

The rare malformation holoprosencephaly: pathogenesis, association with pregestational diabetes and the possible link with food pollutants

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Abstract

Background. Holoprosencephaly is a rare (1/16,000 livebirths) and severe brain malformation occurring during early embryogenesis. The malformation originates from absent or incomplete forebrain division and is associated with altered embryonic patterning.

Objectives. A narrative review to identify and assess the evidence on non-genetic risk factors.

Results. Genes involved include sonic hedgehog, Zinc finger protein, SIX homeobox 3. Pregestational diabetes, with periconceptual hyperglycaemia, is the main non-genetic risk factor; increased oxidative stress in neuroectoderm, in particular neural crest cells, appears as the main mechanism. Several widespread pollutants, including inorganic arsenic, PFAS and PCBs, may increase the risk of pregestational diabetes by altering metabolic factors, including lipids and insulin. A scenario “widespread exposures-rare outcomes in susceptible subjects” suggests that exposure to dietary pollutants may increase the risk of pregestational diabetes, hence of holoprosencephaly in susceptible embryos.

Conclusions. This complex pathway is plausible and worth being investigated; moreover, it highlights the importance of assessing risk factors, and the associated uncertainties, in order to support primary prevention strategies for multifactorial malformations.

Key words

- malformation
- multifactorial
- endocrine disruptors
- risk factors
- pathogenesis

INTRODUCTION

Obesity, type 2 diabetes (T2D) and other metabolic diseases clustered in the “metabolic syndrome” are major health problems worldwide [1]; in addition, obesity and T2D represent important risk factors for other human diseases, including congenital anomalies (CA). Indeed, the European recommendations for the CA primary prevention rank obesity and pre-gestational T2D among the main risk factors to be targeted by community actions [2]. Noticeably, gestational diabetes, i.e., glucose intolerance usually occurring at the second-third trimester of pregnancy, is also an important risk factor for several adverse pregnancy outcomes, such as prenatal overgrowth, stillbirth and prematurity [3]; however, it is not a main risk factor for CA as it occurs after completion of organogenesis. The modes of action by which pre-gestational T2D can increase the CA risk

include increased oxidative stress and perturbed glucose metabolism [4, 5]. Meanwhile, beyond being a general risk factor, epidemiological evidence indicates a role in enhancing the risk of specific malformations. One case is represented by holoprosencephaly, a severe early disturbance of brain and craniofacial development. Holoprosencephaly is rare in livebirths and it causes early death and/or different degrees of severe disability [6, 7].

The role of dietary patterns as risk factor for the various manifestations of the metabolic syndrome is recognized since decades; yet, growing evidence highlight a role for chemical pollutants as well. So-called “western type” dietary styles, matched with insufficient physical exercise and the aging of population, can interact with specific environmental pollutants able to alter glucose and lipid metabolic pathways. The contribution by specific, widespread pollutants to actual burden of meta-

bolic syndrome manifestations such as T2D cannot be overlooked, because of both relevant modes of action and targets and multiple exposure routes, including diet. While exposure can occur along the whole lifespan, the prenatal programming appears as the most susceptible lifestage [1].

Hence a biologically plausible link between holoprosencephaly, T2D and specific widespread pollutants is worth being explored. Under this respect, the conceptual framework of adverse outcome pathways (AOP) might help assessing the interconnections between molecular markers, specific congenital anomalies and risk factors [8]. The growing data-set on AOP and related key events are being used to evaluate the biological plausibility of associations between exposures and some adverse developmental outcomes, e.g., pesticides and developmental neurotoxicity [9]. Meanwhile, AOP have been rarely applied to CA: a recent example is the assessment of a food contaminant, the mycotoxin fumonisin B1, as risk factor for neural tube defects [10]. While no AOP does exist for holoprosencephaly, so far, elements of the AOP approach might support discussing the relevance of some molecular and cellular events to holoprosencephaly and its risk factors. The present work intends to review and discuss the biological basis of a potential relationship between holoprosencephaly, T2D and selected food pollutants.

HOLOPROSENCEPHALY. PATHOGENESIS AND RISK FACTORS

Holoprosencephaly (HPE) is a complex brain malformation with a wide spectrum of associated craniofacial defects (sometimes very severe, such as cyclopia), all characterized by midline defects: the pathogenesis stems from incomplete cleavage of the prosencephalon, occurring between the 18th and the 28th day of gestation in humans [5]. Whereas the pathogenesis is common, the HPE includes a range of phenotypes whose increasing severity depends on the degree of cleavage impairment: lobar, semi-lobar and alobar HPE. The malformation is rare in newborns, with estimated incidence 1/16,000 live births; however due to high mortality in utero, the incidence is much higher in conceptuses, 1/250. In livebirths, HPE mainly leads to early mortality or severe disability; milder forms with minor anomalies and symptoms are also reported. Interestingly, HPE patients frequently present endocrine defects, often more than one in a single individual, including diabetes insipidus, and adrenal hypoplasia [11].

HPE has a well-established embryogenetic origin, yet the molecular basis of the disease is still uncertain in most cases. The main genes involved include Sonic hedgehog (SHH) [12], Zinc finger protein (ZIC2), SIX homeobox 3 (SIX3), as well as other genes involved in the embryonic patterning processes [11]. Noticeably, the sonic hedgehog pathways involved in HPE are also essential in pituitary formation; as a consequence, HPE patients frequently show pituitary deficiency and diabetes insipidus [13].

In a putative AOP perspective, the above genes, as well as others with similar functions, may be target of HPE-relevant molecular initiating events, i.e., may rep-

resent critical starting points for the pathway(s) leading to the adverse phenotype. SHH knock-out mouse embryos develop a severe HPE phenotype, with fusion of the telencephalic vesicle and optic cup, deriving from the loss of the ventral midline portion of the neural tube [14]. ZIC2 is critical for the organization of the anterior notochord, in particular for providing maintenance signal to the prechordal plate; in its turn, this structure promotes, through SHH, the formation of the two hemispheres in the developing forebrain [15, 16]. The transcription factor SIX3 is essential for the development of the forebrain by establishing the anterior-posterior identity of brain vesicles and has a key role in pathways leading to retinal and lens morphogenesis [17, 18]. Noticeably, ZIC2 and SIX3 also inhibit Wnt signaling; in vertebrate embryos, formation of anterior neural structures requires suppression of Wnt signals emanating from the paraxial mesoderm and midbrain territory [16, 19, 20]. Overall, these critical genes point out the main embryonic districts targets of early events in putative pathway(s) leading to HPE, namely: the anterior notochord and the dependent forebrain induction, as well as a direct impairment of forebrain patterning. Inhibition of Wnt signalling immediately consequent to SIX3 or ZIC-2 dysregulation might feature as an early key event in the stream eventually leading to HPE [16, 19, 20]. As shown by animal models, the subsequent cascade of events leading to the craniofacial alterations typical of HPE would involve the pathways orchestrated by Bone morphogenetic protein, Fibroblast growth factor, and Nodal signalling [21].

Altered lipid and cholesterol metabolism may also play a role in HPE pathogenesis. The malformation has been associated with mutations of TG interacting factors (Tgifs) 1 and 2, which are homeodomain proteins; loss of Tgif function can disrupt the SHH signalling pathway [22-24]. Tgifs 1 and 2 interact with the ligand binding domain of retinoid X receptor α ; in its turn, this is a main heterodimeric partner of other nuclear receptors that are key regulators of lipid and cholesterol pathways, liver X (LXRs) and peroxisome proliferator-activated receptors (PPARs). Indeed, Tgif1 and Tgif2 may act as transcriptional regulators of pathways activated by lipids [25]. Unfortunately, to our best knowledge, no further data have elucidated the connection between the risk of HPE and the perturbation of lipid and/or cholesterol metabolism.

HPE is largely recognized as a multifactorial malformation spectrum, where environmental factors interplay with gene defects/mutations. HPE has been defined as a polygenic perturbation of anterior midline formation, with environmental factors, as well as embryonic stochasticity, influencing the phenotypic outcome [26]. Maternal pre-gestational T2D is consistently identified as a major risk factor in humans [11, 12, 27-29]. The largest case-control study on birth defects carried out in the USA, the National Birth Defects Prevention Study, reported a higher rate of pre-gestational diabetes in mothers of case infants (775/31,007, 2.5%) compared to controls (71/11,447, 0.6%); gestational diabetes during the index pregnancy showed also a slight increase (5.3% vs 4.7% in controls). Pregestational diabetes had a

strong impact on the risk of several malformations, HPE showing the second highest increase (adjusted odds ratio, 13.1; 95% confidence interval, 7.0-24.5) [27].

Interestingly the highest increase was observed for another rare malformation, sacral agenesis, a malformation of distal body axis, also associated with sonic hedgehog pathway [30]. Conversely, gestational diabetes was not associated with an increased HPE risk (adjusted odds ratio 1.2, 95% confidence interval, 0.6-2.4); hence, the findings clearly identify the critical role of the glycaemic level before pregnancy through to the early phase of organogenesis [27]. The role of the glycaemic level itself, rather than of the type of diabetes, has been highlighted by a recent study, nested on the same dataset, that found no substantial differences between the increased risks associated with pregestational T2D and pregestational type 1 diabetes. The pathogenesis of type 1 diabetes is characterized by reduced insulin production, rather than weakened insulin balance and control as in T2D; meanwhile, both diabetes types result in periconceptional hyperglycaemia when occurring in women at fertile age [31]. The association between pregestational diabetes and HPE has been confirmed by a worldwide systematic analysis of 59 population-based studies (1990-2021), overall involving more than 80 million participants. In this data-set, pregestational and gestational diabetes showed almost the same prevalence, 3.0% and 2.9% respectively: while pregestational diabetes increased the risk for a number of malformations, HPE showed the highest relative risk (18.18, 95% confidence intervals 4.03-82.06). Conversely, the increase associated with gestational diabetes was significantly smaller in regard of overall malformations: in particular, no increased risk was observed for HPE [29]. The specific increase of risk due to pregestational diabetes may partly reflect the early developmental window for HPE induction: an altered glycaemic status and associated metabolic disruption would need to be already present at organogenesis start. In fact, based on available evidence, gestational diabetes does not elicit a major risk for HPE. Meanwhile some other factors might have contributed to the findings reported in [29]. As the authors point out, the included studies show heterogeneity due to the lack of consensus and uniformity in the screening and diagnostic criteria for both pre- and gestational diabetes. An overestimation of the risk for total birth defects still observed for gestational diabetes might derive from the presence of unrecognized pregestational diabetes discovered only during pregnancy; on the hand, the inclusion of gestational diabetes cases occurring after the early pregnancy – the vulnerable window for birth defects – might lead to an underestimation of the risk. In addition, most studies included live births only; information on stillbirths and terminations of pregnancy for birth defects could provide a more accurate estimate of the impact by the different forms of diabetes on HPE risk [29].

A recent case control study compared 92 HPE cases with 56 patients of a rare genetic syndrome, the Williams-Beuren syndrome, with manifestations very different from HBE and no known environmental risk factors [28]. The results confirmed the importance of maternal pregestational diabetes, present in the 9.2% of cases and

0% of controls ($p=0.02$). In addition, the findings suggest the potential relevance of dietary/lifestyle factors (higher consumption of alcohol, cheese and espresso coffee). The actual role of these dietary factors, e.g., flagging associated nutritional imbalances and/or exposures to toxicants, needs to be further investigated.

Another group of risk factors is the use of consumer products and biocides such as personal insect repellents, insecticides and acaricides for pets, and household pest control products [28]. While this data definitely deserve attention, more accurate exposure data will be needed to evaluate the association (including precise definition of chemicals and possibly biomarkers of exposure).

Veterinary medicine offers a unique example of HPE due to an exogenous dietary factor. Outbreaks of cyclopia were observed in lambs from sheep kept on mountain pastures in western USA. The alkaloid-containing forage grass corn lily (*Veratrum californicum*) was the feed ingredient involved; the main teratogenic agent was the steroidal alkaloid cyclopamine, an inhibitor of the SHH signal transduction pathway and an antagonist of the transmembrane receptor Smoothed [32, 33]; in its turn, the receptor is a key transducer in the SHH pathway [34].

In humans, no known natural or man-made toxicants have been firmly associated till now with HPE; pregestational diabetes is by far the major risk factor identified. As recognized by the World Health Organization, diet – in particular the excess of sugar and saturated fats as well as the excess caloric intake with low physical activity – is a major trigger for the onset and severity of T2D, which is by far the most common form [35].

The pathways involved in the HPE pathogenesis are summarized in *Table 1*.

PREGESTATIONAL DIABETES AND HOLOPROSENCEPHALY: MECHANISMS INVOLVED

The increased risk of malformations in general, and specifically of HPE, by pregestational diabetes may involve several pathways. One might postulate that embryos with mutations (or epigenetic alterations) of genes relevant to HPE onset will be highly vulnerable to such mechanisms.

The hyperglycaemia characteristic of diabetes increases oxidative stress in maternal-embryonic environment. In turn oxidative stress can cause genotoxic damage in cranial neural crest cells, a compartment particularly active in the early embryonic development, with proliferation, differentiation, apoptotic and migration processes. Neural crest cells are pivotal for craniofacial development, which show a wide spectrum of anomalies in HPE, up to cyclopia; stochasticity of damage might be involved in the observed phenotypic variability [36]. The oxidative damage in neural crest cells, associated with pregestational diabetes, may result in craniofacial defects also by targeting the ribosomal RNA, hence the, post-transcription translational process [36, 37].

Two additional risk factors for oxidative stress in the spectrum of changes associated with T2D need being mentioned. One is represented by glycated products: persistent hyperglycemia elicits a widespread non-enzymatic glycation reaction with cell components, such

Table 1

Summary of main genes and pathways involved in the pathogenesis of holoprosencephaly

Gene	Pathway/function	References
Zinc finger protein (ZIC2)	Organization of the anterior notochord, providing maintenance signal to the prechordal plate that promotes, through SHH, the forebrain patterning. With SIX3, suppression of Wnt signals from the paraxial mesoderm and midbrain territory	[11, 15, 16, 19, 20]
SIX homeobox 3 (SIX3)	Organization of the anterior- posterior identity of brain vesicles; initiation of retinal and lens morphogenesis. With ZIC2, suppression of Wnt signals from the paraxial mesoderm and midbrain territory	[11, 16-20]
Sonic hedgehog (SHH)	Patterning of telencephalic vesicle and optic cup from ventral midline portion of the neural tube; pituitary formation	[11, 13, 14]
Bone morphogenetic protein	Craniofacial patterning	[21]
Fibroblast growth factor	Craniofacial patterning	[21]
TG interacting factors (Tgifs) 1 and 2	Homeodomain proteins: regulation of SHH signalling; interaction with the ligand binding domain of retinoid X receptor α ; transcriptional regulators of (LXR and PPAR-regulated) lipid pathways	[22-25]

LXR: liver X receptor; PPAR: peroxisome proliferator-activated receptor.

as proteins, lipids and nucleic acids. The by-products, named advanced glycated end products, activate a number of pathways that enhance oxidative stress [38]. Another important factor for enhanced, systemic oxidative stress is the low-grade but persistent inflammatory status of adipose tissue when T2D is associated with obesity as it frequently occurs; remarkably, pathways activated by glycation products are important also for obesity-related inflammation [39].

One component of the multi-faceted effect of increased oxidative stress is the perturbation of the cholesterol homeostasis, regulated via LXR activation, with increased cellular cholesterol efflux [40]. This effect was observed in human fetoplacental endothelial cells, representing a later pregnancy phase compared to developmental window for HPE, nevertheless, there are no reasons to discount a perturbation of cholesterol homeostasis in the early post-implantation embryo, too. Noticeably, mutations of Tgifs 1 and 2, transcriptional regulators of lipid pathways, mainly LXR and PPAR-regulated, are associated with HPE [22-25].

Overall, the available scientific evidences indicate that pathways leading from pregestational diabetes to the increased risk of HPE all pass through the glycemia-induced oxidative stress. In its turn, oxidative stress may damage the DNA and/or RNA of cellular populations involved in the anterior midline formation and craniofacial development: notochord, prosencephalon, cranial neural crest. Additionally, oxidative stress can disrupt the metabolome by altering the lipid and cholesterol pathways. Several factors may influence the onset and severity of HPE, a clinically multi-faceted CA: the amount of insult, favouring genetic predispositions as well as the induction of changes in the relevant genes/pathways, likely has a stochastic component.

DIETARY SUBSTANCES AND HOLOPROSENCEPHALY-RELEVANT PATHWAYS

Cannabinoids inhibit hedgehog signalling; the cannabinoid $\Delta 9$ -tetrahydrocannabinol (THC) induced HPE

in Cdon mutant mice, a strain with a subclinical deficit in hedgehog signalling; this effect is not observed in other mouse strains without the signalling defect. THC can exert this effect directly, even in cells devoid of cannabinoid receptor-type 1, the typical THC receptor [41].

THC, a well-known psychoactive drug, is also an undesirable substance of dietary relevance, due to its presence, albeit at low levels, in hemp used as human food as well as animal feed, whence it can pass to milk and dairy products [42, 43]. The existence of a teratogenic risk, if any, in human consumers would depend from intake levels and the threshold in genetically-susceptible individuals. Nevertheless, these data flag that even natural substances, present, e.g., in supplements and novel foods may induce mechanisms relevant to HPE.

From a risk assessment perspective, the most interesting aspect is that several pollutants widespread in food commodities may represent indirect risk factors for HPE by acting as metabolic disruptors, thus enhancing the risk of T2D (as well as obesity and other metabolic disorders), mainly by acting on endocrine-regulated pathways [1, 44]. Several such substances are persistent chemicals accumulating in food chains and representing important dietary contaminants. A recent systematic review and meta-analysis of available human studies (both cohorts and case controls) estimated the relationship between the risk of gestational diabetes mellitus and persistent, bioaccumulating pollutants such as the lipophilic polychlorinated biphenyls (PCBs) and poly-brominated diphenyl ethers (PBDEs) and per- and polyfluoroalkyl substances (PFAS), that persist in the body mainly by binding to serum proteins. All three families of persistent pollutants were associated with a moderate but statistically significant increases of the risk with average odds ratios ranging 1.32-1.06 [45]. Similar results were obtained also for phthalates, which are widespread, lipophilic but relatively non-persistent chemicals mainly released in foods from food contact materials [46]. Phthalates may increase the risk of diabetes through activation of PPARs, thus dysregulating

both the lipid and glucose homeostasis and the development and progression of pancreatic β cells [46].

A significantly increased risk for gestational diabetes of a similar magnitude order as in [43] was observed by a recent meta-analysis focused on PFAS. In this case the responsible of the significant association was PFOA, one of the most widespread and most investigated PFAS [47]. As already specified, gestational diabetes is not associated with HPE [27]. Nevertheless, the results reported in [45-47] indicate a potential for unbalancing insulin homeostasis by several main food contaminants, prompting to further investigation on the contribution of toxicant exposures to pregestational diabetes and its impact as teratogenic factor.

Noticeably PFAS are also activators of PPAR- α [48]. A thorough *in vitro* study on the human hepatocyte cell line HEP-G2 was carried out on PFOA, a PFAS involved in major contamination hotspots worldwide. The results show that PFAS can increase the risk of glucose intolerance acting at hepatic level. PFOA, at the concentration of 0.1 ng/mL reduced glycogen synthesis: impaired membrane translocation of the glucose transporter Glut-4 upon insulin stimulation and early uncoupling of insulin receptor activation from downstream were considered early events involved in low grade chronic inflammation-related insulin resistance [49]. PFAS exposure can also worsen the clinical phenotype in organisms already affected by diabetes, as shown by dose-related changes in lipidomics and pancreatic insulinitis in non-obese diabetic (NOD) mice caused by the exposure to the PFAS perfluoroundecanoic acid in drinking water. NOD mice represent a spontaneous animal model of immune diabetes, related to human diabetes type 1 [50]. Nevertheless, the possible relationship between PFAS and pregestational diabetes is not straightforward. Artificial intelligence was used to build an AOP-based investigation on the associations between PFAS and adverse metabolic outcomes. With a remarkable burden of uncertainties, the association is more plausible with dyslipidaemia and obesity rather than with insulin resistance [51]. This finding does not rule out the possibility that PFAS may aggravate a pre-existing hyperglycaemic status or may increase the risk of non-insulin-dependent diabetes [52]; indeed, since the critical aspect appears to be the pregestational hyperglycaemia, type 1 diabetes is a risk factor for HPE comparable to the more frequent and later-onset insulin-resistant T2D [29, 31].

The two main long-chain PFAS, PFOS and PFOA, are being globally phased-out as new entries in the Persistent Organic Pollutant group [53]. These chemicals will remain as "legacy contaminants" in ecosystems and agri-food chains: pervasive presence is associated to their persistence as well to their widespread industrial uses (e.g., textiles) due to their tensioactive and hydrorepellent properties. In its 2020 evaluation the EFSA concluded that, in the European Union, fish, fruits and eggs – and the related products – are the main contributors to the dietary intake and that the exposure levels of parts of the general population are of concern [54]. Preliminary studies on fluorinated compounds proposed as alternatives indicate that at least some of them, such as the

6-chlorinated perfluoroalkyl ether sulfonic acids, have the potential to alter glucose homeostasis markers [55].

Another main example of persistent pollutants associated with diabetes is represented by polychlorinated biphenyls (PCB), a widespread group of chemicals widely employed for industrial uses till their ban in the 80s, due to their persistence and bioaccumulation, PCB still feature as significant food pollutants, even decades after ban. PCBs are lipophilic and contaminate mainly foods of animal origin, such as fatty fishes followed by eggs, milk and dairy products and meat [56]. Several human studies associate PCB serum levels with abnormal glucose metabolism and the risk of diabetes. A recent, large (>4,000 subjects) cohort study carried out in China evidenced the positive relationships of serum PCBs concentrations with fasting plasma glucose values and the risk of diabetes. While no association was found with an oxidative DNA damage biomarker (urinary 8-hydroxy-2'-deoxyguanosine), a clear, significant association was found between PCB exposure and the lipid peroxidation biomarker urinary 8-isoprostane. The highly persistent PCB 153 showed the strongest association with glucose disturbance and lipid peroxidation among the seven main congeners tested [57]. Interestingly, PCB congeners may trigger different toxicity pathways, and PCB 153 belongs to a congener cluster mainly modulating genes involved in steroid and lipid synthesis [58].

Inorganic arsenic, an important pollutant worldwide, is another major dietary contaminant associated with T2D. The main environmental sources are either man-made and geochemical; the main contributors to dietary intake are rice and rice-based products as well as drinking and household water (e.g., used for cooking) in contaminated hotspots. Conversely, organic arsenic metabolites (arsenobetaine, arsenosugars, arsenolipids) accumulate in fish and seafood, but they are generally considered to be of low toxicity [59]. Arsenic appears to disrupt directly insulin-regulated pathways by eliciting several, confluent early events: beta-cell dysfunction (though enhanced apoptosis and inhibited proliferation), impaired glucose tolerance by the reduction of glucose-stimulated insulin secretion, both directly and by increasing oxidative stress through disrupted mitochondrial function; downregulated insulin synthesis by decreasing insulin mRNA transcription [60]. Arsenic also alters the microRNA profile of pancreatic beta cells, especially targeting miR-29a. An *in vitro* study on INS-1 832/13 beta cells showed that genes downregulated by inorganic Arsenic(III) were enriched in insulin secretion and T2D pathways. On the other hand, such effects were not seen with the methylation product methyl arsenite, which downregulated genes enriched in cell cycle and critical beta cell maintenance factors. Overall, post-transcriptional control in pancreatic beta cells may be a main target of inorganic arsenic [61]. In humans, there is evidence that populations living in areas with high environmental arsenic have a greater risk of T2D [62]. On the other hand, it is less clear whether current exposure levels in industrialized world areas, with more severe regulations toward pollutants, are associated with an enhanced risk; however, growing

evidence [63-65] show that higher levels of biomarkers of exposure to arsenic are associated with increased T2D-relevant biomarkers, including altered cholesterol levels [65]. While arsenic likely interacts with several other risk factors, e.g., genetic polymorphisms [63], the available data indicate that the potential link between arsenic exposure, pregestational diabetes and the risk of HPE, and other malformations, deserves attention.

CONCLUSIONS

No studies till now have investigated the possible link-mediated through the enhanced risk of pregestational diabetes between a rare and severe malformation of early nervous system patterning, such as HPE, and widespread food pollutants, such as inorganic arsenic, PFAS and PCBs. Up-to-date scientific evidence lend support to the biological plausibility of such link, indicating that further investigation is worthwhile. Several widespread food contaminants alter metabolic pathways relevant to insulin and/or lipid regulation and increase the risk of pre-gestational diabetes, which is a recognized major risk factor for HPE. The main pathway by which pre-gestational diabetes increases the risk of HPE appears to be increased oxidative stress in neuroectoderm, in particular neural crest cells. The onset of HPE requires a genetic predisposition, including stochastic mutations in genes involved in embryonic patterning. Thus, the available evidence point to a plausible working hypothesis, based on a multifactorial pathway; the widespread exposure to dietary pollutants increases the risk of gestational diabetes which leads to an increased HPE risk in susceptible embryos. This hypothesis identifies a scenario “widespread exposures-

rare outcomes in susceptible subjects”, which presents a number of difficult issues. At the moment, limited investigations have been carried on the complexity of factors and pathways leading to rare malformations: a recent example is the use of the AOP approach in order to assess the biological plausibility of the link between exposure to a food pollutant, the mycotoxin fumonisin B1, and neural tube defects [10]. More insights are expected from the growing use of integrated transcriptomic/proteomic/metabolomics analysis in epidemiological studies, including those on the relationships between type 2 diabetes and environmental chemical exposures [66]. In the case of HPE, the use of integrated omics may help clarify the role of maternal and/or environmental factors and related biomarkers, e.g., the relevance of enhanced glycation products [38, 39]. Definitely, the issue of complexity of rare multifactorial conditions implies a number of uncertainties, which have to be tackled using interdisciplinary approach and structured uncertainty analysis [67]. Meanwhile, understanding pathogenetic pathways and associated risk factors paves the way to evidence-based primary prevention strategies [2]. In conclusion, the available data point out to a potential link between widespread pollutants and HPE, through enhanced diabetes risk, that is worth being further explored.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

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