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Migration and Synaptic Aspects of Neurons Derived from Human Induced Pluripotent Stem Cells from Patients with Focal Cortical Dysplasia II

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Abstract—Malformations of cortical development (MCDs) include many different Central Nervous System (CNS) disorders related to a complex process of cortex formation. In children with refractory epilepsy to drug treatment undergoing surgery, focal cortical dysplasia (FCD), one of the MCDs, is considered the most common structural brain lesion found. This study aimed to study the possible alterations in neural differentiation process of human induced pluripotent stem cells (hiPSCs) related to migration and synaptic aspects from fibroblasts of two individuals affected by FCD type IIb (45-year-old male and 12-year-old female) and normal individuals. At the days 14th, 22nd and 35th, hiPSCs were neural differentiated and analyzed. Using qRT-PCR approach, the expression of 9 genes associated with synaptic and neural migration were quantified. Diagnostic of both patients was consistent with FCD type IIb. Our results showed that in all processes and groups, individuals with dysplasia presented alterations in most part of the genes in relation to control individuals. According to our results, it is suggested that the different expressions are mainly involved in alterations of the expression of receptors and capture sites, timing, coupling of synaptic vesicles with the presynaptic membrane, regulation of ion channel and synaptic exocytosis, imbalance of the apoptosis process and abnormal microtubules that may also contribute to delays in synaptogenesis. Thus, brain formation with dysplasia is probably influenced by these genes studied. **© 2019 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: focal cortical dysplasia type IIb, synaptic, migration, hiPSCs, neural differentiation.

INTRODUCTION

Malformations of cortical development (MCDs) include many different Central Nervous System (CNS) disorders related to a complex process of cortex formation. In children with refractory epilepsy to drug treatment undergoing surgery, focal cortical dysplasia (FCD), one of the MCDs, is considered the most common structural brain lesion found (Palmini et al., 2004). Whether the pathogenesis of FCD results from migration, with an early disturbance and final arrangement of the cortical architecture of immature neurons is a study in progress (Rowland et al., 2012).

The generation of specific human-induced pluripotent stem cells (hiPSC) from fibroblasts from FCD patients is an important approach that allows a better understanding of the mechanisms of neurogenesis. Here, hiPSC-derived neurons were obtained from FCD type IIb and control patients by reprogramming fibroblasts using a Sendai Reprogramming kit. A gene profiling analyses related to synaptic aspects and neural migration of these differentiated neurons was performed using qRT-PCR.

EXPERIMENTAL PROCEDURES

Patients

After signing the informed consent form, at the Epilepsy Surgery Program of the Hospital São Lucas, biopsies were obtained from two FCD type IIb patients. They were free from infectious diseases and undergoing surgical treatment for medically refractory epilepsy. Skin residual fragment from adult patient were obtained to compose the control group. They were treated in the Plastic Surgery Program of the Hospital São Lucas from PUCRS and also sign the informed consent form. They were healthy, free of infectious and neurological disease. All the study was in accordance with protocol number 915.598 (CAAE: 37977114.3.0000.5336) released by the Ethics Committee for Analysis of Research Projects of Hospital São Lucas from PUCRS. In Marinowic

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et al. (2017) is described all patients features, the obtaining of fibroblasts from biopsies, histologic analysis of dysplastic tissue and reprogrammation of hiPSCs. Tissue from healthy "brain" was obtained from RNA extracted from a parallel project.

hiPSCs neural differentiation and evaluation

The neural differentiation protocol was performed according to Song and Sanchez-Ramos (2008). Genes associated with neurogenesis process in brain tissue, fibroblasts, hiPSCs cells, during different periods of neural differentiation and in neural differentiated hiPSC cells from patients with FCD and control patients were molecularly analyzed. Briefly, the neural differentiation protocol can be found in Majolo et al. (2018), including the extraction of RNA and cDNA synthesis process.

Molecular analysis through qRT-PCR

Through real-time Polymerase Chain Reaction (PCR) technique, analysis of gene expression was performed using StepOne Plus (Life Technologies) equipment. Amplifications were done from 20 ng of cDNA. GAPDH was used as an endogenous expression control. The primer sequences are presented below (Table 1).

RESULTS

Patients

Diagnostic of both patients was consistent with type II FCD, more precisely, IIb. Both individual uses antiepileptic's without seizure control. Above, laboratory and clinical features of the participants.

Individual 1: Male (45 years), related to the epilepsy surgery, were done the resection of the epileptogenic zone. Magnetic resonant imaging showed FCD in the right frontal region.
Individual 2: Female (12 years), surgical resections were performed of the left frontal epileptogenic zone. In the left orbitofrontal region, magnetic resonance imaging showed pericyst signal change and an unequivocal dysplastic lesion.

hiPSC differentiation into neurons

After the cellular reprogramming with exposure of the fibroblasts to the viral vectors with consequent formation of the clones, we performed the differentiation of hiPSCs into neurons. With 35 days it was possible to see morphological characteristics very similar to nerve cells and neural networks. Also, with 35 days, the neural differentiated cells showed positive labeling for the FluoroPan Neuronal Marker antibody.

Molecular analysis

In relation to *DLG4* gene dysplastic cells hiPSCs expressed the gene 2 times more compared to control individuals. Both the brain tissues of individuals with FCD and controls had very close levels of expression, using fibroblast as a reaction calibrator (Fig. 1A). During the neural differentiation protocol for 14 and 22 days the expression values were also similar between the groups, using the hiPSCs cells as a calibrator. In neurodifferentiation for 35 days, the subjects expressed dysplastic about 4 times less the gene compared to control cells (Fig. 2A).

hiPSCs from dysplastic and control subjects showed similar *FGF2* gene expression values. Brain tissue from control subjects showed an increase of 17 times more compared to dysplastic individuals (Fig. 1B). During the three neural differentiation periods, 14, 22 and 35 days, the expression of the gene in the control subjects were, respectively, 75, 69 and 195 times less expressed in comparison to the dysplastic individuals (Fig. 2B).

Expression of *STX1A* gene in hiPSCs cells of control subjects were 4 times higher compared hiPSCs dysplastic cells of individuals using fibroblast reaction as calibrator. Expression of the gene in brain tissue was similar between the groups (Fig. 1C). During the neural differentiation protocol for 14 days, the dysplastic individuals expressed 10 times the gene in relation to the control individuals. In the dysplastic group, gene expression in neurodifferentiation cells for 35 days was less expressed in comparison to the control group (Fig. 2C).

Table 1. Primers for hiPSC and differentiated neuron gene markers.

	Forward	Reverse
ASCL1	5′-AAGCAAGTCAAGCGACAGCG-3′	5′-AGTCGTTGGAGTAGTTGGGG-3′
DCX	5′-CATCCCCAACACCTCAGAAG-3′	5′-GGAGGTTCCGTTTGCTGA-3′
DLG4	5′-TCCTCACAGTGCTGCATAGC-3′	5′-TGTCTTCATCTTGGTAGCGG-3′
FGF2	5′-AGAGCGACCCTCACATCAAG-3′	5′- TCGTTTCAGTGCCACATAC-3′
NEFL	5′-TGAACACAGACGCTATGCGCTCAG-3′	5′-CACCTTTATGTGAGTGGACACAGAG-3′
NEUROD6	5′-CTGAGAATCGGCAAGAGACC-3′	5′-CTGCACAGTAATGCATGCCG-3′
NEUROG2	5′-AGGAAGAGGACGTGTTAGTGC-3′	5′-GCAATCGTGTACCAGACCCAG
NRCAM	5′-TTGTGCAAAGAGGGAGCATG-3′	5′-GGGCAGTTCCCTGTTGTCCT-3′
STX1A	5′-ATCGCAGAGAACGTGGAGGAG-3′	5′-AGCGTGGAGTGCTGTGTCTTC-3′
GAPDH	5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′	5′-CATGTGGGCCATGAGGTCCACCAC-3′

* ASCL1 (Achaete-scute family bHLH transcription factor 1); DCX (Doublecortin); DLG4 (Discs large homolog 4); FGF2 (Fibroblast growth factor 2); NEUROD6 (Neuronal differentiation 6); HES1 (Hes family bHLH transcription factor 1); NEFL (Neurofilament light chain); NEUROD6 (Neuronal differentiation 6); NEUROG2 (Neurogenin 2); NRCAM (Neuronal cell adhesion molecule); STX1A (Syntaxin 1A).

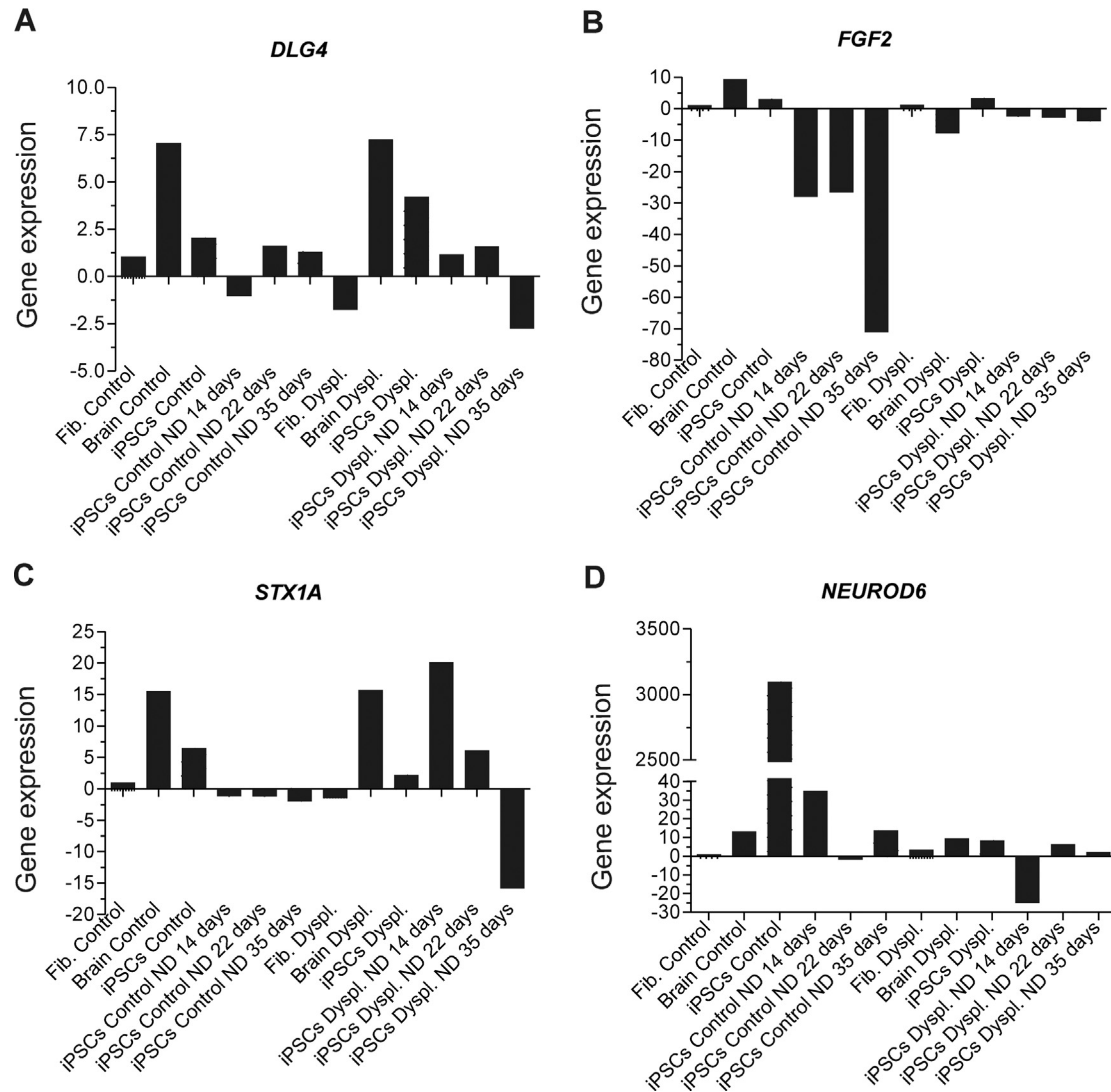

Fig. 1. Result of relative quantification through qRT-PCR of the genes and all study groups. The results are presented using as calibrator fibroblasts from the control group. A) *DLG4,* B) *FGF2,* C) *STX1A,* D) *NEUROD6,* E) *ASCL1,* F) *DCX2,* G) *NEUROG2,* H) *NEFL,* I) *NRCAM.*

There was an increase in the expression of *NEUROD6* gene about 3084 times in the cells of hiPSCs controls compared hiPSCs of dysplastic individuals. In relation the brain tissue from control subjects, there was a greater expression of 4 times more compared to the dysplastic group (Fig. 1D). During neural differentiation for 14 and 35 days, the dysplastic group showed a decrease in gene expression in relation to the control group (77,308 and 1267 times less, respectively). Already in the 22 days neural differentiation, control subjects presented a decrease of 5512.51 times less in relation to the dysplastic group (Fig. 2D).

The relative expression of *ASCL1* gene into dysplastic brain tissue showed an increase of 142 times when compared to control brain tissue. Already the control hiPSCs cells expressed 50 times more compared dysplastic hiPSCs using control group fibroblasts as calibrator (Fig. 1E). During the neurodifferentiation protocol, in FCD subjects, a decrease of about 6, 197 and 10 times less, respectively, was observed in the expression of *ASCL1* in neurodifferentiated cells for 14, 22 and 35 days compared to the control individuals these same days, using the undifferentiated hiPSCs as a calibrator (Fig. 2E).

The levels of *DCX* expression in the control hiPSCs cells were 1235 times higher than the dysplastic hiPSCs. Dysplastic brain tissue expressed 24 times more in relation to the control brain tissue (Fig. 1F). In the control subjects there was a decrease in gene expression during the neurodifferentiation protocol for 22 and 35 days, being approximately 471 and 538 times less expressed compared to the dysplastic group. Cells neurodifferentiated for 14 days showed similar values between groups (Fig. 2F).

Control hiPSCs cells expressed 2705 times the *NEUROG2* gene when compared to dysplastic hiPSC cells. The brain tissue presented higher expression values in dysplastic individuals (3 times more) (Fig. 1G). During the neurodifferentiation protocol, for 14, 22 and 35 days, the dysplastic group presented

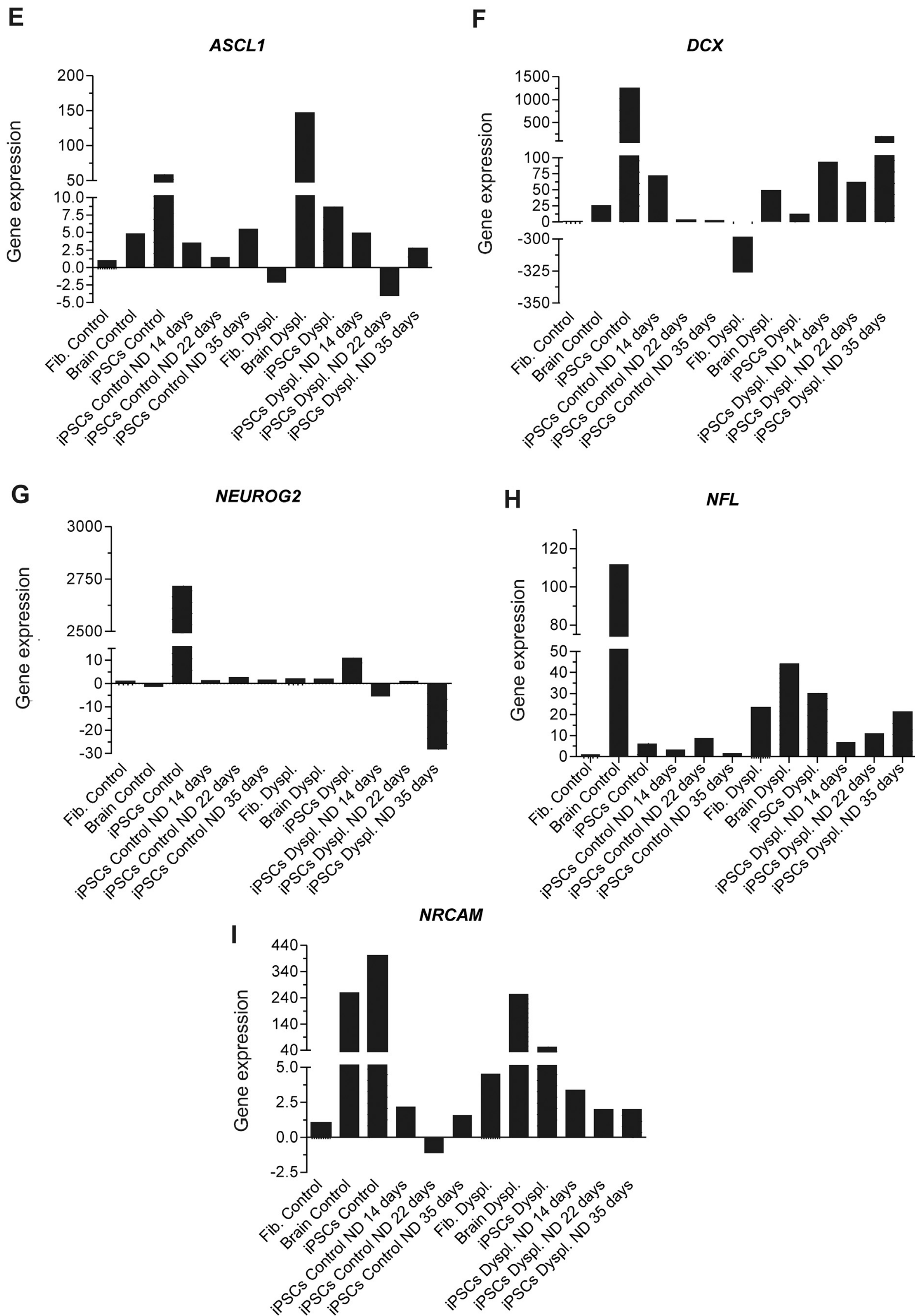

Fig. 1. (*continued*).

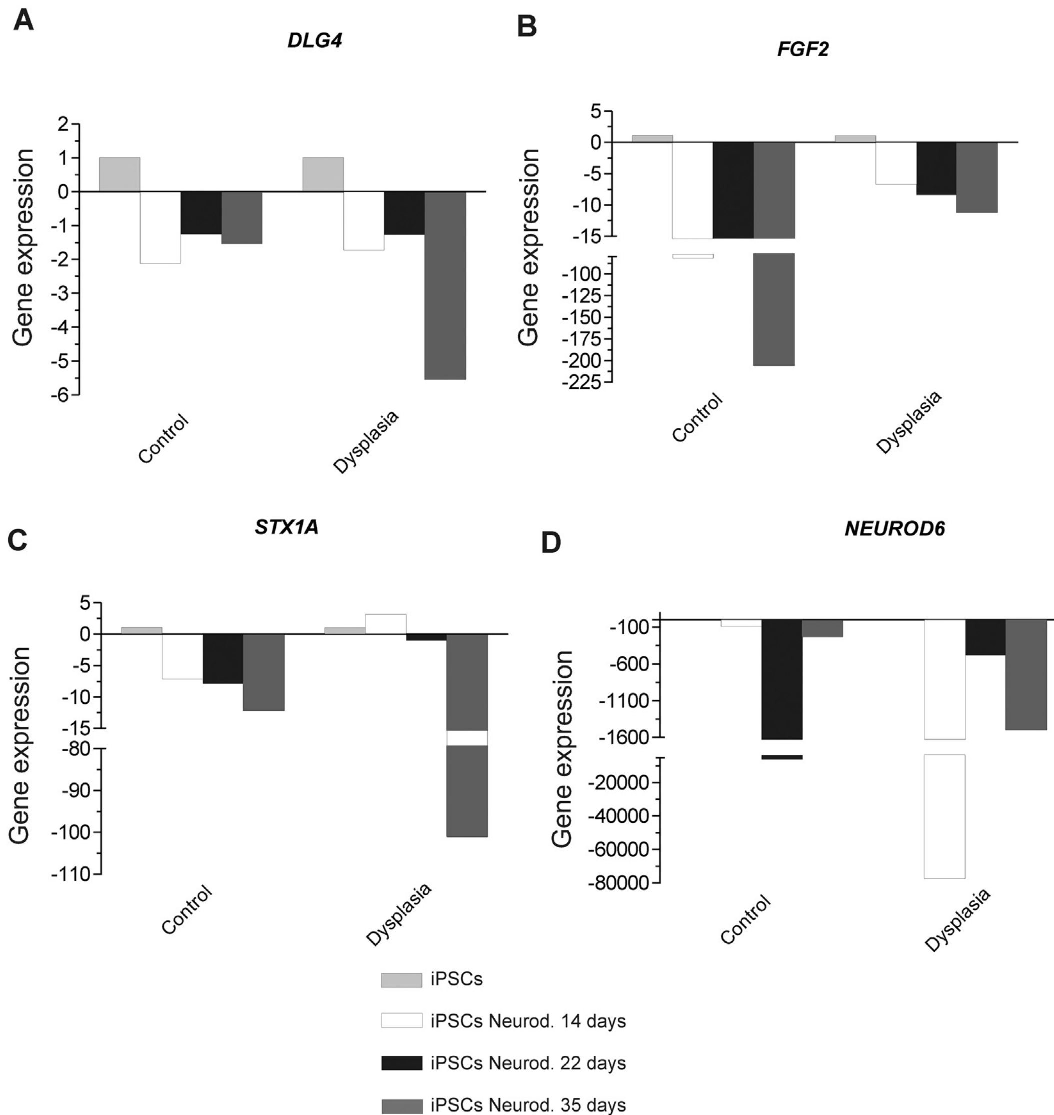

Fig. 2. Result of relative quantification through qRT-PCR of the genes during the neural differentiation of hiPSCs. The results are presented using undifferentiated hiPSCs as the calibrator. A) *DLG4,* B) *FGF2,* C) *STX1A,* D) *NEUROD6,* E) *ASCL1,* F) *DCX2,* G) *NEUROG2,* H) *NEFL,* I) *NRCAM.*

a decrease of 13,152, 1333 and 74,747 times less the expression of the *NEUROG2* gene in relation to the control group (Fig. 2G).

Analysis of the *NEFL* gene presented higher values of expression in the brain tissue of control subjects compared to the brain tissue of subjects with FCD (68 times more expressed). The dysplastic hiPSC cells, however, expressed 24 times more compared to control hiPSC cells (Fig. 1H). In neurodifferentiation for 14 days, the dysplastic group expressed 3 times the gene and returned to similar values in gene expression during neurodifferentiation for 22 days. Already in the neurodifferentiation for 35 days, the dysplastic group presented an increase in relation to the control group (7 times more expressed) (Fig. 2H).

Control hiPSCs cells had an increase in *NRCAM* gene expression compared to dysplastic hiPSCs (346 times). The expression in the brain tissue of both groups was similar (Fig. 1I). During neurodifferentiation for 14, 22 and 35 days, the control group decreased, respectively, 66, 251 and 48 times less the gene expression in comparison to the dysplastic group (Fig. 2I).

DISCUSSION

To date more than 100 different genes have been associated with MCDs, associated with cell proliferation, cell cycle regulation, neuronal migration, late cortical organization and structure and function of the cytoskeleton (Parrini, 2017; Lee et al., 2012; Guerrini and Parrini, 2015). Consequently, these changes in the CNS can affect not only the pattern of the cortical malformation but also the place where the cortex can be affected (Kuzniecky, 2015). In the present study, we approached 9 different genes that were analyzed during neurogenesis and embryonic neurodifferentiation, involved in neural migration.

The skin fibroblasts of control patients are from abdominoplasty, so there is the influence of environmental exposure, such as the sun, if the person is bald or not, different from the child. All this should be considered in obtaining this material. Already the fibroblasts obtained from patients affected by the disease come from the surgical incision, therefore, the environmental exposure is completely different, and the age is also different. One patient lived with solar radiation for 12 years, and the other for 45 years. The piece of fibroblasts we receive from surgeries is extremely small. If the insult is able to genetically alter that embryo in formation, during fertilization where the first cells are generated, it will be present in the hiPSCs and in the fibroblast, now if it is a change at the epigenetic level or at a temporal level, there will be a normal embryology. This study is very preliminary so that an opinion can be formed against a more molecular and genetic or environmental origin.

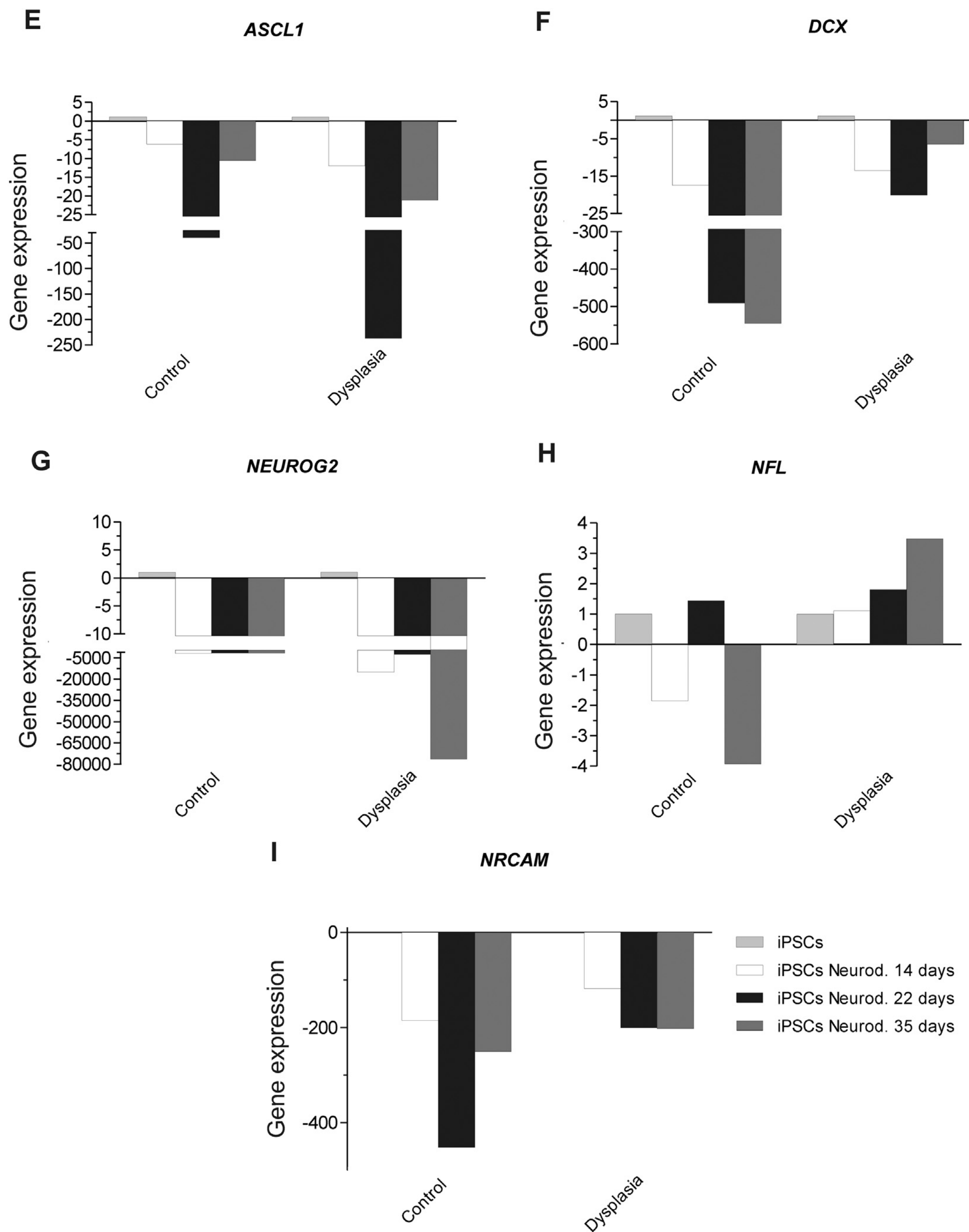

Fig. 2. (*continued*).

from two patients and their ages and genders (45-year-old male and 12-year-old female), there may be differences in responses of cells from young patients than from adults. Due to the very small sample size, since they are surgical patients because of drug refractoriness, we decided not to explore analysis and use the results as suggestive of an alteration without any more conclusive statement. The data presented in this study are original and useful as a pilot study from two patients and would justify further studies of a larger cohort for statistical analyzes, taking into account the pairing of ages and gender.

In relation to the molecular analysis in the hiPSCs cells, lower expression values were present in dysplastic individuals compared to control individuals in *STX1A* and *NEUROD6* genes. The *FGF2* gene presented similar values between the groups. In relation the neural differentiation protocol, the *DLG4* and *STX1A* genes (35 days), and the *NEUROD6* gene (14 and 35 days) showed lower expression values in dysplastic individuals compared to controls. The genes *FGF2* (14, 22 and 35 days), *STX1A* (14 days) e *NEUROD6* (22 days) presented higher values of expression in the dysplastic individuals than in the control individuals. In relation to the *DLG4* gene neural differentiated for 14 and 22 days, the expression was similar between the dysplastic and control groups. In brain tissue, *FGF2* and *NEUROD6* genes presented higher values of expression in the dysplastic individuals in comparison to the control group. Both *DLG4* and *STX1A* genes showed similar expressions.

Histopathology and immunohistochemistry is compatible with a diagnosis of FCD Type IIb with cortical dyslamination and large, dysplastic neurons, with balloon cells (Blümcke et al., 2011; Marinowic et al., 2017). Balloon cells possess a poorly defined membrane with single or multiple nuclei and an eosinophilic cytoplasm, which are characteristics of neuronal and glial cells (Taylor et al., 1971; Kabat and Krol, 2012; Marinowic et al., 2017). These cells are of mixed neural lineage, expressing at the same time neuronal and glial proteins, though proportions differ in different balloon cells in the same biopsy specimen and between different patients (Yasin et al., 2010). Considering the fact that the fibroblasts studied were

The differences found in the expression levels of hiPSCs cells can have consequences for the development of the three germ layers, resulting in different malformations, including cortical dysplasia. During corticogenesis, the expression of *NEUROD6* is triggered on the 11th day of embryonic development and contributes to the specification of multipotential progenitor cells, which possibly justifies the level of gene expression in dysplastic hiPSC cells to be lower compared to control hiPSC cells, contributing to the formation of the disease (Shimizu et al., 1995; Uittenbogaard et al., 2010a). As the brain tissue, which may be associated with FCD, since, in healthy individuals, *NEUROD6* gene expression remains in areas with higher cognitive functions in the adult brain, such as in the hippocampus, neocortex, and entorhinal cortex. These are the first area to be affected in Alzheimer disease (Shimizu et al., 1995; Bartholomä and Nave, 1994; Uittenbogaard et al., 2010b).

The altered levels of the *DLG4* gene may be related to the altered expression of neuroreceptors and capture sites described in several studies involving individuals affected by FCD and probably contributes to the disease through the development of immature neurons and the intrinsic excitability of FCD (Marin-Valencia et al., 2014). Changes in neuroreceptors and uptake sites have also been demonstrated in the *STX* gene in the present study, which may possibly be explained by a question of "altered timing", since "timing" is fundamental in cellular processes, noting that altered expression of neuroreceptors and capture sites has been repeatedly demonstrated in FCD, and the *STX* gene is directly involved in the coupling of synaptic vesicles with the presynaptic plasma membrane, regulation of ion channel and synaptic exocytosis (Lopes et al., 1999, Sarnat et al., 2015). Much like the immature cytoskeletal system observed in the FCD tissue, the expression profile of neuroreceptors may also reflect immature neurons in both cytomorphic and balloon cells, based on immunohistochemically analysis and mRNA (Cepeda et al., 2006; Crino et al., 2001).

Though this is not the technique of this present study, the sequence of synaptogenesis in the developing human fetal brain also is demonstrated in tissue sections by immunohistochemistry of synaptophysin, a synaptic vesicle glycoprotein and that synaptogenesis may be delayed or even precocious in the cortex and hippocampus in certain cerebral malformations (Sarnat et al., 2010; Sarnat and Flores-Sarnat, 2013; Sarnat and Flores-Sarnat, 2014; Sarnat, 2015). Abnormal timing thus is demonstrated by another technique, which supports the premise in this present manuscript that genetic mutation of genes of synaptogenesis affect timing in ontogenetic development.

The alterations found in the studied genes can be explained by an imbalance in the apoptosis process considering that members of the NeuroD subfamily, like *NEUROD6* gene, plays an important role in the onset of neuronal differentiation, simultaneously promoting neuronal survival through the expression of anti-apoptotic regulators that preserve mitochondrial integrity (Uittenbogaard and Chiaramello, 2005). Inhibition of programmed cell death during neural differentiation may have a role in regulating the amount of precursor cells during the cell proliferation phase as well as in the other stages of brain formation.

In normal development of the NS of mammals, apoptosis extensively occurs, and has been observed in neural precursor cells, differentiated post mitotic neurons and also glial cells (Buss et al., 2006; Jacobson et al., 1997; Oppenheim, 1991; Kristiansen and Ham, 2014). These programmed cell deaths are essential for the establishment with correct proportions of neural populations and glia. Around one-third of the cells normally die from apoptosis during the first two weeks after birth in a normal mammalian development (Kristiansen and Ham, 2014; Wright et al., 1983).

The study of Gonzalez et al (1996) demonstrated that *FGF2* and one of its receptors, FGFR1, are widely expressed within human fetal tissues early in the second trimester (Gonzalez et al., 1996). In cultures derived from the CNS, including cortex, hippocampus, cerebellum, septum, mesencephalon, and spinal cord, *FGF2* enhanced the survival and neurite outgrowth of neurons, (Zhou et al., 1996).

Synaptogenesis, including formation of the dendritic column, is associated with the time of release of different molecules. Changes in timing may influence the formation of late or even early synapses. Also contributing to delays in synaptogenesis, the abnormal microtubule-related proteins, including phosphorylated Tau protein are up regulated in FCD type II, hemimegalencephaly, ganglioma and Tuberous Sclerosis Complex (Sarnat et al., 2012; Sarnat and Flores-Sarnat, 2015). Epigenetic may also alter through secretion of inappropriate hormonal or also by fetal exposure to neurotoxins (Sarnat and Flores-Sarnat, 2015; Castejón, 2008).

The genes that were most expressed in dysplastic individuals in relation to the control group, may therefore have caused delay during the brain maturational where the axoplasmic synaptophysin may remain reactive more than necessary (Sarnat and Flores-Sarnat, 2015). In neurons of the cortex and caudate nucleus, for example, "de-saturation" may happen by prenatal or perinatal hypoxic / ischemic encephalopathy, with delay in synaptic surfaces impairment (Mcclendon et al., 2014; Back and Miller, 2014; Sarnat and Flores-Sarnat, 2015).

The hiPSCs cells of *ASCL1, DCX, NRCAM* and *NEUROG2* genes presented higher expressions in the control individuals compared to the dysplastic individuals. Neural precursor cells suffer asymmetric and symmetric cellular divisions (Pang et al., 2008). Consequently, changes in Notch and Numb concentrations occur within the cell, stimulating it to become mature for migration or to remain as a precursor cell (Qin et al., 2002). Probably changes in the concentrations of Notch and Numb explain the increased expression of *ASCL1, DCX, NRCAM* and *NEUROG2* genes in the hiPSCs cells, which represent the embryonic cells, in the control individuals compared to the dysplastic individuals. This intense proliferative activity of the gene triggered during the embryonic period may compromise the development of the three embryonic leaflets as a whole, causing severe damage to NCS development and resulting in cortical dysplasia as well as different MCDs.

During the neurodifferentiation protocol, ASCL1 and NEUROG2 genes had lower expression values in dysplastic

individuals than in the control ones. Already, *NRCAM* and DCX genes presented lower expression values in the control individuals than in the dysplastic ones. However, during neurodifferentiation for 14 days, DCX gene presented values similar to the dysplastic individuals. The NEFL gene presented oscillations, for 14 days of neurodifferentiation, dysplastic individuals presented higher expression values. In neurodifferentiation for 22 days the expression was similar between the groups. Already in neurodifferentiation for 35 days, the control individuals had higher expression values. Possibly these differences can be explained by the following reasons and characteristics of the FCD: the migratory cells formed a heterotopia with groups of neurons or neurons individuals within the subcortical white matter as a consequence that these cells stopped before and did not reach the target place in the cerebral cortex. In others words, maturational arrest before radial migratory neuroblasts reach the cortical plate (Sarnat and Flores-Sarnat, 2015). As well as for reasons of maturational arrest due to neuroblast adhesion to defective radial glial fibers, including, a molecule with defective in cell adhesion of the intercellular matrix (Magliocco et al., 1992; Sarnat and Flores-Sarnat, 2015).

Early expression at high levels during the neurodifferentiation of dysplastic individuals demonstrates an increase in the early migration potential dysplastic cells, which can lead to damage to cell migration. Through this scenario, we can infer that, during the cortical neurogenesis, the neuroblasts of individuals with FCD begin their migration phase before the physiologically typical period. Therefore, these cells are probably not suitable for migration or the structure of the radial framework is not yet fully formed for the process, making it difficult for these cells to reach their specific destination.

During migration, neuroblasts provides its direction by the ability to adhere the radial glia and follow it. The correct orientation is done by the contact made by the neuroblasts with the ventricular surface before migrating to the cortical plate (Anderson et al., 1997; Pang et al., 2008). When they reach the cortical plate, all migration neurons must stop in their proper laminar position (Kriegstein and Noctor, 2004; Métin et al., 2006; Pang et al., 2008). In relation to brain tissue, the genes *ASCL1, DCX, NEUROG2* presented higher expression values in dysplastic individuals than control individuals. The *NRCAM* gene presented similar values between the groups. A relevant aspect to be considered to explain the altered expression in brain tissue is the destruction of nerve cells and radial glia, interrupting migratory cells in the middle of their journey, unable to travel beyond the proper site due to the consequences of epileptic seizures (Sarnat, 1992). What also may have happened is the formation of early neurons that do not reach their destination, forming the aberrant cortex with the presence of giant or balloon cells in the white matter. It is important to highlight that timing is fundamental and involved not just in the initiation but also in the synchronization of each developmental stage during morphogenesis. Neurological dysfunctions can be consequences of matrix arrest, delay and precocity, thus they can result in different malformations. In somatic mutations, the time of mitotic cycles, for example, may differ FCD type II from hemimegalencephaly. Thus, timing should always be considered in the interpretation of brain dysgenesis and may be directly related to the alterations found in the studied genes (Sarnat and Flores-Sarnat, 2015).

The different levels of gene expression of dysplastic individuals in relation to the control individuals may also be explained by alterations in the PI3K pathway, essential in the developing CNS, including cell survival, control of apoptosis, neuronal migration, morphological development of neurons and in the process of synaptic neurotransmission. Others authors found similar results, where, in FCD type IIb, molecules associated with phosphorylation of PI3K and AKT or PKB may be activated (Baybis et al., 2004; Sato, 2010; Ljungberg et al., 2006; Takei and Nawa, 2014; CRINO, 2011; Poduri et al., 2013; Schick et al., 2007). It is also important to highlight that there is more than one mechanism involved in the pathogenesis of dysmorphic neurons in mTOR activation disorders (Rossini et al., 2017). Five initial ‘stages’ of human neuroepithelial cell transition to neuroblast may be identified, based upon weighted gene co-expression or “transcriptome analysis” of cell cultures (Yuanyuan et al., 2017), and the mTOR pathway is critical to early cellular lineage and differentiation.

From the analysis of the genes involved in synaptic and neural migration aspects, in the fibroblasts, brain tissue, hiPSCs and neural differentiated cells, we present fundamental data's that contributes for the understanding of the pathogenesis, especially in the neurogenesis and neural differentiation process that are in agreement with the prevailing hypothesis that FCD originates from the abnormal migration during the embryonic period (Ying et al., 2005; Najm et al., 2007). In relation to the control group, when we treat a non-inheritable mutation, fibroblasts would not be the best alternative. However, there is only speculation about FCD. We do not know if these somatic mutations occur due to a fragility or even when they occur (embryonic development at a later stage – corticogenesis, for example, up to about 30 weeks' gestation). This model is precisely for this, it would not surprise us if there were no differences compared to controls, but even so, we would describe something innovative. Also, there is still the mosaicism of their brain, another pertinent situation when we think of reprogramming. All these findings are important to better understand the expression levels, action and involvement of these genes in essentials processes in the nervous system development, including neural migration. Thus, the pattern of the cortical malformation and the place where the cortex can be influenced by these changes in the CNS (Kuzniecky, 2015).

Based on our findings, it is suggested that the different expressions are mainly involved in alterations of the expression of receptors and capture sites, timing, coupling of synaptic vesicles with the presynaptic membrane, regulation of ion channel and synaptic exocytosis, imbalance of the apoptosis process and abnormal microtubules that may also contribute to delays in synaptogenesis. Also, FCD individuals may have neural precursor cells more sensitive to stimuli, consequently, showing alterations in cell survival, apoptosis control, neuronal migration, neuronal morphological development and synaptic neurotransmission during the embryonic neurogenesis Thus, individuals with dysplasia,

probably, during brain formation were influenced by these explored genes.

With this study we showed important results using hiPSCs approach, deriving neurons from fibroblasts associated with epilepsy in FCD type IIb patients. Here we model the disease mechanisms allowing to translate these findings to the development of new potential therapeutic targets.

ABBREVIATIONS

FCD focal cortical dysplasia
FCD II focal cortical dysplasia type 2
hiPSCs human induced pluripotent stem cells
MTOR mammalian target of rapamycin
PI3K phosphoinositide 3-kinase

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA MATERIALS

Not applicable.

FUNDING

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COMPETING INTERESTS

None.

AUTHORS' CONTRIBUTIONS

FM and DRM carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. ALFP, DCM and JCC conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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