systematic review concluded that although the overall microbiota is altered by various dietary interventions, more research is needed to support the assumption that *A. muciniphila* can be targeted with dietary intervention because the results from this approach have not been consistent between studies[3]. As a matter of fact, we demonstrated in two human studies that either supplementation with isolated inulin and/or oligofructose (16 g/day)[4] or increasing the consumption of selected vegetables to naturally boost the intake of prebiotic fibers (15 g/day) induces a marked shift in the gut microbiota composition (e.g., a consistent increase in *Bifidobacterium* spp. and *Faecalibacterium prausnitzii* and a decrease in other specific taxa)[4,5]. However, although both types of regimens are associated with a strong impact on gut microbiota composition, none of these dietary approached induced changes in *A. muciniphila* levels[4,5].

Because the intake of all the nutrients mentioned by Janket et al.[1] were listed among our exclusion criteria, these confounding factors were excluded in our study[2].

We believe that our study cannot be viewed as a phase 1 trial, as such studies are designed differently and are used to find the best compromise in regard to the safety, tolerability, and pharmacodynamics (PD) and pharmacokinetics (PK) of an absorbed compound, and also normally include dose ranging to determine at what dose adverse effects begin to appear. Although we designed our nutritional study with all best efforts to achieve high quality standards, such as are used in testing pharmaceutical compounds, our study obviously did not include any escalation of doses to determine the minimal toxic dose, and it would have been impossible to assess PK and PD with this sort of nutritional approach.

Janket et al.[1] also point out that in our previous study in mice[6], we found that "the health benefits of the *A. muciniphila* membrane protein Amuc_1100 were more pronounced than those of live *A. muciniphila*." They suggest that supplementation with Amuc_1100 rather than live bacteria will be more appropriate in future research. The protein indeed replicated part of the effects of the live bacterium; however, we wish to highlight that the effects of the protein matched those observed with the pasteurized bacteria, which had greater efficacy than the live bacteria. Thus, using pasteurized *A. muciniphila* probably has different effects than simply increasing the levels of endogenous live bacteria or supplementing with a high dose of live bacteria.

In conclusion, the fact that many potential confounding factors were indeed taken into account and monitored clearly imply that Simpson's paradox may not apply to our study. ❐

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Competing interests

P.D.C. is an inventor on patent applications (PCT/EP2013/073972; PCT/EP2016/071327, and PCT/EP2016/060033 filed with the European Patent Office (EP) and the patent offices of Australia (AU), Brazil (BR), Canada (CA), China (CN), Eurasian Patent Organization (EA), Israel (IL), India (IN), Hong Kong (HK), Japan (JP), South Korea (KR), Mexico (MX), New Zealand (NZ), and the United States (US)) dealing with the use of *A. muciniphila* and its components in the context of obesity and related disorders. P.D.C. is a co-founder of A-Mansia Biotech SA.

A critical analysis of the neurodevelopmental and neurosensory outcomes after 2 years for children with in utero Zika virus exposure

To the Editor — We have read with interest the recent work by Nielsen-Saines et al.[1]. In this prospective study, the authors present the neurodevelopmental and neurosensory outcomes within the second year of life of 216 newborns born from mothers with a laboratory-confirmed Zika virus (ZIKV) infection. A total of 67 children (31.0%) demonstrated abnormal neurodevelopmental scores or isolated hearing or visual disturbances, of whom 18 (12.3%) had severe developmental delay. Factors associated with poorer outcomes were early infection during pregnancy, prematurity, male sex and abnormal fundoscopic examination.

ZIKV has recently been recognized as a teratogenic agent associated with fetal and neonatal anomalies, which have now been amalgamated into the well-described congenital ZIKV syndrome[2]. Long-term outcomes associated with congenital ZIKV infection, however, remain unknown[3]. As in other congenital infections, such as cytomegalovirus, it is expected that some apparently normal newborns may develop late-onset visual, hearing and neurocognitive disorders[4].

Pomar et al. have recently published on the early outcomes (up to the first week of life) in 291 exposed fetuses during the recent ZIKV epidemic in French Guiana[5]. In this cohort, 222 neonates (76.3%) were asymptomatic at birth. A reassuring finding is that up to 69% of the exposed children in the Nielsen-Saines et al. cohort remained asymptomatic at 2 years of age, suggesting that only a minority of apparently normal newborns will develop late-onset symptoms.

We suspect that not all cases of abnormal neurodevelopment presented by Nielsen et al. are attributable to ZIKV exposure in utero, which may have led to an overestimated risk. Indeed, in the study by Pomar et al., a congenital infection was confirmed in 76 of 291 fetuses (26.1%), highlighting the fact that only a minority of exposed fetuses will become infected in the case of maternal infection[5], as with other congenital infections. Unfortunately, no information regarding ZIKV neonatal testing is provided by Nielsen-Saines et al., impairing any conclusions on the exact

contribution of ZIKV congenital infection. Furthermore, the population attributable fractions of congenital ZIKV infection reported by Pomar et al. for any adverse and severe adverse outcomes were 47% and 61%, respectively, suggesting the importance of other contributing factors to the congenital ZIKV symptomatology[5]. No control group was included by Nielsen-Saines et al., further limiting the conclusions. In agreement, prematurity, which may be an important confounding factor, was significantly associated with an abnormal developmental score and may have contributed to a potential overestimation of the risk (see Supplemental Table 1 in ref. [1]). Maternal coinfection may have played the same role. This further emphasizes the need for controlled studies with information about other potential contributing factors and the necessity to systematically test exposed newborns at birth.

Overall, we believe that this first prospective analysis of long-term outcomes by Nielsen-Saines et al. provides reassuring information and highlights the fact that the overall risk of severe adverse outcomes in cases of maternal ZIKV infection remains low and similar to what is known for other congenital infections[4]. ❐

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Author contributions

All authors contributed equally to the discussion regarding the Nielsen-Saines et al. study. M.V. and D.B. wrote the draft. D.B. supervised the work.

Competing interests

The authors declare no competing interests.

Reply to 'A critical analysis of neurodevelopmental and neurosensory outcomes after 2 years for children with in utero Zika virus exposure'

Nielsen-Saines et al. reply — In reference to the observations made by Vouga et al.[1], 31.5% of children with PCR-confirmed antenatal Zika virus (ZIKV) exposure had below-average neurodevelopment and/or hearing or visual deficits in our cohort[2]. We should emphasize that all children in our study were born to women with symptomatic ZIKV infection and confirmed ZIKV viremia or viruria during gestation (i.e., a high-risk cohort of children). There were 28 preterm infants (13%); 18 (two-thirds of preterm children) were born between 35 and 37 weeks. Fourteen preterm children had below-average Bayley-III results[3]; the others were developmentally normal. Three preterm children had severe neurologic abnormalities with structural brain defects characteristic of congenital Zika syndrome, and one additional child also had macular hypoplasia. Hearing deficits were noted in six preterm children, including one child with structural brain defects. Five preterm children scored 2 s.d. below average (developmental delay) and nine scored 1 s.d. below average (at risk for developmental delay) in one or more Bayley-III domains[3,4]. An association between prematurity and Bayley-III scores ≤ -1 s.d. was noted ($P = 0.008$)[1], while a statistically significant association was not seen for scores ≤ -2 s.d. ($P = 0.09$). We concur prematurity may influence neurodevelopment. Nevertheless, eight preterm children had other findings suggestive of congenital infection (characteristic neuroimaging and/or hearing loss). Preterm infants in the cohort by definition had ZIKV in utero exposure, so we cannot rule out one condition versus the other. Because ZIKV antenatal exposure induces poor neurodevelopmental outcomes in a subset of children, it is possible delayed neurodevelopment was potentiated in infants already at risk. We acknowledge the absence of a control group as a study limitation. However, because Zika was epidemic in Rio de Janeiro, it was very challenging to rule out antenatal ZIKV exposure in a control pediatric population of the same age and from the same environment, as control group mothers could have had asymptomatic ZIKV infection during pregnancy. This is further complicated by the difficulty in diagnosing ZIKV infection retrospectively because of the narrow period of viremia and short-lived IgM responses, plus serologic cross-reactivity with dengue viruses[5]. It is important to stress, however, that we observed a very skewed distribution towards below-average neurodevelopment in our pediatric cohort. In a general population comprising healthy children, a normal bell-shaped curve distribution across Bayley-III domains ranging from very above average, above average, average, below average and very below average (>2 s.d. to < −2 s.d.) would be generally anticipated[2], with most children falling within 1 to −1 s.d. of the normal range (average development) and similar proportions of children falling in above average and below average categories. Our findings emphasize the need for long-term follow-up of ZIKV-exposed children. We have established a cohort of children born to women with no symptoms of ZIKV in pregnancy during the time of the Rio epidemic. We are in the process of performing extensive serologic testing to ensure only ZIKV-unexposed children are followed as controls. For future neurodevelopmental assessments, we will report results in both populations. In regard