·临床研究·

合并选择性宫内生长受限的单绒毛膜双羊膜囊早产双胎的早期营养与生长及DNA甲基化比较

冯嘉丽[1]，陈培松[2]，余慕雪[1]
(1. 中山大学附属第一医院儿科，广东 广州 510080；2. 中山大学附属第一医院医学检验科，广东 广州 510080)

摘　要：【目的】分析合并选择性宫内生长受限(sIUGR)的单绒毛膜双羊膜囊(MCDA)早产双胎的早期营养和生长情况，探索DNA甲基化调控宫外生长的可能机制。【方法】纳入2019年3月至2022年2月在中山大学附属第一医院新生儿科住院治疗的MCDA合并sIUGR早产双胎共48例，按出生体质量大小分为出生体质量较大组24例及出生体质量较小组24例。采用t检验、ANOVA、χ^2检验/Fisher确切概率法分析比较两组出生后第1周的每日营养摄入量、住院期间新生儿并发症发生率、出生至校正24月龄体格生长情况，使用甲基化芯片分析比较两组DNA甲基化差异并通过焦磷酸测序方法验证差异位点。【结果】两组出生后第1周每日碳水化合物、蛋白质、脂肪及能量的摄入量差异无统计学意义(P值均>0.05)。两组出生窒息、新生儿呼吸窘迫综合征、支气管肺发育不良、早产儿视网膜病、坏死性小肠结肠炎的发生率差异均无统计学意义(P值均>0.05)。出生至校正24月龄出生体质量较大组体质量、身长、头围Z评分均高于出生体质量较小组(P值均为0.000)。双胎体质量Z评分差值及身长Z评分差值在校正胎龄40周、校正6月龄、校正12月龄与出生时相比差异无统计学意义(P值均>0.05)；校正18月龄及24月龄低于出生时($P_{18\text{月龄体质量}}=0.009$，$P_{18\text{月龄身长}}=0.032$，$P_{24\text{月龄体质量}}=0.026$，$P_{24\text{月龄身长}}=0.004$)。双胎头围Z评分差值在校正胎龄40周时与出生时相比差异无统计学意义(P值>0.05)；校正6月龄、校正12月龄、校正18月龄以及校正24月龄时均低于出生时($P_{6\text{月龄}}=0.001$，其余P值均为0.000)。校正胎龄40周、校正6月龄、校正12月龄、校正18月龄以及校正24月龄的双胎体质量、身长、头围差异程度均较出生时减小($P_{40\text{周体质量}}=0.001$，$P_{40\text{周身长}}=0.007$，$P_{40\text{周头围}}=0.001$，其余P值均为0.000)。两组存在18个显著DNA甲基化差异性的位点。出生体质量较小组在*NFATC1*基因体上的一个位点甲基化程度高于出生体质量较大组($P=0.043$)。【结论】MCDA合并sIUGR的早产双胎早期营养及新生儿期并发症发生率无差别，至校正24月龄出生体质量较小者较出生体质量较大者生长快，可能与出生体质量较小者在*NFATC1*基因上甲基化上调有关。

关键词：单绒毛膜双羊膜囊双胎；选择性宫内生长受限；DNA甲基化；早期营养；生长
中图分类号：R722.6　　**文献标志码**：A　　**文章编号**：1672-3554(2026)02-0328-08
DOI：10.11714/jsysu.med.YX20250194

Early Nutrition, Growth Parameters and DNA Methylation Differences of Preterm Monochorionic Diamniotic Twins Combined with Selective Intrauterine Growth Restriction

FENG Jiali[1], CHEN Peisong[2], YU Muxue[1]
(1. Department of Pediatrics, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China;
2. Department of Laboratory Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China)
Correspondence to: YU Muxue; E-mail: yumuxue@mail.sysu.edu.cn

Abstract: 【Objective】 To investigate early nutrition and growth of preterm monochorionic diamniotic (MCDA) twins combined with selective intrauterine growth restriction (sIUGR) and explore the potential role of DNA methylation in

收稿日期：2025-12-22　　**录用日期**：2026-03-10
基金项目：广东省自然科学基金(2019A1515011663)
作者简介：冯嘉丽，第一作者，研究方向：新生儿疾病，E-mail：fengjli8@mail.sysu.edu.cn；余慕雪，通信作者，主任医师，E-mail：yumuxue@mail.sysu.edu.cn

regulating extrauterine growth.【Methods】Twenty-four pairs of preterm MCDA twins combined with sIUGR hospitalized in the Department of Neonatology of The First Affiliated Hospital of Sun Yat-sen University from March 2019 to February 2022 were enrolled, divided into larger twins group (n=24) and smaller twins group (n=24) according to birth weight. The comparison of daily nutritional intakes, the incidence of neonatal complications and physical growth from birth to 24 months of corrected age between two groups was performed using t-test, analysis of ANOVA, Chi-square test or Fisher's exact test. Differential DNA methylation analysis was performed using the methylation microarrays and differentially methylated site was validated using pyrosequencing method.【Results】There were no significant differences in the daily intake of carbohydrate, protein, fat and energy between two groups (all P>0.05). There were no significant differences in the incidence of asphyxia, neonatal respiratory distress syndrome, bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis between two groups (all P>0.05). Z-scores for weight, length and head circumference were significantly lower in the smaller twins group compared with the larger twins group from birth to 24 months of corrected age (all P =0.000). Difference in Z-scores for weight and length at term, 6 months, 12 months of corrected age did not differ significantly from that at birth (all P>0.05) while difference in Z-scores for weight and length between the twins at 18 months and 24 months of corrected age were significantly lower than that at birth (P = 0.009, P = 0.032, P = 0.026, P = 0.004). Difference in Z-scores for head circumference at 6 months, 12 months, 18 months, 24 months of corrected age were significantly lower than at birth (P = 0.001, others P = 0.000) except at term (P>0.05). The differences in weight, length and head circumference between the twins at term, 6 months, 12 months, 18 months and 24 months of corrected age significantly reduced (P = 0.001, P = 0.007, P = 0.001, others P = 0.000). Eighteen differentially methylated sites were identified and the methylation level of *NFATC1* gene in the smaller twins group was significantly higher than that in the larger twins group (P = 0.043).【Conclusion】MCDA twins combined with sIUGR shares similar early nutrition and neonatal complications, while smaller twins at 24 months of corrected age grow faster than larger twins, which might be related to a higher methylation level of *NFATC1* gene in smaller twins.

Key words: monochorionic diamniotic twins; selective intrauterine growth restriction; DNA methylation; early nutrition; growth

[J SUN Yat-sen Univ(Med Sci),2026,47(2):328-335]

单绒毛膜双羊膜囊(monochorionic diamniotic，MCDA)双胎由一个受精卵分裂发育形成，共用一个胎盘[1-2]，易合并早产[2]，且可能因为胎盘份额不均或其他原因出现体格生长差异合并选择性宫内生长受限(selective intrauterine growth restriction，sIUGR)[3-5]。研究表明出生1周内理想的蛋白质和能量的摄入有助于生长发育[6-8]。双胞胎的研究发现轻出生体质量者可较重出生体质量者生长更快[9-10]。遗传背景一致的MCDA双胎出现生长轨迹不同，不改变基因序列而通过DNA甲基化的方式调控基因表达的表观遗传机制可能参与调控[11]。MCDA双胎是研究生长发育中DNA甲基化表观遗传调控的理想模型，本研究拟分析合并sIUGR的MCDA早产双胎的早期营养和生长情况，探索DNA甲基化调控宫外生长的可能机制。

1　材料与方法

1.1　研究对象

本研究经中山大学附属第一医院临床科研和实验动物伦理委员会批准(伦审[2020]24号)，对2019年3月至2022年2月在中山大学附属第一医院新生儿科住院治疗的MCDA合并sIUGR双胎的临床资料进行分析；纳入标准为：出生胎龄＜37周、生后24 h内入院的合并sIUGR的MCDA双胎；患儿监护人签署知情同意书。排除双胎或双胎之一宫内死亡或住院期间死亡，或合并染色体异常、遗传代谢性疾病者。根据双胎出生体质量分为出生体质量较大组、出生体质量较小组。

1.2　方　法

1.2.1　临床资料收集　记录住院资料：出生胎龄、性别、出生体质量、出生身长、出生头围等；出生后第1周的每日营养摄入量；新生儿期并发症，包括出生窒息、呼吸窘迫综合征(respiratory distress syndrome，RDS)、支气管肺发育不良(bronchopulmonary dysplasia，BPD)、视网膜病变(retinopathy of prematurity，ROP)、确诊期和进展期的坏死性小肠结肠炎(necrotizing enterocolitis，NEC)。随访研究对象在校正胎龄40周、校正6月龄、校正12月龄、校正18月龄以及校正24月龄的体质量、身长及头围。

新生儿期疾病诊断参照《实用新生儿学》第5版[12]。采用2013年Fenton早产儿生长曲线及其软件计算早产儿出生相应胎龄体格生长指标Z评分[13]。采用2006年世界卫生组织儿童生长标准[14]评估校正胎龄40周、校正6月龄、校正12月龄、校正18月龄以及校正24月龄的体格生长指标Z评分。出生至校正24月龄6个时间点双胎体格指标Z评分差值=出生体质量较大组Z评分−出生体质量较小组Z评分。出生至校正24月龄6个时间点双胎体格生长指标差异程度=[(出生体质量较大组体格生长指标−出生体质量较小组体格生长指标)/出生体质量较大组的体格生长指标]×100%。

1.2.2 甲基化检测 收集研究对象的临床检测剩余静脉血标本,提取DNA并进行重亚硫酸盐处理,后使用Illumina Infinium Human Methylation 850K BeadChip(850K甲基化芯片)筛选显著甲基化差异位点,通过Gene Ontology功能注释[15]、KEGG通路分析[16]对甲基化差异位点对应的15个基因进行生物学信息分析,选择与生长相关的基因的甲基化位点通过焦磷酸测序进行验证。

1.3 统计学方法

用SPSS 20.0统计软件进行统计学分析。正态分布的计量资料采用均数±标准差($\bar{x}\pm s$)表示,出生体质量较大组和出生体质量较小组两组间各指标的比较采用配对t检验;出生至校正24月龄间6个时间点双胎体格指标Z评分差值、差异程度的比较采用单因素方差分析(ANOVA),2个时间点间双胎体格指标Z评分差值、差异程度的比较采用LSD−t检验。计数资料采用例数和百分率表示,两组新生儿并发症发生率比较采用χ^2检验或Fisher确切概率法。检验采用双侧检验,$P<0.05$表示差异有统计学意义。用R软件进行甲基化芯片结果分析。采用pool.t-test方法筛选组间的差异甲基化位点,β作为衡量该位点甲基化程度的值,筛选标准为$\Delta\beta>0.1$,$P<0.05$。

2 结 果

2.1 双胎的新生儿期资料

2.1.1 基本资料 共纳入MCDA合并sIUGR的早产双胎24对,其中男性双胎13对(占54.17%),女性双胎11对(占46.83%),平均出生胎龄为(32.71±2.20)周。

2.1.2 出生后第1周营养摄入情况 出生后第1周,出生体质量较大组与出生体质量较小组每日碳水化合物、蛋白质、脂肪及能量的摄入量符合正态分布,方差齐,两组差异无统计学意义(P均>0.05,表1)。

2.1.3 新生儿期合并症情况 如表2所示:新生儿期两组出生窒息、RDS、BPD、ROP、NEC的发生率差异均无统计学意义(P值均>0.05;表2)。

2.2 双胎的生长随访

2.2.1 双胎的体格生长指标Z评分 双胎的体格生长指标Z评分符合正态分布,具有方差齐性。如表3所示,从出生至校正24月龄,出生体质量较大组的体质量Z评分、身长Z评分及头围Z评分均高于出生体质量较小组,差异有统计学意义(P值均为0.000)。

2.2.2 双胎的体格生长指标Z评分差值及体格生长指标差异程度的变化 各个随访时间点双胎体格指标Z评分差值=出生体质量较大组Z评分−出生体质量较小组Z评分。双胎的体格生长指标Z评分差值均符合正态分布,具有方差齐性。如表4所示:与出生时相比,双胎体质量Z评分差值及身长Z评分差值在校正胎龄40周、校正6月龄、校正12月龄时差异无统计学意义(P值均>0.05);校正18月龄及24月龄低于出生时($P_{18\text{月龄体质量}}=0.009$,$P_{18\text{月龄身长}}=0.032$,$P_{24\text{月龄体质量}}=0.026$,$P_{24\text{月龄身长}}=0.004$),差异有统计学意义。与出生时相比,双胎头围Z评分差值在校正胎龄40周时差异无统计学意义(P值>0.05);校正6月龄、校正12月龄、校正18月龄以及校正24月龄时均低于出生时($P_{6\text{月龄}}=0.001$,其余P值均为0.000),差异有统计学意义。

各个随访时间点双胎体格生长指标差异程度=[(出生体质量较大组体格生长指标−出生体质量较小组体格生长指标)/出生体质量较大组的体格生长指标]×100%;差异程度均符合正态分布,具有方差齐性。如表5所示:与出生时相比,校正胎龄40周、校正6月龄、校正12月龄、校正18月龄以及校正24月龄的双胎体质量、身长、头围差异程度均减小($P_{40\text{周体质量}}=0.001$,$P_{40\text{周身长}}=0.007$,$P_{40\text{周头围}}=0.001$,其余P值均为0.000),差异有统计学意义。

2.3 双胎的DNA甲基化差异

2.3.1 双胎的DNA甲基化差异位点 850 K芯片共对865 253个位点进行了DNA甲基化检测及比较,筛选出18个存在显著甲基化差异的位点,其中15个位点存在对应基因,3个位点位于基因间区

表1　出生后第1周双胎出生体质量较大组与出生体质量较小组碳水化合物、氨基酸、脂肪和能量的每日摄入量比较

Table 1　Comparison of daily intake of carbohydrate, protein, fat and energy for the first week of life between larger twins group and smaller twins group　($\bar{x} \pm s$)

Item	Day	Larger twins group (n = 24)	Smaller twins group (n = 24)	t	P
Carbohydrate/(g·kg^{-1}·d^{-1})	1	7.48±0.92	7.68±1.05	−0.941	0.357
	2	8.33±1.09	8.56±1.30	−0.881	0.387
	3	9.12±1.33	9.63±1.41	−1.785	0.087
	4	10.03±1.54	10.29±1.44	−0.761	0.455
	5	10.93±1.56	11.13±1.37	−0.723	0.477
	6	11.44±1.45	11.76±1.40	−1.091	0.287
	7	11.92±1.47	12.27±1.29	−1.189	0.246
Protein/(g·kg^{-1}·d^{-1})	1	1.53±0.24	1.53±0.34	−0.049	0.961
	2	1.86±0.38	1.87±0.39	−0.117	0.908
	3	2.11±0.35	2.16±0.44	−0.697	0.493
	4	2.35±0.43	2.45±0.55	−1.064	0.298
	5	2.60±0.51	2.81±0.54	−1.960	0.062
	6	2.84±0.46	3.04±0.58	−1.468	0.156
	7	3.01±0.53	3.24±0.57	−1.589	0.126
Fat/(g·kg^{-1}·d^{-1})	1	2.08±0.51	1.94±0.37	1.718	0.099
	2	2.63±0.68	2.50±0.57	1.383	0.180
	3	3.12±0.66	2.95±0.71	2.078	0.050
	4	3.68±0.67	3.43±0.75	2.059	0.052
	5	4.12±0.78	3.97±0.79	1.211	0.238
	6	4.59±0.85	4.43±0.83	1.241	0.227
	7	5.04±0.78	4.81±0.78	1.924	0.067
Energy/(kcal·kg^{-1}·d^{-1})	1	54.71±5.00	54.40±4.78	0.688	0.498
	2	64.43±8.38	64.17±7.25	0.426	0.674
	3	73.89±6.99	73.75±9.62	0.179	0.860
	4	82.59±7.27	81.99±10.67	0.525	0.605
	5	91.34±8.79	91.35±10.25	−0.021	0.983
	6	98.45±8.05	99.05±8.63	−0.987	0.334
	7	105.19±7.74	105.49±8.30	−0.515	0.612

（表6）。通过Gene Ontology功能注释、KEGG通路分析对甲基化差异位点对应的15个基因进行生物学信息分析，发现*NFATC1*基因参与多个信号通路如破骨细胞分化，与生长发育相关性大。

2.3.2　焦磷酸盐测序验证双胎的DNA甲基化差异位点　在*NFATC1*基因上存在DNA甲基化差异位点，出生体质量较小组平均甲基化程度为(45.58±7.16)%，高于出生体质量较大组的平均甲基化程度(40.65±9.30)%，差异具有统计学意义(P = 0.043)。

3　讨 论

3.1　合并选择性宫内生长受限的单绒毛膜双羊膜囊早产双胎的DNA甲基化差异

近年来DNA甲基化的研究持续受到关注，同

表2 双胎出生体质量较大组与出生体质量较小组新生儿期并发症比较

Table 2 Comparison of incidence of neonatal complications between larger twins group and smaller twins group

[n(%)]

Item	larger twins group (n = 24)	smaller twins group (n = 24)	χ^2	P
Asphyxia at birth	5(20.83)	8(33.33)	0.949	0.330
RDS	18(75.00)	17(70.83)	0.105	0.745
BPD	6(25.00)	11(45.83)	2.277	0.131
ROP*	0(0)	2(8.33)	–	0.489
NEC*	0(0)	1(4.17)	–	1.000

RDS: neonatal respiratory distress syndrome; BPD: bronchopulmonary dysplasia; ROP: Retinopathy of prematurity; NEC: necrotizing enterocolitis. * Fisher′s exact test.

表3 出生至校正24月龄双胎出生体质量较大组与出生体质量较小组的体质量、身长、头围Z评分比较

Table 3 Comparison of Z-scores for weight, length and head circumference from birth to 24 months of corrected age between larger twins group and smaller twins group

[($\bar{x} \pm s$), (n=24)]

Corrected age	Z-scores	Larger twins group	Smaller twins group	t	P
Birth	For weight	−0.03±0.60	−1.67±0.68	15.577	0.000
	For length	−0.31±0.84	−1.69±0.97	7.977	0.000
	For head circumference	0.34±0.82	−1.16±1.09	6.679	0.000
Term	For weight	−0.43±0.96	−2.31±1.07	9.320	0.000
	For length	−0.54±0.69	−1.78±0.96	9.568	0.000
	For head circumference	−0.18±0.81	−1.31±0.73	6.958	0.000
6 months	For weight	0.00±0.97	0.17±1.11	7.228	0.000
	For length	0.04±0.97	−1.11±1.31	6.326	0.000
	For head circumference	−0.00±0.93	−0.82±0.97	7.861	0.000
12 months	For weight	0.17±1.01	−1.00±1.21	6.470	0.000
	For length	0.31±1.21	−0.68±1.52	5.333	0.000
	For head circumference	0.11±0.88	−0.66±0.77	7.448	0.000
18 months	For weight	0.24±1.00	−0.76±1.21	5.899	0.000
	For length	0.13±1.11	−0.73±1.11	5.176	0.000
	For head circumference	−0.02±0.85	−0.52±0.85	4.751	0.000
24 months	For weight	0.12±0.92	−0.66±1.10	4.904	0.000
	For length	0.05±1.03	−0.65±1.20	5.100	0.000
	For head circumference	−0.01±0.74	−0.41±0.74	4.946	0.000

卵双胞胎为研究DNA甲基化调控的理想模型[17]，包括DNA甲基化与疾病的发生的研究[17-19]及DNA甲基化对衰老的作用[20-21]，但DNA甲基化对生长的调控作用如何尚不明确。研究生长不一致的同卵双胎有助于了解DNA甲基化对生长的调控。我们发现合并sIUGR的MCDA双胎在某些基因位点上存在DNA甲基化差异。有研究报道出生体质量差异20%以上的双胎胎盘组织整体DNA甲基化无差异，但存在差异性位点，如*LRAT*、*SLC19A1*、*DECR1*基因等[22-23]。在本研究中，上述基因的位点没有发现显著DNA甲基化差异，但发现了尚未报道的差异性位点；其中*ITH5*基因[24]、*MLLT3*基因[25]等多个

表4　出生至校正24月龄双胎间体质量、身长、头围*Z*评分差值的变化

Table 4　Differences between the twins in *Z*-scores for weight, length and head circumference from birth to 24 months of corrected age

[$(\bar{x} \pm s)$, $(n=24)$]

Difference in *Z*-scores	Corrected Age						*F*	*P*
	Birth	Term	6 months	12 months	18 months	24 months		
For weight	1.64±0.51	1.88±0.99	1.37±0.93	1.17±0.88	1.00±0.83$^{*\#}$	0.78±0.92$^{*\#\&}$	5.783	0.000
For length	1.37±0.84	1.24±0.64	1.15±0.89	0.99±0.86	0.86±0.82*	0.70±0.67$^{*\#}$	2.391	0.041
For head circumference	1.51±1.11	1.13±0.80	0.82±0.51*	0.77±0.50*	0.50±0.52$^{*\#}$	0.41±0.40$^{*\#\&}$	8.626	0.000

One-way ANOVA. *compared with birth, $P<0.05$; $^{\#}$compared with term, $P<0.05$; $^{\&}$compared with 6months, $P<0.05$.

表5　出生至校正24月龄双胎间体质量、身长、头围差异程度的变化

Table 5　Differences in weight, length and head circumference between the twins from birth to 24 months of corrected age

[$(\bar{x} \pm s)$, %]

Difference	Corrected Age						*F*	*P*
	Birth	Term	6 months	12 months	18 months	24 months		
In weight	32.36±10.30	23.51±10.90*	14.23±8.83*	12.22±8.50*	10.61±8.13$^{*\#}$	8.43±7.70$^{*\#\&}$	24.358	0.000
In length	8.30±4.91	5.67±2.97*	3.82±3.09*	3.24±3.05*	2.95±2.84$^{*\#}$	2.53±2.51$^{*\#}$	10.490	0.000
In head circumference	7.31±5.28	4.51±3.15*	2.36±1.42$^{*\#}$	2.18±1.37$^{*\#}$	1.43±1.48$^{*\#}$	1.16±1.14$^{*\#}$	17.652	0.000

One-way ANOVA. *compared with birth, $P<0.05$; $^{\#}$compared with term, $P<0.05$; $^{\&}$compared with 6months, $P<0.05$.

表6　双胎差异性甲基化位点

Table 6　Differentially methylated regions between the twins

Probe ID	*P*	Methylation Difference	Gene	Chromosome	Position
cg00989059	0.035	−0.14	*ITIH5*	chr10:7621718-7621950	S_Shore
cg01591526	0.045	0.11	*MLLT3*		
cg02532022	0.024	0.13	*VPS33B*	chr15:91565454-91565920	S_Shore
cg02837600	0.027	0.14	*LOC642852*	chr21:46714984-46715206	N_Shore
cg03787013	0.003	−0.11	*PRMT1*	chr19:50180010-50180907	Island
cg08437576	0.018	−0.10	*PAQR5*		
cg08549810	0.009	0.11	*ODF2L*	chr1:86861566-86862083	N_Shore
cg11522044	0.045	0.13		chr12:46776731-46777856	S_Shelf
cg11787544	0.023	−0.47			
cg12145624	0.041	0.41	*MAST4*	chr5:65891877-65893539	N_Shore
cg13438944	0.015	0.54	*C14orf179*	chr14:76452079-76452542	Island
cg18084442	0.017	−0.11	*LOC100506688*		
cg19360943	0.024	−0.18	*ING4*		
cg20205061	0.032	0.11	*MSL3*		
cg20341535	0.011	−0.13	*SCN4B*	chr11:118016237-118016984	S_Shore
cg23239647	0.000	−0.77		chr2:105275588-105275940	N_Shelf
cg24398381	0.038	0.11	*NFATC1*	chr18:77272584-77273603	S_Shelf
cg27339550	0.014	0.15	*ZNF853*	chr7:6654745-6655860	Island

基因与肿瘤有关，*C14orf179*基因、*MSL3*基因、*ZNF853*基因尚未有研究报道明确功能，而*NFATC1*基因与生长发育相关。

3.2 合并选择性宫内生长受限的单绒毛膜双羊膜囊早产双胎的出生体质量较小者在NFATC1基因体的甲基化上调

*NFATC1*即活化T细胞核因子1蛋白，可作为转录因子参与多个通路调控生长发育。多项研究对*NFATC1*的功能进行了探究。*NFATC1*可调节骨质形成和骨代谢[26]；有研究发现诱导破骨细胞分化的RANKL信号通路可促进*NFATC1*表达，*NFATC1*基因缺失的干细胞不能在RANKL刺激下分化为破骨细胞，说明*NFATC1*在RANKL信号通路下游诱导破骨细胞分化[27]；*NFATC1*缺陷小鼠除了破骨细胞发育受损外，还存在颅骨形成缺陷，成骨细胞中*NFATC1*活性增强可以引起骨质增加[28]。*NFATC1*对成骨细胞和破骨细胞均有调控作用，可影响生长。此外，*NFATC1*还参与骨骼肌的发育、心脏的发育以及淋巴细胞的增殖和分化等各种通道的调控[29-31]。关于*NFATC1*基因的甲基化与基因表达的关系目前尚无研究。

本研究发现，MCDA合并sIUGR早产双胎在*NFATC1*基因的基因体上存在甲基化差异位点，而目前认为基因体的甲基化程度与基因的表达呈正相关[32]，出生体质量较小者在*NFATC1*基因体的甲基化上调可能使其表达上调，从而促进生长。

3.3 合并选择性宫内生长受限的单绒毛膜双羊膜囊早产双胎的早期营养与生长

本研究纳入的早产双胎给予规范营养管理，早期开始肠内营养，不足部分予肠外营养补充[33-34]，以合理的早期营养保证早产双胎的宫外生长，与其他强调改善新生儿早期营养以促进宫外生长情况的研究目的一致[35-36]。本研究中合并sIUGR的MCDA双胎早期营养一致，新生儿期并发症无明显差异，双胎体质量、身长及头围的差异出生后逐渐缩小，出生体质量较小者早期宫外生长较出生体质量较大者快，可能出生体质量较小者在*NFATC1*基因体的甲基化上调引起其表达上调，通过促进骨骼肌形成、调节骨骼发育来促进生长加速。

3.4 本研究的局限性

本研究存在一定局限性，一是在MCDA合并sIUGR早产双胎中发现了甲基化差异位点，未与非sIUGR双胎中该位点的甲基化差异程度进行对比；二是未进一步明确该位点的甲基化差异对基因表达的作用。我们将继续进行更长期的随访，并进一步研究*NFATC1*基因甲基化对表达的调控作用，探究参与调节的可能通路。

综上所述，MCDA合并sIUGR的早产双胎早期营养及新生儿期并发症发生率无差别，至校正24月龄出生体质量较小者较出生体质量较大者生长更快，可能与出生体质量较小者在*NFATC1*基因上甲基化上调有关。

参考文献

[1] Trevett T, Johnson A. Monochorionic twin pregnancies [J]. Clin Perinatol, 2005, 32(2): 475-494.

[2] Qiu T, Cheng W, Chen Y, et al. Clinical outcome of monochorionic diamniotic twins with intrauterine growth restriction[J]. Am J Perinatol, 41(10): 1321-1328.

[3] Bennasar M, Eixarch E, Martinez JM, et al. Selective intrauterine growth restriction in monochorionic diamniotic twin pregnancies [J]. Semin Fetal Neonatal Med, 2017, 22(6): 376-382.

[4] Gou C, Li M, Zhang X, et al. Placental characteristics in monochorionic twins with selective intrauterine growth restriction assessed by gradient angiography and three-dimensional reconstruction [J]. J Matern Fetal Neonatal Med, 2017, 30(21): 2590-2595.

[5] Lin Z, Wang X, Li L, et al. A study on the correlation of placental anastomosis and superficial vascular branches of selective fetal growth restriction in monochorionic diamniotic twins[J]. BMC Pregnancy Childbirth, 2023, 23(1): 827.

[6] Stephens BE, Walden RV, Gargus RA, et al. First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants [J]. Pediatrics, 2009, 123(5): 1337-1343.

[7] Schneider J, Fischer Fumeaux CJ, Duerden EG, et al. Nutrient intake in the first two weeks of life and brain growth in preterm neonates[J]. Pediatrics, 2018, 141(3): e20172169.

[8] Nagel EM, Gonzalez V JD, Bye JK, et al. Enhanced parenteral nutrition is feasible and safe in very low birth weight preterm infants: a randomized trial [J]. Neonatology, 2023, 120(2): 242-249.

[9] Groene SG, Gremmen IJ, van Zwet EW, et al. Fetal growth restriction inhibits childhood growth despite catch-up in discordant identical twins: an observational cohort study [J].

Eur J Endocrinol, 2023, 189(2): 183-189.
[10] Cortez Ferreira M, Mariano IM, Marinhas A, et al. Growth trajectories in monochorionic and dichorionic twins with weight discordance: a cohort study[J]. Eur J Pediatr, 2025, 184(5): 319.
[11] Peixoto P, Cartron PF, Serandour AA, et al. From 1957 to nowadays: a brief history of epigenetics[J]. Int J Mol Sci, 2020, 21(20): 7571.
[12] 邵肖梅, 叶鸿瑁, 丘小汕. 实用新生儿学(第5版)[M]. 北京: 人民卫生出版社, 2019: 390-1039.
Shao XM, Ye HM, Qiu XS. Practice of Neonatology (5th edition)[M]. Beijing: People's Medical Publishing House, 2019: 390-1039.
[13] Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants[J]. BMC Pediatr, 2013, 13: 59.
[14] WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age[J]. Acta Paediatr Suppl, 2006, 450: 76-85.
[15] Pitarch B, hagoyen M, Ranea JAG, et al. A review on gene ontology evaluations[J]. Database (Oxford), 2025, 2025: baaf058.
[16] Kanehisa M, Furumichi M, Tanabe M, et al. KEGG: new perspectives on genomes, pathways, diseases and drugs[J]. Nucleic Acids Res, 2017, 45(D1): D353-361.
[17] Sun ML, Li Y, Man RX, et al. DNA methylation in monozygotic twins discordant for acute lymphoblastic leukemia: a case report and systematic review[J]. Clin Epigenetics, 2025, 17(1): 94.
[18] Heikkinen A, Esser VFC, Lee SHT, et al. Twin pair analysis uncovers links between DNA methylation, mitochondrial DNA quantity and obesity[J]. Nat Commun, 2025, 16(1): 4374.
[19] Vecellio M, Paraboschi EM, Ceribelli A, et al. DNA methylation signature in monozygotic twins discordant for psoriatic disease[J]. Front Cell Dev Biol, 2021, 24, 9: 778677.
[20] Kim S, Wyckoff J, Morris AT, et al. DNA methylation associated with healthy aging of elderly twins[J]. Geroscience, 2018, 40(5-6): 469-484.
[21] Hong X, Cao H, Cao W, et al. Trends of genetic contributions on epigenetic clocks and related methylation sites with aging: a population-based adult twin study[J]. Aging Cell, 2025, 24(3): e14403.
[22] He Z, Lu H, Luo H, et al. The promoter methylomes of monochorionic twin placentas reveal intrauterine growth restriction-specific variations in the methylation patterns[J]. Sci Rep, 2016, 6: 20181.
[23] Roifman M, Choufani S, Turinsky AL, et al. Genome-wide placental DNA methylation analysis of severely growth-discordant monochorionic twins reveals novel epigenetic targets for intrauterine growth restriction[J]. Clin Epigenetics, 2016, 21, 8: 70.
[24] Kosinski J, Sechi A, Hain J, et al. ITIH5 as a multifaceted player in pancreatic cancer suppression, impairing tyrosine kinase signaling, cell adhesion and migration[J]. Mol Oncol, 2024, 18(6): 1486-1509.
[25] Li Y, Liu H, Li J, et al. MLLT3 regulates melanoma stemness and progression by inhibiting HMGB1 nuclear entry and MAGEA1 M^5C modification[J]. Adv Sci (Weinh), 2025, 12(10): e2408529.
[26] Zhang S, Gao M, Song S, et al. Unraveling the mechanisms that regulate osteoclast differentiation: a review of current advances[J]. Genesis, 2025, 63(1): e70012.
[27] Takayanagi H, Kim S, Koga T, et al. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts[J]. Dev Cell, 2002, 3(6): 889-901.
[28] Winslow MM, Pan M, Starbuck M, et al. Calcineurin/NFAT signaling in osteoblasts regulates bone mass[J]. Dev Cell, 2006, 10(6): 771-782.
[29] Ehlers ML, Celona B, Black BL. NFATc1 controls skeletal muscle fiber type and is a negative regulator of MyoD activity[J]. Cell Rep, 2014, 8(6): 1639-1648.
[30] de la Pompa JL, Timmerman LA, Takimoto H, et al. Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum[J]. Nature, 1998, 392(6672): 182-186.
[31] Giampaolo S, Wójcik G, Klein-Hessling S, et al. B cell development is critically dependent on NFATc1 activity[J]. Cell Mol Immunol, 2019, 16(5): 508-520.
[32] Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond[J]. Nat Rev Genet, 2012, 13(7): 484-492.
[33] Dutta S, Singh B, Chessell L, et al. Guidelines for feeding very low birth weight infants[J]. Nutrients, 2015, 7(1): 423-442.
[34] Fudehope D, Vento M, Bhutta Z, et al. Nutritional requirements and feeding recommendations for small for gestational age infants[J]. J Pediatr, 2013, 162(3 Suppl): S81-89.
[35] Martin CR, Brown YF, Ehrenkranz RA, et al. Nutritional practices and growth velocity in the first month of life in extremely premature infants[J]. Pediatrics, 2009, 124(2): 649-657.
[36] Guellec I, Lapillonne A, Marret S, et al. Effect of intra- and extrauterine growth on long-term neurologic outcomes of very preterm infants[J]. J Pediatr, 2016, 175: 93-99.e1.

(编辑　余　菁)