity and prevent water loss.<sup>20</sup> The metabolomic analysis also revealed a lesser release of phospholipids such as phosphatidylethanolamine and lysophospholipids in baby foreskin keratinocytes as compared to adult abdominal keratinocytes (Figure 2B). Although phospholipids are not major constituents of the stratum corneum, they are present and serve as reservoirs of free fatty acids, which are metabolized from phospholipids by phospholipases to contribute to skin homeostasis and barrier integrity.<sup>21</sup> This decreased production of phospholipids in BFK could in part explain barrier immaturity usually observed in babies.

In addition, phosphatidylcholine and phosphatidylethanolamine are major components of the plasma membrane of basal keratinocytes and lamellar bodies that are secreted by keratinocytes for permeability barrier homeostasis.<sup>21</sup> Thus, degradation of the plasma membrane as expected during keratinocyte differentiation

or secretion of lamellar bodies to maintain the protective barrier functions would result in the release of phosphatidylcholine and phosphatidylethanolamine. The dysregulation of those phospholipids as observed in baby foreskin keratinocytes could be linked to a still-developing skin barrier in babies.

Finally, the ECM and proteins of the integrin family play a role in the balance between proliferation and differentiation to maintain skin homeostasis.<sup>22</sup> The down-regulation of both the extracellular matrix organization pathway and integrins observed in our study in BFK might thus also explain the immature skin barrier (Figure S4).

## 5 | CONCLUSIONS AND PERSPECTIVES

In conclusion, there is a metabolic switch between keratinocytes from baby donors and those from adults. Under the tested cell culture conditions, baby foreskin keratinocytes primarily seem to use amino acid metabolism fuelling the TCA cycle and oxidative phosphorylation as an energy source, encouraging cell differentiation, while adult abdominal keratinocytes seem to privilege glycolysis to promote cell proliferation. A better understanding of the physico-chemical properties of baby skin stands as the keystone for enhancing knowledge on the development of baby skin barrier.

### CONFLICTS OF INTEREST

All authors were employees of Johnson & Johnson Santé Beauté France when the research was performed.

### AUTHOR CONTRIBUTIONS

Claire Mangez and Pierre-François Roux performed the research. Claire Mangez, Pierre-François Roux and Cécilia Brun analysed the data, Georgios Stamatas, Thierry Oddos and Cécilia Brun designed the study and coordinated it. Georgios Stamatas and Cécilia Brun drafted the manuscript. All authors were involved in data interpretation, and have read and approved the final manuscript.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Fig S1

Fig S2

Fig S3

Fig S4

Table S1

Table S2

Supplementary Material

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