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SPECIAL ISSUE*: Prebiotics and probiotics in paediatrics*

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Foreword: All things considered about probiotics, prebiotics and ıntestinal microbiota in children – from bench to bedside

There are numerorus published and ongoing experimental/clinical studies about probiotics and prebiotics, intestinal microbiota and nutrition. Three years ago, at the first International Symposium of Probiotics Prebiotics in Paediatrics in Istanbul (2012) we highlighted the 'paediatric perspective' on these issues and brought together more than 40 global key opinion leaders and 400 attendants to have a chance to extensively discuss the past, present and the future. In 2014, the second state of art congress, held in Antalya, aimed to discuss the gut microbiota and microbiotics and their impact through lifespan. Selected papers of this conference are presented in this special issue 'prebiotics and probiotics in paediatrics' of Beneficial Microbes. A summary of the conference results is provided below.

In the light of previous studies, it is well known that the alteration of intestinal microbiota during early infancy period (delivery mode, lack of breastfeeding, early use of antibiotics, etc.) results in clinical conditions which affects whole life. Erica Isolauri summarised the recently proposed extended hygiene hypothesis which suggests that modern civilisation is faced with a progressive increase in immune-mediated health problems due to reduced microbial contact at an early age (Isolauri and Salminen, 2015). This hypothesis emphasises the intimate interrelationship between diet, the immune system and microbiome: while allergic diseases comprise the most common chronic disease in childhood, obesity is the most prevalent nutritional disorder among children throughout the world. The composition of the gut microbiota and its modification by specific probiotics or prebiotics early in life may have an impact on the risk of diseases in the childhood. Probiotic effects have been attributed to restoration of increased intestinal permeability, improvement of the intestine's immunological barrier functions, alleviation of the intestinal inflammatory response, and reduced generation of proinflammatory cytokines characteristic of local and systemic allergic inflammation. Sanja Kolaček's presentation highlighted a trilateral system of gut flora: intestinal epithelial cells, gut microbiota and intestinal immunity which has paramount importance in immune response in health and disease. This complicated and yet partly investigated system has undergone co-evolution through millions of years, shaping each other with the ultimate goal of achieving the maximal benefit and security without providing a major harm to other components (Kolacek, 2014). Recent studies enable interactions and cross-talk of this trilateral system with environmental factors such nutrition, infections and antibiotics. Further studies are needed to clarify this system since the human race is now facing the increasing prevalence of chronic disorders, such as autoimmune, atopic, inflammatory and metabolic syndrome. Sanja Kolaček provided clues about cross-communication of the gut flora with our innate immune system and how this communication influences adaptive immune responses. She also asked 'how our commensal flora can exert different immune responses varying from anergy over tolerance to 'tonic' inflammation' and 'what are the possible genetically influenced causes for the epithelial barrier/microbiome miscommunication, resulting in chronic diseases'.

Yvan Vandenplas answered when the healthy gastro-intestinal (GI) microbiota is challenged in early infancy and what our precautions might be. The increasing number of clinical and laboratory studies made us recognise the importance of a healthy GI flora, particularly during the infancy period. An unbalanced GI flora predisposes to the development of allergy and chronic inflammation. Not only the early use of antibiotics has a high impact on healthy microbiota, but also medications, such as anti-acid drugs like proton pump inhibitors and H2-blockers that are in the top 10 of the most prescribed medications in young infants (Vandenplas, 2015). The modern infant, particularly if caesarean section-delivered and devoid of the recommened exclusive breast-feeding, may lack sufficient stimulation of the mucosal immune system to generate a tolerogenic immune milieu and they are prone to develop chronic inflammatory conditions: allergic and autoimmune disease or obesity. Zvi Weizman reviewed the vast majority of clinical research to date regarding the clinical efficacy of probiotic microorganisms that has focused on the treatment of daycare infections. Infants

and children attending childcare centers demonstrate a significantly higher risk of respiratory and gastrointestinal infections (Weizman, 2015). The studies focusing on prevention of common childhood infections in day-care centers showed that major clinical benefits include reduced morbidity incidence as well as shorter illness duration. Daily probiotic supplementation has been promising in reducing both the incidence and duration of common acute illnesses but additional studies are necessary to evaluate a wider spectrum of probiotic microorganisms comparing strain-specific effects in various settings.

Probiotic use in children for the treatment of acute infectious diarrhoea, prevention of antibiotic associated diarrhoea and prevention of daycare infections are major well-described/studied indications. Numerous clinical trials from different clinical settings with different strains and doses are known. For routine use of the selected strains during childhood, evidence-based evaluation of previous studies is the key issue. The best known and studied indication, acute gastroenteritis management, was summarised by Hania Szajewska with the recommendations of the Working Group on Probiotics and Prebiotics of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN). ESPGHAN Working Group formulated the inclusion criteria: if at least two randomised controlled clinical trials that used a given probiotic (with strain specification) were available and choosing a probiotic from a manufacturer who has a regulated quality control of factors including the composition and content of the agent (Szajewska, 2015). According to this report, two strains received strong recommendation, although with a low quality of evidence: *Lactobacillusa rhamnosus* GG (LGG) and *Saccharomyces boulardii* in childhood period. Lynne McFarland showed that paediatric antibiotic associated diarrhoea (AAD) occured more frequently in hospitalised children compared to outpatient children, with an average duration of AAD ranging from 2 to 9 days. On the other hand, the prevalence of *Clostridium difficile* infection in paediatric cases is lower (0-8%) than expected. The challenge for paediatricians is to decide which probiotic product is more effective and which are safe for use in children (McFarland, 2015). She declared that further studies are needed for more randomised, controlled trials of existing probiotic strains or newer strains for the paediatric population.

Clinical effects of probiotics for the prevention and/or treatment of paediatric functional GI disorders are are widely studied. Flavio Indrio focused on the clinical and socioeconomic impact of prevention of functional GI disorders in neonates. Functional gastrointestinal disorders are defined as a variable combination of chronic or recurrent gastrointestinal symptoms that are not explained by structural or biochemical abnormalities: infantile colic, gastro-esophageal reflux and constipation are the most common examples (Indrio *et al.,* 2015). Yvan Vandenplas, in his second presentation, summarised amazing algorithms for the management of infants who were admitted to physicians with frequent gastro-intestinal complaints. In his presentation, he updated his previously published algorithms with the latest data in the available literature. His paper in this special issue will be an excellent guide for paediatricians and family physicians keeping in mind that these algorithms are not 'evidence-based guideline', but result of a consensus based on the available final literature (Vandenplas and Alarcon, 2015). Current evidence has already shown that a low-grade mucosal inflammation and immune/motor alteration present in these infants. Probiotics could play a crucial role in the modulation of intestinal inflammation and may represent a new strategy for preventing these conditions, at least in predisposed children. Stefano Guandalini, clarified the rationale for probiotic use in patients with inflammatory bowel disesae. The endogenous intestinal microbiota in fact plays a central role in their development, and there is an extensive literature supporting the efficacy of various strains in experimental models of inflammatory bowel disease (Guandalini *et al.,* 2015). However, the findings vary extensively in Crohn's disease (no beneficial effect neither in children nor in adults), however data is more promising in ulcerative colitis.

The safety and efficacy of prebiotics (as naturally occurring and manufactured oligosaccharides), probiotics or synbiotics in infant feeding are one of key research area in nutrition. Christine Edwards, explained how to assess GI health benefits of prebiotics by focusing 'microbial fermentation and metabolism'. As stated in the literature, some bacterial metabolites are generally accepted as beneficial to the host, whereas others are considered as potentially toxic (Edwards, 2014). Protein fermentation metabolites (phenol, p-cresol, indole, ammonia), typically considered as harmful metabolites, do not induce toxic effects at the concentration ranges encountered in the colon. End-products of saccharolytic fermentation, small chain fatty acids, may have very different effects on colonic health, host physiology, lipoprotein metabolism and appetite. There is insufficient evidence to use changes in levels of individual bacterial metabolites as markers in the assessment of prebiotic effectiveness. Integration of metabolomic approaches with metagenome data and potentially faecal water toxicity data might advance the field. Sertac Arslanoglu, discussed the short and long term health outcomes of prebiotic supplementation early in life. Intestinal microbiota acquired during early postnatal period is one of the essential factors for the appropriate development of immune system in terms of immune regulation and maintenance of tolerance to environmental and self antigens. Since it is well-known that the composition of gut microbiota differs between healthy and allergic infants, studies for modifying the gut microbiota

and thereby modulating the immune response in infancy have started (Arslanoglu, 2014). Bifidogenic modification of the gut microbiota in infancy showed benefits on functional bowel disorders in short term, and decreased allergy prevelance and common childhood infection episodes in long term. Marked reduction in the occurrence of atopic dermatitis with prebiotic supplementation might lead us to consider utilisation of prebiotics as primary prevention strategy for formula-fed infants.

As previously mentioned, the alterations of intestinal microbioata with different factors result in allergic disorders and in turn necessitates the key research area: prevention of allergic disorders with pre and/or probiotics. Erick Forno summarised the current achievements about the probiotics, atopy and asthma in childhood. The prevalence of asthma and atopic diseases have significantly increased around the world over the past several decades; approximately 20% of the global population suffers from some forms of atopic disorder. The 'hygiene hypothesis' postulated that increased 'cleanliness', reduced family size and decreased childhood infections have led to a rise in atopic and auto-immune diseases by lowering our normal exposure to microbes (reduced microbial diversity), which play a critical role in the maturation of the immune system. Probiotic strains themselves or perhaps the balance they help achieve seem to have beneficial effects to prevent or reduce atopy (Forno, 2014). This effect may happen via improved balance between Th1 and Th2 immune responses, toll-like receptor activation, modulation of inflammatory signals or other mechanisms. Probiotics are effective in decreasing levels of total immunoglobulin E and the reduction was more pronounced with longer periods of follow-up. There was no beneficial effect of probiotics on childhood asthma or wheezing. Future studies looking at probiotics and asthma should be careful in selecting the strains of probiotics used, and should follow children for longer periods of time to allow for an effect to take place. Eduardo Lopez-Huerta primarily focused on the importance of appropriate synbiotic formulation effects on common infections in infancy period. Two previous studies presented in the symposium showed that synbiotic formulation was well tolerated and there were no serious adverse reactions (Lopez-Huerta, 2015). The administration of the synbiotic formula may be useful for the prevention of community-acquired paediatric infections.

Prevention and treatment of cow milk allergy (CMA) will undoubtedly be one of the most frequently discussed topic in nutrition era in up-coming years. Simon Murch focused on the elements of the gut microflora that appear critical in the establishment of mucosal and systemic immune tolerance. The development of oral tolerance to dietary antigens, including cow's milk, is underpinned by a coordinated response to the flora that induce commitment of dendritic cells and subsequently T cells to a regulatory phenotype (Treg cells). Infants with intestinal food allergies, such as CMA, have been shown to have reduced numbers of circulating and mucosal Treg cells that display reduced regulatory response upon antigen challenge, i.e. reduced expression of the key regulatory cytokine transforming growth factor-β (Murch, 2014). The question thus arises whether this process of induced Treg generation could be accelerated by bacterial flora that favour their generation. Recent studies prove that modulation of the flora represents a potentially viable pathway to enhance mucosal tolerance and inhibit allergic disease of various kinds, though the results are variable from study to study. Johan Garssen summarised the new dietary concepts for allergy management regarding the role of pro-, pre- and synbiotics. Prebiotic oligosaccharides, probiotics and synbiotics are increasingly accepted as immunomodulatory ingredients for the prevention and or treatment of immune related disorders such as infections, allergies, obesity and even some brain/behaviour disorders (Garsen, 2014). During the pregancy period the immune system is highly susceptible to both positive as well as negative triggers affecting a healthy immune development. The intriguing feature of immune development is that it is a continuous process which never ends. Current research indicates that early events on immune development might have serious consequences on immune related diseases at adult ages, such as allergies, asthma and even autoimmunity.

Roberto Berni Canani reviewed how to target gut microbiota to induce oral tolerance in children with CMA. There were qualitative and quantitative differences in the composition of gut microbiota between patients affected by CMA and healthy infants and so it has been proposed that specific beneficial bacteria could potentially restore intestinal homeostasis and prevent or treat food allergy (Cosenza *et al*., 2015). Recent study demonstrated that an extensively hydrolysed casein formula containing LGG was able to significantly accelerate the development of tolerance acquisition in infants affected by CMA. The results of this study might open the way to a possible 'nutritional immunology approach' in these patients. Els van Hoffen underlined that food allergy (FA) and atopic dermatitis (AD) are both manifestation of the atopy syndrome. FA is the result of an aberrant immune response towards harmless food antigens, indicating that the induction or oral tolerance to food is disturbed. For education of the immune system to discriminate between 'the good and the bad' and for maintaining gut barrier function, the gut microbiota is very important for the creation of a tolerogenic environment (Van Hoffen, 2014). Similarly, the skin is colonised with microbiota that play a role in the protection and maintenance of the homeostasis of the skin. Also, gut microbiota composition in infants with FA and AD is different from healthy infants. The Cochrane reviews show that primary prevention of AD is the most promising effect of supplementation, whereas treatment

of AD is not effective. Surprisingly, in contrast to AD, prevention or treatment of FA by pre- or probiotics in infants with AD seemed much less promising according to current literature. More detailed characterisation of probiotic strains is important, for example, by gene-trait matching, as an essential step in establishing their potential mechanism of action.

Gut-brain axis interactions, alterations of intestinal microbiota in patients with neuro-psychiatric disorders, and potential effects of probiotics on the disease course are the most promising scientific era today and in the near future. Stephen Collins summarised the current achievement about the complex and yet-to-be-discovered system between brain and intestinal microbiota, which is a bi-directional communication system that involves neural, endocrine and immunological signalling. Several lines of evidence demonstrating the ability of the microbiota to influence the brain can be read in detail in his brilliant review. Paul Forsythe evaluated how gut microbiota effects the immune and enteric nervous systems. The intestinal bacterial ecosystem is also involved in the regulation of the endocrine system and this 'forgotten organ' is responsible for the maturation and development of the immune system (Bienenstock, 2014). As far as the immune system is concerned, certain beneficial commensal bacteria, such as probiotics, promote anti-inflammatory and immunoregulatory pathways both locally in the intestine, and as well as systemically. Probiotics and other bacteria promote fermentation, especially in the large intestine, and their products, such as acetate, propionate and butyrate have themselves been shown to have powerful immune and nervous system effects. Several of the beneficial bacteria have direct and indirect effects on the enteric nervous system and the spinal afferent and vagus nerves, which carry all forms of sensation to the brain. Some of them have analgesic effects and have been shown to attenuate visceral pain in both clinical and experimental situations. Mechanistics and functional explanations of how these effects are occurring may offer new therapeutic approaches to the maintenance of health and the prevention and treatment of disease.

Like the gut brain axis issue, interaction between alterations of intestinal microbiota and obesity is another important promising scientific issue. Koen Venema summarised the differences in energy extraction by the microbiota of lean and obese individuals. The gut microbiota can influence body weight because it affects the nutritional metabolism of the host in several ways: by inducing release of satiety hormones in the intestinal tract and producing short-chain fatty acids which are energy substrates for the host (Venema, 2014). Surprisingly, the obese microbiota did not in all cases extract more energy and actually about 50% of the substrates used by the obese microbiota of the studied animals showed a lower energy extraction per gram of substrate than the lean microbiota. This is why the role of the microbiota in obesity is a complex one and it is far from clear how these different mechanisms interact in the host. For potential evaluation of effects of probiotics or the alterations of intestinal microbiota, we need systems and equipment simulating the real gut function in human being. Massimo Marzorati presented an *in vitro* technology platform to assess the mechanism of action of pre- and probiotics in the gastrointestinal tract. The intestine is frequently considered as a black box and the better an *in vitro* system can simulate the real gut situation, the higher is the physiological significance of the obtained information (Marzorati, 2014). As a result, the last decade saw a number of dynamic *in vitro* simulators that attempt to reproduce the physiological parameters that could influence GI microbial community and its metabolic activity. With these potential systems, to assess the potential mechanism of action of a given functional ingredient, the targeted delivery of probiotics, the area of fermentation of a fibre in the GI tract might be possible.

During the 2nd International Symposium of Probiotics Prebiotics in Paediatrics, well-planned clinical and experimental trials were firstly presented. L.M.J. Knippels and colleagues presented two experimental studies. Their previous experimental studies indicated that maternal supplementation with non-digestible carbohydrates might beneficially affect maternal and foetal immune status in mice. Therefore, maternal supplementation during pregnancy was studied to observe whether any long-term programming effects on the immune response in the offspring of both sensitized and non-sensitized dams would be. The results showed that maternal supplementation with a specific mixture of oligosaccharides during pregnancy leads to a significant decrease in allergic symptoms in the off-spring of non-sensitized mice, suggesting a beneficial programming effect. Results from this study might indicate a role for regulatory T cells. The second part of the study showed that these sensitized dams had significantly different shock scores and temperature changes. He claimed that these results would suggest that maternal supplementation with a specific mixture of oligosaccharides could have a beneficial programming effect on the immune response of off-spring of sensitized mice. L.F. Harthoorn presented the results of a prospective, randomised, double-blind controlled study about the functional effects of an amino-acid based formula with synbiotics in CMA infants. They enrolled full-term infants diagnosed IgE and/or non-IgE mediated CMA who received a commercially available amino-acid based formula with or without synbiotics for 16 weeks. They concluded that their study showed an amino-acid formula with synbiotics was equally tolerated and supported normal growth. Infants receiving the synbiotic blend had softer stools, less reported infections and less antibiotic use. They suggested that addition of these synbiotics to an aminoacid formula might improve

resistance to infections. B. Cristina showed that the combination of oligofructose and inuline might reduce the severity of chronic nonspecific childhood diarrhoea. The underlying cause of chronic nonspecific diarrhoea was not clear, including mechanisms such as bowel motility disorders or dietary factors (low fat diet, osmotic fluids, very high fluid intake) and prebiotics might have improved symptoms in these patients acting through gut microbiotia. Randomised, placebo controlled clinical trial in subjects aged 1 to 5 years old, with a combination of oligofructose and inuline 4 g/day for a 3 months period, was significantly effective and improved quality of life.

After two successful meetings in Turkey, the organisational team of IS3P decided to move this symposium to another location. The third international symposium will be held in Brussels, Belgium in 2016 and promises to bring new and exciting advances in 'Probiotics, Prebiotics in Paediatrics'.

Metehan Ozen[1] and Ener Cagri Dinleyici[2]

[1]Acıbadem University, Faculty of Medicine, Department of Paediatrics, Istanbul, Turkey; metehanoz@yahoo.com
[2]Eskisehir Osmangazi University, Faculty of Medicine, Department of Paediatrics, Eskisehir, Turkey; timboothtr@yahoo.com

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References

Arslanoglu, S., 2014. Prebiotic supplementation early in life: short and long term health outcomes. IS3P 2014 Congress Book 1: 31.

Bienenstock, J., 2014. Gut microbiome: effects on the immune and enteric nervous systems. IS3P 2014 Congress Book 1: 34.

Cosenza, L., Nocerino, R., Di Scala, C., Di Costanzo, M., Amoroso, A., Leone, L., Paparo, L., Pezzella, C., Aitoro, R. and Berni Canani, R., 2015. Bugs for atopy: the *Lactobacillus rhamnosus* GG strategy for food allergy prevention and treatment in children. Beneficial Microbes 6: 225-232.

Edwards, C., 2014. How to assess gastrointestinal health benefits of prebiotics: focusing on 'microbial fermentation and metabolism'. IS3P 2014 Congress Book 1: 30.

Forno, E., 2014. Probıotıcs, atopy, and asthma – present and promise. IS3P 2014 Congress Book 1: 39.

Garssen, J., 2014. New dietary concepts for allergy management: the role of pro-, pre- and synbiotics. IS3P 2014 Congress Book 1: 25.

Guandalini, S., Cernat, E., Moscoso, D., 2015. Prebiotics and probiotics in irritable bowel syndrome and inflammatory bowel disease in children. Beneficial Microbes 6: 209-218.

Lopez-Huertas, E., 2015. Safety and efficacy of human breast milk *Lactobacillus fermentum* CECT 5716. A mini-review of studies with infant formulae. Beneficial Microbes 6: 219-224.

Indrio, F., Di Mauro, A., Riezzo, G., Panza, R., Cavallo, L., Francavilla, R., 2015. Prevention of functional gastrointestinal disorders in neonates: clinical and socioeconomic impact. Beneficial Microbes 6: 195-198.

Isolauri, E. and Salminen, S., 2015. The impact of early gut microbiota modulation on the risk of child disease: alert to accuracy in probiotic studies. Beneficial Microbes 6: 167-172.

Kolacek, S., 2014. Gut flora in immune response in health and dısease. IS3P 2014 Congress Book 1: 23.

Marzorati, M., 2014. An *in vitro* technology platform to assess the mechanism of action of pre- and probiotics in the gastrointestinal tract. S3P 2014 Congress Book 1: 32.

McFarland, LV., 2015. Deciphering meta-analytic results: a mini-review of probiotics for the prevention of paediatric antibiotic-associated diarrhoea and *Clostridum difficile* infections. Beneficial Microbes 6: 189-194.

Murch, S., 2014. Modulating the gut microbiota to aid tolerance induction in CMA children. IS3P 2014 Congress Book 1: 26.

Szajewska, H., 2015. What is new on the term probiotics and the role of probiotics for the management of acute gastroenteritis. Beneficial Microbes 6: 185-188.

Van Hoffen, E., 2014. Food allergy, atopic dermatitis and pre- or probiotics: therapy or prevention?. IS3P 2014 Congress Book 1: 41.

Vandenplas, Y., 2015. Healthy gut microbiota and long term health. Beneficial Microbes 6: 173-180.

Vandenplas, Y. and Alarcon, P., 2015. Updated algorithms for managing frequent gastrointestinal symptoms in infants. Beneficial Microbes 6: 199-208.

Venema, K., 2014. Differences in energy extraction by the microbiota of lean and obese individuals. IS3P 2014 Congress Book 1: 35.

Weizman, Z., 2015. The role of probiotics and prebiotics in the prevention of infections in child day care centres. Beneficial Microbes 6: 181-184.