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The gut microbiota as a therapeutic target in children with autism spectrum disorder – current literature review

Mikrobiom jelitowy jako cel terapeutyczny u dzieci z zaburzeniami ze spektrum autyzmu – aktualny przegląd piśmiennictwa

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Abstract

The high prevalence of autism spectrum disorder (ASD), the specificity of paediatric patients and the determination of their parents give rise to the growing interest of scientists in potential therapeutic approaches. There are multiple aetiological factors involved, and more will probably be discovered. Currently, the involvement of genetic and environmental factors in the aetiology is widely accepted. Growing evidence has been presented for the link between gut dysbiosis and autism. Analysing recent scientific reports, attention was drawn to the proportion and composition of gut microbiota in children with this diagnosis. These are mainly *Bacteroidetes*, *Firmicutes* and *Actinobacteria*, all of which were more abundant in children with ASD than in controls. In turn, children with ASD showed significantly higher counts of *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium* and *Phascolarctobacterium* and lower percentages of *Coprococcus* and *Bifidobacterium*. If the initial hypotheses support microbial involvement in ASD-related symptoms, clinical trials with microbiota as a therapeutic target would be highly desirable. Recently, researchers have also been interested in faecal microbiota transplantation and microbiota transfer therapy. However, given the inconsistency in results between different centres, final conclusions cannot be reached. Differences in the composition of microbiota arise from, among others, the large age range of patients, which has a general impact on the variability of the microbiome composition over the years of life, environmental diversity with its dietary and cultural habits affecting the unique composition of the microflora, as well as food selectivity that is typically seen in ASD.

Keywords: autism spectrum disorder, microbiota, child

Streszczenie

Powszechność zjawiska, jakim jest zaburzenie ze spektrum autyzmu (*autism spectrum disorder*, ASD), specyfika pacjentów pediatrycznych oraz determinacja rodziców chorych dzieci stale napędzają wzrost zainteresowania badaczy tematem potencjalnych form leczenia ASD. Czynników etiologicznych wpływających na rozwój ASD jest wiele i zapewne w miarę rozwoju nauki będą poznawane kolejne. Obecnie powszechnie przyjmuje się udział czynników zarówno genetycznych, jak i środowiskowych w etiologii. Coraz więcej dowodów wzmacnia istnienie związku między dysbiozą jelit a autyzmem. W ostatnich doniesieniach naukowych uwagę zwraca proporcja i skład mikrobioty jelit dzieci z tym rozpoznaniem. Są to głównie bakterie *Bacteroidetes*, *Firmicutes* i *Actinobacteria*, z których wszystkie były liczniejsze u dzieci z ASD niż w grupie kontrolnej dzieci zdrowych. Z kolei dzieci z ASD wykazywały znacznie większą liczebność *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium* i *Phascolarctobacterium* oraz niższy odsetek *Coprococcus* i *Bifidobacterium*. Gdyby wstępne hipotezy potwierdziły udział drobnoustrojów w objawach związanych z ASD, badania kliniczne z mikroflorą jako cel terapeutyczny byłyby wysoce pożądane. W ostatnim czasie przedmiotem zainteresowania badaczy jest również przeszczep flory bakteryjnej i terapia transferu mikrobioty. Biorąc jednak pod uwagę niespójność w wynikach pomiędzy różnymi ośrodkami, nie można

sformułować ostatecznej tezy. Różnice w składzie mikroflory wynikają m.in. z dużej rozpiętości wiekowej badanych pacjentów, która ma ogólny wpływ na zmienność składu mikrobiomu z biegiem lat życia, różnorodności środowiskowej z jej nawykami żywieniowymi i kulturowymi mającymi wpływ na unikalny skład mikroflory, jak również dominującej w ASD wybiórczości pokarmowej.

Słowa kluczowe: zaburzenia ze spektrum autyzmu, mikrobiota, pediatria

INTRODUCTION

Autism spectrum disorder (ASD) is neurodevelopmental dysfunction characterised by an early onset and heterogeneous aetiology. The prevalence of ASD has been steadily increasing in recent years, perhaps in part due to greater awareness among medical professionals allowing for identification of the condition and a wider knowledge of the problem among modern parents. It is currently estimated that nearly 1–2% of children worldwide are affected by ASD, which means that approximately 10–20 million children are diagnosed with the disorder[1]. Studies have shown that the first symptoms of ASD develop as early as between 6 and 12 months of age. From this period onwards, symptoms can be seen in the form of diminished responding to hearing one's own name, avoiding eye contact, inadequate calibrating of one's own emotional reactions to others' emotional reactions, attachment difficulties, poor vocalisation, atypical responses to sensory stimuli, stereotypical behaviour, atypical cognition of objects and developmental arrest or regression. Statistically, alarming symptoms are noticed as early as in the first year of a child's life by 30–50% of parents, and during the first two years by 80%, at which point specialist help is sought[2].

NOMENCLATURE AND CLASSIFICATION

To begin with, it is worth noting some major changes in nomenclature in the form of merging several diagnostic entities (i.e. autistic disorder, Asperger's syndrome, childhood disintegrative disorder and other holistic developmental disorders not included in the other diagnostic categories) into one common diagnosis, namely ASD. It is no coincidence that the word "spectrum" refers to the wide range of symptoms within a single diagnosis. Furthermore, the new 11th version of ICD has distinguished this diagnosis under the name "autism spectrum disorder" since 2022 (6A02 according to ICD-11). The changes introduced are due to the fact that the diagnostic criteria in the old classification were based solely on the presence of stereotypic behaviours and deficits in social and communication skills. It is now emphasised that symptoms in these areas must be present in early childhood; however, their absence at later stages of life does not exclude the diagnosis. The consideration of abnormalities within the patient's sensory profile are an important element added to the diagnostic criteria.

AETIOLOGY

There are many aetiological factors involved in the development of ASD and it is likely that more will be identified as the science progresses. It is now widely recognised that both genetic and environmental factors are engaged in the aetiology of ASD. Although genetic risk is mainly influenced by the prenatal period and, in particular, by the process of neurogenesis and neuronal cell migration during the early development of the cerebral cortex, mainly in glutaminergic neurons, interneurons and non-neuronal cells have also been linked to some genes implicated in the development of ASD. ASD is highly genetically heterogeneous, with about 100 genes involved in its development detected so far[3], including *CNTNAP2* (contactin-associated protein-like 2)[4] and *CHD8* (chromodomain helicase DNA binding protein 8), encoding the chromodomain helicase DNA-binding (CHD) protein[5]. Both *de novo* mutations and deletions in *SHANK3* (SH3 and multiple ankyrin repeat domains 3) have been particularly linked with autistic symptoms[6]. In addition to genetic influences, environmental factors increasing the risk of the disorder are also important. Key factors include exposure to air pollution, pesticides, stress, medications and diet. Infections, antibiotic use, maternal diabetes, levels of folic acid, iron and maternal consumption of polyunsaturated fatty acids (PUFA) also play an important role in the prenatal period.

GUT MICROBIOTA COMPOSITION IN CHILDREN DIAGNOSED WITH ASD

The microbiota is the totality of microbes (predominantly bacteria, but also viruses and fungi) that reside in the human body, mainly in the gastrointestinal (GI) tract. The composition of the gut microbiome changes throughout life. In the neonatal period and infancy, it depends, among other things, on the gestational age, the route of delivery, the type of nutrition (breast milk, formula) or the antibiotic therapy implemented. In adulthood, diet, past infections, stress, hygiene and medications are important. Maintaining both quantitative and qualitative balance within the microbiome ensures the maintenance of health. Dysbiosis, which is shift in the composition and function of the microbiota, may contribute to the ASD symptomatology and, consequently, to the growing interest in novel pharmacological options. Its likely impact on ASD phenotype may be explained by the gut–brain axis,

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which is a two-way biochemical pathway between the GI tract and the central nervous system (CNS), essential for maintaining homeostasis, but also likely to be involved in the pathogenesis of mental disorders. Neurogenic, endocrine and immunological mechanisms, which may be modified by the gut microflora, are involved in the modulation of the gut–brain axis.

A 2020 meta-analysis identified 18 reliable monographs with high-quality methodology among 708 scientific papers. Together, they provided data on the composition of the microbiome in a group of almost 500 children with ASD and 400 healthy controls[7]. An analysis of pooled results from these studies (Tab. 1) found that the microbiota in the children included in the study consisted mainly of two main types of bacteria, i.e. *Bacteroidetes* (consisting of three classes of Gram-negative, non-spore-forming, anaerobic or aerobic bacteria inhabiting the GI tract and the skin surface, such as the bacteria of the genera *Bacteroides* and *Bifidobacterium*) and *Firmicutes* (mostly Gram-positive bacteria, e.g. in the classes *Bacilius* and *Clostridium*, which include some bacilli, diplococci and lactobacilli). These were followed by *Actinobacteria*. The counts of *Proteobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Fusobacteria* and *Tenericutes* were significantly lower or even close to zero in both ASD children and controls.

As for the main types of bacteria inhabiting the GI tract in ASD children, the overall findings indicated that they had higher abundance of all of the above microbes compared to neurotypical children; however, some analyses were contradictory. In the case of *Bacteroidetes*, most of the analyses indicated their higher relative abundance in ASD children[8,9], while other studies obtained opposite results[10,11]. The same was true for *Firmicutes*, with higher counts in ASD children indicted by some analyses[10,11], and higher abundance in controls shown in others[12,13], while some studies showed no differences between groups[8,9]. In contrast, no significant differences were found between groups for *Actinobacteria*. At the level of bacterial genera, children with ASD showed significantly increased abundance of *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium* and *Phascolarctobacterium* compared to healthy children. In contrast, neurotypical children had higher counts of *Bifidobacterium* and *Coprococcus*.

Many other studies have reported a shift in the ratio of the main bacterial types mentioned, i.e. *Bacteroidetes* and *Firmicutes*. ASD children had a lower *Bacteroidetes*:*Firmicutes* ratio[14,15]. Finally, it is worth noting the increased proportion of *Clostridium* compared to controls across many studies[14,15]. A double-blind study conducted at the University of Oxford showed increased levels of *Clostridium histolyticum* in ASD children compared to healthy controls[16]. Furthermore, the increased abundance of faecal *Clostridium perfringens* in ASD children has been confirmed in several other scientific reports[17]. With regard to the above observations, it is worth mentioning at this point that the possible role of *Clostridium* in the pathogenesis of ASD was first suggested

	ASD	Controls	*p*
	95% CI	95% CI	
Bacteroidetes	↑ 14.33	10.97	**0.002**
Bacteriodes	↑ 9.04	4.69	**<0.001**
Parabacteroides	↑ 0.32	0.04	**<0.001**
Firmicutes	↑ 13.42	10.77	**<0.001**
Anaerostipes	0.01	0.02	0.620
Anaerotruncus	0.23	0.14	0.430
Blautia	1.52	1.91	0.610
Faecalibacterium	6.84	5.00	0.040
Ruminococcus	2.90	2.21	0.170
Veillonella	0.07	0.13	0.460
Clostridium	↑ 0.74	0.16	**<0.001**
Coprococcus	↑ 0.11	0.24	**0.004**
Dialister	0.54	0.65	0.760
Dorea	0.42	0.21	0.110
Phascolarctobacterium	↑ 0.13	0.01	**0.020**
Roseburia	0.11	0.09	0.630
Proteobacteria	↑ 0.09	0.02	**<0.001**
Sutterella	0.11	0.22	0.480
Actinobacteria	0.53	0.43	0.360
Bifidobacterium	↑ 0.46	0.89	**<0.001**
Cyanobacteria	0.00	0.01	0.440
Fusobacteria	0.02	0.04	0.430
Verrucomicrobia	0.04	0.07	0.430
Akkermansia	0.04	0.55	0.280
Tenericutes	0.06	0.00	97.7

Tab. 1. A meta-analysis on the gut microbiota composition in ASD children vs. controls[7]

in 1998 by Ellen Bolte, a mother of an autistic child, who observed significant neurobehavioural changes after repeated courses of antibiotics and consequent chronic diarrhoea[17]. Furthermore, the hypothesis of *Clostridium* as a potential risk factor for ASD is supported by a study which administered vancomycin (effective against *Clostridium*) to autistic children for 6 weeks, achieving a significant improvement in symptoms, as reported by parents[18].

Considering the contribution of fungi to the composition of the human intestinal microbiota, where *Candida* is one of the most common types, it is worth recalling a study that showed a twofold increase in the prevalence of *Candida* in ASD children compared to controls[18]. The inconsistency of the results of current studies is probably due to methodological inadequacies ($N < 50$). Indeed, analyses involving more than 100 participants found no association between ASD and the microbiome. It is worth noting that ASD patients often have a less varied diet (due to food selectivity and co-occurring GI symptoms), and the dietary composition of each individual patient differs. Limitations that are present in the aforementioned meta-analysis[18] include the large age disparity of the study participants, which has a general impact on the variability of microbiome composition over the years of life, and the

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environmental diversity with its dietary and cultural habits affecting the unique composition of the microbiota. The inclusion of studies from all over the world may therefore be the reason for the heterogeneous differences between them. Another limitation was the inability to assess bacterial diversity at the species level, as only few studies included such data. Additionally, all included studies were based on assessing the composition of the gut microbiota from faecal samples, which fails to show the actual microbial gut diversity as a result of assessing only those that are excreted in the stool[7]. The accuracy of the analysis could be improved by taking intestinal samples[19], however, such a procedure, possibly requiring anaesthesia in children, especially in those with ASD, would be difficult with risks associated with both the procedure itself and excessive stress for children and their parents. Due to the above-mentioned limitations, it is unclear whether the results of the available reports on the link between ASD symptoms and gut microbiota can be conclusive and clinically relevant.

FACTORS AFFECTING THE COMPOSITION OF THE GUT MICROBIOTA IN CHILDREN WITH ASD

Given the above cited factors that have a global impact on the microbial contribution to the gut microbiota, the study of the microbiome in ASD children should expand on the issue of the impact of diet, which differs from the diet of neurotypical individuals and is characterised by the relatively common phenomenon of food selectivity.

Healthy children, especially at pre-school age, are often labelled as non-eaters and prefer only selected foods in their diet, rejecting those suggested by their parents. This phenomenon occurs around the age of 6 years and is usually a norm variant for this developmental age. However, this selectivity is more pronounced and begins at a very young age in ASD children. Additionally, problems with food intake tend to remain at a constant level for a very long time, leading to negative consequences for the health, nutritional status and overall development of the young organism. As a result, this may give rise to malnutrition with both insufficient and excessive calorie intake. According to research in ASD children, food selectivity is influenced by factors such as texture (69%), appearance (58%), taste (45%), smell (36%) and temperature (22%)[20]. The way food is presented or the use of specific cutlery and crockery may also be important. A strong preference for starchy foods, snacks and highly processed products with a concomitant rejection of vegetables, fruit and protein is also particularly common. Increased consumption of high-calorie foods can lead to excessive weight gain and related lifestyle diseases, but also to nutritional deficiencies. Food selectivity and inadequate nutrient supply have been shown to be associated with changes in the composition and diversity of the gut microbiome compared to neurotypical children[20].

THE IMPACT OF MICROBIOME ON THE SYMPTOMS IN ASD CHILDREN

There is a hypothesis on the association between gut microbiome disorders and ASD, which derives from the observation of a wide range of GI symptoms such as abdominal pain, diarrhoea, constipation, vomiting and reflux. Studies have shown that up to 40% of ASD children present with GI symptoms and, according to a clinical meta-analysis, they are more likely to develop GI dysfunction than healthy children[21]. Many functional disorders are known to also arise from the composition of the intestinal microflora. It is of key importance to maintain balance in the composition of the microbiota for the proper functioning of the entire body, the immune and nervous systems in particular. Its disruption allows potentially pathogenic bacteria to colonise the GI tract to the extent that dysbiosis promotes the pathogenesis of metabolic or psychiatric disorders[22]. It has even been hypothesised that gut dysbiosis associated with GI symptoms in a child genetically predisposed to develop ASD may facilitate phenotypic expression or increase the severity of neurobehavioural symptoms[22]. In practice, children with co-occurring ASD and GI symptoms may exhibit more severe anxiety, irritability and social withdrawal compared to asymptomatic children. It was shown in CHARGE (Childhood Autism Risks from Genetics and Environment) study that the prevalence of GI symptoms was significantly associated with increased autistic behaviour, as measured with the Aberrant Behavior Checklist (ABC). On the other hand, ASD symptom severity, as measured with the Autism Treatment Evaluation Checklist (ATEC), was correlated with higher severity of gastrointestinal symptoms. Findings that expand the view of dysbiosis and ASD are in line with the concept of the gut–brain axis, which implies a two-way interaction between gut bacteria and the brain. An increasing number of scientific reports have hypothesised that the different expression of inflammatory markers in ASD children correlates with both neurobehavioural and gastroenterological symptoms. This hypothesis is based on the theory that disrupted intestinal epithelial barrier, which is pathophysiologically related to the ability of molecules to migrate from the GI tract into the bloodstream, may lead to altered intestinal permeability[23]. The microbiota and its metabolites are crucial in maintaining the integrity of the intestinal epithelial barrier, and therefore dysbiosis in ASD patients may alter intestinal permeability[23]. This may allow the penetration of microbes and toxins, such as lipopolysaccharides, or bacterial metabolites, triggering the immune response and increasing the release of inflammatory markers. The activated immune system, by releasing inflammatory cytokines and chemokines, may modulate CNS function and contribute to the pathogenesis of ASD by influencing early brain development. Furthermore, short-chain fatty acids such as propionate, butyrate, acetate

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and succinate, which are metabolic by-products of gut-colonising microorganisms, may also be involved in ASD symptoms[24]. It was found in an animal model of ASD that intracerebroventricular (ICV) injections of propionate (PPA) stimulated hyperactivity, stereotypies, impaired social behaviour, seizures, elevated oxidative stress markers, and activated inflammatory responses in the CNS. The impact of PPA is very likely as it rapidly passes through the gut–blood and blood–brain barriers. It can reach the CNS either passively or actively, through cell membranes, as well as it can enter the cells[25]. Propionate and butyrate prompted extensive modifications in the expression of host genes responsible for the production of neuronal cell adhesion molecules, neurotransmitters, oxidative stress, lipid metabolism and mitochondrial function in ASD[26]. Additionally, the microbiome plays a key role in the maturation of the immune system, by modulating innate and acquired immunity, regulatory T cells in particular[23].

MICROBIOME-BASED THERAPEUTIC OPTIONS IN ASD CHILDREN

There are currently no treatments available for ASD that would be 100% effective. Recommended and well-investigated therapeutic options are limited to holistic developmental support, including behavioural therapies, collaboration between a psychiatrist and psychologist, rehabilitation, sensory integration classes, educational therapies, and speech therapy. This unfortunately prompts parents to constantly seek alternative approaches, even those not supported by sound scientific data.

Dietary interventions, such as gluten-free or ketogenic diets, which are commonly used in ASD children can be potentially harmful. Restrictive diets further limit the already advanced food selectivity, resulting in the risk of even greater micro- and macronutrient deficiencies. The rationale behind the likely efficacy of a gluten-free diet is the theory that ASD arises from disrupted metabolic processes. Food-derived peptides with opioid activity penetrate the intestinal wall with impaired permeability and enter the CNS, affecting neurotransmission and thus ASD symptoms. According to a theory, breakdown products of gluten, among others, act as opioid receptor agonists. Therefore, a gluten-free diet appears to reduce urinary levels of opioid peptides and the severity of behavioural symptoms, but the evidence supporting this theory is still weak and limited. The results of studies on the efficacy of a gluten-free diet are contradictory. Improved control of neurobehavioural symptoms as a result of ketogenic diet (classified as a high-fat, low-carbohydrate diet that is effective in, for example, epilepsy) has also been reported in children with mild to moderate ASD. Unfortunately, adverse reactions in the form of constipation, hypercholesterolaemia, vomiting and dehydration were observed in the study group. The adverse reactions and the increased counts of pathogenic gut bacteria, as well as the limited number of encouraging results, have led researchers to improve the study design to avoid negative effects on the patients, with a view to their safety.

Probiotics may be a better and safer therapeutic alternative to dietary interventions as, in addition to their generally known safety profile and good tolerability, probiotics may have beneficial effects on the intestinal mucosa, seal the intestinal epithelial barrier through mucin production and increase the production of digestive enzymes and antioxidants. In a cohort study, a group of 22 children with ASD were supplemented with *Lactobacillus acidophilus* twice daily for two months. Although their ability to concentrate and follow instructions improved, there was no effect on behavioural or emotional disorders[26]. In two cases, daily administration of VSL#3, a mixture of ten probiotic strains, initiated regression of ASD symptoms, but this was reversed when supplementation was discontinued. In a 2017 prospective study, 30 children with ASD were given strains of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium longum* for three months and then assessed for ASD symptoms using ATEC. The overall results indicated reduced severity of symptoms, especially in terms of speech, language and communication[27]. A randomised double-blind study was also conducted, in which the *Lactobacillus plantarum* strain WCFS1 was supplemented for three weeks. The patients were divided into two groups. One was given the probiotic treatment first and then a placebo for a further three weeks, while the other was first given the placebo and then the probiotic. Symptom improvement was only seen at the beginning of the study in both groups[28]. Furthermore, there is an ongoing randomised trial assessing the effects of 6-month supplementation with a probiotic mixture (Vivomixx) in ASD children with and without GI symptoms. In conclusion, the possible beneficial effect of probiotics on neurobehavioural symptoms in ASD children is still controversial and requires further well-designed studies.

Faecal microbiota transplantation (FMT) and microbiota transfer therapy (MTT) have recently attracted the interest of researchers due to their efficacy in the treatment of recurrent *Clostridium difficile* infections and their promising role in the treatment of inflammatory bowel disease (IBD). MTT appears to be a promising therapeutic approach for intestinal dysbiosis in ASD. In an open-label clinical trial, a modified FMT protocol was developed that consisted of 14 days of oral vancomycin, followed by 12–24 hours of intestinal cleansing and, finally, oral or rectal administration of standardised human gut microflora for 7–8 weeks. Post-treatment improvements in behavioural ASD symptoms were observed and persisted for 8 weeks post-treatment[29]. Furthermore, post-MTT observations indicate that faecal metabolite profiles become more similar to those seen in a typically developing cohort. There was an 82–88% decrease in the median difference between the ASD and control group[30]. It should be noted that the control group only provided stool samples and did not receive any treatment. It was found that ASD patients had a unique metabolic

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profile at baseline. The ASD group had significantly lower baseline levels of eight metabolites (nicotinamide riboside, inosine 5'-monophosphate, iminodiacetate, methyl succinate, galactonate, valylglycine, sarcosine and leucylglycine), and higher caprylate and heptanoate levels. The same group of researchers found in the longitudinal analysis that difference in levels of social withdrawal measured in individuals at different time points correlated with the degree of change in gut microbiome composition, and that speech deterioration between time points was associated with reduced gut microbiome diversity. This was the first study to relate the severity of behavioural symptoms to gut microbiome composition over time and suggested a dynamic relationship between ASD-associated symptoms and gut microbes[30]. A randomised double-blind placebo-controlled study would be needed to confirm these promising results.

CONCLUSIONS

Increasing evidence suggests a link between dysbiotic gut microbiota and autism. In the cited meta-analysis, the microflora of ASD children consisted mainly of *Bacteroidetes*, *Firmicutes* and *Actinobacteria*, all of which were more abundant than in the control group. There was also a significantly higher abundance of *Bacteroides*, *Parabacteroides*, *Clostridium*, *Faecalibacterium* and *Phascolarctobacterium* and lower counts of *Coprococcus* and *Bifidobacterium* compared to controls. At present, however, the inconsistency of results between different centres makes it possible to generate a definitive thesis that microbiota contributes to ASD symptoms. If this were the case, clinical trials with microbiota as a therapeutic target for the treatment of ASD would be very promising and highly desirable. The prevalence of ASD, the specificity of paediatric patients and the determination of their parents obliges us to continue to explore knowledge and design studies that will clarify, rather than multiply, the discrepancies that accumulate from year to year.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organisations which might negatively affect the content of this publication and/or claim authorship rights to this publication.

Author contribution

Original concept of study: PG, MS, AD, TP. Collection, recording and/or compilation of data: PG, MS, AD. Analysis and interpretation of data: PG, MS, AD. Writing of manuscript: PG, MS. Critical review of manuscript: PG, AKD, ICK, AS, TP. Final approval of manuscript: PG, AKD, AS, TP.

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