REVIEW ARTICLE

Formaldehyde and Brain Disorders: A Meta-Analysis and Bioinformatics Approach

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Abstract

While there is significant investigation and investment in brain and neurodegenerative disease research, current understanding of the etiologies of illnesses like Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and brain cancer remains limited. Environmental exposure to the pollutant formaldehyde, an emerging neurotoxin widely used in industry, is suspected to play a critical role in mediating these disorders, although findings are limited and inconsistent. Focusing on highly exposed groups, we performed a meta-analysis of human epidemiological studies of formaldehyde and neurodegenerative disease ($N = 19$) or brain tumors ($N = 12$). To assess the biological plausibility of observed associations, we then conducted a bioinformatics analysis using WikiPathways and the Comparative Toxicogenomics Database and identified candidate genes and pathways that may be related to these interactions. We reported the meta-relative risk (meta-RR) of ALS following high exposures to formaldehyde was increased by 78% (meta-RR = 1.78, 95% confidence interval, CI 1.20–2.65). Similarly, the meta-RR for brain cancer was increased by 71% (meta-RR = 1.71; 95% CI 1.07–2.73) among highly exposed individuals. Multiple sensitivity analyses did not reveal sources of heterogeneity or bias. Our bioinformatics analysis revealed that the oxidative stress genes superoxide dismutase (*SOD1*, *SOD2*) and the pro-inflammatory marker tumor necrosis factor (*TNF*) were identified as the top relevant genes, and the *folate metabolism*, *vitamin B₁₂ metabolism*, and the *ALS* pathways were highly affected by formaldehyde and related to the most brain diseases of interest. Further inquiry revealed the two metabolic pathways are also intimately tied with the formaldehyde cycle. Overall, our bioinformatics analysis supports the link of formaldehyde exposure to ALS or brain tumor reported from our meta-analysis. This new multifactorial approach enabled us to both interrogate the robustness of the epidemiological data and identify genes and pathways that may be involved in these interactions, ultimately lending strong evidence and potential biological plausibility for the association between formaldehyde exposure and brain disease.

Keywords Neurodegenerative disease · Amyotrophic lateral sclerosis · Brain tumor · Human exposures · Comparative toxicogenomics database · WikiPathways

Abbreviations

5MTHF	5-Methyltetrahydrofolate
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ALS-PDC	ALS-parkinsonism-dementia complex
BT	Brain tumor
CNS	Central nervous system
CNKI	Chinese National Knowledge Infrastructure
CQVIP	Chongqing VIP Information
CTD	Comparative Toxicogenomics Database
CI	Confidence interval
CST3	Cytostatin

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GSEA   Gene set enrichment analysis
GBC/GSC   Glioblastoma stem-like cells
IARC   International Agency for Research on Cancer
L-BMAA   Beta-aminomethyl-L-alanine
Meta-RR   Meta-relative risk
MAPT   Mictorubule associated protein tau
MS   Multiple sclerosis
NDD   Neurodegenerative disease
NOS   Newcastle-Ottawa Scale
OMP   Olfactory marker protein
OSM   Oncostatin M
oNDD   Other neurodegenerative disease
PD   Parkinson's disease
ppm   Parts per million
PNS   Peripheral nervous system
PRISMA   Preferred Reporting Items for Systematic Reviews and Meta-Analysis
RR   Relative risk
SOD2   Superoxide dismutase 2
SNAP25   Synaptosomal-associated protein 25
THF   Tetrahydrofolate
TNF   Tumor necrosis factor

Background and Significance

Formaldehyde Exposure is Universal and Carcinogenic

Formaldehyde is ubiquitous in the environment and our bodies. In most organisms, including humans, formaldehyde is endogenously produced through amino acid and methanol metabolism, lipid peroxidation, demethylation of DNA, RNA, and histones (EFSA 2014; Swenberg et al. 2011) and is either consumed as an intermediate in the "one-carbon pool" (Neuberger 1981) or used for the biosynthesis of purines, thymidine, and some amino acids. It is reportedly kept at a concentration of approximately 80–100 μM in the blood (Casanova et al. 1988; Heck et al. 1985) with a delicate equilibrium maintained by endogenous metabolic pathways. Commercially, formaldehyde is indispensable for many products and processes that are important to the world's economy (Tang et al. 2009). From its use in embalming to hair relaxation to the manufacture of plastics and composite wood products, the global formaldehyde production is rapidly growing and is projected to reach 36.6 million tons in 2026 (Bhisey 2026). Thus, chronic exogenous exposure to formaldehyde, a major public health concern, is known to cause nasopharyngeal cancer and myeloid leukemia in humans (IARC 2012). However, potential links between the exposure and brain cancers and other neural disorders are less understood.

Formaldehyde Has the Potential to Damage the Brain

Exposure to formaldehyde via inhalation is known to impair memory (Bach et al. 1990; Kilburn et al. 1987) and cognitive functions (Kilburn et al. 1985; Perna et al. 2001) in humans, to cause deficits in learning and memory (Qiang et al. 2014), neuronal damage (Sarsilmaz et al. 2007), and oxidative stress in the cerebellum (Songur et al. 2008) of experimental animals, and to induce misfolded neuronal tau and related proteins (Nie et al. 2007) in vitro. These formaldehyde-associated alterations and neurotoxicity have raised questions about its role in modulating diseases of the central nervous system (CNS), such as neurodegenerative disease (NDD) and brain tumor (BT).

Although the two are distinct pathological disorders, NDD and BT are suspected to share common mechanisms of genetic and molecular abnormalities (Du and Pertsemlidis 2011), indicating that the mechanisms of these seemingly dichotomous diseases may converge in the dysregulation of gene expression and at the post-translational level. For example, increased lipid peroxidation, a biomarker of oxidative stress, was found in animal models of Alzheimer's disease (AD) (Pratico et al. 2001) and Huntington's disease (Perez-De La Cruz et al. 2009), and in patients with Parkinson's disease (PD) (Dexter et al. 1989) and brain cancer (Popov et al. 2003).

Formaldehyde Inhalation and Olfactory Function

A major route of exogenous exposure to formaldehyde in humans is via inhalation, where it could come in contact with and damage the olfactory bulb, an outgrowth of the forebrain specialized for the processing of signals that give rise to the sense of smell (Shepherd and Greer 1998). Repeated formaldehyde inhalation exposure impairs olfactory function in humans (Kilburn et al. 1985; Holmstrom and Wilhelmsson 1988; Hisamitsu et al. 2011; Edling et al. 1988), which has been linked to PD (Doty et al. 1988; Tissingh et al. 2001), amyotrophic lateral sclerosis (ALS) (Viguera et al. 2018), AD (McCaffrey et al. 2000; Morgan et al. 1995), and BT (Daniels et al. 2001). Given formaldehyde's ability to damage the olfactory system that appears to be intimately tied to both NDD and brain cancer, here, we will focus on the effect of inhalation to formaldehyde on the brain in this study.

Formaldehyde Exposure and Brain Disorders

We previously reported the growing evidence that formaldehyde exposure may contribute to the risk of brain cancer (Zhang and Rana 2018a) and NDD (Zhang and Rana 2018b). A number of epidemiological studies conducted

with professional workers, such as anatomists, pathologists, embalmers, and funeral home workers, and in industrial workers have analyzed the association between formaldehyde exposure and brain cancer, although the findings remain limited and inconsistent. In 2012, the International Agency for Research on Cancer (IARC) noted that despite several studies identifying statistically significant positive associations between exposure to formaldehyde and brain cancer, the results are inconsistent (IARC 2012). To the best of our knowledge, no authoritative entities have evaluated formaldehyde's ability to cause NDD, though some studies have been suggestive.

Investigators in China have shown an association between mean endogenous levels of formaldehyde in normal, mild cognitive impairment, and AD patients (Tong et al. 2017). Other studies have reported the genesis of AD-like neuropathology in primates treated with methanol (Yang et al. 2014), a precursor of formaldehyde. The Western Pacific ALS-parkinsonism-dementia complex (ALS-PDC) disorder (Guam, Kii Peninsula, Honshu Island, Japan, and Papua, Indonesia on the island of New Guinea) is strongly associated with exposure to cycad seed genotoxins, namely methylazoxymethanol (the aglycone of cycasin) and beta-aminomethyl-L-alanine (L-BMAA), both of which are metabolized to formaldehyde (Spencer 2019; Spencer et al. 2012). Most recently, investigators in China reported that excess formaldehyde impaired memory in patients with mutations in formaldehyde metabolism enzyme ALDH2 and in the ALDH2 deficient mice (Ai et al. 2019).

To determine whether exogenous exposure to formaldehyde is associated with increased risk of NDD or brain cancer, we conducted a meta-analysis of all studies of formaldehyde-exposed workers. We then applied a bioinformatics analysis to identify candidate genes and pathways associated with both formaldehyde exposure and NDD/BT with the aim of investigating underlying mechanisms.

Meta-Analysis Approach to Evaluate the Human Evidence

Meta-analysis Objective

Given formaldehyde's documented neurotoxicity in vivo (Sarsilmaz et al. 2007; Lu et al. 2008; Aslan et al. 2006; Tulpule and Dringen 2011) and in vitro (Nie et al. 2007; Tulpule et al. 2012; Song et al. 2010; Tang et al. 2013), we hypothesized formaldehyde plays a role in NDD and possibly brain cancer. We investigated this process by carefully reviewing the epidemiological evidence and performing a meta-analysis to examine the association between human exposure to high levels of formaldehyde and NDD and

brain cancer. Meta-analysis provides a method for reviewing the current literature on a topic of interest, identifying heterogeneity in the literature and its possible sources, and evaluating major aspects of causal inference including the magnitude of the association, dose-response, and the possible presence and extent of bias and confounding.

Previously, two meta-analyses have reported mixed findings on formaldehyde exposure and brain cancer (Blair et al. 1990; Bosetti et al. 2008) and no meta-analysis has been conducted to date on formaldehyde-associated NDD. Our meta-analysis on brain tumors differs from previous reports by including a number of updated cohort studies (Beane Freeman et al. 2013; Coggon et al. 2014; Hauptmann et al. 2009; Meyers et al. 2013), multiple new analyses of heterogeneity and bias, and a focus on groups with higher formaldehyde exposure if available by employing an a priori hypothesis targeting exposure magnitude (A Priori Approach and Exposure Categories).

Searching and Identifying Relevant Human Studies

The literature search was conducted according to the guidelines of the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis* (PRISMA, Moher D, Liberati A, Tetzlaff J, Altman DG, Group P 2009). The screening process and results are shown in Fig. 1. We conducted a systematic electronic literature review using PubMed in July 2018, which was updated in May 2019. We performed electronic searches of online databases (PubMed, Web of Science Core Collection, Biosis Previews, Embase, Google Scholar, ToxNet, Chinese National Knowledge Infrastructure, CNKI, Wanfang Data, and Chongqing VIP Information, CQVIP) using the search terms, inclusion, and exclusion criteria outlined in Supplementary Section A1.

Selection of NDD Studies

We identified 394 studies from PubMed, 32 studies from ToxNet, and 5 studies from scanning bibliographies. After 28 duplicates were removed, 403 studies were primarily screened. Of these studies, 371 were excluded because they were reviews, correspondence, animal, mechanistic or para-occupational studies, or did not include the relevant exposure or outcome of interest (Fig. 1). When the final 32 studies were identified, 13 studies were further excluded for lacking clear formaldehyde exposure data or relative risk (RR) estimates. Finally, 19 NDD studies were selected, among which there were nine (6 + 3, detailed in Exposure Assessment and Estimate) studies on ALS, six on PD, one on AD, and three on multiple sclerosis (MS).
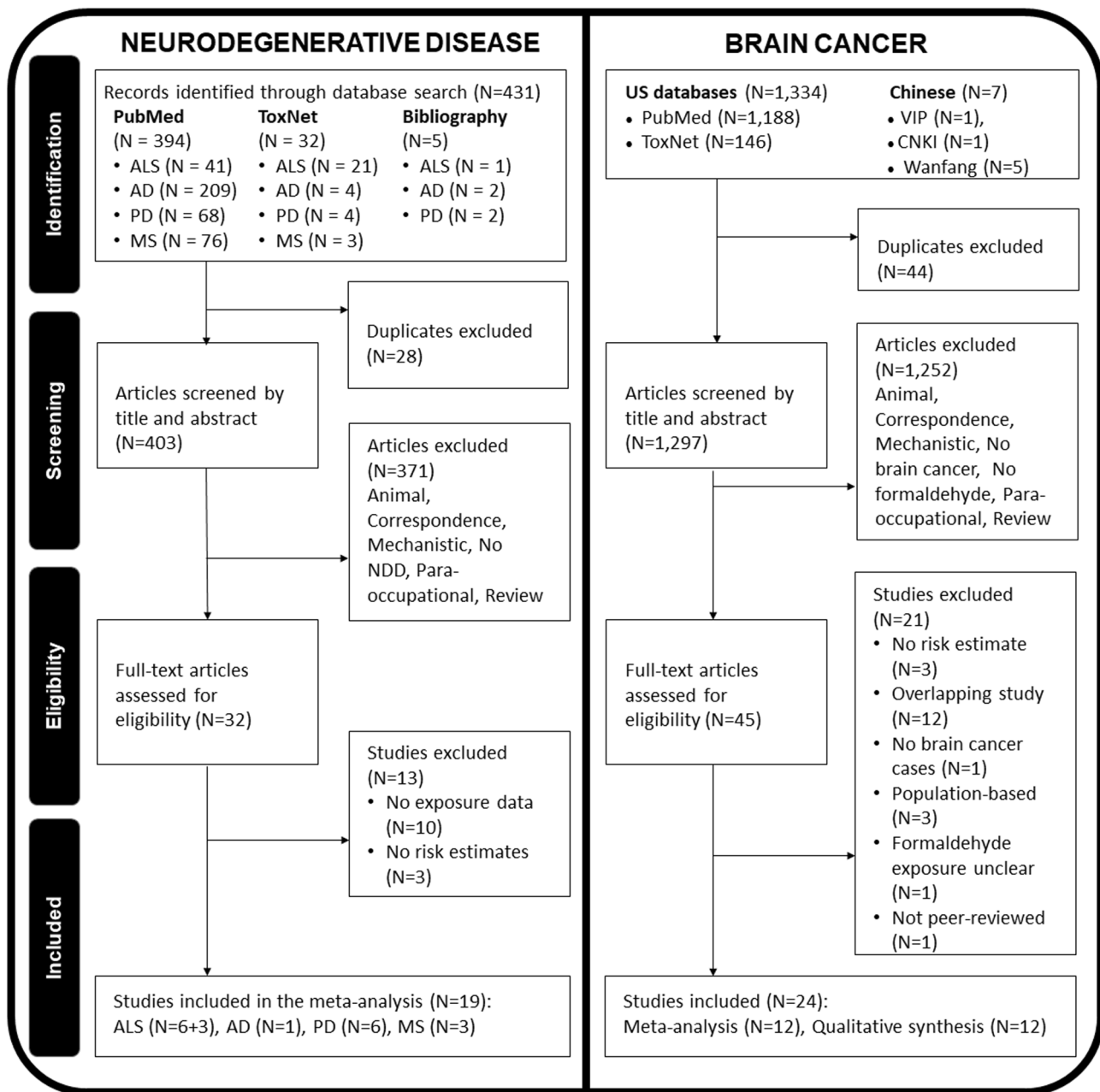
Fig. 1 Study selection process for meta-analyses using PRISMA guidelines

Exposure Assessment and Estimate

The NDD studies selected for the meta-analysis are described in Table 1. Six of the ALS studies conducted an exposure assessment specific to formaldehyde, defined as having a structured interview (Fang et al. 2009), job exposure matrix (Peters et al. 2017; Roberts et al. 2016; Seals et al. 2017), standardized questionnaire (Weisskopf et al. 2009), or studying a cohort of workers with known formaldehyde exposure (Pinkerton et al. 2013). An additional three studies of ALS lacked a formaldehyde exposure assessment (Chiò

et al. 1991; Deapen and Henderson 1986; Gunnarsson et al. 1991), instead reporting only job title as "ever exposed" (Table 1).

Similarly, all epidemiological studies of PD, MS, and AD lacked a clear formaldehyde exposure assessment, relying on job titles, census data, hobbies, or occupational/residential exposure to activities with known formaldehyde exposure (Table 1). Given these individuals were likely exposed to formaldehyde and that the frequency and intensity of these exposures are unknown, we defined these groups as having "formaldehyde exposure by proxy." The analogous group

Table 1 Selected studies and their results used in our meta-analyses of neurodegenerative disease and brain tumors

Study	Location	Population	Design	Type	RE	CI_L	CI_U	Cases	Exposure level	Weight[a]
Amyotrophic lateral sclerosis (N = 9)										
Fang et al. (2009)	USA	NEMC and BWH	CC	OR	3.0	0.7	12.9	4	> 60,000 h weighted	6.04
Peters et al. (2017)	Sweden	National Patient Register	CC	OR	1.22	0.95	1.57	123	> 0.013 mg/m^3	19.22
Pinkerton et al. (2013)	USA	Garment workers	Cohort	SMR	0.94	0.19	2.75	3	> 10-year duration	6.94
Roberts et al. (2016)	USA	National Longitudinal Mortality Study	Cohort	HR	4.43	1.16	16.85	2	High intensity/probability	6.93
Seals et al. (2017)	Denmark	Danish National Patient Register	CC	rr	1.3	1.1	1.5	266	> 0.28 ppm	29.28
Weisskopf et al. (2009)	USA	ACSCPSII	Cohort	rr	4.1	2.2	7.1	13	> 10-year duration	31.58
Chiò et al. (1991) b	Italy	Textile workers	CC	OR	1.2	0.44c	2.61c	6	Ever based on job title	N/A[b]
Deapen and Henderson (1986) b	USA	Plastics manufacturing	CC	OR	3.7	1	20.5	11	Ever based on job title	N/A[b]
Gunnarsson et al. (1991)[b]	Sweden	Male textile workers	CC	OR	0.1	0.3	0.7	1	Ever based on job title	N/A[b]
		Male carpenter, woodworker	CC	OR	1.0	0.6	1.5	55	Ever based on job title	N/A[b]
		Female textile workers	CC	OR	0.4	0.1	1.6	3	Ever based on job title	N/A[b]
Parkinson's disease (N = 6)										
Chaturvedi et al. (1995)	Canada	Hobby exposure plastic resins	CC	OR	8.79	2.43	25.89	9	Ever based on hobby	0.9
Li et al. (2009)	Sweden	Male textile workers	Coh	SIR	1.03	0.61	1.63	18	Three censuses (job title)[c]	5.1
		Male woodworkers	Coh	SIR	1.29	1.08	1.54	126	Three censuses (job title)[c]	39.5
		Female textile workers	Coh	SIR	1.03	0.87	1.21	146	Ever based on job title	45.7
		Female woodworkers	Coh	SIR	0.66	0.30	1.26	9	Ever based on job title	2.4
Nee et al. (1991)	USA	Plastic monomers, additives worker	CC	OR	5.25	2.79^d	8.98^d	13	Ever based on job title	3.6
Hertzman et al. (1990)	Canada	Planer mills workers	CC	OR	4.11	0.91	18.5	8	Ever based on job title	0.5
Vanacore et al. (2005)	Europe	Wood and furniture worker	CC	OR	0.43	0.04	5.17	1	Ever based on job title	0.7
		Textile and clothing worker	CC	OR	2.19	0.56	8.52	7	Ever based on job title	N/A
Tanner et al. (1989)	China	O/R textile manufacture	CC	OR	1.90	0.79	4.67	unk	Ever based on O/R	1.6
		O/R lumber	CC	OR	0.87	0.13	2.19	unk	Ever based on O/R	N/A[b]
		O/R plastic	CC	OR	1.62	0.61	4.31	unk	Ever based on O/R	N/A[b]
		O/R paper mills	CC	OR	1.08	0.17	6.76	unk	Ever based on O/R	N/A[b]
Multiple sclerosis (N = 3)										
Li et al. (2008)	Sweden	Male textile workers	Coh	SIR	0.31	0.00	1.80	1	Three censuses	56.0
		Female textile workers	Coh	SIR	1.34	0.75	2.21	15	Three censuses	16.6
		Male woodworkers	Coh	SIR	0.88	0.51	1.41	17	Three censuses	18.7
Dubrow and Gute (1988)	USA	Male textile workers	Coh	PMR	1.83	0.67^c	3.98^c	6	Ever based on job title	6.1
Zorzon et al. (2003)	Italy	O/R formalin	CC	OR	0.5	0.1	1.5	4	Ever based on job title	2.6
		Woodworkers	CC	OR	1.9	0.6	6.5	8	Ever based on job title	N/A[b]
Alzheimer's disease (N = 1)										
Tyas et al. (2001)	Canada	Liquid plastics and rubbers workers	Cohort	RR	1.01	0.12	8.38	1	Ever based on job title	N/A[b]
		Glues, adhesives workers	Cohort	RR	1.41	0.49	4.05	5	Ever based on job title	N/A[b]

Table 1 (continued)

Study	Location	Population	Design	Type	RE	CI_L	CI_U	Cases	Exposure level	Weight[a]
Brain tumor: professional workers ($N = 5$)										
Hall et al. (1991)	UK	Pathologists	Cohort	SMR	2.18	0.80	4.75	6	Ever	10.87
Hauptmann et al. (2009)	USA	Embalmers	CC	OR	1.7	0.5	5.3	12	Peak $\geq$ 9.3 ppm	8.38
Levine et al. (1984)	Canada	Undertakers	Cohort	SMR	1.15	0.24	3.51	3	Ever	7.25
Stroup et al. (1986)	USA	Anatomists	Cohort	SMR	3.9	1.0	9.9	9	Gross anatomists	8.64
Wang et al. (1989)	China	Anatomists	Cohort	SMR	8.27	1.00	29.86	2	Ever	5.34
Brain tumor: industrial workers ($N = 7$)										
Andjelkovich et al. (1995)	USA	Iron foundry workers	Cohort	SMR	0.62	0.07	2.23	2	Ever	5.20
Beane Freeman et al. (2013)	USA	Industrial plant workers	Cohort	SMR	0.87	0.49	1.56	20	Peak $\geq$ 4.0 ppm	14.05
Coggon et al. (2014)	UK	Chemical factory workers	CC	SMR	0.56	0.24	1.11	8	> 2.0 ppm	12.10
Fondelli et al. (2007)	Italy	Library restoration workers	Cohort	SMR	10.03	1.22	36.24	2	Ever	5.35
Jiang et al. (1990)	China	Resin factory workers	Cohort	SMR	3.9	0.05	21.7	1	Ever	2.10
Meyers et al. (2013)	USA	Garment plant workers	Cohort	SMR	1.72	0.86	3.08	11	Duration 10+ years	13.43
Wong and Gibson (1983)	USA	Formaldehyde plant workers	Cohort	SMR	2.13	0.43	6.23	3	Hired before 1961	7.28

ACSCPII American Cancer Society Cancer Prevention Study II, *BWH* Brigham and Women's Hospital, *CC* case-control, *CIL* lower 95% confidence interval, *CIU* upper 95% confidence interval, *CS* cross-sectional, *hr* hour, *N/A* not applicable, *NEMC* New England Medical Center, *OR* odds ratio, *O/R* occupational or residential exposure, *RR* relative risk, *rr* rate ratio, *SIR* standardized incidence ratios, *SMR* standardized mortality ratio, *unk* unknown.

[a]Weight given to each study in the random effects model

[b]Evaluated in sensitivity analyses only

[c]Data for multiple censuses only reported for men

[d]Calculated by us

in studies with a formaldehyde exposure assessment would be "ever exposed," defined as individuals with any type of exposure to formaldehyde at any level.

Due to the limited number of studies on NDD, we chose to include studies with estimated exposure assessment (proxy exposures). The studies that assessed formaldehyde exposure by proxy were used in (1) the ALS sensitivity analysis; (2) the analysis of PD and NDD, as no other studies of these outcomes; and (3) in an extended meta-analysis of all NDDs involving workers in occupations known to have formaldehyde exposure, namely studies of individuals with exposure to plastics (Deapen and Henderson 1986; Chaturvedi et al. 1995; Nee et al. 1991; Tanner et al. 1989; Tyas et al. 2001), woodwork (Gunnarsson et al. 1991; Tanner et al. 1989; Li et al. 2008, 2009; Vanacore et al. 2005; Zorzon et al. 2003; Hertzman et al. 1990), and textiles (Chiò et al. 1991; Gunnarsson et al. 1991; Tanner et al. 1989; Li et al. 2008, 2009; Vanacore et al. 2005; Dubrow and Gute 1988).

Regions of NDD Studies Selected

For ALS, five studies were conducted in the USA, two were conducted in Sweden, one was conducted in Italy, and one was conducted in Denmark. For PD, two studies were in Canada, one study was conducted in China, one was in Sweden, one was in the USA, and one was conducted across many countries in Europe. For MS, one study was in Sweden, one study was in the USA, and one study was in Italy. The only study of AD was in Canada.

Selection of Brain Tumor Studies

Following thorough searches from multiple resources and excluding 44 duplicates, we screened a total of 1297 articles by title and abstract (Fig. 1). We excluded 1,252 articles that used animal or mechanistic study designs, lacked the exposure or outcome of interest, had para-occupational exposure, or were correspondence or a review, leaving 45 articles for full text review. Of these studies, 12 studies with 67,819 participants were eligible for inclusion in the meta-analysis. Table 1 also summarizes these selected brain cancer studies. Because brain cancer is a diverse cancer that may be defined differently among studies, the ICD codes from these studies have been reported in Supplementary Table 1.

There were five studies of professional workers and seven studies of industrial workers (Table 1). Two studies were case-control design (Coggon et al. 2014; Hauptmann et al. 2009), and ten were cohort studies. Six studies were conducted in the USA, one study was in Canada, two studies were in the UK, one study was in Italy, and two studies were in China.

Study Quality Evaluation

The methodological quality of the cohort and case-control studies included in the meta-analyses was assessed using the Newcastle Ottawa Scale (NOS) (Wells et al. 2009). Cohort studies (Supplementary Table 2) were evaluated based on the representativeness of the cohort, selection of the controls, ascertainment of exposure, outcome diagnosis at start of study, comparability of cohort on the basis of controlling for age, assessment of disease outcome, sufficiency of follow-up length, and response rate. Case-control studies (Supplementary Table 3) were evaluated on the validation of cases, representativeness of cases, selection of controls, absence of disease in the controls, whether the study controlled for age, exposure assessment, concordance of method among cases and controls, and similarity of response rate among both groups. A total of twelve points were available, and full results of the quality analysis are reported in Supplementary Section A2.

A Priori Approach and Exposure Categories

Our focus on groups with high formaldehyde exposure is based on the principle that, in general, if a true association exists, *higher* exposures are likely to yield *higher* relative risk estimates (Rom and Markowitz 2007). Including people with very low exposure in the exposed group can dilute relative risk estimates. All else being equal, higher relative risks are associated with greater statistical power and are less likely to be due to relatively minor biases or confounding (Hill 1965). Since our main goal is to evaluate the potential association of the exposure and risk of the disease outcomes and not to conduct a precise dose-response assessment or to evaluate risks in people with low exposures, our focus was on the groups with the highest available formaldehyde exposure. This a priori approach has been previously employed to estimate meta-risks for benzene (Steinmaus et al. 2008), formaldehyde (Zhang et al. 2009; Duong et al. 2011), and other agents (Welling et al. 2015; Zhang et al. 2019; Smith et al. 2017). Although the focus was on higher exposures (e.g., occupationally exposed cohorts), studies involving lower exposures (e.g., studies done in the general population) were also evaluated in sensitivity analyses.

Some studies reported multiple RRs or ORs for different exposure categories (high, "ever exposure," or proxy exposures; see Exposure Assessment and Estimate). Based on our a priori hypothesis, when multiple RRs or ORs were given, we selected estimates in the following order: (1) peak exposure, (2) average exposure intensity, (3) cumulative exposure, (4) exposure duration, (5) earliest year of hire, and (6) ever exposure. We prioritized *peak exposure* because metrics like average intensity and cumulative exposure may be less accurate

Table 2 Meta-analysis results of amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases (NDD)

Analysis	N	Fixed effects model			Random effects model[a]			Heterogeneity	
		Meta-RR	CI_L	CI_U	Meta-RR	CI_L	CI_U	X^2	P
Amyotrophic lateral sclerosis (ALS)									
Main-highest peak exposure	6	1.37	1.21	1.56	1.78	1.20	2.65	19.08	< 0.01
Ever exposure[b]	6	1.25	1.17	1.33	1.17	0.93	1.47	20.59	< 0.01
Including studies with proxy exposure[c]	11	1.10	0.98	1.23	1.18	0.65	2.17	158.33	<0.01
Exclude one ALS study[d]									
Fang et al. (2009)	5	1.36	1.20	1.55	1.73	1.14	2.60	17.96	< 0.01
Weisskopf et al. (2009)	5	1.30	1.14	1.48	1.31	1.08	1.59	4.96	0.29
Pinkerton et al. (2013)	5	1.37	1.21	1.56	1.89	1.24	2.88	18.77	< 0.01
Roberts et al. (2016)	5	1.36	1.19	1.54	1.65	1.12	2.45	16.10	< 0.01
Peters et al. (2017)	5	1.43	1.23	1.65	2.24	1.09	4.61	17.98	< 0.01
Seals et al. (2017)	5	1.52	1.22	1.90	2.21	1.05	4.66	17.75	< 0.01
Other NDD subtypes									
Parkinson's disease[b]	9	1.23	1.10	1.38	1.70	1.17	2.48	46.49	< 0.01
Multiple sclerosis[b]	5	0.54	0.43	0.68	0.80	0.37	1.73	35.33	< 0.01
Total NDD in EE studies									
Plastics	5	4.06	2.63	6.25	3.64	1.84	7.19	7.44	0.11
Wood workers[b]	8	1.19	1.02	1.38	1.10	0.86	1.42	9.35	0.23
Textile workers[b]	12	0.73	0.65	0.83	0.77	0.44	1.34	158.22	< 0.01

CI confidence interval, *EE* exposure estimated, *NDD* neurodegenerative disease, *N* number of studies, *meta-RR* meta-analysis relative risk

[a]Random effects model was used when X^2 heterogeneity statistic > degrees of freedom (number of studies minus 1)

[b]Not reported in Roberts et al. 2016 and was calculated by the authors

[c]Includes additional papers where formaldehyde exposure was not clearly defined but included professions with known exposure (plastics manufacturing, woodwork, and textile work)

[d]One study excluded at a time.

measures of true exposure if workers and/or professionals (such as embalmers) with periods of very high exposure also have intervening time periods with little or no exposure. We evaluated the impact of our a priori exposure selection criteria in sensitivity analyses. We conducted a separate meta-analysis of individuals with "ever exposure," to assess the magnitude of potential bias caused by adding subjects with low exposures in the current literature. We also compared "ever exposure" with our a priori hypothesis to assess whether a representative dose-response relationship might be identifiable. Table 1 reports the results and weights of the studies used in the NDD and brain tumor meta-analyses.

Statistical Methods

We calculated overall summary estimates and weights of the studies using both the fixed effects inverse variance method (Greenland 1998) and the random effects method (DerSimonian and Laird 1986). Heterogeneity was evaluated using the general variance-based method (Petitti 1994). If heterogeneity was present, the random effects model was used. One benefit of the random effects model

is the ability to incorporate between study variance into the summary variance estimate and confidence intervals (CI), which may help prevent artificially narrow CIs resulting from use of the fixed effects model in the presence of between-study heterogeneity (Petitti 1994). We evaluated publication bias through funnel plots, Egger's test, and Begg's test (Begg and Mazumdar 1994; Egger et al. 1997). All meta-analysis and statistical analysis were conducted with Stata IC 15.1 (StataCorp 2017) and Microsoft Excel 2013 (Corporation 2013).

Meta-analysis Findings

We first report our major findings for formaldehyde-associated ALS then the results of sensitivity analyses for ALS. Other subtypes of NDD, such as PD and MS, are also reported. Next, we report the findings of the meta-analysis on brain cancer with a detailed sensitivity analysis. A greater portion of this section is devoted to this discussion because we have identified more studies on brain tumor and there are previous meta-analyses present for comparison.

Table 3 Major findings from brain cancer and formaldehyde exposure meta-analysis and sensitivity analysis

Analysis	N	Fixed effects model			Random effects model[a]			Heterogeneity	
		Meta-RR	CI_L	CI_U	Meta-RR	CI_L	CI_U	X^2	P
Exposure									
Main-highest peak exposure	12	1.43	1.07	1.91	1.71	1.07	2.73	23.73	0.01
Highest average intensity	12	1.51	1.13	2.03	1.77	1.11	2.83	23.36	0.02
Highest cumulative exposure	12	1.51	1.13	2.03	1.72	1.09	2.70	21.46	0.03
Longest duration	12	1.42	1.06	1.90	1.75	1.07	2.84	24.29	0.01
Ever exposure	12	1.22	1.04	1.44	1.82	1.20	2.75	41.77	<0.01
Professionals vs. industry									
Professional workers[b]	5	2.41	1.43	4.08	2.42	1.41	4.17	4.46	0.37
Industrial workers[c]	7	1.14	0.81	1.61	1.32	0.72	2.44	14.01	0.03
Add population-based exposure/SPIR studies									
Add Dell and Teta 1995[d]	14	1.29	0.99	1.69	1.47	0.96	2.25	27.55	0.01
Add Lacourt et al. 2013[e]	13	1.32	1.04	1.69	1.56	1.05	2.33	24.68	0.02
Add Hansen and Olsen 1995[f]	13	1.39	1.10	1.75	1.59	1.08	2.34	23.87	0.02
Add all three	16	1.25	1.03	1.53	1.35	0.99	1.85	27.96	0.02
Hall 1991 [98]									
Only male subjects from Hall 1991[g]	12	1.45	1.08	1.93	1.73	1.08	2.78	24.16	0.01
Study location									
North America	7	1.38	0.97	1.95	1.43	0.95	2.16	7.47	0.28
Europe/Asia	5	1.56	0.93	2.61	2.75	0.83	9.14	16.10	<0.01
Study design									
Cohort	10	1.67	1.21	2.30	2.00	1.21	3.29	17.01	0.05
Case-control	2	0.78	0.41	1.48	0.89	0.30	2.59	2.39	0.12

CI confidence interval, N number of studies, meta-RR meta-analysis relative risk, SPIR standardized proportionate incidence ratio, USMR US Mortality Rate.

[a]Random effects model was used when X^2 heterogeneity statistic > degrees of freedom (number of studies minus 1)

[b]Professionals are defined as anatomists, pathologists, embalmers, or funeral home directors

[c]Industrial workers are defined as laborers in factories or restorative projects

[d]Relative risk estimates presented in Dell and Teta 1995; since data are only reported for salaried workers and short-term workers separately, $N = 2$

[e]Relative risk estimates presented in Lacourt et al. 2013

[f]Relative risk estimates presented in Hansen and Olsen 1995

[g]Cohort narrowed down to only male pathologists

## Formaldehyde Exposure Increases Risk of NDD

Major ALS Findings

The findings of our meta-analysis of NDD are presented in Table 2. As discussed above in Exposure Assessment and Estimate, only six of nine studies of ALS had formaldehyde exposure assessments (Fang et al. 2009; Peters et al. 2017; Roberts et al. 2016; Seals et al. 2017; Weisskopf et al. 2009; Pinkerton et al. 2013). When examining the most highly exposed groups, we found a meta-relative risk (meta-RR) of 1.78 with a 95% CI of 1.20 to 2.65 (Fig. 2a). While there was somewhat high heterogeneity, we note that large majority of studies (5 of 6) have RRs that are greater than 1.0.

The funnel plot revealed some evidence of asymmetry consistent with publication bias (Fig. 2b), although the number of studies is small and there was no evidence of bias in the in Egger's ($p = 0.204$) or Begg's tests ($p = 0.188$).

ALS Sensitivity Analysis

Examining "ever exposure" revealed a lower meta-RR of 1.17 (95% CI 0.93–1.47), in comparison with our a priori "high exposures" (meta-RR = 1.78, 95% CI 1.20–2.65; Table 2). Therefore, there was a 61% increase in meta-RR, suggesting the presence of an exposure-response relationship (Fig. 3a).

Amyotrophic Lateral Sclerosis

a

Brain Cancer
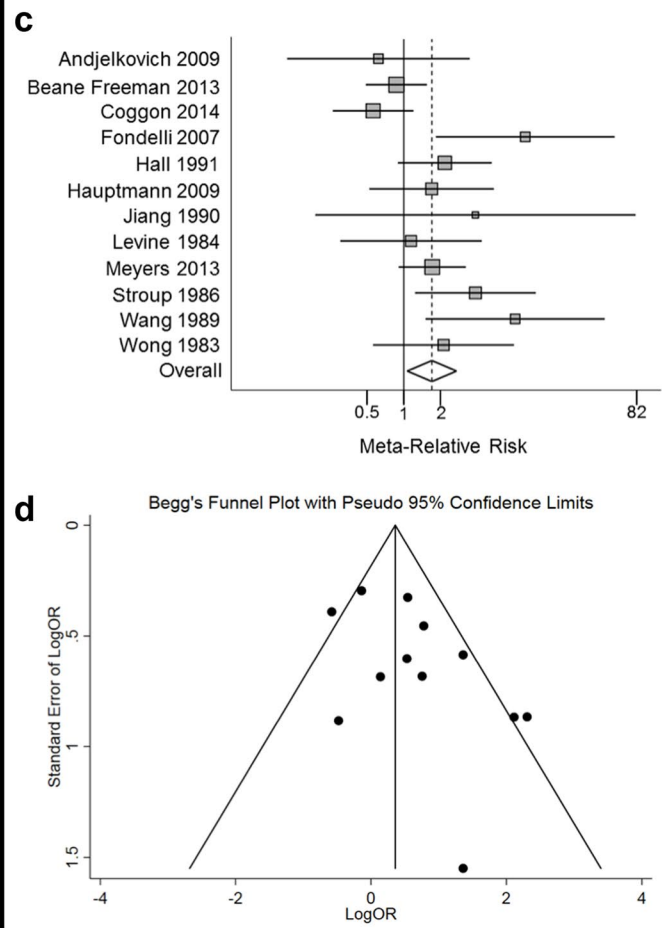
c

b

d
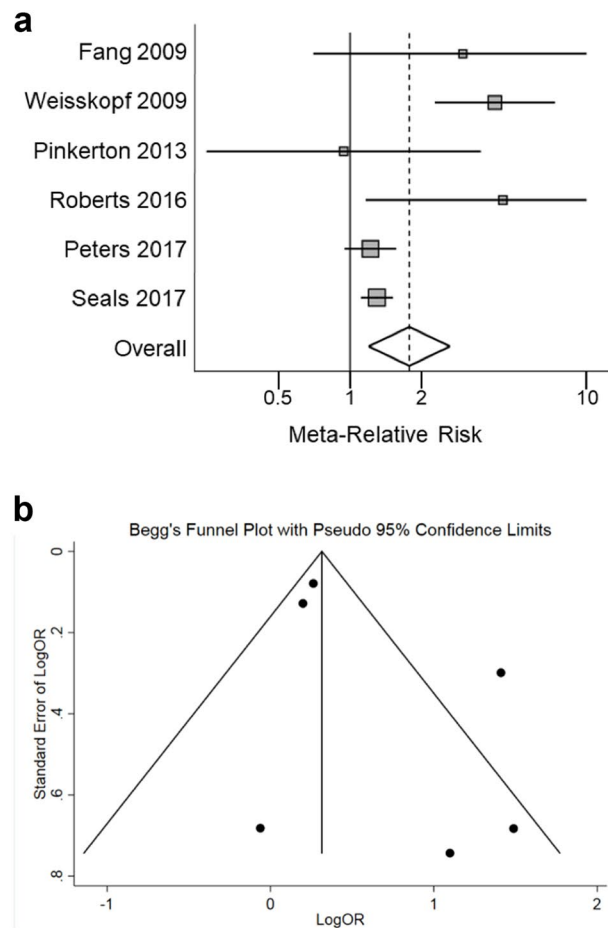
Fig. 2 Forest plot of ALS meta-analysis using random effects model **a** and funnel plot of ALS studies **b**. Forest plot of brain tumor meta-analysis using random effects model **c** and funnel plot of brain tumor **d**

To determine the effects of adding the three ALS studies that assessed exposure by proxy, we included additional studies of individuals with known exposure to plastics, woodwork, and textiles (Chiò et al. 1991; Deapen and Henderson 1986; Gunnarsson et al. 1991) and found the risk of ALS was lower (meta-RR = 1.18, 95% CI 0.65–2.17; Table 2). When we excluded any one individual study included in our main analysis (meta-RR = 1.78), the meta-RRs of ALS in the sensitivity analysis fluctuated but did not change substantially (range 1.30 to 2.24).

## Parkinson's Disease and Alzheimer's Disease

While the six ALS studies had adequate exposure assessments, all studies of PD and MS reported formaldehyde exposure by proxy (see Exposure Assessment and Estimate). PD appeared to be associated with jobs known to have

formaldehyde exposure (meta-RR = 1.70, 95% CI 1.17–2.48). Although the meta-RR of PD is quite similar to the one from ALS (1.78), due to the lack of the appropriate formaldehyde exposure assessment, it remains uncertain whether the observed risk was truly attributable to formaldehyde and not to other confounding factors. Thus, one needs to interpret the PD results with caution. We could not perform a meta-analysis for AD because we only identified one AD study.

Multiple Sclerosis

We found no association for MS. It should be noted that MS, a chronic inflammatory demyelination disease of the CNS, is primarily considered an autoimmune disorder that can progress into a neurodegenerative disease (Schaeffer et al. 2015). MS was included in NDD in the meta-analysis for completeness but excluded from NDD

Fig. 3 Meta-relative risk of ALS mortality **a** and brain cancer mortality **b** by level of formaldehyde exposure using random effects model. The relative risks are indicated by the black lines in the middle, and the 95% confidence intervals are indicated by the upper and lower vertical black lines
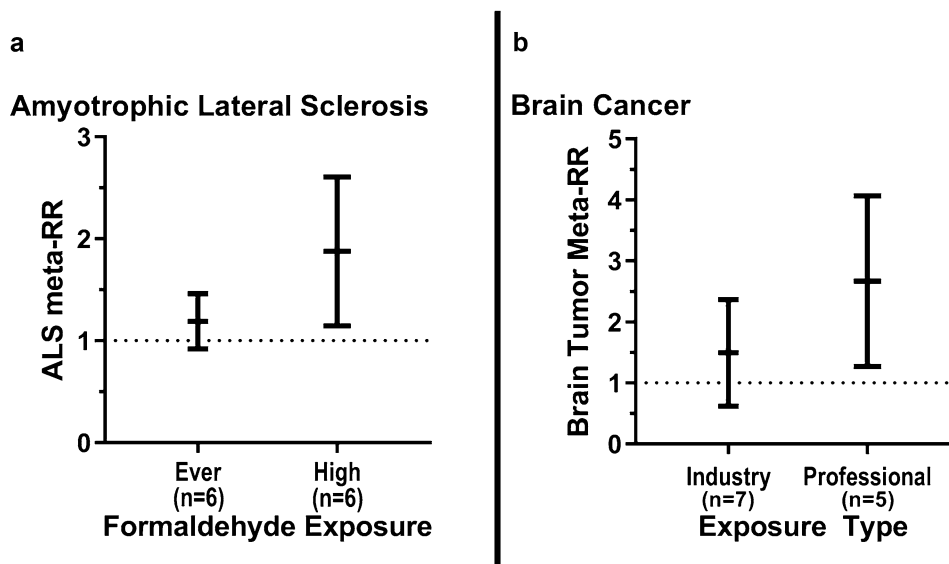
Plastics Workers Have Increased Risk of NDD

When studies were stratified by type of industry, we observed a meta-RR for all NDD outcomes of interest (AD, ALS, MS, PD) of 3.64 (95% CI 1.84–7.19) for individuals exposed to plastics, a meta-RR of 1.10 (95% CI 0.86–1.42) for woodworkers, and no association for textile workers (Table 2). The lack of appropriate exposure assessments in these studies indicate these results must be interpreted with caution. Interestingly, these findings are consistent with formaldehyde exposure data in US industries, which reported average short-term formaldehyde exposures for miscellaneous plastic work, lumber, and textile industries to be 0.28, 0.27, and 0.20 mg/m^3, respectively (Lavoue et al. 2008). Overall, they provide support for our hypothesis that higher formaldehyde exposure increases NDD risk.

Increased Meta-relative Risk of Brain Cancer

Next, we performed the meta-analysis and stratified sensitivity analyses on brain cancer; the results are reported in Table 3, and the forest plot is displayed in Fig. 2c. The meta-RR for all studies combined was 1.71 (95% CI 1.07–2.73). Figure 2 d shows the funnel plot for publication bias. Among all studies, there was no evidence of asymmetry consistent with obvious publication bias. Egger's ($p = 0.076$) and Begg's ($p = 0.337$) tests similarly did not show evidence of publication bias.

We conducted a series of sensitivity analyses below to evaluate the impact of including or excluding each individual study or other possible studies. In general, results were similar across all analyses, demonstrating the robustness of our findings.

Alternative Exposure Criteria

We evaluated the impact of our a priori selection of peak exposure. When the relative risk for the highest average intensity was prioritized, the meta-RR changed nominally (meta-RR = 1.77, 95% CI 1.11–2.83). Similarly, when the relative risk for the highest cumulative exposure was selected first, the meta-RR was 1.72 (95% CI 1.09–2.70). When longest duration was used, the meta-RR remained relatively constant at 1.75 (95% CI: 1.07–2.84). When "ever exposure" was used, the meta-RR was elevated to 1.82 (95% CI 1.20–2.75; Table 3).

Professionals vs. Industrial Workers

Many of the brain tumor studies distinguished their study subjects either from professional or industrial working settings. Formaldehyde was associated with increased risk of brain cancer in professional workers (meta-RR = 2.42; 95% CI 1.41–4.17). Elevated risks of brain cancer among industrial workers were detected (meta-RR = 1.32; 95% CI 0.72–2.44), though not statistically significant. Overall, professionals had a 110% greater meta-relative risk of developing brain cancer than industrial workers, among the most highly exposed groups (Fig. 3b).

Similar to what we found earlier in formaldehyde-associated leukemia (Zhang et al. 2009), higher risk of

brain cancer among professionals may be due to exposure level and pattern, as an embalmer or anatomist is more likely to experience high-probability, high-intensity exposure than an individual involved with garment work. Embalmers are exposed at 1.32–2.86 parts per million (ppm) (Hiipakka et al. 2001) and anatomists/pathologists are exposed at levels between 0.7–3.7 ppm (Dias-Teixeira et al. 2016). In addition to inhalation, professionals are more likely to absorb formaldehyde through their skin during embalming or specimen preparation, which may introduce another route of exposure (Boeniger and Stewart 1992).

Lower risks among industrial workers may be because these groups experience far lower exposures over longer periods of time; for example, garment workers are typically exposed between 0.09–0.20 ppm (Pinkerton et al. 2004). Further, industrial workers hold a wide variety of jobs which may result in a broader range of actual exposure levels, making it difficult to compare different cohorts of industry workers. The variance in exposure pattern and intensity may account for the differences in meta-relative risks.

Lastly, not only does our meta-analysis indicate that professionals are at the highest risk for brain tumor development, two previous meta-analyses (Blair et al. 1990; Bosetti et al. 2008) also consistently reported the similar findings (Table 4) that are detailed in Comparison with Previous Meta-analyses on Brain Tumors.

Population-Based Exposure Studies and SPIR Studies Added

We analyzed studies with population-based exposure and studies that reported standardized proportionate incidence ratios (SPIR; Supplementary Section A1). As shown in Table 3, when population-based exposure studies (Dell and Teta 1995; Lacourt et al. 2013; Hansen and Olsen 1995) were added one at a time, the meta-RR decreased slightly.

When we added the study that reported SPIR (Hansen and Olsen 1995), the meta-RR decreased to 1.59 (95% CI 1.08–2.34). Adding all three studies decreased the meta-RR to 1.35 (95% CI 0.99–1.85).

Study Exclusion

To ensure one study was not artificially inflating the risk estimate, we excluded all studies one at a time and found that they all nominally changed the meta-RR (Supplementary Fig. 1). Although Fondelli et al. (2007) had the highest RR, removal of this study did not significantly impact on the results (meta-RR = 1.51, 95% CI 0.98–2.34). Similarly, removal of any individual study included in our analysis (meta-RR = 1.71) of brain tumors did not significantly change our meta-RR (range 1.51 to 1.91).

Study Subpopulation in Males Only

In our meta-analysis, we used the relative risk from the Hall et al. (1991) study that included both males and females. Since all of the brain cancer cases in Hall et al. (1991) were male, the article also presented a relative risk for just male subjects. When this relative risk for male subjects only was used, the meta-RR remained unchanged at 1.73 (95% CI 1.08–2.78).

Study Location and Design

Studies from North America had a meta-RR of 1.43 (95% CI 0.95–2.16), whereas studies from Europe and Asia had a meta-RR of 2.75 (95% CI 0.83–9.14). This difference may have been driven by studies conducted in China before 1990, when the Chinese Ministry of Health's maximum allowable concentration for formaldehyde was very high (3 mg/m^3, MOH China).

Table 4 Comparison of current and previous meta-analyses on brain cancer

Group	Blair et al. 1990		Bosetti et al. (2008)		Current meta-analysis (2020)	
	N	Meta-RR (95% CI)	N	Meta-RR (95% CI)	N	Meta-RR (95% CI)
Professionals	9	1.5 (1.2–2.0)[a,b]	7	1.56 (1.24–1.96)	5	2.42 (1.41–4.17)
Industry workers	3	0.9 (0.7–1.1)[b]	4	0.92 (0.75–1.13)	7	1.32 (0.72–2.44)
Overall	12	1.29 (0.95–1.76)[c]	11	1.26 (1.01–1.59)[c,d]	12	1.71 (1.07–2.73)

CI confidence interval, *meta-RR* meta-analysis relative risk

[a]Reported white and nonwhite separately, so N = 2 Hayes et al. 1990

[b]Confidence interval calculated by us

[c]Meta-RR calculated by us using random-effects model because X^2 heterogeneity statistic > degrees of freedom (number of studies minus 1)

[d]Risk estimates used the numbers from Bosetti et al. 2008. When the numbers from original studies were used to calculate meta-RR, the result was slightly different as 1.24 (0.99–1.55)

Comparison with Previous Meta-analyses on Brain Tumors

While there are no previous meta-analyses of formaldehyde and NDD for comparison, two meta-analyses of formaldehyde and brain cancer have been published to date (Blair et al. 1990; Bosetti et al. 2008). Both studies conducted separate analyses for professional groups and for industrial workers but did not perform a meta-analysis of all studies together. Table 4 compares our present findings with the two previously published meta-analyses. The earliest meta-analysis by Blair et al. (1990) evaluated ten studies on formaldehyde exposure and found an increased RR of 1.5 (95% CI 1.2–2.0) in professionals and no excess mortality in three studies of industrial workers (RR = 0.9, 95% CI 0.7–1.1; Blair et al. 1990). Note that 95% confidence intervals were not reported but were calculated by us using the fixed effects model.

Similarly, the more recent meta-analysis of brain cancer among professionals (n = 7) performed by Bosetti et al. (2008) reported an overall statistically significant increased meta-RR of 1.56 (95% CI 1.24–1.96) in professionals, but not in industrial workers (RR = 0.92, 95% CI 0.75–1.13, n = 4, Bosetti et al. 2008). Both Blair et al. (1990) and Bosetti et al. (2008) did not report an overall meta-RR including both groups, so we calculated it using the random effects model to be 1.29 (95% CI 0.95–1.76) and 1.26 (95% 1.01–1.59), respectively. A comparison of the individual studies and relative risks used in our meta-analysis with the two previous studies is presented in Supplementary Table 4.

Our new meta-analysis differs from the previous reports for two reasons: (1) we used four recently updated studies that were not available when the previous meta-analyses were conducted, and (2) we employed an a priori selection of the highest exposure groups. Consequently, we detected a 2.42 increased meta-relative risk among professional workers, and 1.32 increased meta-relative risk among industrial workers.

Strengths and Limitations of Meta-Analyses

Strengths of Meta-Analyses

The strengths of our meta-analyses are the inclusion of several updated cohort studies, use of multiple sensitivity analyses to evaluate heterogeneity and bias, and our novel a priori hypothesis. Selection of the highest exposure group in each study, when reported, appeared to improve our ability to detect the presence of an exposure-disease association particularly in the NDD analysis. Multiple sensitivity analyses conducted among occupational subgroups and high versus low exposure groups consistently revealed strong, positive associations, indicating formaldehyde may contribute to the risk of both brain tumor and ALS.

Another strength was the inclusion of multiple cohort studies, which are typically considered the gold standard in epidemiology (Rothman 2012; Rothman et al. 2008). The meta-RR of cohort studies (N = 10), was 2.00 (95% CI 1.21–3.29), which was notably higher than that of case–control studies (see Table 3).

Bias and Differential Risk

One potential source of bias is exposure misclassification, as many studies did not have complete information regarding the specific levels and duration of exposure, length of employment, and concomitant exposures. This misclassification was likely non-differential among cohort studies (exposure status equally misclassified among cases and controls) which tends to attenuate the risk (Pearce et al. 2007), and differential in case-control studies due to recall bias (exposures may have been remembered differently by cases or their proxies than controls).

Our brain tumor analysis was more likely to suffer from biases resulting from non-differential misclassification, as ten of the 12 studies were cohort design. Furthermore, the ascertainment of the brain tumor diagnosis (primary vs. metastatic origin) may have been questionable. While many of the tumors may have been primary malignancies, some death certificates may reflect misdiagnoses, as the brain is a common site for metastases of other primary cancers (Thomas and Waxweiler 1986). This non-differential misclassification of disease status would also bias results toward the null, thus indicating that our main meta-RR could potentially underestimate the risk for brain tumors.

Despite these limitations, our results suggest an increased risk of ALS and brain tumors among individuals highly exposed to formaldehyde.

Summary of Key Findings

Our meta-analyses have revealed for the first time that high exposure to formaldehyde increased ALS meta-relative risk by 78% (meta-RR = 1.78); individuals with higher exposures to formaldehyde had an increased meta-RR compared with those with low/"ever exposures" (Fig. 3a). This finding is also supported by elevated total NDD risk among plastics workers than other industrial workers (Table 2). Additionally, we reported high exposure to formaldehyde increased the relative risk of developing brain cancer (meta-RR = 1.71). Evidently, the increased risk was far greater among professionals (meta-RR = 2.42) compared with industrial workers (1.32) (Table 3 and Fig. 3b) likely due to their different exposure patterns and levels, which is comparable with and supported by previous meta-analyses (Table 4). In summary, inhaled formaldehyde can potentially damage the brain to cause ALS or tumors.
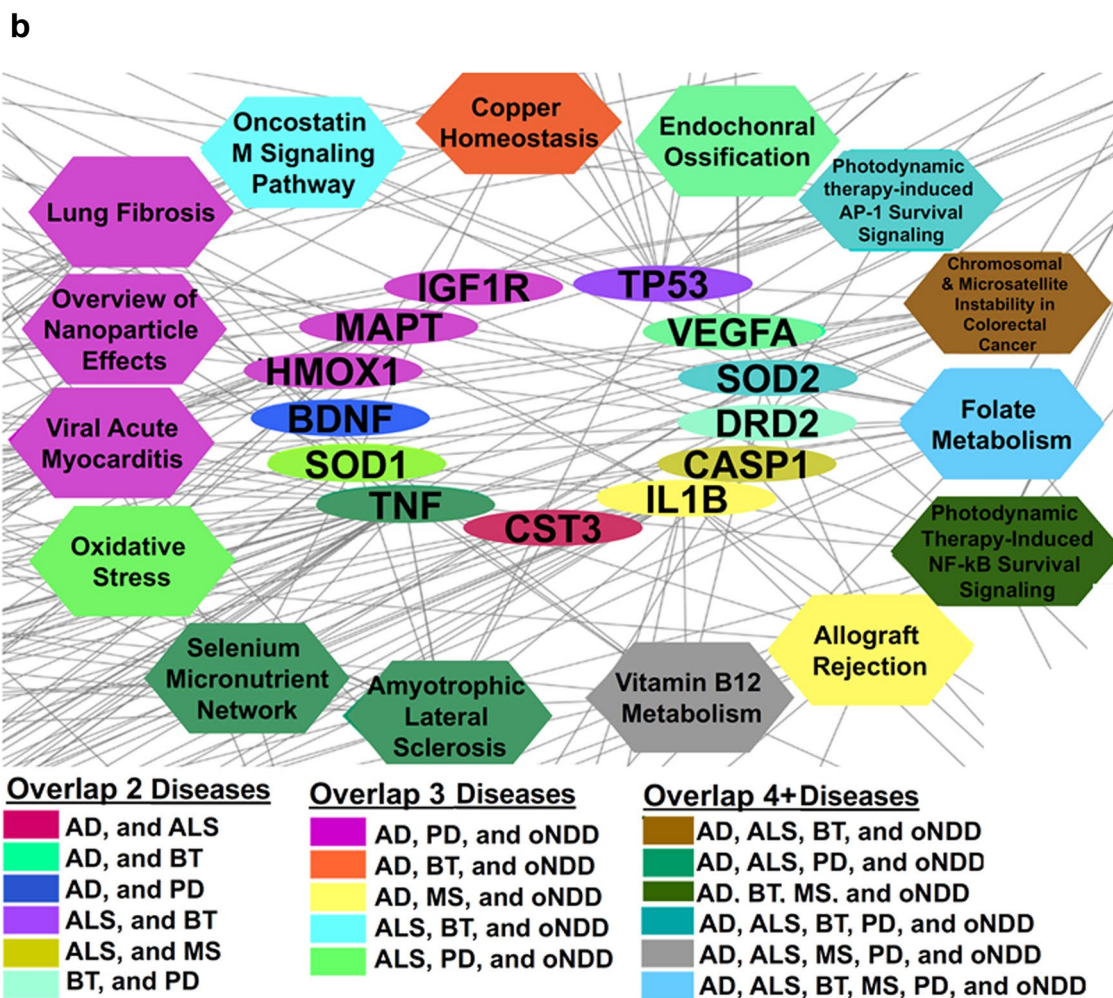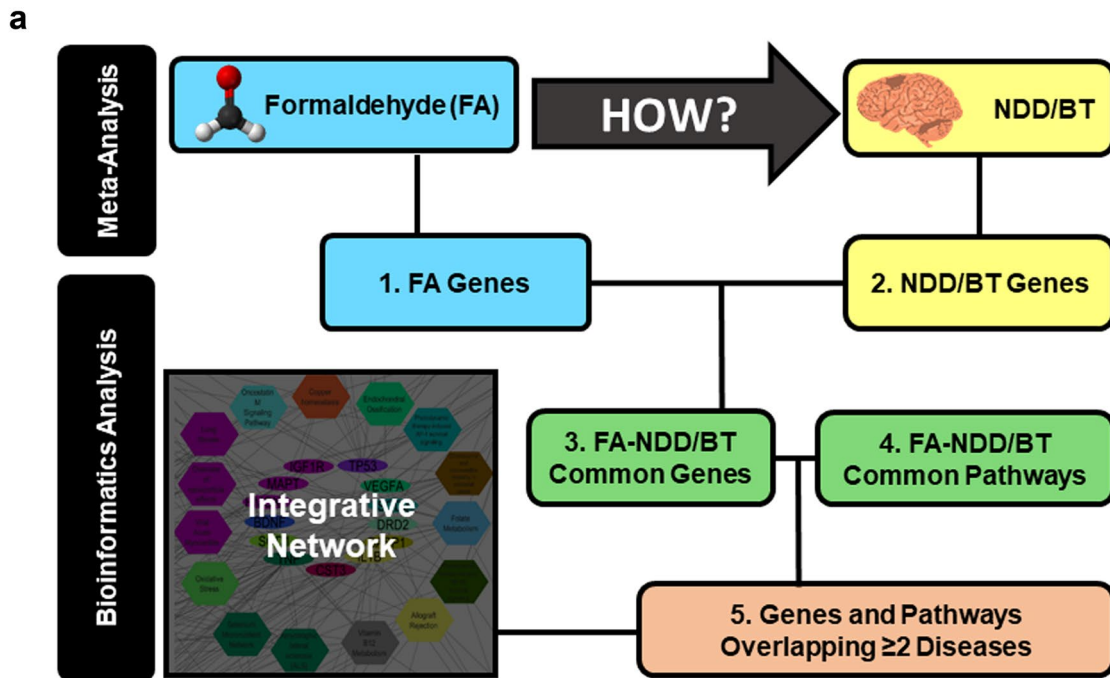
◄**Fig. 4** Summary of bioinformatics analysis approach. **a**. Definitions of numbers are explained in "Bioinformatics Approach" in the text. Close-up of the integrative network depicted in Supplementary Fig. 2, which contains the genes and pathways that were overlapping between two or more NDDs of interest **b**. The color of the nodes depicts the respective combinations of the different disease types. The turquoise gene *SOD2* (in the middle) and the gray pathway Vitamin B_{12} Metabolism (at the bottom), for example, are both observed to be affected in AD, ALS, brain tumor, PD, as well as in oNDD after formaldehyde exposure **b**

Potential Mechanisms Investigated with Bioinformatics

To examine our findings from human studies further, we investigated the biological plausibility of how inhaled formaldehyde could exert its toxic effects in the brain. Given these associations have historically been neglected, there is a paucity of studies investigating mechanisms underlying formaldehyde-mediated brain cancer and neurodegeneration. To address this limitation, we employed a bioinformatics approach to identify candidate genes and pathways that may be responsible for mediating these interactions.

Bioinformatics Approach

To deepen our understanding of the epidemiologic association between formaldehyde exposure and brain cancer and NDDs, we used gene-association data to identify (1) formaldehyde-associated genes, (2) the genes associated with the diseases of interest (ALS, AD, PD, MS, BT), (3) overlapped common genes from formaldehyde and NDD/BT, and (4) inferred formaldehyde–gene–NDD/BT associations and their associated pathways. Lastly, (5) we overlapped the genes and pathways identified from the inferred chemical–gene/pathway–disease associations to create an integrated network of candidate genes and pathways that could have played a role in mediating the associations observed in the human epidemiological studies (Fig. 4a).

Integration of Gene-Association Data from the CTD

First, to investigate the relationship between formaldehyde exposure and NDD or BT, the Comparative Toxicogenomics Database (CTD) (Davis et al. 2017) was queried to obtain appropriate gene association data. Details on the CTD and how the curated FA-genes and NDD/BT-genes were selected may be found in Supplementary Section A3.1.

Identifying Common Biomarker Genes

Second, to identify possible disease biomarkers following formaldehyde exposure, the obtained chemical–gene and gene–disease lists were compared using Venn Diagrams (Table 5). Details of how these resulting chemical-gene-disease associations were inferred may be found in Supplementary Section A3.2.

Performing Gene Enrichment Analysis

Next, in order to gain more information into the biological processes that are enriched for the formaldehyde-induced disease genes, we performed gene set enrichment analysis (GSEA, detailed in Supplementary Section A3.3) using the Cytoscape (version 3.6.1), (Lotia et al. 2013) app ClueGO (version 2.5.2), (Bindea et al. 2009), a network visualization and analysis tool, in combination with the WikiPathways pathway repository containing 467 human pathways comprising 6235 human genes (version Oct 11, 2018) (Slenter et al. 2018).

Integrating Formaldehyde-Associated NDD/BT Genes and Pathways

Lastly, to understand what genes and which specific pathways are involved in the formaldehyde-associated NDD/BT, we created an integrative network of genes and pathways using Cytoscape (major findings reported in Fig. 4b). The full network, which is presented in Supplementary Fig. 2, allowed us to visualize similarities and differences between formaldehyde-related subtypes of NDD and brain tumors to further understand how these diseases are modulated.

Results of Bioinformatics Approach

Formaldehyde-Induced Brain Disorder Genes

When comparing gene lists for formaldehyde exposure versus the brain disorder of interest (Table 5), we identified the genes and pathways that overlapped the greatest number of diseases. The formaldehyde-associated genes that overlap three or more disease outcomes are reported in Table 5. Superoxide dismutase 2 (*SOD2*) was found for formaldehyde from 5 of 6 total brain-related disorders (other neurodegenerative disease [oNDD], AD, ALS, PD, and BT). Tumor necrosis factor (*TNF*) was found in formaldehyde from 4 NDDs (oNDD, AD, ALS, and PD).

Table 5 Chemical gene and pathway association data from the Comparative Toxicogenomics Database (CTD), related with formaldehyde exposure, were compared with curated gene-disease associations for AD, ALS, brain tumor, MS, PD, and oNDD

Name	NDD				MS	BT	Total
	oNDD	AD	ALS	PD			
Genes							
Superoxide dismutase 2 (SOD2)	✓	✓	✓	✓		✓	5
Tumor necrosis factor (TNF)	✓	✓	✓	✓			4
Superoxide dismutase 1 (SOD1)	✓		✓	✓			3
Heme Oxygenase 1 (HMOX1)	✓	✓		✓			3
Insulin Like Growth Factor 1 Receptor (IGF1R)	✓	✓		✓			3
Microtubule Associated Protein Tau (MAPT)	✓	✓		✓			3
Interleukin 1 Beta (IL1B)	✓	✓			✓		3
Pathways							
Folate Metabolism	✓	✓	✓	✓	✓	✓	6
Photodynamic therapy-induced AP-1 survival signaling.	✓	✓	✓	✓		✓	5
Vitamin B12 Metabolism	✓	✓	✓	✓	✓		5
Photodynamic therapy-induced NF-kB survival signaling	✓	✓			✓	✓	4
Amyotrophic lateral sclerosis (ALS)	✓	✓	✓	✓			4
Selenium Micronutrient Network	✓	✓	✓	✓			4
Chromosomal and microsatellite instability in colorectal cancer	✓	✓	✓			✓	4
Oncostatin M Signaling Pathway	✓		✓			✓	3
Copper homeostasis	✓	✓				✓	3
Oxidative Stress	✓		✓	✓			3
Viral Acute Myocarditis	✓	✓		✓			3
Lung fibrosis	✓	✓		✓			3
Overview of nanoparticle effects	✓	✓		✓			3
Allograft Rejection	✓	✓			✓		3

The complete formaldehyde-gene list all overlapping brain disorders can be found in Supplementary Table 5. As stated above in Multiple Sclerosis and Integration of Gene-Association Data from the CTD, because MS is a chronic inflammatory demyelinating disease that may progress into a NDD, it was analyzed separately.

Biological Pathways Affected in Formaldehyde-Induced Brain Disease

Using the respective formaldehyde-induced NDD gene lists, we conducted GSEA and identified significantly enriched pathways for formaldehyde and the NDD subgroups or brain tumor related genes (Supplementary Table 6). These unique pathways are affected by any of the NDD subgroups or brain cancer. The obtained lists of biological pathways were compared between individual NDD and with oNDD as a group. Fourteen pathways were found to be enriched by gene association data from three or more brain disorders (Table 5). The *folate metabolism* pathway was identified for all brain disorders of interest. Other pathways that were highly enriched included the *photodynamic therapy-induced AP-1 survival signaling* pathway, the *vitamin B$_{12}$ metabolism* pathway, the *ALS* pathway, and the *oxidative stress* pathway.

An Integrative Network to Visualize Formaldehyde-Induced Effects on Brain Disorders

To summarize the results, an integrative network depicting the overlapping and unique genes and pathways was created in Cytoscape (Supplementary Fig. 2). The network is divided in two parts: (1) NDD and (2) brain tumors plus MS. The left panel of the figure depicts the unique genes and pathways that were found for PD, AD, ALS, and oNDDs (NDDs excluding AD, ALS, and PD). The upright panel of the network depicts the genes and pathways that were found for brain tumor and MS. The clusters of pathways and genes depicted next to each NDD are those overlapping with the oNDD group.

The most important results are depicted in Fig. 4b, which is the bottom right corner of Supplementary Fig. 2. This cluster of multicolor nodes represents the overlapping genes and pathways between two or more brain disorders (as were mentioned in Bioinformatics Approach and Results of Bioinformatics Approach). The genes presented in this network have been filtered because they could be involved in either of the enriched pathways (filtered list in comparison to Table 5). Figure 4b shows that the *folate metabolism* pathway overlapped all brain diseases of interest: PD, AD, ALS, MS, and brain tumors (5/5), as well as the oNDD group. Similarly, the *vitamin B$_{12}$ metabolism* is enriched for gene association data related with AD, ALS, MS, and PD (4/5) plus the oNDD group.

Pathways Significantly Affected by Formaldehyde Exposure

The genes and pathways that were used to generate the network are reported in Supplementary Table 7. Using these data, we further analyzed the 14 pathways overlapping 3 + diseases to see which were significantly impacted by formaldehyde. To do so, we divided the number of genes affected by formaldehyde by the total number of genes involved in the pathway to calculate the percentage of the pathway affected (Supplementary Table 8). The *ALS* pathway had the greatest percent affected at 34%, lending support to our findings in the meta-analysis of human data. Other notable pathways included *overview of nanoparticle effects*, *NF-kB survival signaling*, *folate metabolism,* and *oxidative stress*, in which 32%, 31%, 23%, and 20% of genes were affected, respectively. A schematic of the formaldehyde genes affected in the *ALS* pathway is depicted in Supplementary Fig. 3. Another interesting pathway was the *oncostatin M* (OSM) *signaling* pathway, a homeostatic regulator in the central nervous system, in which 17% of genes were affected.

Genes and Pathways Involved in Formaldehyde-Induced Brain Disorders

We analyzed the results of the bioinformatics screen to determine concordance with known mechanisms of NDD/BT, including oxidative stress, abnormal protein aggregation, and inflammation, and to identify novel pathways that may be dysregulated.

Oxidative Stress and Lipid Peroxidation

One well-characterized feature of NDD and BT is oxidative stress. *SOD2*, depicted in the network to be affected within AD, ALS, brain tumor, and PD, plays a role in neutralizing oxidative stress. Additionally, some other genes listed in Table 5 and Supplementary Table 5, such as *HMOX1* and *GSTP1*, can also be encoded to antioxidant enzymes to detoxify oxidative stress. Formaldehyde has been reported to induce oxidative stress not only in the brain but also in other tissues such as the bone marrow, spleen, liver, and testes of exposed mice (Ye et al. 2013).

Abnormal Protein Aggregation

Aggregation of proteins (such as amyloid-β and tau) and inclusion body formation is considered a hallmark of NDD (Ross and Poirier 2004). Cytostatin C (*CST3*), a formaldehyde-associated gene that was affected in ALS and AD, is a cysteine protease inhibitor that prevents amyloid-β deposition in mice (Mi et al. 2007). We also identified microtubule-associated protein tau (*MAPT*) in AD and PD; *MAPT* mutations can cause hyperphosphorylation and deposition of tau proteins into aggregates (Dujardin et al. 2018).
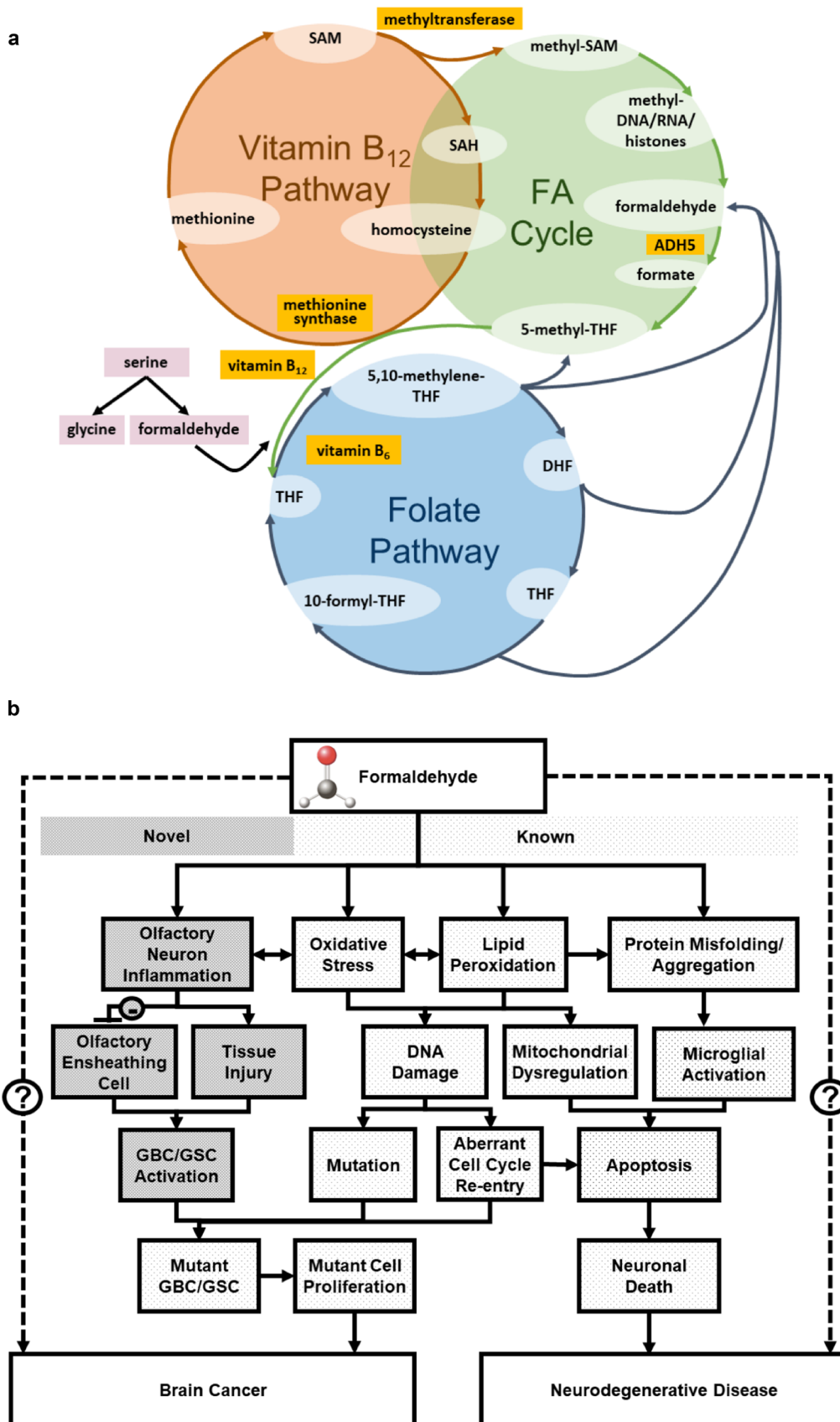
◄**Fig. 5** Intersection of folate driven one-carbon pool, formaldehyde cycle, and vitamin B$_{12}$ pathway **a**. Potential mechanisms underlying formaldehyde-induced neurodegenerative disease and brain cancer **b**

Mitochondrial Dysfunction and Vitamin B$_{12}$ Metabolism

Mitochondrial dysfunction is another common mechanism in NDD pathology, manifesting as decreased energy production, excitotoxicity, impaired calcium buffering, and increased mitochondrial membrane permeability (Beal 1998). Deficiency in *vitamin B$_{12}$*, a pathway overlapping ALS, AD, PD, and MS is known to cause mitochondrial toxicity as a result of citric acid cycle inhibition (Toyoshima et al. 1996) and predicts worsening mobility in PD patients (Christine et al. 2018).

Low *vitamin B$_{12}$* levels inhibit the enzyme *methionine synthase* that catalyzes formation of methionine from homocysteine, leading to increased homocysteine (Fig. 5a). Hyperhomocystemia can cause the dysfunction or death of cells in the nervous system by impairing DNA repair mechanisms and inducing oxidative stress (Kruman et al. 2000, 2002; Obeid and Herrmann 2006). This mechanism was demonstrated in PC12 cells as a model for neuronal secretion and differentiation (Wagner et al. 1993), where formaldehyde exposure induced oxidative stress and inhibited hydrogen sulfide production, contributing to neurotoxicity induced by homocysteine (Tang et al. 2013). In human epidemiological studies, hyperhomocystemia has been strongly linked to development of dementia, AD (Seshadri et al. 2002), and cognitive decline in PD patients.

Folate Metabolism

The only pathway identified through our bioinformatics analysis that overlapped every brain disorder of interest (ALS, BT, AD, PD, and MS) was the *folate metabolism* pathway, also known as the one-carbon cycle (Fig. 5a). Interestingly, the *vitamin B$_{12}$*-dependent formation of methionine from homocysteine (discussed in detail in Mitochondrial Dysfunction and Vitamin B12 Metabolism) is also a major intersecting branch point in the one-carbon cycle, as it uses 5-methyltetrahydrofolate (5MTHF) from the *folate metabolism* pathway as a methyl donor.

The one-carbon metabolism is involved in many biochemical reactions such as the generation of DNA precursors, which may affect neuroprogenitor cell proliferation. Dysregulation of the one-carbon pool mediated by folate deficiency has been shown to inhibit adult hippocampal neuroprogenitor cell proliferation in vivo, a central function of neurogenesis in the olfactory bulb, subventricular zone of the lateral ventricle, and in the hippocampus (Kruman et al. 2005).

Formaldehyde Cycle Links Both Vitamin B$_{12}$ and Folate Pathways

Metabolisms of vitamin B$_{12}$ and folate (discussed in Mitochondrial Dysfunction and Vitamin B12 Metabolism and Folate Metabolism, respectively) are intimately linked with each other and connected with the recently discovered endogenous formaldehyde cycle (Burgos-Barragan et al. 2017) (Fig. 5a). In the *folate metabolism* pathway, it was previously known that formaldehyde generated from the enzymatic cleavage of serine serves as an intermediate in the cycle, where it rapidly reacts with tetrahydrofolate (THF) to form 5,10-methylene-THF. Formaldehyde has also been identified as a product of the one-carbon pool (Burgos-Barragan et al. 2017). This endogenously produced formaldehyde can react with glutathione and ultimately be hydrolyzed into formate, which is free to re-enter the canonical one-carbon metabolic pathway. Any excessive formaldehyde that is not recycled may induce DNA damage. Interestingly, our most current study using a CRISPR screen reported that genes encoding components of DNA damage response/repair and the formaldehyde-involved one-carbon metabolism were also identified as top candidate genes whose disruption altered FA cytotoxicity and conferred increased sensitivity to formaldehyde (Zhao et al. 2020a).

Peripheral and Neural Inflammation

Recent studies indicate peripheral inflammation in the body may be transmitted to the brain to induce neuroflammation (Cabrera-Pastor et al. 2019). We identified *TNF*, a gene overlapping AD, ALS, and PD, is a pro-inflammatory marker that has been shown to be increased significantly in mice after 15.5 mg/kg/day formaldehyde exposure (Liu et al. 2018). The *IL1β* gene encodes a cytokine protein that is an important mediator of the inflammatory response; increased production of *IL1β* causes a number of auto-inflammatory syndromes (Masters et al. 2009; Bensi et al. 1987). Additionally, we identified photodynamic therapy-induced NF-kB and AP-1 signaling pathways (Table 5 and Supplementary Table 8) that involve with many other pro-inflammatory or inflammatory genes and intimately associated with formaldehyde-related NDDs and BT.

We summarize the mechanisms described in this section in Fig. 5b; many of which are well-known, and the novel pathway with olfactory neuron inflammation is discussed next in Potential Mechanisms: Neural Damage via Olfactory Bulb. It should be noted that the pathways denoted in the figure are interconnected and sometimes bidirectional; for example, both inflammation and oxidative stress are known to promote microglial activation which can then cause lipid peroxidation.

Potential Mechanisms: Neural Damage via Olfactory Bulb

As described above, we have established both an epidemiological association between formaldehyde exposure and brain cancer/NDD and described in detail the putative genes and pathways intersecting formaldehyde and multiple brain disorders of interest. Indeed, the question of how formaldehyde reaches these targets and modulates these different classes of disease remains; perhaps the common link is the nose, or more precisely, the olfactory bulb or olfactory neuron inflammation (Fig. 5b).

Formaldehyde and Olfactory Impairment

Although it is commonly postulated that most inhaled airborne formaldehyde is detoxified upon contact with the mucosal surfaces of the mouth and nose, formaldehyde encounters and could damage the olfactory bulb (Formaldehyde Inhalation and Olfactory Function). Repeated formaldehyde inhalation exposure impairs olfactory function in humans (Kilburn et al. 1985; Holmstrom and Wilhelmsson 1988; Hisamitsu et al. 2011; Edling et al. 1988) and in rats. (Zhang et al. 2014) Our recently published study shows that formaldehyde inhalation inhibits the growth of hematopoietic stem and progenitor cells in the olfactory mucosa of exposed mice in vivo and ex vivo (Zhao et al. 2020b). Exposure to formaldehyde also decreased glutamate, GABA, and nitric oxide synthase expression (Li et al. 2010) and reduced expression of synaptosomal-associated protein 25 (SNAP25) in the olfactory bulb, as well as mature and immature olfactory sensory neuron markers, olfactory marker protein (OMP), and Tuj-1 in rats (Zhang et al. 2014). Decreases in SNAP25 are predictive of neuron loss and decreased synaptogenesis (Washbourne et al. 2002).

Olfactory impairment has been linked to PD (Doty et al. 1988, 1995; Tissingh et al. 2001), ALS (Viguera et al. 2018), and AD (McCaffrey et al. 2000; Morgan et al. 1995). In particular, AD specific neuropathology has been detected in the olfactory epithelium (Yamagishi et al. 1994; Lee et al. 1993; Arnold et al. 1998), with phosphorylated tau and neurofilament proteins present in the axons and dendrites of olfactory neurons (Talamo et al. 1989; Reyes et al. 1993). AD patients also have altered olfactory evoked response potentials (Warner et al. 1986). Reduced olfactory performance has also been reported in patients with brain tumors (Daniels et al. 2001).

Interestingly, the mammalian olfactory epithelium has a unique capacity to replace olfactory receptor neurons throughout life (Graziadei and Graziadei 1979; Schwob 2002) using olfactory ensheathing cells (OECs) that wrap olfactory axons and support their continued regeneration (Su et al. 2013). When activated, these OECs have the ability to travel from the olfactory bulb in the peripheral nervous system (PNS) to primary tumor sites in the CNS and along the invasive tumor border where they can selectively target glioblastoma stem-like cells (GBC/GSC), which are thought to initiate glioblastomas (Carvalho et al. 2019).

Formaldehyde-Induced Toxicity in Olfactory Neurons and/or OECs

We hypothesize that formaldehyde could damage these OECs, which may in turn promote brain tumor proliferation. Olfactory neuronal inflammation may also promote oxidative stress, lipid peroxidation, and DNA damage acting along the apoptotic pathway, promoting neurodegenerative disease (Fig. 5b).

As reported above in Pathways Significantly Affected by Formaldehyde Exposure, the *OSM signaling* pathway was both affected by formaldehyde (17% of genes affected; Supplementary Table 8) and overlapped formaldehyde and both ALS and brain cancer (Table 5), the disorders for which we uncovered the strongest human epidemiological evidence. The OSM is a member of the interleukin-6 cytokine family. It modulates the homeostasis of neural precursor cells, which are responsible for the production of new neural cells in the olfactory bulb, among other brain regions (Houben et al. 2019). OSM has also been demonstrated to exhibit neuroprotective and anti-tumorigenic effects (Beatus et al. 2011), though its full role in the CNS is not completely understood.

Conclusion

In this systematic review and detailed meta-analysis, we describe the epidemiological evidence that appears to support an association between formaldehyde inhalation and the development of ALS and brain cancer. A similar association was also reported for PD, but the lack of rigorous exposure assessment in these studies indicates these results should be interpreted with caution. Indeed, the question of biological plausibility for the formaldehyde-associated brain damage (NDD and BT) remains.

Using multiple bioinformatics analyses, we reported that our new findings suggest formaldehyde may mediate these brain diseases through dysregulation of the *ALS* and *folate metabolism* pathways, among others. While cancer cells and neurons are distinct, as the former rapidly divide and the latter are non-replicating, there is evidence that supports common genetic mechanisms involved in brain cancer and NDD progression. Our findings highlight that formaldehyde exposure, particularly at high levels, may manifest in the initiation and

progression of different neural diseases by mis-regulating common genes or pathways. We recommend conducting an animal exposure study employing CRISPR technology to knockdown critical genes identified from our bioinformatics analysis to further understand potential mechanisms supported by empirical data.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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