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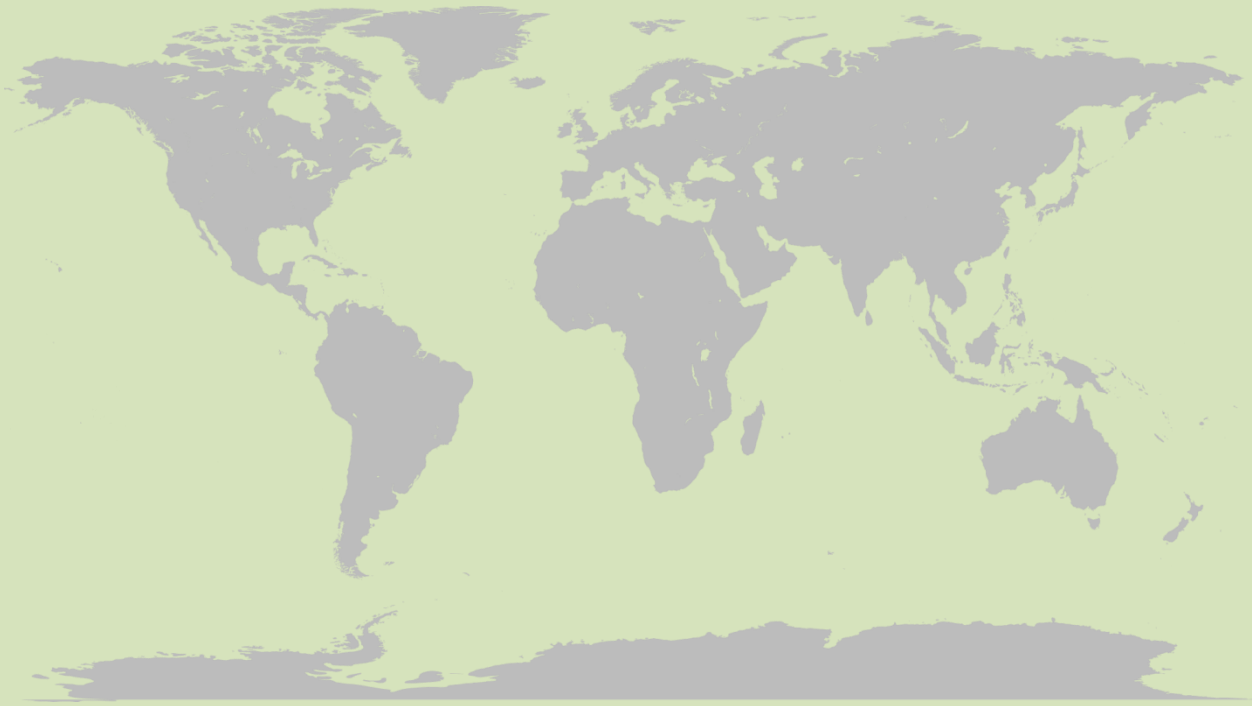
Global Clinical And Translational Research

Toward an integration of genomic, environmental, and social medicine



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The journal of *Global Clinical and Translational Research* is published and printed quarterly by the Global Clinical and Translational Research Institute, Inc, MD, USA.

GUIDE FOR AUTHORS

Aims and Scopes
Global Clinical and Translational Research is a new peer-reviewed, a multidisciplinary scientific journal dedicated to publishing articles regarding all areas of clinical and translational research in humans or animals.

The journal aims to promote a unified platform on research communications for basic scientists, medical doctors, clinical and health professionals, social scientists and social workers to share the most recent advances in all areas of clinical and translational sciences.

The journal accepts original research articles, reviews, mini-reviews, correspondence, case reports, short notes, and rapid communications covering all aspects of clinical and translational research. Papers about novel applications of statistical methods or data science are also welcomed. Specific fields of the papers to be published include but not limited to

- Clinical research in human or animals, including identification of genetic or molecular markers associ-

ated with diseases or traits that have clinical implycations;
- Translational research that based on clinical research findings. For example, research findings provide evidence for health professionals to improve human health conditions (T1 translational research), and provide targets for basic scientists to study the biological mechanism (T2 translational research);
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- Paper title
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Abstract
Not exceed 200 words, should be structured as below for all research articles; other articles should use non-structured abstract.
- Background:
- Methods:
- Results:
- Discussion:
- Conclusion:

Keywords (3-5 keywords)

Maintext
Introduction. This section should be succinct, with no subheadings.
Methods. Should contain all procedures with enough details so that they can be repeated.
Results. Both descriptive and analytic results.
Discussions. This should describe the implications and significance of the findings, also highlighting the limitations of the study.
Conclusion (optional): This should clearly explain the main conclusions of the work highlighting its importance and relevance.

Conflict of interest.
If there is no conflict of interest, authors should state, "The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper."

Acknowledgments (optional, including funding support if available).

References
Authors are responsible for ensuring that the information in each reference is complete and accurate. All references must be numbered consecutively and citations of references in the text should be identified using numbers in square brackets (e.g., "as discussed by Smith [1]"; "as discussed elsewhere [3, 4]"). All references should be cited within the text; otherwise, these references will be automatically removed. GCTR uses "Vancouver" style, as outlined in the ICMJE sample references (https:// www.nlm.nih.gov/bsd/uniform _requirements.html).

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Tables should be cited consecutively in the text. Every table must have a descriptive title and if numerical measurements are given, the units should be included in the column heading. Vertical rules should not be used. A table should be well annotated and easy to read and interpret with little reference to the text.

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Global Clinical and Translational Research aims to comply with the recommendations of the International Committee of Medical Journal Editors (ICMJE) on trials registration. Therefore, authors are requested to register the clinical trial presented in the manuscript in a public trials registry and include the trial registration number at the end of the abstract. Trials must be registered prospectively before patient recruitment has begun. We promote transparency in clinical trial reporting.

In addition, the journal endorses the Principles and Gudelines for Reporting Preclinical Research by the National Institutes of Health, which a considerable number of journals have agreed to endorse (https://www.nih.gov/research-training/rigor-reproducibility/principles-guidelines-reporting-preclinical-research). The journal also promotes the core set of standards for rigorous reporting of study design (Adapted from Landis et al., Nature 2012).

CONTENTS

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A brief summary of the papers appearing in this issue

Beginning with an editorial by the Editors-in-Chief, Dr. Claude Hughes and Dr. Fengyu Zhang, this first issue of the journal published seven papers, which features three areas that the journal aims to focus, including genomic, environmental and social medicine. The first paper is on gastroschisis, a congenital abdominal wall defect that is largely caused by the large environment; through genome-wide association study, the second paper purely investigates the genetic variants associated with multiple neuropsychiatric disorders. Two papers focus on the social aspects of human disorders including cervical survivor and depression in older adults — lastly, two review papers discussed gene-environment risk factors and altered gut microbiome in autism spectrum disorders (ASD).

The increasing prevalence of gastroschisis: associated factors, possible mechanisms, and potential mitigative interventions

The incidence of gastroschisis has increased globally over recent decades, but the cause of this increase is not elucidated and etiology of gastroschisis has not determined. **Hughes and Adibe** conducted a selective review of literature on risk factors of gastroschisis including maternal illnesses, medication use, and substance abuse, environmental exposure or agricultural chemicals. They hypothesized two possible modes-of-action hypotheses: 1) mechanical forces – mechanosensitivity and mechanotransduction signaling, and 2) Ephrin-Eph receptor signaling, which could be highly attractive research and development opportunities, including preventive and mitigative intervention.

Common genetic variants shared among five major psychiatric disorders: a large-scale genome-wide combined analysis

Genetic correlation and pleiotropic effects among psychiatric disorders have been implicated. **Xia *et al*.** conducted a large genome-wide combined analysis of p-value for about 8 million single nucleotide polymorphisms (SNPs) in samples about 151,672 cases of schizophrenia, bipolar, major depressive disorder, attention deficit-hyperactivity disorder, and autism spectrum disorder and equivalent 284,444 controls of European ancestry based on the data from the latest genome-wide association studies; they found that SNPs mapped to 336 loci were shared by three adult psychiatric disorders (schizophrenia, bipolar and major depressive disorder), 73 loci shared by childhood disorders, and 47 genes by all five disorders at a genome-wide significance. A large number of SNPs were found in the extended major histocompatibility complex (MHC) for three adult disorders, but none of them was shared by two childhood disorders. The SNPs shared by all five disorders were located in the regions that have been identified as containing copy number variation associated with autism and had largely neurodevelopmental functions. In addition, some of those genes have been implicated for Alzheimer's diseases (AD) and Parkinson's disease (PD). This study provides a valuable list of genes from which to investigate genetic mechanism or gene-gene interaction on the development of neuropsychiatric disorders.

Consequences of cervical cancer treatment on sexual health in cancer survivors: a qualitative study

There has been little information on the attitudes and perceptions of cervical cancer survivors (CCS) toward sexual activity subsequent to a diagnosis of cervical cancer and its treatment. **Ye *et al*.** conducted a qualitative study of 20 patients after surgical treatment; they found that uncertainty, fear, and worry dominated the attitudes and behaviors of CCS-related to sexual activity. Patient-centered information on the change in sexual life and strategies to cope with the physical and psycho-sexual sequelae of treatment are needed.

An overview of genetic and environmental risk of autism spectrum disorder

It is evident that both genes and environment contribute to the etiology of Autism Spectrum Disorder (ASD). **Ou *et al*.** provided an overview of the genetic and environmental risk factors that have been associated with ASD. They proposed that genes and environmental factors, as well as their interactions, should be considered in the future study, with the expectation that epigenetic studies will lead to understanding the link between the environment and risk of ASD.

Altered gut microbiome in autism spectrum disorder: potential mechanism and implications for clinical intervention

A large number of individuals with ASD have gastrointestinal problem, and recent studies demonstrate that the endogenous gut microbiota has a close relationship with ASD. **Li *et al*.** reviewed the reports of microbial dysbiosis in ASD and discussed the recent evidence of biological interactions among microbiota, metabolism, immunity, neurodevelopment, behaviors, and the role of gut microbiome in the link between ASD and environmental risk factors. They suggest adjuvant treatments to consider in attempts to correct autistic behaviors.

Childhood adversity and depression among older adults: results from a longitudinal survey in China

Depressive symptoms in older adults are often mistaken for symptoms of healthy aging and so may have not draw timely attention clinically; and individual with depression may not receive appropriate treatment. Using data from the China Health and Retirement Longitudinal Study (CHARLS), **Li *et al*.** analyzed to examine the association of childhood adversity and depression among older adults; and found that the likelihood of depression was significantly associated with poor parental mental status, physical abuse, and emotional abuse during childhood. Our study adds to research in the area of adverse childhood events and its effect on adult psychological and physical well-being.

Editorial

New Journal Launch: *Global Clinical and Translational Research*

Accepted October 30, 2018

We are proud to announce the launch of *Global Clinical and Translational Research*, a new journal that aims to promote clinical and translational research in the genomic era from a global perspective. The journal will (1) provide a unified platform of research communication for basic scientists, medical doctors, other clinical health professionals, social scientists and social workers to share the most recent advances in all areas of clinical and translational sciences; (2) introduce new techniques and methodology such as genomics-based techniques and approaches to design and perform a new generation of clinical research; (3) foster collaborative clinical research from a global perspective.

In the first two decades of the 21st century, strategies for conducting biomedical research have been profoundly influenced by the completion of the Human Genome Projects (HGP) [1]. The HGP is properly regarded as the major groundbreaking accomplishment in human biology as we moved from the 20th into the 21st century. Related projects such as the International Haplotype Map of the Human Genome Project (HapMap) and the development of the tools of bioinformatics computer programs, especially the invention of genome-wide association techniques, have made it possible to dissect genetic architecture of complex human diseases and health, and to identify genetic variants that may impact the response of individuals or populations to pharmaceutical treatments, environmental exposures to chemicals or toxicants and psychosocial stressors. The findings from such research over the past decade have accelerated discoveries such as identification of molecular targets of drugs and other environmental or dietary compounds as well as novel targets for further research and development of other ligands that may become new therapeutic medications including and offer new evidence for population-based interventions, or early and precision diagnostics.

With the increasingly available tools of bio-technology in genomics and other "omics," the then U.S. President Obama announced the precision medicine initiative in 2015. The goal of this initiative is to pioneer a new model of biomedical and health research that promises to *accelerate biomedical discoveries* and *translate them into new tools*, *knowledge, and therapies* for clinicians to select which treatments will work best for which patients and for public health professionals to better decide when and how preventative measures should be taken. The concept of precision medicine is to develop treatment and prevention strategies that take individual variability [2] into account, which may involve the individual's genetic heterogeneity, life course experiences, and lifestyle. Identifying those individual factors will require a series of research activities across different populations to accommodate variation in the genetic background including ethnicity, as well as local or regional environmental and dietary factors.

This new journal will be a vehicle to communicate those new findings of clinical and translational research in the era of genomics or other "omics." Without a doubt, following the precision medicine initiative, clinical and translational research will be a robust global enterprise over the next few decades; therefore, with wide application of the new tools of biotechnology, more and more studies will focus on discovery in human subjects through observational and experimental approaches. Findings from such studies can be translated not only into basic research hypotheses that should lead to novel ideas for mechanistic biological research or biology but also into clinical practices such as precision diagnosis and treatment, preventive interventions at a population level or enhanced environmental health for entire communities [3]. The scope of translational research must greatly extend beyond the traditionally defined "from bench to bedside and back again" to a broad multidisciplinary and multilayered process of discovery, implementation, and global public health impact.

The journal will be edited by a multidisciplinary team lead by Drs. Claude Hughes and Fengyu Zhang. Dr. Hughes' broad experience includes pharmaceutical clinical trials; practice of Obstetrics & Gynecology and Reproductive Endocrinology & Infertility; modeling and biomarker development; basic, clinical and population research in the health of women across the lifespan, and the impact of dietary and environmental chemicals in reproductive, developmental and translational toxicology. Dr. Zhang's research expertise focus conducting multidisciplinary research on genetic and environmental determinants of complex human disorders and population health, clinical trial-based pharmacogenomics of treatment response, as well as biomarker discovery; and he has a strong background in quantitative and data science.

The journal accepts original research articles, reviews, mini-reviews, case reports, short notes, and rapid communications covering all aspects of clinical and translational research. Papers about novel applications of statistical methods or data science are also welcomed.

Editors-in-Chief
Claude Hughes, MD PhD
Fengyu Zhang, PhD MS

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Article

The Increasing Prevalence of Gastroschisis: Associated Factors, Possible Mechanisms, and Potential Mitigative Interventions

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ABSTRACT

Background: Gastroschisis has increased globally over recent decades and this increase is not explained by demographic changes in maternal age. Implicated risk factors for this increase include lifestyle behaviors, environmental exposures, lower socioeconomic status, lower body mass index, poor nutrition, smoking tobacco, using illicit drugs, alcohol, or analgesics and genitourinary infections.

Methods: Selective review of the literature.

Results: Present hypotheses would only suggest avoidance of suspect exposures as protective interventions. To identify safe and efficacious protective therapies, new cellular/molecular modes-of-action need to be considered. Plausible developmental modes-of-action include a) changes in epigenetic programming of relevant stem or progenitor cells; b) mechanical forces (cellular mechanosensitivity and mechanotransduction) signaling; and c) ephrin–Eph receptor multimodal signaling. These developmental modes-of-action present plausible options for "druggable" molecules that could be developed into protective or mitigative therapeutic agents for gastroschisis.

Conclusion: Possible interventions for modifiable factors in gastroschisis include 1) Delay childbearing. 2) Improve nutrition for younger gravidas. 3) Pre-conceptional counseling to reduce embryonic exposures to the range of implicated lifestyle, environmental and medical factors. 4) Urge research colleagues to investigate the cellular and molecular mechanisms underlying gastroschisis and to translate those insights into one or more safe and efficacious preventive or mitigative therapies.

KEYWORDS

Druggable molecules; ephrin–Eph receptor; exposures; gastroschisis; mechanosensitivity and mechanotransduction; protective therapeutics; risk factors; translational toxicology

OUTLINE

I. INTRODUCTION

The alarming demographic trend in gastroschisis

Gastroschisis (GS) is a congenital abdominal wall defect in which the intestines, and sometimes, other organs, protrude outside of the fetal abdomen through an opening in the abdominal wall. The prevalence of gastroschisis is on the rise, increasing two to four times in the last 45+ years in several regions around the world. Data spanning the years of 1970-2015 demonstrate some variation ov-

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er time, but in general, this worrisome trend shows a moderate ongoing increase [1; 2; 3; 4; 5; 6; 7].

While the observed increases in gastroschisis prevalence are not explained by demographic changes in maternal age or race/ethnicity, gastroschisis is strongly associated with young maternal age. The association of gastroschisis with young maternal age is most apparent among mothers aged younger than 20 years. Nonetheless, Jones et al. [3] documented significant increases in prevalence in all age groups during 2006–2012 compared with 1995 –2005. These investigators also noted the most significant increases occurred among younger Black mothers even though the prevalence in Black mothers remains lower than in White or Hispanic mothers.

The underlying cause or causes of these increases have not been definitively established. Some proposed risk factors include lifestyle behaviors, environmental exposores, lower socioeconomic status, and lower body mass index, poor nutrition, smoking tobacco, using illicit drugs, alcohol, or analgesics as well as genitourinary infections.

Clinical care, surgery and outcomes

The outcome for a fetus or infant who has gastroschisis may be a stillbirth, neonatal death or uncomplicated surgical correction. The Centers for Disease Control and Prevention (CDC) estimate is that about 1,900 babies are born each year in the United States with gastroschisis [1]. While 1-year survival rates may be approximately about 70% in some regions [5], if delivery of the infant occurs at a tertiary care center with immediate neonatal intervention, life expectancy for infants with gastroschisis can exceed 90%. In virtually all cases, care of affected infants exacts a significant emotional toll on parents and other family members and imposes significant financial/health care costs.

Keys et al. [8] performed a retrospective analysis in the United Kingdom of all patients admitted to a tertiary neonatal surgical center with gastroschisis from January 1996 to December 2005. The primary outcome measures were incidence, length of hospital stay, and the total cost for all patients each year.

Over that interval of ten years, the incidence of gastroschisis rose 3-fold while the median cost per patient remained relatively constant. Due to the increased incidence of this malformation, the estimated annual cost to the National Health Service (NHS) to care for this condition in England and Wales had risen from £3.6 million in 1996 to more than £15 million in 2005.

Gastroschisis is defined as a full-thickness congenital abominal wall defect usually situated on the right side of the umbilicus, with intestines protruding into the amniotic fluid without any protective membrane. The amniotic fluid creates an inflammation of the bowel wall, called perivisceritis. Associated with intestinal abnormalities are malrotation and a degree of short bowel, volvulus, perforation and atresia may be found [9]. The optimal management of neonates with gastroschisis is unclear, and there is significant morbidity [10]. Surgical management includes techniques for primary repair in which the intestinal contents are immediately reduced into the abdomen, or silo placement and delayed repair if the abdominal cavity is not able to accommodate the volume of the intestine [11].

Infants with gastroschisis often need other complementary treatments including intravenous nutrients, prophylactic antibiotics, and careful control of body temperature. *In utero* exposure of the fetal intestine to the amniotic fluid may cause inflammation and bowel injury, resulting in significant gastrointestinal problems during the neonatal period after closure of the defect. Complications include prolonged ileus, sepsis, associated intestinal atresia, malabsorption, wound infection, and necrotizing enterocolitis [11].

Kassa and Lilja [10] conducted a single-center retrospective analysis of 79 neonates with gastroschisis spanning 1989-2009. Length of hospital stay (LOS), days of parenteral nutrition (PN), and survival were outcome measures. Overall survival was 92%, and primary closure was achieved in 80%. Median LOS was 25 days, and median duration of PN was 17 days. Intestinal atresia, "vanishing" gastroschisis, delayed repair, and prematurity all affected outcome. Route of delivery and associated malformations were not related to poorer outcome. Necrotizing enterocolitis did not occur in any of these patients. Outcomes were favorable as measured by survival, LOS, and days of PN. Primary predictors of poor outcome were factors related to short bowel syndrome and delayed repair.

Bergholz et al. [12] compared the outcome of newborns with simple (sGS) and complex gastroschisis (cGS: gastroschisis with intestinal atresia, necrosis, perforation, and/or volvulus) by conducting a systematic database search, quality assessment and meta-analysis of relevant articles which evaluated the mortality and morbidity of newborns with cGS versus sGS. Of 19 identified reports, 13 eligible studies were included. The mortality of infants with cGS (16.67%) was significantly higher than sGS (2.18 %, RR: 5.39). Infants with cGS are started on enteral feedings later and they take longer to full enteral feedings with a subsequent longer duration of parenteral nutrition. Their risk of sepsis, short bowel syndrome and necrotizing enterocolitis is higher. They stay longer in the hospital and are more likely to be sent home with enteral tube feedings and parenteral nutrition.

de Buys Roessingh et al. [9] performed a retrospective study covering the period from January 2000 to December 2007. The following variables were analyzed for each child: weight, sex, APGAR (Appearance, Pulse, Grimace, Activity, and Respiration) score, perforations, atresia, volvulus, bowel length, subjective description of perivisceritis, duration of parenteral nutrition, initiation of enteral nutrition, time to full enteral intake, necrotizing enterocolitis, the average period of hospitalization, and mortality. Sixty-eight cases of gastroschisis were studied that included 9 cases of perforations, 8 of volvulus, 12 of

atresia and 49 with a subjective description of periviscceritis (72%).

The mortality rate was 12% (eight deaths) and the average duration of total parenteral nutrition was 56.7 d (min -max: 8-950; median: 22), with five cases of necrotizing enterocolitis. The average length of hospitalization for 60 patients was 54.7 d (min-max: 2-370; median: 25.5). The presence of intestinal atresia was the only factor correlated with prolonged parenteral nutrition, delayed time to full enteral intake, and more extended hospitalization. In this study, intestinal atresia was the primary predictive factor for severity of gastroschisis.

Surgical repair should be offered promptly, commonly on the first day after delivery to minimize infection risks. Flap closure is an alternative to fascial closure for gastroschisis. Youssef et al. [13] performed a systematic review and meta-analysis of outcomes comparing these surgical techniques. These investigators analyzed the following outcomes: mortality, ventilation days, feeding parameters, length of stay (LOS), wound infection, resource utilization, and umbilical hernia incidence. Twelve studies were included allowing evaluation of 1124 patients of which 350 underwent flap closure (210 immediately; 140 post-silo). Their meta-analysis revealed no significant differences in mortality, LOS, or feeding parameters between groups. Flap patients had fewer wound infections (OR=0.40; 95% CL: 0.22-0.74; and P=0.003). Flap patients had an increased risk of an umbilical hernia, but they were less likely to undergo repair (19% vs. 41%; P =0.01). These investigators concluded that flap closure has equivalent or superior outcomes to fascial closure for patients with gastroschisis and suggest that with its potential advantages of bedside closure and reduced sedation requirements; flap closure may represent the preferred closure strategy.

Developmental factors and genomic MOAs

Gastroschisis is a unique birth defect due to its association with young maternal age and increasing global prevalence. In the absence of evidence of a specific genetic cause or environmental teratogen, the underlying pathophysiological mechanisms are undefined. A thoughtful commentary by Opitz [14] considered whether gastroschisis is better characterized as a primary or secondary malformation.

Opitz profiled primary malformations as a "developpmental field defect" defined based on three cardinal criteria as follows:

- Heterogeneity; namely, the demonstration of causal heterogeneity of a malformation as evidence of identical reactivity to different endogenous causes on an embryonic primordium, common to all the clinical entities under discussion.
- Homology in that the embryonic primordia in humans capable of being malformed have the identical morphogenetic counterpart in more or less closely related mammals or vertebrates with corresponding molecular induction systems.
- Phylogeneity; from the concept of homology, it follows that corresponding anatomical structures in various species arising in response to more or less identical molecular induction cascades.

This is in contrast to a secondary malformation, which would be observed in an individual who was presumably genetically normal at conception but then experienced altered morphogenesis due to exposure to some exogenous stressor(s) such as chemical, physical, infectious, and maternal metabolic or psycho-social factors.

Some non-genetic factors have been implicated in the occurrence of gastroschisis, but no single factor stands out as a likely predominant cause. Drongowski et al. [15] reviewed the antenatal history of 19 infants with gastroschisis and 54 control infants born with a congenital anomaly unrelated to gastroschisis. When compared to controls, mothers of infants with gastroschisis were more likely to have used aspirin during pregnancy, to be taking oral contraceptives at the time of conception or to use an illegal drug, particularly cocaine.

Payne et al. [16] studied growth restriction in gastroschisis to consider if placental factors might be a cause. These investigators compared to birth weight (BW), crown-heel length (LT), occipitofrontal circumference (OFC) and ponderal index (PI) in 179 gastroschisis cases and 895 matched controls. Fetal ultrasounds (n=80) were reviewed to determine the onset of growth restriction and placental histology was examined in 31 gastroschisis patients and 29 controls. Gastroschisis cases weighed less than controls (BW, 2400±502 g vs. 2750±532 g; p<0.001) and they had lower BW as a group compared to controls. Intrauterine growth restriction was symmetric with gastroschisis patients having a shorter LT, smaller OFC but larger ponderal index compared to controls. Growth deficits appeared early in the second trimester and worsened as gestation increased. Placental chorangiosis was more common in gastroschisis patients than controls, even after removing all small for gestateonal age patients.

Mac Bird et al. [17] investigated associations between potential maternal factors with the risk of gastroschisis and omphalocele within a large population-based sample of participants enrolled in the National Birth Defects Prevention Study between October 1997 and December 2003. Data were collected on 485 cases of gastroschisis, 168 cases of omphalocele, and 4967 controls. These investigators found the expected higher risk in younger women and lower risk in Black women but also found a moderately increased risk of gastroschisis in women who had smoked tobacco, taken ibuprofen or consumed alcohol.

Duong et al. [18] compared mothers of infants with birth defects (n=10,825) and mothers of infants without birth defects (n=6795) who participated in the multisite National Birth Defects Prevention Study between 1997 and 2005. They found that mothers of infants with gastroschisis were significantly more likely to report any use of a hot tub in early pregnancy. Among the mothers who reported using a hot tub more than once in the exposure

period and remaining in it for more than 30 min, they found significantly elevated risk (OR≥2.0) for gastroschisis and some other birth defects.

In a related report, Agopian et al. [19] assessed the relationships between bathing habits during pregnancy and the risk of 13 non-syndromic birth defects in the National Birth Defects Prevention Study delivered during 2000-2007. These investigators found that the risk for gastroschisis in offspring was increased among women who reported showers lasting more than 15 compared to less than 15 minutes. It is at least plausible that this observation might relate to the duration of exposure to high water temperatures for somewhat extended episodes during early pregnancy.

Lin et al. [20] assessed maternal occupation and the risk of significant birth defects. For gastroschisis, an increased risk was found for maternal occupations of entertainers or athletes. In addition, they found a decreased risk of gastroschisis for the maternal occupation of being a non-preschool teacher.

Ortega-García et al. [21] raised the possibility of psychosocial stress as a potential risk factor for gastroschisis. In a case-control study of gastroschisis in Spain from December 2007 to June 2013, these investigators found that mothers of children with gastroschisis were younger, smoked more cigarettes, used more illegal drugs, and suffered domestic violence more frequently than the controls.

In 2015, Skarsgard et al. [22] reported on their investigation of the threefold increase in gastroschisis in Canada over the previous 10 years. They compared 692 gastroschisis pregnancies from the Canadian Pediatric Surgery Network and 4708 pregnancies from the Canadian Community Health Survey. Younger maternal age, smoking tobacco, a history of pregestational or gestational diabetes, and use of medication to treat depression all showed significant associations with increased risk of gastroschisis.

Genetic, genomic, gene variants, epigenetics MOAs

Bugge et al. [23] published the first case report on female monozygotic (MZ) twins discordant for gastroschisis. They found no family history of gastroschisis and no suspicious exposures during the pregnancy. Zygosity of the infants was verified by DNA analysis using highly polymorphic microsatellites. Albeit only one case, this observation does not support an explanation via a simple inherited gene etiology.

There is an inbred mouse strain (HLG) that shows a high incidence of gastroschisis after X-ray exposure of the zygotes with about 10% of fetuses having this malformation after irradiation with 1 Gy. Assessment of data from crossbreeding studies [24] suggests that a single-locus inheritance is not a good fit, and two or three gene loci are likely to be involved. Remarkably, additional evidence suggests that the elevated risk of gastroschisis can be transmitted to the next mouse generation [25] and suggests that the induced genomic instability might be a factor in the known familial risk of gastroschisis.

In another mouse model of gastroschisis, for the AEBP1 (adipocyte enhancer binding protein) gene, isoform Aclp (-/-) mice demonstrate a ventral wall defect that is similar to gastroschisis in humans [26].

Feldkamp et al. [26] reasoned that Aclp is a potential candidate gene for gastroschisis due to its developmental expression, association with the extracellular matrix and is essential for abdominal wall development. From this mechanistic perspective, Feldkamp et al. proceeded with assessing AEBP1 gene variants in 40 cases of infants with gastroschisis. These investigators identified several novel variants in AEBP1, but the frequency of these variants did not significantly differ between the cases and the control group. Additionally, they used bioinformatics programs to predict the likely amino acid changes, and these changes were predicted to be benign.

With the hypothesis that genes related to vascular compromise may interact with environmental factors to affect the risk of gastroschisis, Padula et al. [27] conducted a population-based case-control study of 228 infants in California. They evaluated 75 genetic variants in 20 genes and the risk of gastroschisis associated with maternal age, race or ethnicity, vitamin use, and tobacco smoking exposure. These investigators found 11 gene variants with increased risk and four variants with decreased risk of gastroschisis for heterozygous or homozygous variants genotypes and suggested that NOS3, ADD1, ICAM1, ICAM4, and ICAM5 warrant further investigation and may interact with environmental exposures.

Makhmudi et al. [28] sought to assess the hypothesized dual vascular/thrombotic pathogenesis of gastroschisis in which it is argued that normal right umbilical vein involution forms a possible site for thrombosis adjacent to the umbilical ring. Accordingly, these investigators measured the frequency of three common prothrombotic polymerphisms, MTHFR c.677C>T, F2 c.20210G>A, and F5 Leiden in 46 Indonesian gastroschisis patients and in 89 ethnicity-matched controls. While MTHFR c. 677C>T showed a significant association with gastroschisis (OR= 2.1), no affected infants had risk alleles for either F2 c. 20210G>A or F5 Leiden. This finding lends support to the thrombotic pathogenesis hypothesis for gastroschisis.

As a robust alternative to the vascular pathogenesis hyp-othesis for the occurrence of gastroschisis, an umbilical ring defect theory has been proposed, based on embryological studies. [29; 30] The embryological argument is that if a vascular insult to the vitelline artery were to be the proximate cause of gastroschisis, then the entire midgut should be injured rather than the abdominal wall, which gets its vascular supply from the intersegmental arteries. In turn, if insults to these vessels caused gastroschisis, then there is no explanation for the preponderance of gastroschisis occurring on the right rather than equally on the left. If the occurrence of gastroschisis does indeed derive from disordered development or inadequate maintenance of the structural integrity of the umbilical

ring and its amnion-epithelial connection, then cellular and molecular systems that regulate tissue organization, cellular signaling, and movements while maintaining the structural integrity of adjacent tissues should be evaluated.

II RISK FACTORS

In 1990, Goldbaum et al. [31] reported on their assessment of risk factors that might explain an observed increase in gastroschisis in residents of Washington State during the interval 1984-1987. They reviewed birth certificates for 62 infants born with gastroschisis and 617 randomly selected unaffected infants matched for birth year. The four risk factors that "stood out" were a birth month (higher risk in January, February, and March); mothers age younger than 25 years and especially age younger than 20 years; tobacco smoking during pregnancy; and mothers receiving inadequate prenatal care. These investigators suggest that it is likely that other unidentified behaviors and environmental exposures could explain the risk associations with birth month, young maternal age, and limited prenatal care.

In a different U.S. region, Chabra & Hall [32] assessed a single center cluster of 10 cases of gastroschisis at the Neonatal Intensive Care Unit at the University of Kentucky Medical Center in 1996. These investigators then retrospectively sought environmental or genetic causes. They reviewed the maternal and patient medical records for 36 neonates with gastroschisis admitted from January 1992 to December 1996. While there was evidence that many of the mothers were teenagers, primiparous, and had an increased frequency of tobacco smoking, they found no evidence of temporal or spatial clustering in the gastroschisis cases.

In an excellent comprehensive review about a decade ago, Rasmussen and Frias [33] summarized what was known about non-genetic risk factors for gastroschisis. These authors highlighted several key observations that in turn suggested directions for future research opportunities. With some degree of disappointment, research needs mostly remain current and we hope to suggest some new directions that merit attention.

Rasmussen and Frias noted that the etiology of gastroschisis is unknown, but the familial increased risk (approximately 3.5%) in the families with a previous child with gastroschisis suggested that genetic factors play a role in its causation. While that genetic relationship is logical, it is also true that cohabiting family members share many of the same environmental exposures, so the same reasoning could apply to support the notion that a familial pattern of risk may be attributable to sharing a common multifactorial “risky” environment.

Beyond the widely demonstrated risk of younger maternal age, other robust individual exogenous risk factors are not as consistently found. There is some evidence that exposures such as sociodemographic factors, maternal medication use, substance use/abuse, workplace or environmental chemical exposures do correlate with some increased risk of gastroschisis. Since a prevailing hypothesis is that gastroschisis is often due to some vascular disruption during early development, it can be argued that all of these are candidates for clinical or public health protective or mitigative interventions. For most of these exposures, it is reasonable to suppose that gene-environment interactions are key to manifestation of the outcome of gastroschisis, and there is some evidence that this notion is applicable to the risk factor of maternal tobacco smoking and the occurrence of variant alleles of genes such as NOS3, NPPA, and ICAM1 involved in the VEGF-NOS3 pathway [34].

Maternal illnesses, medication use, and substance abuse

Several groups of investigators have studied the possible impact of several exogenous factors in the risk of gastroschisis. Quite logically the efforts have focused on maternal exposures in the periconception and early gestational intervals when key embryonic developmental events might be influenced in a cause and effect manner. Several factors, including maternal demographics, medical illnesses, environmental exposures, use of prescribed and over-the-counter medications, consumption of ethanol, and use of recreational drugs, have been assessed to some extent in the risk of gastroschisis.

Werler et al. [53] analyzed data from a case-control surveillance program of birth defects from 1976-1990. They compared maternal demographic, reproductive, and medical factors and first-trimester environmental expoures between 76 gastroschisis cases and 2,581 malformed controls. They found the expected strong inverse association with maternal age but also detected a relationship of increased risk of gastroschisis with maternal ethanol consumption, roughly doubling or trip-ling the risk.

In another study of multiple possible risk factors for gastroschisis, Torfs et al.[36] reported on several associations for maternal medications and environmental exposures as follows:

- Hobby or occupational exposures to solvents (odds ratio (OR)=3.8 or colorants (OR=2.30);
- Use of medications aspirin (OR=4.7) and ibuprofen (OR=4.0) but not for acetaminophen;
- Periconceptional exposure to X rays (OR=2.5);
- Use of antibiotics, antinauseants, sulfonamides, or oral contraceptives were not associated;
- Use of decongestants, pseudoephedrine (OR=2.1), phenylpropanolamine (OR=10.0), group of all decongestants including oxymetazoline and ephedrine (OR=2.4).

From these observations, the authors remarked that since most of these associations were for vasoactive substaances, their results support a vascular hypothesis for the pathogenesis of gastroschisis.

Carrying forward the idea of vascular disruption as a critical component in the events that may lead to gastroschisis, Hume et al. [37] considered the potential effect of prenatal cocaine exposure in gastroschisis. These investigators performed a retrospective case-control study

based upon more than 68,000 delivery records at a single hospital for 9 years. Transverse limb defects and gastroschisis were defined as cases, and non-disruption anomalies served as controls. In 190 cases of limb anomalies, abdominal wall defects, and cleft lips, 119 cases had information regarding maternal cocaine use during pregnancy. Hume et al. found 7 of 34 vascular disruption cases associated with cocaine exposure versus 12 of 85 other malformations controls yielding an odds ratio for cocaine exposure and vascular disruption of 1.58 (95% CL: 0.55-4.47). Although there are limitations to these data, these results did not seem to support the idea that prenatal cocaine exposure might influence gastroschisis risk via a vascular disruption effect.

A few reports suggest an association between other drugs of abuse and the occurrence of gastroschisis. In two studies by one group of investigators [38; 39], maternal exposures to recreational drugs were ascertained by measurement of individual recreational drugs in maternal hair samples timed for the period of conception and in different stages of pregnancy in expectant mothers with a diagnosis of fetal gastroschisis and in a group of wowen with a normal fetus as controls. Overall, these investigators found evidence of recreational drug use in 18% of women with fetal gastroschisis and confirmed the association of gastroschisis with young maternal age. In another recent report, a higher prevalence of gastroschisis was found in regions with the US where rates of opioid prescriptions were highly indicative of an association between opioid use during pregnancy and gastroschisis. [40]

While most reports on gastroschisis derive from clinical investigations, Burdan et al. [41] have presented data in an animal model to compare the effects of drugs within a single category, namely cyclooxygenase inhibitors. The effects of prenatal exposure to various selective and non-selective cyclooxygenase-2 (COX-2) inhibitors on abdominal wall defects in the rat were assessed by a retrospective analysis of laboratory data from several teratological studies with COX-inhibitors (aspirin, DFU, DuP-697, ibuprofen, paracetamol, piroxicam, propyphenazone, tolmetin) throughout 1997-2004. In 6744 live-born fetuses, abdominal wall defects were found in four animals exposed to different non-selective COX inhibitors and one case of gastroschisis in a rat exposed to a selective COX-2 inhibitor. In their analysis of the various drugs, only aspirin statistically increased the risk of abdominal wall defects in rat fetuses with an expected ratio for aspirin of 56 per 10,000 offspring.

A cluster of gastroschisis cases (n=14) was observed in April 2007- April 2008 in Reno, Nevada. Elliott et al. [42] performed a case-control study to identify potential risk factors. In comparison to controls (n=57; matched 4:1 to the case mothers by maternal date of birth within 1 year) , gastroschisis was associated with the prepregnancy use of methamphetamine (OR=7.15) or any vasoconstrictive recreational drugs (methamphetamine, amphetamine, cocaine, ecstasy) with OR of 4.46. These findings support the notion that use of vasoconstrictive recreational drugs is a risk factor for gastroschisis.

The commonly used drugs of alcohol (ethanol), tobacco, and cannabis have also been implicated as risk factors for gastroschisis. First, in the large multicenter National Birth Defects Prevention Study (6622 control infants and 1768 infants with birth defects born in 1997-2005), Richardson et al. [43] performed a case-control study to assess any association between periconceptional alcohol consumption and several birth defects including omphalocele and gastroschisis. These investigators found that periconceptional alcohol consumption was associated with omphalocele (OR=1.50; 1.15-1.96) and gastroschisis (OR=1.40; 95% CL: 1.17-1.67). Second, as regards maternal smoking, Hackshaw et al. [44] conducted a meta-analysis using data from 172 articles with a total of 173,687 malformed cases and 11,674,332 unaffected controls and reported a significant positive association of maternal smoking with gastroschisis (OR=1.50, 95% CL:1.28-1.76). Additionally, it has been hypothesized that differences in genetic susceptibility may exist regarding this association of smoking with gastroschisis. In one study that investigated this possibility, Jenkins et al. [45] analyzed five single nucleotide polymorphisms in three genes (CYP1A1, CYP1A2, and NAT2) that encode for enzymes involved in metabolism of some cigarette smoke constituents in 108 non-Hispanic white and 62 Hispanic families who had infants with gastroschisis, and 1,147 non-Hispanic white and 337 Hispanic families who had liveborn infants with no significant structural birth defects (controls). While these investigators did identify three suggestive associations among 30 potential associations, they concluded that these data did not support the occurrence of effect modification between periconceptional maternal smoking and most of the xenobiotic metabolizing enzyme gene variants assessed. Third, using multiple logistic regression, van Gelder et al. [46] reanalyzed associations between periconceptional cannabis use and 20 specific birth defects using data from the National Birth Defects Prevention Study from 1997-2005 for 13,859 case infants and 6,556 control infants and found that after correction for exposure misclassification, cannabis use was associated with gastroschisis with an OR of 1.7.

A modest number of prescription medications have also been implicated as risk factors for gastroschisis when mothers have used such drugs at various intervals from preconception through approximately the third month of pregnancy. It must be noted that any association of gastroschisis risk may be due either to a) the underlying maternal disease for which a drug was prescribed, b) use of the drug *per se* or c) the co-exposure of the embryo/fetus to the maternal disease and the drug in question. Nonetheless, the drugs for which there is some evidence of an associated risk include venlafaxine [47], antiherpetic medications (acyclovir, valacyclovir or famciclovir) [48], levonorgestrel/ethinylestradiol [49], antidepressants [50], topical antivirals [50], and continuation of oral contraceptives in early pregnancy [50]. As noted by Given et al. [50], "While it is difficult to disentangle the

effects of the medication and underlying indication, our results add to the evidence base on preventable risk factors for gastroschisis. These risk factors may contribute to the higher risk among young mothers, and geographical and temporal variation in prevalence." Additionally, another infectious agent, chlamydia trachomatis, has been suggested by Feldkamp et al. [51] as a risk factor for gastroschisis based on observation of the unusual finding of vacuolated amniotic epithelium with lipid droplets in gastroschisis placentas, combined with some experimental evidence of the trafficking of host lipids into the chlamydia intracellular inclusions.

Maternal nutrition and metabolic milieu

In order to diminish the risk and/or severity of gastroschisis, other than identifying and limiting exposures to possible exogenous hazards, endogenous maternal factors that could be favorably modified should be considered. These factors, which include various exogenous-endogeenous (gene-environment) interactions, encompass at least metabolic, nutritional, vascular, and inflammatory components.

In a past animal study of gastroschisis, interactions of nutritional factors and a smoking-related component were studied. Singh [52] maintained pregnant CD-1 mice on diets with two levels of protein and three levels of zinc, and exposed half of each diet subgroup to either air (control) or to 500 ppm carbon monoxide (CO) from gestation days (GD) 8-18. At necropsy, fetal mortality and malformations were increased by protein and zinc deficiencies, and CO exposure increased fetal mortality. The incidence of gastroschisis in the low protein/zinc deficient/CO exposed group was 47%, and 60% of the litters were affected. The incidence of gastroschisis in the rest of the low protein/zinc diets/air or CO groups was zero. In this model, gastroschisis is caused by the combination of protein-zinc deficiencies plus CO exposure during gestation and thus may be relevant to human populations who may have nutritional deficiencies and exposure to CO via environmental or maternal smoking (tobacco or cannabis).

In another past study of 57 cases of gastroschisis and 506 controls, Torfs et al. [53] tested for DNA polymorphisms of 32 genes representing enzymes involved in angiogenesis, blood vessel integrity, inflammation, wound repair, and dermal or epidermal strength. These investigators found that several gene polymorphisms were associated with an increased risk for gastroschisis for heterozygotes (ICAM1 gly241arg; NOS3 glu298asp; NPPA 2238 T>C; and ADD1 gly460trp) and that for the NPPA and ADD1 single-nucleotide polymorphisms (SNPs), the homozygote variants had a significantly higher risk than the heterozygotes. Additionally, three SNPs (NOS3; ICAM1; and NPP) showed a strong interaction for risk with maternal smoking, supporting the hypothesis that gene-environmental interactions are a part of the vascular compromise that is plausibly involved in the etiology of gastroschisis.

Maternal nutrition and either low maternal body mass or maternal obesity have been considered in some studies of gastroschisis. Waller et al. [54] assessed the relationship between maternal obesity, overweight and underweight status, and 16 categories of structural birth defects in mothers enrolled in the National Birth Defects Prevention Study who had index pregnancies between October 1, 1997, and December 31, 2002. These investigators found that mothers of offspring with omphalocele were significantly more likely to be obese than mothers of controls (ORs between 1.33 and 2.10) while mothers of offspring with gastroschisis were significantly less likely to be obese than mothers of controls. These results suggested a strong inverse association of obesity with gastroschisis. In another study, Siega-Riz et al. [55] evaluated the joint effects of maternal pre-pregnancy body mass index and maternal age on the risk of gastroschisis. These investigators reported that younger women who had lower BMI were at highest risk of having an infant with gastroschisis. For example, a 15-year-old woman with a BMI of 17 has seven times the odds of having an offspring with gastroschisis compared with a 24-year-old woman with a BMI of 23. Finally, Paranjothy et al. [56] emphasized the importance of maternal nutrition in the etiology of gastroschisis in their study. These investigators assessed high maternal alcohol consumption and poor diet in the first trimester as risk factors in a case-control study in the UK. Their results showed that high consumption of fruits and vegetables during the first trimester (OR=0.2), taking folic acid for at least 6 weeks during the first trimester (OR=0.3) and increased body fat percentage of total maternal body weight (OR=0.9) were independently associated with reduced risk and that cigarette smoking (OR=2.7) was an independent factor for increased risk.

In summary, beyond the apparent need to reduce or avoid smoking by women in the prepregnancy and gestational intervals, interventions to attain better nutritional status in terms of both diet quality (intake of fruits and vegetables) and adequacy of caloric intake particularly for younger gravidas, must be part of the public health effort to reduce the risk of gastroschisis in infants.

Environmental exposures

One general hypothesis for any disease showing an increase over time is that changing environmental, dietary or occupational exposures might be a causal factor. Therefore, a modest number of studies have tried to determine if various exposures from those sources might be implicated in gastroschisis.

A few studies have assessed the risk of various birth defects including gastroschisis in populations residing near hazardous waste landfill sites. In the EUROHAZCON study, Dolk et al. [57] studied the risk of congenital anomalies near hazardous-waste landfill sites in Europe. For overall congenital anomalies, residence within 3 km of a landfill site was associated with a significantly raised risk (295 cases/511 controls living 0-3 km from sites, 794/1855 living 3-7 km from sites) with an OR=1.33. For gastroschisis alone, the risk was only borderline signifi-

cant with OR=3.19 [0.95-10.77]). In another study in the UK, Fielder et al. [58] studied the health of residents living near the Nanty-Gwyddon landfill site. While their neonatal data showed some complexity, they reported that there was no consistent difference in the proportion of low birth-weight infants before and after the opening of the landfill site. Additionally, among resident living near the site, there was an increased risk of newborns having a congenital abnormality, but this was seen both before (relative risk, RR=1.9; 95% CL: 1.3-2.85; P<0.001) and after (RR=1.9; 95% CL: 1.23-2.95; P=0.003) its opening. However, they noted that an observed cluster of cases of gastroschisis was seen only after opening of the site. Finally, a broader study in the UK also assessed the risk of adverse birth outcomes in populations living near landfill sites. Elliott et al. [59] identified populations living within 2 km of 9,565 landfill sites operational at some time between 1982 and 1997 and included more than 8.2 million live births, 43,471 still-births, and 124,597 congenital anomalies (including terminations). For all anomalies combined, the relative risk of a residence near landfill sites was not convincingly associated with risk (slight decrease for unadjusted OR=0.92; slight increase for adjusted OR=1.01). However, for abdominal wall defects and surgical correction of gastroschisis and omphalocele, they found adjusted risks with OR=1.08 (95% CL: 1.01-1.15) and OR=1.19 (95% CL: 1.05-1.34) respectively. In summary, if there is any association of residence near waste landfill sites and gastroschisis, then the association is quite weak. The strength of evidence does not support a public health advisory to relocate residences away from such landfill sites to mitigate the risk of gastroschisis.

Another hypothesized route of exposure that might influence gastroschisis risk is drinking water. One study [60] in North Carolina considered this possibility. Root and Emch traced drinking water to its sources and how those sources related to the locations of textile mills. These investigators found no association between prenatal exposure to upstream textile mill effluent and risk of gastroschisis; however, they did report an increased risk in women who relied on public water systems that drew from a surface water source rather than a ground-water source.

Seemingly, limited consideration has been given to possible maternal occupational exposures as a risk factor for gastroschisis. In a single report, Lupo et al. [61] studied maternal occupational exposure to polycyclic aromatic hydrocarbons (PAHs) in the National Birth Defects Prevention Study. In this large data set, the prevalence of estimated occupational PAH exposure was 9.0% in case mothers (27 of 299) compared to 3.6% in control mothers (107 of 2993). Remarkably, they found a significant association between occupational PAHs and gastroschisis among mothers at or older than 20 years of age (adjusted OR=2.53; 95% CL: 1.27-5.04); however, they did not find such an association in mothers younger than 20 years (adjusted OR=1.14; 95% CL: 0.55-2.33). The investigators noted that this observation might be meaningful since, on the one hand, young maternal age is the most potent known risk factor for gastroschisis while on the other hand, most gastroschisis cases are born to mothers at age of more than 20 years.

The plausibility of dietary or environmental chemicals as teratogens that can cause congenital abdominal wall defects including gastroschisis is generally supported by studies in animal models [62; 60]. In attempting to translate/correlate data from animal models with observations in human populations, at least one caution is waranted. There may be discrepancies in the nomenclature used by laboratory teratologists and that used by physicians and epidemiologists. These differences may substantially matter since evidence broadly suggests that in human populations, rates of omphalocele have not changed while gastroschisis rates have increased. With this provision, compounds that have been implicated in animal models include mycotoxins [62] as well as a wide range of other compounds, reported by van Dorp et al. [63] to include induction of umbilical cord hernia by 8, omphalocele by 9 and gastroschisis by 22 teratogens.

Distinct exposure category – agricultural chemicals

One distinct category of potential chemical exposures that can be distinguished by possibly having shared occupational, residential and/or dietary routes of exposure is agricultural chemicals such as herbicides, fertilizers, fungicides, pesticides, and petroleum products. Indeed, a few reports suggest that some such exposures may be risk factors for gastroschisis.

Waller et al. [64] conducted a retrospective, case-control study using Washington State Birth Certificate and US Geological Survey databases in 805 cases defined as all live-born singleton infants with gastroschisis and 3616 controls. Gastroschisis occurred more frequently among those who resided 25 km from a site of high atrazine concentration (OR=1.6). The risk was related inversely to the distance between the maternal residence and the closest toxic atrazine site. In multivariate analysis, nulliparity, tobacco use, and spring conception were significant predictive factors for gastroschisis. Based on these data, these investigators remarked "maternal exposure to surface water atrazine is associated with fetal gastroschisis, particularly in spring conceptions." In a subsequent study conducted in Texas using similar study techniques, Agopian et al. [65] evaluated the relationship between maternal atrazine exposure and gastroschisis risk by maternal age in 1,161 gastroschisis cases and 8,390 controls. In this latter study [65], there was no association of maternal atrazine exposure and gastroschisis risk in women under 25 years of age; however, there was an increased risk for gastroschisis in offspring of women at or older than 25 years with counties of residence that had higher levels of potential residential atrazine exposure. These investigators noted that these results suggest that the etiology of gastroschisis may vary based on maternal age.

In an interesting study in an animal model, the possibility of a derivative of two different classes of agricultural chemicals (atrazine and nitrates) was assessed for inducti-

on of embryonic malformations. Given the findings cited above about atrazine as a possible risk factor for gastroschisis, consider as well that the U.S. Geological Survey states on its website [The USGS Water Science School; https://water.usgs.gov/edu/nitrogen.html] "Nitrate can get into the water directly as the result of runoff of fertilizers containing nitrate." Thus, Joshi et al. [66] studied the effects of developmental exposure of the chicken to the potential reaction product of nitrate and atrazine N-nitrosoatrazine (NNAT). These investigators treated fertilized eggs with 0.06-3.63 μg NNAT and continued incubation for five more days. With 90% survival, 23% of embryos were malformed. Multiple malformations were documented, among these gastroschisis.

Finally, two investigations have attempted to take a broader approach to assess the potential risk of gastroschisis in association with exposures to various multiple agricultural chemicals. Using data from the National Birth Defects Prevention Study among employed women, Kielb et al. [67] conducted a multi-site case-control analysis that included the following designations: any occupational exposure (yes/no) to pesticides, to insecticides only, to both insecticides and herbicides (I +H) and to insecticides, herbicides and fungicides (I+H+F). The data showed that occupational exposure to I+H+F was associated with the risk of gastroschisis among infants of women at or older than 20 years (OR=1.88) but not for women younger than 20 years of age. Shaw et al. [68] studied 156 cases (infants/ fetuses with gastroschisis) and 785 controls (infants without birth defects) regarding early pregnancy agricultural pesticide exposures and risk of gastroschisis in the San Joaquin Valley of California. The investigators analyzed associations of gastroschisis with 22 chemical pesticide groups and 36 specific pesticide chemicals. No association was found with any of the pesticide groups, and among the specific pesticide chemicals, only exposure to petroleum distillates was associated with an elevated risk (OR=2.5; 95% CL: 1.1-5.6).

In summary, there are some data suggesting associations between exposure to a few agricultural chemicals and risk of gastroschisis. As a general public health approach, it would be prudent to minimize exposure of women pre-pregnancy and during early weeks/months of gestation to commonly used agricultural chemicals for which there is some concerning data.

III. POTENTIAL MOA HYPOTHESES

A few mode-of-action (MOA) hypotheses have been proposed as explanations for associations of gastroschisis with various exposures. As cited earlier in this article, several investigators have argued that a vascular compromise MOA for gastroschisis seems to cogently link several observations [27; 28; 33; 34; 36; 37; 53].

Other plausible hypotheses for gastroschisis were reviewed and two new hypotheses were advanced in back-to-back publications [69; 70] a few years ago. Feldkamp M et al. [69] carefully considered existing alternative embryonic hypotheses for the occurrence of gastroschisis as follows:

a) Failure of mesoderm to form in the body wall;
b) Rupture of the amnion around the umbilical ring with subsequent herniation of bowel;
c) Abnormal involution of the right umbilical vein leading to weakening of the body wall and gut herniation; and
d) Disruption of the right vitelline (yolk sac) artery with subsequent body wall damage and gut herniation.
e) These investigators commented that in their view, that these hypotheses were not adequate to explain how gastroschisis could occur, and thus proposed an alternative embryonic hypothesis; namely,
f) Abnormal folding of the body wall results in a ventral body wall defect through which the gut herniates, leading to the clinical presentation of gastroschisis.

In the second of these two publications, Chambers et al. [70] described conduct of a case-control study to compare the prevalence of change in paternity with the index pregnancy in 102 mothers of gastroschisis cases to that in 117 mothers of non-malformed infants and in 78 mothers of infants with neural tube defects or oral clefts. After adjustment for maternal age, change in paternity in multigravid gastroschisis case mothers was higher (OR =7.81; 95% CL: 2.80–21.88) relative to multigravid mothers of malformed and non-malformed controls combined. Based on these results, the investigators suggest support for an additional hypothesis that maternal immune factors may play a causative role in gastroschisis.

These several hypothesized MOAs are plausible but do not offer the degree of detail about cellular or molecular processes, signaling cascades and/or feedback (dys) regulation that is fully satisfying for a deep understanding of the basis for the occurrence of this malformation. Additionally, at present these hypotheses do not suggest potential protective (preventive or mitigative) interventions other than the general notion of avoidance of suspect exposures. To take meaningful translational toxicology/teratology steps and strive toward identifying one or more safe and efficacious protective nutritional, lifestyle or pharmaceutical therapies, new cellular/ molecular MOAs need to be considered and investigated. We suggest three as follows:

a) most tentatively, changes in epigenetic programing of relevant stem or progenitor cells;
b) mechanical forces MOA meaning the mechanobiology of collective cell behaviors; and
c) ephrin–Eph signaling as a developmental MOA.

As a dynamic field of study, epigenetics is seemingly involved in or relevant to almost every area of biological regulation. We may have overlooked some reports, but we have not seen published evidence suggesting that epigenetic programming has been explicitly investigated in gastroschisis. To illustrate the range of potential effects that epigenetic mediation of exposures could exert, consider the comments of Bateman et al. in a recent review

[71]. These authors noted that several environmental chemicals often-called endocrine disrupting chemicals (EDCs) might induce the changes in mesenchymal stem cells (MSCs). Such actions may include alterations in adipogenic differentiation, osteogenic differentiation, activation of pro-inflammatory cytokines, oxidative stress, trophic factor production, the immune-modulatory capacity of MSCs, differentiation into appropriate cellular lineages, and paracrine signaling in wound healing. Several of these effects could be a clue with relevance to basic mechanisms by which gastroschisis occurs, at least in some cases.

MOA - mechanical forces – mechanosensitivity and mechanotransduction signaling

A potentially robust MOA underlying gastroschisis could be in the mechanobiology of collective cell behaviors [72; 73; 74; 75; 76; 77]. Cells and cell collectives respond to multiple non-mechanical signals and gradients such as gradients of diffusible molecules and electrical fields but also mechanical signals. Cells sense chemical and mechanical signals in their local microenvironment and both classes of signals regulate gene expression programs in the nucleus. With consideration of possible disordered signaling in the occurrence of gastroschisis, the fates of cells and cell collectives in development are subject to mechanical and morphological cues that function as critical signaling mechanisms. Ligands provide cues in the extracellular matrix (ECM), but also physical properties including ECM stiffness, cell shape, cell-substrate adhesion, cell-cell adhesions, and cytoskeleton architecture all of which inform cells and cellular collectives of their respective surrounding locale.

Mechanical properties of the external environment influence the coordinated behaviors of cells during key biological processes such as morphogenesis and tissue remodeling [72; 73; 74; 75; 76; 77]. Collections of cells interact with both the surrounding extra-cellular matrix and with neighboring cells. The behavior of cellular collectives depends upon active interactions among cells to affect their movements. Mechanosensitive adhesion complexes regulate such collective movements at the cell-substrate interface as well as cell-cell junctions. Both types of conections permit cellular responses but also propagate physical signals. From Vining and Mooney [75], "Mechanical forces regulate cell fate decisions during organogenesis as progenitor cells are directed to diverse specialized functions in fetal organs.

Complex patterning depends on cell–ECM interactions. Biochemical cues initiate morphogenesis, but the formation of cell layers that become organized into defined structures in organs requires physical traction forces on the ECM, the physical properties of which provide a template for organ growth.

The concerted action of biochemical signals, cell-intrinsic forces, and cell–ECM interactions result in highly organized patterns of development, such as fractal patterns observed in branching morphogenesis. As development progresses, intrinsic forces exerted by cells transition from largely cell–cell to more cell–extracellular matrix (ECM) transmission because of matrix content in tissues increases."

There is a growing understanding of the mechanobiology of collective cell movements. Single cells use actomyosin contractility to exert traction forces on the extracellular matrix (ECM) at integrin-based adhesions. Adhesion complexes play a role in mechanosensitivity and mechanotransduction signaling in collective cell behaveiors. Additionally, various physical properties of the cellular environment can regulate collective cell behaviors, tissue organization and cell-generated forces that encompass molecular, cellular and tissue levels. Single cells and cells migrating in cell collectives polarize by extending lamellipodia at their contact with ECM or with other cells at the boundary. On the scale of collective cell movements, individual cells may exert traction on either the ECM or on neighboring cells.

Cell division and extrusion also alter tissue movement and contribute to the propagation of strain and velocity waves driven by mechanobiochemical signals. Cells on a leading edge also form focal adhesions.

Focal adhesions depend upon some key functional proteins that regulate mechanical coupling of cells. A prominent group consists of the cadherins including epithetlial (E)-cadherin, neuronal (N)-cadherin, placental (P)-cadherin and vascular-endothelial (VE)-cadherin as well as cadherin 6, cadherin 7 and cadherin 11. The junctional protein afadin interacts with nectins in adherens junctions, which seem to support tissue integrity during collective cell migration and morphogenesis [75; 76; 77; 78; 79]. Additionally, zonula occludens proteins ZO1, ZO2, and ZO3, desmosomes and intermediary filaments, in tight junctions also appear to contribute to intercellular mechano-coupling [80].

From Ladoux and Mege [72], "Substrate geometry influences the mode of collective migration. In areas of positive curvature (for example, the tips of finger-like structures pointing into the gap), cells predominantly move by active crawling. In areas of negative curvature (that is, where the gap bows into the tissue), prominent actomyosin cables are formed. Actomyosin contractility and active cell crawling operate additively in gap closure, leading to faster tissue velocity in regions of negative curvature. Cellular coordination, which is at the basis of various phenomena, most prominently including tissue shaping during morphogenesis, is a mechanoregulated, multiscale process integrating events on the molecular, cellular and multicellular scales that can be observed at a wide range of timescales, from milliseconds to days. Cellular mechanosensitivity and mechanotransduction signaling is intimately integrated with transcriptional programming, epigenetic modifications, and biochemical differentiation. Together these signals interact to influence cell fates. Tatapudy et al. [73] have summarized interrelationships among metabolism, reactive oxygen species (ROS), intracellular pH (pHi), and cell morphology as follows:

"Metabolic inputs regulate epigenetics and cell signaling to promote changes in cell fate. Glycolysis produces metabolic intermediates that feed into the folate and one carbon metabolism cycle to

a) Produce S-adenosylmethionine (SAM), which is a cofactor for DNA methyl-transferases (DNMTs) and histone methyl-transferases (HMTs).
b) Glucose-derived acetyl-CoA enters the tricarboxylic acid (TCA) cycle to form citrate, which can be converted back to acetyl-CoA by ATP-citrate lyase. This source of acetyl-CoA (but not acetyl-CoA derived from fatty acid oxidation) contributes to the pool of nuclear acetyl-CoA that is essential for histone acetylation by histone acetyl-transferases (HATs).
c) a-ketoglutarate (a-KG), which is produced in the TCA cycle and the cytoplasm, is an essential cofactor for TET and Jumonji C enzymes, which demethylate DNA and histones, respectively.

Increased oxidative phosphorylation also generates reactive oxygen species (ROS), which promote oxidation, carbonylation, and hydroxylation as well as increase the levels of JNK and p38/MAPK pathway activity."

The three-dimensional organization of chromosomes in the nucleus regulates gene expression patterns, and chromosomal organization is regulated by nuclear mechano-transduction [74]. These signals are sensed and transduced in the nucleus through the cytoskeleton, and its constituents of actin, microtubules and regulatory molecules wherein actin exerts contraction and microtubules exert compression. This regulatory relationship has been hypothesized by Uhler and Shivashankar [74] as follows: "…mechanosensing of the extracellular signals from the microenvironment results in both activation of specific transcription factors and modulation of the cytoskeleton–nucleus links, leading to the arrangement of a particular chromosome and gene neighborhoods. The particular chromatin spatial configurations and post-translational modifications are important for guiding transcriptiton factors to their target genes and obtaining optimal transcriptional outputs to maintain cellular homeostasis."

Mechanical coupling enables stem cells to respond to their local environment and to store information over time [75]. For example, changes in ECM induced by cells early in development can mechanically trigger changes in interacting cells at a later stage. These interactions regulate cell behavior and influence cell fates in development both in that locale and more distantly. In addition to intrinsic cell-generated forces, extrinsic shear, tension, and compression forces can be sensed by signaling molecules such as ion channels, modified receptor–ligand and mechanical changes in the cytoskeleton.

Mechanosensitivity signaling primarily depends upon cell-cell adhesion complexes in early development. As progenitor cells differentiate later in development, they produce and adhere to ECM. As the ECM content of tissues increases during development, cellular mechano-sensing also increases allowing cells to respond to changes in ECM characteristics such as stiffness, adjacent deposition, and local degradation.

MOA – Ephrin-Eph rceptor signaling

Eph proteins are transmembrane Tyr kinase receptors that interact with ephrins, which are membrane-tethered ligands. Eph receptors are grouped into EphA or EphB subfamilies depending on whether they preferentially bind to ephrin-As (membrane-anchored) or ephrin-Bs (transmembrane ephrin ligands). This system provides short-distance cell-cell signaling that affects the cellular cytoskeleton, leading to cell-cell repulsion and sometimes cell-cell adhesion. This ephrin-Eph receptor mechanism appears to play an important role in pattern formation and morphogenesis by influencing cell sorting and positioning during developpment [84]. Since short-distance signaling between neighboring cells can be mediated by Eph receptors and ephrin ligands, this system can direct cell repulsion, cell-cell adhesion, cell proliferation, tissue boundary formation, and cell migration. Regarding possible relevance to the occurrence of gastroschisis, ephrin–Eph signaling regulates developmental cell sorting at tissue compartment boundaries which is plausibly important in normal abdominal wall formation.

Eph receptor-ephrin signaling is multimodal [84]. While Eph proteins behave as classical receptors and ephrins as their ligands, signaling also occurs in the counter-direction with Eph receptor proteins acting as ligands for the respective ephrins. This evokes simultaneous bidirectional intracellular signals in the respective cells. The complex pattern of Eph-ephrin signaling can be sumarized as follows:

a) Forward signaling – signal transduction goes from ephrins to Eph receptors;
b) Reverse signaling – signal transduction goes from Eph receptors to ephrins;
c) Bidirectional signaling – signal transduction simultaneously activates downstream pathways for both Eph receptors and ephrins;
d) Parallel signaling - signal transduction in which Eph receptors and ephrins on the same cell; activate in response to their respective ephrins and Eph receptors on a neighboring cell;
e) Anti-parallel signaling – signal transduction in which there is simultaneous ephrin-Eph receptor forward signaling but in counter directions.

Regarding molecular behavior on cell surfaces, Eph receptor-ephrin signaling depends upon interactions as multimers in signaling clusters. These signaling clusters appear to be important in cellular boundary formation during embryonic development. On the one hand, some embryonic boundaries are unstable and thus permit cell movements, but later stable boundaries allow segmentation of groups of cells and establishment of distinct tissues. A balance of adhesive and repulsive forces permit formation of embryonic boundaries such as separation of the cardinal embryonic germ layers, embryonic segmentation and fractal-like development of the branched tub-

ular networks including blood vessels, lymphatics, and pulmonary architecture.

The erythropoietin-producing hepatocellular (Eph) family of receptor tyrosine kinases regulate a multitude of physicological and pathological processes. Noberini et al. [85] performed a high throughput screen of small molecules as potential ligands for the extracellular domain of the EphA4 receptor. These investigators found that a 2, 5-dimethyl-pyrrolyl benzoic acid derivative, as well as a number of other molecular ligands, could inhibit the interaction of EphA4 with a peptide ligand and with natural ephrin ligands. Among the investigational compounds, two isomers acted as competitive inhibitors selectively at the Eph-A4 and the closely related EphA2 receptor [85; 86]. These findings demonstrated that small inhibitory molecules could selectively target the Eph receptor-ephrin signaling system.

IV. PROSEPCTS FOR PROTECTIVE/MITIGATIVE THERAPIES: RESEARCH DEVELOPMENT NEEDS

The core "forward-looking" question is as follows:

- Are the exogenous risk factors for gastroschisis modifiable?

Key corollary questions include

- How early can gastroschisis be diagnosed?
- Could early intervention allow mitigation that would reduce the severity of individual cases by the institution of some safe and at least partially effective therapy? In other words, could a therapy shift complex cases to be manifest as more straight-forward cases of gastroschisis?
- Ultimately in the future, could true preventive (risk reduction) interventions be proven and implemented to reduce some occurrences altogether?

While accelerated and delayed early embryonic growth *in utero* can be measured by ultrasound between 6 and ten gestational weeks [87], Khan et al. [88] summarized the current prospects for *in utero* gastroschisis imaging *per se*. Since gastroschisis represents a herniation of abdominal contents through a paramedian full-thickness abdominal fusion defect usually to the right side of the umbilical cord, then in early pregnancy, loops of bowel may be seen floating in the amniotic fluid. While gastroschisis is usually detected before 20 weeks of gestation by ultrasound, with transvaginal ultrasound, the diagnosis can be made as early as 12 weeks of gestation. Although antenatal ultrasound imaging is the primary means for diagnosis, detection rates are only about 70%. Presumably, diagnostic performance is limited by both being operator dependent and due to artifacts in imaging such that visualization of a 2-5 cm defect in the right paraumbilical region to make the diagnosis of gastroschisis can be missed. Finally, while the anterior abdominal wall and umbilical cord insertion can be readily recognized on ante-natal scanning, the inner aspect of the anterior abdominal wall can be challenging to distinguish from the abdominal viscera.

If we focus on our two hypothesized molecular/cellular MOAs of Mechanosensitivity and Mechanotransduction Signaling and Ephrin-Eph Receptor Signaling, then what are the plausible "druggable" molecules that might play a role in mediating the occurrence of gastroschisis, implying some role in development of skin, subcutaneous tissue, fascia, muscle, peritoneum, possibly smooth muscle and/or combinations of those tissues? Some unevaluated possibilities exist. One facile resource in such a search for druggable molecular targets is the Druggable Proteome section within the Human Protein Atlas [89; https://proteinatlas.org/humanproteome/druggable].This resource is readily searchable for various functional and structural categories of human proteins. For the current purpose of seeking molecules that might be critical to normal or disordered development of the embryonic/fetal abdominal wall, we would pose queries to seek membrane-associated proteins that may relate to

a) linkage among adjacent cells or to extraellular constituents (Mechanosensitivity and Mechanotransduction Signaling) and
b) Ephrin-Eph Receptor Signaling molecules, and for which there are data indicating substantial expression in relevant tissues such as skeletal muscle or skin.

Using those simple cues, a modest number of druggable molecules can be nominated as candidates for further research and potential development as protective or mitigative therapeutics.

This database tool groups targets for FDA approved drugs by function as classes of enzymes, transporters, voltage-gated ion channels, G-protein coupled receptors, nuclear receptors, and CD markers. For the purpose of translational research in gastroschisis, cellular localization of targets may offer an important corollary clue as to potential relevance for this developmental disorder. If such structural and locational information heightens relevance, then integral membrane (IM; n=250), single pass transmembrane (SPTM; n=101) and IM/SPTM (n=9) would be worthy of prioritizing for research attention. Among those groups (IM, SPTM and IM/SPTM), a few molecules appear to have some background data suggesting relevance to abdominal wall development/maldevelopment.

We do not claim that these nominees are known to mediate gastroschisis, nor would we exclude many other potential molecular pathways or key cellular mediators. Indeed, we hope to evoke new inquiries by investigators with a range of expertise and research skillsets to address the challenge and opportunity that these mechanistic prospects present.

As suggested by the prior section on Ephrin-Eph receptor signaling, we suggest that this pathway may be involved in mediating abdominal wall development and could be modulated by medications or dietary component. In addition to reports [85, 86] about small molecule ligands for the EphA4 and EphA2 receptors, there are other candidates as small molecule Eph receptor ligands. The endogenous human compound lithocholic acid is a bile acid produced by gut bacterial flora. Giorgio et al. [90] have identified lithocholic acid (LCA) as a reversible comp-

titive ligand that inhibits EphA2 receptor-ephrin-A1 binding and that LCA inhibited EphA2 phosphorylation induced by ephrinA1-Fc in several cell lines without affecting cell viability or other receptor tyrosine-kinase (EGFR, VEGFR, IGFR1b, IRKb) activity. They noted that structurally related bile acids neither inhibited Eph-ephrin binding nor Eph phosphorylation.

Table 1. Potentially druggable membrane-associated human proteins in gastroschisis related to hypothesized MOAs of mechanosensitivity and mechanotransduction signaling or Ephrin-Eph receptor signaling

Postulated MOA	Nominated Druggable Molecule(s)	Substantial expression in relevant tissue(s)
Mechanosensitivity and Mechanotransduction Signaling[1]		
	Integrin subunit alpha V	Skin, smooth muscle
	Integrin subunit beta 1	Smooth muscle
	Integrin subunit beta 7	Skeletal muscle, skin
Ephrin-Eph Receptor Signaling[2]		
	Ephrin A3	Skin
	Ephrin A4	Skeletal muscle, skin
	Ephrin A5	Skeletal muscle, skin
	Ephrin B1	Skeletal muscle, skin
	Ephrin B2	Skeletal muscle, skin
	Eph receptor A1	Skeletal muscle, skin
	Eph receptor A3	Skeletal muscle
	Eph receptor A4	Skin
	Eph receptor A7	Skeletal muscle, skin
	Eph receptor A10	Skeletal muscle, skin
	Eph receptor B2	Skeletal muscle, skin
	Eph receptor B3	Skin
	Eph receptor B4	Skeletal muscle
Other(s)[3]		
Matrix Metallopeptidases (MMPs)[4]		
	MMP 14	Skin
	MMP 15	Skeletal muscle, skin
	MMP 16	Skeletal muscle, skin

[1]Selected from 25 Integrin molecules.
[2]Selected from 22 Ephrin and Eph receptor molecules.
[3]Various other groups of molecules could be considered; MMPs are shown as an illustrative group.
[4]Selected from 23 MMPs.

A final speculative suggestion where one or more small molecules might mediate protection against gastroschisis occurrence is a recent report about gastroschisis and maternal intake of phytoestrogens [91]. In that report, Wadhwa et al. evaluated whether the risk of gastroschisis was associated with maternal dietary intake of phytoestrogens. In the National Birth Defects Prevention Study, these investigators analyzed data on mothers of 409 gastroschisis cases and 3,007 controls with births in 2005-2010. From validated maternal food frequency questionnaire data, logistic regression analyses for each phytoestrogen was adjusted for maternal energy intake, age, BMI, race/ethnicity, and first-trimester smoking. This analysis showed that biochanin A, formononetin, and coumestrol had significant non-linear associations with gastroschisis. For these compounds, lower intakes were associated with increased risk and associations were not significant for the other phytoestrogens.

Since this profile of potentially protective isoflavones and coumestans is somewhat characteristic of those that occur in the group of legumes that include red clover, we suggest that this group of plants would merit systematic phytopharmacognosy research into prevention of gastroschisis in animal models and ultimately in human preventive clinical trials.

V. SUMMARY

In order to reduce the future occurrence and /or severity of cases of gastroschisis, what public health, lifestyle, clinical care, and therapeutic research and development options appear to be plausible? We offer the following recommendations as worthy of consideration knowing that many of these will surely not be applicable in all circumstances or for all gravidas:

1) Young maternal age – Provide younger adult women and their male partners with information regarding various advantages (including lowered risk of gastroschisis) for delaying childbearing along with access to contraception choices.
2) Pre-pregnancy and gestational nutrition – Adopt interventions to attain better nutritional status regarding both diet quality (intake of fruits and vegetables) and adequacy of caloric intake particularly for younger gravidas.
3) Recasting "prenatal care" to routinely include acessible preconceptional counseling, leading into early access to prenatal care *per se* could

a. reduce embryonic exposures to commonly used medications such as aspirin, oral contraceptives, ibuprofen, decongestants, venlafaxine, antiherpetic medications (acyclovir, valacyclovir or famciclovir), antidepressants, topical antivirals;
b. reduce embryonic exposures to occupational/ residential chemicals (polycyclic aromatic hydrocarbons, solvents, colorants, various agricultural chemicals);
c. guidance to avoid episodes of excessive heat stress;
d. possibly detection and reduction of episodes of domestic violence;
e. optimize pre-conception control of diabetes as well as early detection and control of gestational diabetes;
f. early detection and treatment of chlamydia trachomatis;
g. Very early ultrasound assessment of fetal anatomy is the most plausible means for earliest detection of a gastroschisis defect, which would be a requisite for instituting treatment with any potential or (hopefully) proven therapy that may mitigate the severity of gastroschisis cases.

4) Lifestyle/recreational drugs – Use all valid means to minimize prepregnancy and gestational use of tobacco, alcohol, cannabis and "recreational" drugs (cocaine, methamphetamine, etc.) of abuse.
5) Broadly implore research colleagues to
   a. take on the challenge of defining the underlying cellular and molecular mechanisms by which various environmental factors impact the risk and severity of gastroschisis and
   b. to drive those insights across translational boundaries to provide one or more safe and efficacious preventive or mitigative therapeutics for this developmental disease.

While we have hypothesized that druggable molecules in the Mechanosensitivity and Mechanotransduction Signaling and Ephrin-Eph Receptor Signaling pathways should be highly attractive unique research and development opportunities, the diverse range of exogenous factors implicated in the occurrence of gastroschisis will likely mean that multiple signaling pathways may be relevant in various distinct subsets of causal exposures.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Article

Common genetic variants shared among five major psychiatric disorders: a large-scale genome-wide combined analysis

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ABSTRACT

Background. Genetic correlation and pleiotropic effects among psychiatric disorders have been reported. This study aimed to identify specific common genetic variants shared between five adult psychiatric disorders: schizophrenia, bipolar, major depressive disorder, attention deficit-hyperactivity disorder, and autism spectrum disorder.

Methods. A combined p value of about 8 million single nucleotide polymorphisms (SNPs) were calculated in an equivalent sample of 151,672 cases and 284,444 controls of European ancestry from published data based on the latest genome-wide association studies of five major psychiatric disorder using Stouffer's Z-score method. SNPs that achieved genome-wide significance ($P<5x10^{-08}$) were mapped to loci and genomic regions for further investigation; and gene functional annotation and clustering were performed to understand biological process and molecular function of the loci identified. We also examined CNVs and performed expression quantitative trait loci analysis for SNPs by genomic region.

Results. We found that 6,293 SNPs mapped to 336 loci are shared by the three adult psychiatric disorders, 1,108 variants at 73 loci are shared by the childhood disorders, and 713 variants at 47 genes are shared by all five disorders at genome-wide significance ($p<5x10^{-08}$). Of the 2,583 SNPs at the extended major histocompatability complex identified for three adult disorders, none of them were associated with two childhood disorders; and SNPs shared by all five disorders were located in the regions that have been identified as containing copy number variation associated with autism and had largely neurodevelopmental functions.

Conclusion. We show a number of specific SNPs associated with psychiatric disorders of childhood or adult onset, illustrating not only genetic heterogeneity across these disorders but also developmental genes shared by them all. These results provide a manageable list of anchors from which to investigate epigenetic mechanism or gene-gene interaction on the development of neuropsychiatric disorders and for developing a measurement matrix for disease risk that could potentially be used for new taxonomy for precision medicine.

KEYWORDS

Psychiatric disorders; schizophrenia; bipolar disorder; major depressive disorder; attention deficit-hyperactivity disorder; autism spectrum disorder; genome-wide association study; combined analysis.

INTRODUCTION

The genome-wide association study (GWAS) has emerged as a compelling tool for investigating the genetic architecture and the etiology of complex human diseases over the past decade[1]. Many common genetic variants have been associated with complex human disorders through GWAS since the early studies in type 2 diabetes and inflammatory bowel disease[2, 3]. As the application of the GWAS approach has progressed, more and more gen-

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ome-wide genotyped data have been accumulated, which has made it possible to conduct genome-wide meta-analyses of multiple cohorts of GWAS samples[4]. The number of genome-wide association studies that involve a large number of patients and healthy controls are increasing every year. As of 2017, about 40% of recent studies are with a sample size of more than 50,000 individuals [5], and some even with more than 200,000 [6,7]. With samples of such size, investigators expect to identify variants with lower frequency and smaller effect size, by overcoming genetic heterogeneity[8] to attain adequate power to detect a genetic association. More importantly, with the development of genotype imputation and large whole-genome sequencing datasets available, it is feasible to assess the whole genome common variants for association with common complex human disorders without whole genome sequencing of all sample individuals.

The lessons from GWAS include that association of genetic variants with common human disorders is complex and involves a matrix of polygenic and pleiotropic effects. It has become clear that common complex human disorders are affected by multiple variants in a polygenic way [9]; whereas an individual variant may be associated with multiple diseases or traits (i.e., pleiotropic). Preliminary evidence of pleiotropic effects has been found in immune-related disorders, various types of cancers, or neuropsychiatric disorders[10]. For example, genetic varints at 3p21, 10q24, and SNPs within two L-type voltage-gated calcium channel subunits, *CACNA1C* and *CACNB2* have been found to be shared by multiple psychiatric disorders [11]. Coincidentally, genome-wide SNPs association analysis has revealed that psychiatric disorders might share a moderate to high degree of genetic correlation [12]. Identification of the pleiotropic effects of specific genetic variants on common complex human disorders is a potentially important step in building the knowledge network and a measurement matrix for disease risk for developing a new taxonomy of human diseases, which will play a fundamental role in achieving the goals of precision medicine [13, 14].

METHODS

Dataset and analysis. Individual SNP data used in this study were obtained from the published data releases by the Psychiatric Genomic Consortium that contained the largest genome-wide association studies of schizophrenia (SCZ), bipolar disorder (BD) [15], and major depressive disorder (MDD) [7], attention deficit hyperactivity disorder (ADHD) [16], and autism spectrum disorder (ASD) [17]. Detailed information on the sample individuals, original data process and analysis of individual sample cohort has been described previous in the individual studies. Of note, the ADHD sample mainly include a population-based cohort of ADHD cases and controls from Denmark collected by the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH), and 11 cohorts aggregated by the Psychiatric Genomics Consortium (PGC); the majority of the ASD sample was also from iPSYCH ASD and five family-based samples of European ancestry, which contributed cases and pseudo-controls.

The analysis was performed by calculating the combined p values of individual SNPs across the genome in multiple disorders. The Stouffer's Z-score method (1949), an extension of classical Fisher's combined method (1925) that has been employed in previous GWAS [18], was used to have a two-sided test. The hypothesis would be "multiple psychiatric disorders shared some common genetic variants," and under a null hypothesis, a statistic can be calculated by a combined Z-score, which can be converted into p-value based on the inverse of the standard normal cumulative distribution. The Z-score is defined as $Z=\frac{\sum_{i=1}^{k} Z_i}{\sqrt{k}}$; where k is the number of individual disorders, and the Z_i is the inverse of the standard normal cumulative distribution based on the p value for an individual SNP in the ith disorder.

The analysis was limited to SNPs showing a consistent direction of association at nominal significance ($P< 0.05$) across disorders, to keep internal consistency and reduce heterogeneity, i.e., ruling out SNPs, which is highly significant in one disorder, but not the other or shows opposite allelic directionality across disorders. The combined analysis was performed first for schizophrenia, bipolar and major depressive disorder, i.e., the three adult psychiatric disorders that have been demonstrated a moderate to high genetic correction, then for ADHD and ASD, the two childhood neurodevelopmental disorders, and finally for five disorders combined. A genome-wide significance threshold ($P<5x10^{-08}$) was employed.

SNP functional annotation and eQTL analysis. Individual SNPs were mapped to a locus based on the dbSNP and the HapMap data[19], and then the unmapped SNPs by the database were manually verified with the UCSC genome browser (GRCh37/hg19). Functional annotation of associated loci was performed based on the DAVID Bioinformatics Resource 6.8 using Gene Ontology (GO) term [20]. The eQTL analysis was performed on the GTEx dataset developped by the Genotype-Tissue Expression (GTEx) Project; the gene expressions in human tissues were based on the Human Protein Atlas (HPA) RNA-seq (https://www.proteinatlas.org/).

RESULTS

Table 1 presents the number of sample individuals and SNPs included in this study by the individual disorder. Briefly, the schizophrenia and bipolar analysis consisted of 53,555 cases (20,129 BD, 33,426 SCZ) and 54,065 controls with 8,379,106 SNPs; the MDD study comprised 59,851 cases and 113,154 controls with 13,554,492 SNPs; the ADHD study included 20,183 cases and 35,191 controls with 8,047,420 SNPs; and the ASD study included 9,112,386 SNPs in 18,381ASD cases and 22,664 controls. The combined p values were calculated to identify genetic variants shared by multiple disorders (Methods). While the original data in the MDD study included a fair

number of rare variants, we only focused on the common variants (minor allele frequency, MAF>1%).

Table 1. Samples and number of SNPs by individual disorder

Disorder	Sample	No. variants	Cases	Control
Schizophrenia (SCZ)	PGC	8,379,106	33,426	54,065
Bipolar disorder (BD)	PGC	8,958,989	20,129	54,065
Major depressive disorder(MDD)	PGC	13,554,492	59,851	113,154
Attention deficit hyperactivity disorder(ADHD)	Combined	8,047,420	20,183	35,191
	iPSYCH*		14,584	22,492
	PGC-ADHD		5,499	12,599
Autism spectrum disorder (ASD)	Combined	9,112,386	18,381	27,969
	iPSYCH		13,076	22,664
	PGC -ASD		5,305	5,305
Common SNPs in combined five disorders		6,467,684	151,672	284,444

*Cases and controls from Denmark collected by the Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH); PGC-ASD, Psychiatric Genomic Consortium--Autism spectrum disorders contained five family-based trio samples of European ancestry; PGC-ADHD, Psychiatric Genomic Consortium--Attention deficit and hyperactivity disorder comprised 11 European, North American and Chinese cohorts aggregated by the Psychiatric Genomics Consortium.

Common variants associated with schizophrenia, bipolar and major depressive disorder

We identified 6,293 SNPs shared by three adult-onset disorders at genome-wide significance ($P<5x10^{-08}$). These included 2,583 SNPs in the extended major histocompatability complex (xMHC), which spans a 7.5-Mb region defined by the *SLC17A2* gene at the telomeric end to the *DAXX* gene at the centromeric end of chromosome 6 (Chr6:25,875,084-33,797,216, hg19) and mapped to 421 loci (excluding tRNA genes)according to the gene map of xMHC[21, 22]; and 3,710 SNPs were located in the non-xMHC (Figure 1A). We noted that these SNPs were in strong linkage disequilibrium (LD) and clustered by genomic region. Of the total non-xMHC SNPs, 3,654 SNPs (98.5%) were mapped to 68 genomic regions, defined by which flanking SNPs of any two regions were at least 1-Mb apart. Based on the SNP functional prediction[19], 263 SNPs (8%) were at TFBS, and 109 SNPs (3.3%) were conserved in vertebrates (Table S2).

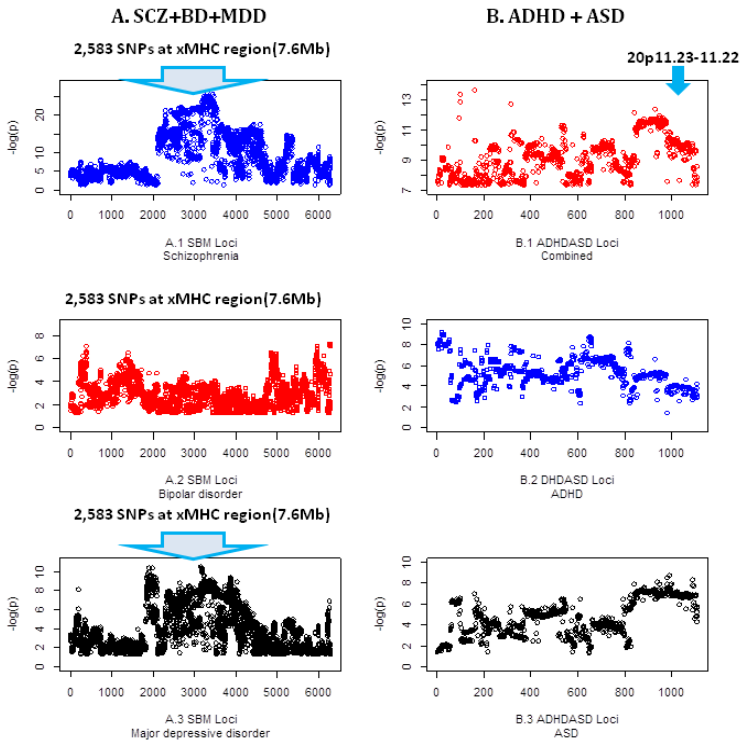

Figure 1. Scatter plots of p values (-log) for SNPs shared by psychiatric disorders. (x-Axis is SNPs in chromosomal order; A.1-A.3, 6,293 SNPs shared by three adult-onset disorders for association with an individual disorder; B.1-B.3, 1,108 SNPs shared by ADHDASD and for associ-ation with ADHD and ASD).

Among the 68 regions at non-xMHC loci, 51 regions did not have an SNP associated with a particular disorder at genome-wide significance; whereas other 17 regions cotained at least one SNPs in each region associated with an individual disorder at genome-wide significance. In the 51 regions that might be novel or specifically shared by the three adult-onset disorders (Table 2), 15 regions contained only a single SNPs; these included chr1: 1636-46390 at *NUF2*, rs7581403 at *MYT1L*, chr2:185676179 at *ZNF804A*, chr2:193934836, rs13078307 at *CNTN4*, rs-7613933 at *WDR49*,chr5:152167615, rs10239423 at *SP4*, rs7905569 at *IFITM8P*, rs4837673 at *DBC1||MIR-147*, rs1828385 at *LOC100120660||LOC751600*, chr18: 26337461, rs72903982 at *KC6*, chr20:43682549 at *STK4*, chr20:48108321 at *RYR2;* and the other 36 regions contained about 80 genes, including *DAMTSL3, ALPK3, ANKS1B, BRAF, COX8A, DCC, ENOX1, FGFR1, GABRA1, GABRG2, GCKR, GRIN2A, KCNB1, KCTD13, NFIX, PCLO, TRIM8,* and *BORCS7*. Meanwhile, SNPs in the other 17 genomic regions, each containing at least one SNP associated with an individual disorder at genome-wide significance, were mapped to 85 genes; and some of which *CACNB2, DRD2*, *CACNA1C, CACNAI1, AS3MT, TSNARE1, KLC1*, and *SOCRS3* have been associated with schizophrenia, and *EP300, VRK2, BAG5*, *APOPT1* have been associated with MDD at genome-wide significance (Table 2) .

Table 2. Genomic regions and loci mapped by the 6,293 SNPs (3,710 at non-xMHC) shared by three adult-onset psychiatric disorders at genome-wide significance.

Locus		Chr	SNP	BP1	BP2	ID	#SNP	Mapped gene loci
1		1	rs200448	6701978	6765079	1	21	*DNAJC11, CAMTA1,*
2		1	rs4949240	30438197	30509225	22	38	*PTPRU||MATN1*
3	**	1	rs2841192	73307627	73856328	60	132	*KRT8P21, LRRC44*
4		1	rs6661796	79186203	79261383	192	43	*IFI44||ADGR4L, ELTD1*
5		1	chr1_16364	163646390		251	1	*NUF2*
6	**	1	rs11587347	239198959	244025999	252	5	*[RGS7, KMO, OPN3, WDR64, EXO1, PLD5], ZNF238*
7		2	rs7581403	2314997	2314997	257	1	*MYT1L*
8		2	chr2_22499	22499207	28290347	258	458	*BRE, C2orf16, GCKR, GPN1, RBKS, SUPT7L, KLHL29, MRPL32, MRPL33, ZNF512*
9	**	2	rs13011472	57961602	58308458	717	10	*VRK2*
10		2	rs56088823	96793024	96905568	727	13	*ASTL, DUSP2, STARD7*
11		2	chr2_18567	185676179		740	1	*ZNF804A*
12		2	chr2_19393	193934836		741	1	*NA*
13		2	chr2_19822	198226403	198940251	742	206	*BOLL, MARS2, PLCL1, RFTN2*
14		2	rs13020196	201071942	201244261	948	56	*ERGIC3, SPATS2L*
15		2	rs2551656	208371637	208531683	1005	84	*CREB1, FAM119A*
16		3	rs13078307	2565355	2565355	1090	1	*CNTN4*
1	**	3	rs6802320	52241835	53475074	1091	523	*ALAS1, DCP1A, DNAH1, GLT8D1,GLYCTK, GNL3, ITIH1, ITIH3, ITIH4, MUSTN1, SFMBT1,*
	**							*TWF2, RFT1, MIR135A1, NEK4, NT5DC2, PBRM1,PHF7, WDR82, SPCS1, STAB1, TLR9, TMEM110*
18		3	rs2176028	80655077	80693369	1613	6	*[ROBO1, GBE1]*
19		3	rs4146338	117493964	117772036	1619	84	*LOC728873||IGSF11*
20		3	rs7613933	167304189	167304189	1703	1	*WDR49*
21		4	rs56089943	48342682	48496368	1704	41	*SLC10A4,TEC||SLAIN2, ZAR1*
22		4	rs1599125	118644885	118753686	1745	82	*NT5C3P1||NDST3*
2	**	5	rs7716818	103684787	104089064	1827	271	*NUDT12||RAB9P1*
24		5	chr5_15216	152167615	152167615	2098	1	*AK123826*
25		5	rs2290732	161324898	161352075	2099	14	*GABRA1, GABRG2*
26	**	6	rs2744301	25323143	25875567	2113	59	*CARMIL1, SCGN, HIST1H2AA, SLC17A1, SLC17A3, HIST1H2APS2, SLC17A4*
27		6	rs36014129	25884519	33797216	2172	2583	*Extended MHC region*
28		6	rs28360639	50783501	50936376	4755	71	*FTHP1, RPS17L4,TFAP2B,TFAP2D*
29		6	rs9360557	73132745	73155701	4826	14	*RIMS1, [KCNQ5]*
30		6	rs12190758	93148341	93176175	4841	4	*[EPHA7]*
31		7	rs10239423	21503450	21503450	4845	1	*SP4*
32		7	rs4291157	24624449	24844736	4846	67	*BLVRA, MRPS24, DFNA5, MPP6, OSBPL3*
33		7	rs2371213	82397302	82482281	4914	35	*PCLO*
34		7	rs10265001	140665521	140777030	4950	43	*BRAF, MRPS33*
35		8	rs6983972	9699144	9728595	4994	5	*MIR597|| MIR124-1*
36		8	chr8_33747	33747954	34021138	4999	5	*DUSP26*
37		8	rs57709857	38290424	38291844	5004	2	*FGFR1*
38		8	rs790569	64621828	64621828	5006	1	*IFITM8P*
39	**	8	rs10098073	143309504	143352779	5007	46	*TSNARE1*
40		9	rs9695226	22759396	22785141	5053	8	*FLJ35282*
41		9	rs4837673	122546769	122546769	5061	1	*DBC1|| MIR147*
42	**	10	rs12777923	18661160	18775255	5062	81	*CACNB2*
43	**	10	rs7893954	104318966	105059896	5143	233	*ARL3, AS3MT, CNNM2, WBP1L, CYP17A1, NT5C2, INA, PCGF6, CXCL12,*
								SFXN2, SUFU, TRIM8, BORCS7
44		10	rs12761679	106512727	106747354	5376	19	*SORCS3*
45		11	rs7946546	63595648	63790521	5395	83	*ATP5LP1,MARK2,MACROD1, RCOR2, NAA40, COX8A, OTUB1*
46	**	11	rs17601612	113317745	113412443	5478	25	*DRD2, TMPRSS5*
47		11	rs402320	131374917	131399805	5504	75	*RREB1*
48	**	12	rs740416	2499892	2514270	5579	8	*CACNA1C*
49		12	rs1319892	99436519	99690240	5587	127	*ANKS1B*
50	**	12	chr12_1236	123639761		5714	1	*SSPN*
51		13	rs9562504	44244876	44351241	5715	11	*ENOX1*
52		14	rs28645341	30174078	30182920	5726	3	*PRKD1*
53		14	rs36341	72390689	72454646	5729	14	*RGS6, SIPA1L1*
54	**	14	rs11160502	99667179	99731731	5743	4	*BCL11B*
55	**	14	chr14_1039	103916280	104319989	5747	247	*BAG5, APOPT1, KLC1, TRMT61A, CKB, XRCC3, PPP1R13B, ZFYVE21*
56		15	rs1828385	36355868			1	*LOC100130660||LOC751603*
57		15	rs2562774	84380096	85393240	5924	65	*ADAMTSL3, ALPK3, ZSCAN2, SEC11A, WDR73, ZNF592,*
58	**	15	rs7168951	91406146	91437388	5989	24	*BLM||FURIN,FES,FURIN*
59		16	rs11648559	9874699	9960879	6013	156	*GRIN2A*
60		16	rs12919683	29943367	30018500	6170	47	*INO80E, DOC2A, KCTD13, TAOK2*
61		18	chr18_2633	26337461		6217	1	*NA*
62		18	rs72903982	39152991		6218	1	*KC6*
63		18	rs7505145	50711776	50870391	6219	30	*DCC*
64		18	rs56096694	52722378	53101598	6249	8	*MAP1LC3P, TCF4*
65		19	rs17706798	13116172	13122612	6257	4	*NFIX*
66		20	chr20_4368	43682549		6261	1	*STK4*
67		20	chr20_4582	45829133		6262	1	*RYR2*
68		20	chr20_4810	48108321	48110279	6263	4	*KCNB1||PTGIS*
69	**	22	rs71799331	39974015	41617897	6267	27	*CACNA11, EP300, L3MBTL2, MCHR1||SLC25A17, MKL1*

**, loci where SNPs were associated with one of three disorders; #SNP, number of SNPs shared by three adult-onset psychiatric disorder at genome-wide significance in each genomic region; [ ], genes nearby; NA, no gene available within about 1- Mb genomic region; SNP is the first flanking SNPs of individual region; BP1 and BP2 are position of two flanking SNPs of individual gene region.

Functional annotation of the genes in the non-xMHC indicated a few top biological processes, including urate metabolic process, protein phosphorylation, and sodium-dependent phosphate transport. These loci are located at a-

xon, postsynaptic membrane and postsynaptic density, mitochondrion, cell junction, voltage-gated calcium channel complex, and mitochondrial inner membrane; and they have molecular functions of sodium phosphate symporter activity, sodium-dependent phosphate transmembrane transporter activity, protein binding, p53 binding, ephrin receptor binding, and voltage-gated calcium channel activity involved in AV node cell action potential. However, few of these clustering survived the correction for multiple testing (Table S3).

ADHD and ASD shared loci

Our combined analysis of 8,047,420 SNPs in 38,266 cases with ADHD or ASD and 63,160 controls identified 1,108 SNPs shared by the two childhood psychiatric disorders at genome-wide significance (Figure 1B). These SNPs were located at 47 genomic regions, of which flanking SNPs of any two adjacent regions were at least 1-Mb apart. Majority of these 1,108 SNPs (88%) were located at loci 1p34.2, 1p21.3, 4q24, 5q14.3, 5q21.2, 6q13, 7q31.1, 10q25.1, 13q31.1, 16q22.2, 17q21.31, 20p11.23-11.22 (Table 3). All these loci have been reported to contain copy number variants (CNVs) associated with ASD in multiple reports or different study populations, according to the Simons Foundation Autism Research Initiative (SFARI) CNV database (Table S4), The associated genes with these CNVs included *HIVP3, RIMS3, DYPD, PTBP2, TET2, MEF2C, RIMS1,* and *SLC25A39*. In addition, of the total 47 genomic regions identified in this analysis, five regions at 1p21.3, 5q14.3, 16q22.2, 10q25.1,20p11.23-11.22 contained at least one SNP associated with either ADHD or ASD at genome-wide significance. The remain-ing 42 regions that did not have an SNP associated with an individual disorder at GWAS significance may harbor novel loci for ADHD and ASD. The detailed estimates of SNPs with a minimum p-value at each genomic region are in the supplementary data (Table S5). However, we did not find any SNPs shared by ADHD and ASD in the xMHC where a large number of SNPs in strong LD were found shared by the three adult-onset disorders.

Table 3. Genomic regions and loci mapped by the 1,108 SNPs common to ADHD and ASD at genome-wide significance.

Region	ID	Chr	SNP1	BP1	SNP2	BP2	#S	Mapped genes	CNVs	
1	1	1	rs35947542	43,983,679	rs3952787	44,323,244	54	*JMJD2A,PTPRF,ST3GAL3*	1p34.2	
2	55	1	rs61783205	46,317,219	rs77881576	46,587,530	7	*MAST2*	1p34.1	
3	62	1	rs222901	96,508,040	rs11307310	96,998,097	102	*RP11-147C23.1,RP5-898J17.1, PTBP2*	1p21.3	*
4	164	1	rs35518820	99,035,830	rs6662897	99,095,611	6	*LOC729987||SNX7*		
5	170	2	rs76504400	1,722,904	rs76504400	1,722,904	1	*PXDN*		
6	171	2	rs77966298	10,984,514	rs77966298	10,984,514	1	*PDIA6*	2p25.1	
7	172	2	rs6711582	159,327,935	rs1548635	159,529,888	14	*PKP4*	2q24.1	
8	186	2	rs75263467	174,601,275	rs75263467	174,601,275	1	*SP3*		
9	187	3	rs74877867	20,493,116	rs14607701	20,621,759	5	*MITF*		
10	192	3	rs56842404	70,253,808	rs62254854	70,266,538	2	*RP11-231I13.2,LOC100128160||FOXP1*		
11	194	3	rs11488172	82,320,385	rs11488172	82,320,385	1	*GBE1*		
12	195	3	rs11710737	107,464,170	rs7634587	107,516,847	4	*BBX*		
13	199	3	rs9855048	128,163,890	rs9855048	128,163,890	1	*EEFSEC||DNAJB8*		
14	200	3	rs20033201	158,001,670	rs11707386	158,152,073	3	*LOC730057||MAGI1,RSRC1*		
15	203	4	rs71297516	2,768,387	rs6422311	2,781,240	8	*TNIP2||SH3BP2*		
16	211	4	rs14510234	31,147,972	rs20072120	31,151,465	4	*RP11-617I14.1*		
17	215	4	rs228619	103,569,283	rs223504	103,635,183	51	*MANBA*	4q24	
18	266	5	rs71613075	87,514,778	rs2009730	87,933,568	95	*CTC-498M16.4,LINC00461,TMEM161B-AS1*	5q14.3	*
19	361	5	rs2635182	92,255,166	rs34523	92,303,352	6	*CTC-458G6.2*		
20	367	5	rs7716818	103,684,787	rs323509	104,082,179	184	*NUDT12||RAB9P1*	5q21.2	
21	551	5	rs6862136	144,495,743	rs6884441	144,512,659	4	*LOC100132712||ASSP10*		
22	555	6	rs9342783	70,852,493	rs3818327	70,861,135	16	*COL19A1*	6q13	
23	572	7	rs4947694	52,181,844	rs4947694	52,181,844	1	*COBL*		
24	573	7	rs34080086	105,017,329	chr7:10506	105,064,665	2	*SRPK2*		
25	575	7	rs34291892	114,058,731	rs7799269	114,290,491	16	*FOXP2*	7q31.1	
26	591	8	rs34458570	745,496	rs1532744	786,916	6	*ERICH1-AS1*		
27	597	8	rs4739249	21,323,694	rs4739249	21,323,694	1	*LOC100129163||GFRA2,*		
28	598	8	rs11445716	144,749,175	rs11445716	144,749,175	1	*ZNF251*		
29	599	9	rs13440322	31,026,272	rs28495892	31,064,791	9	*LOC100130670||LOC138412*	9p21.1	
30	608	10	rs45595836	16,691,399	rs45595836	16,691,399	1	*RSU1*		
31	609	10	rs12769316	104,152,751	rs12772374	104,156,911	2	*GBF1||NFKB2, NFKB2*		
32	611	10	rs61867293	106,563,924	rs11192280	106,776,925	34	*SORCS3*	10q25.1	*
33	645	11	rs5793730	95,309,155	rs5793730	95,309,155	1	*RP11-338H14.1||FAM76B*		
34	646	12	rs704067	89,726,027	rs60474271	89,776,845	13	*DUSP6,POC1B,RP11-13A1.3*		
35	659	13	rs9544757	78,821,529	rs9530779	78,969,536	130	*EDNRB||POU4F1*	13q13.1	
36	789	14	rs14080258	29,419,892	rs17638843	29,524,041	2	*LOC100128215||PRKD1*		
37	791	14	rs36063234	33,409,812	rs4981170	33,412,996	2	*NPAS3*		
38	793	14	rs11263529	94,838,142	rs28929474	94,844,947	2	*SERPINA1*		
39	795	15	rs11857683	87,769,703	rs8042805	87,779,902	3	*LOC100132083||TMEM83*		
40	798	15	rs11854401	93,929,730	rs8042369	93,957,898	8	*UNQ9370||LOC728292*		
41	806	16	rs17606532	72,333,127	rs12924285	72,653,326	13	*LOC390739, LOC645478,UNQ9370*	16q22.2	*
42	819	17	chr17:4396	43,965,129	rs71375338	44,332,793	5	*MAPT*	17q21.31	
43	824	18	rs4144756	39,305,154	rs4144756	39,305,154	1	*KC6, PIK3C3*		
44	825	19	rs13886705	37,439,641	rs13886705	37,439,641	1	*ZNF569*		
45	826	20	rs6047225	21,054,496	rs6035892	21,549,424	280	*KIZ,RPS15AP1,MRPS11P1,RPL24P2,NKX2-2, PAX1,GSTM3P,XRN2,NKX2*	20p11.23	*
46	1106	20	rs11480060	54,230,218	rs11480060	54,230,218	1	*RPL12P4,CBLN4*		
47	1107	21	rs11775706	36,927,870	rs14491176	37,255,329	2	*RUNX1*		

*, Regions where SNPs were associated with either ADHD or ASD; BP1 and BP2 are position for flanking SNP1 and SNP2.

Of the total 1,108 SNPs shared by ADHD and ASD, 1,095 SNPs were mapped to 63 genes including intergenic regions (Table S6) and 912 SNPs (82.3%) were annotated based on the dbSNP[19]. We noted that five SNPs were

non-synonymous (Table S7); 33 SNPs (3.62%) are located at transcriptional factor binding site (TFBS), mostly at 20q11.23-11.22 involving three loci at *RPL24P||C20orf19, C20orf9,* and *RPS15AP1||XRN2*(Table S8); 55 SNPs (6.03%) are conserved in vertebrates and mostly located at 5 genomic regions with CNVs (Table S9). These predicted functional SNPs were located at *MAST2, MANBA, SERPINA, C20orf9, FOXP1, TMEM161B-AS1, GBF1||NFKB2, PTBP2, PKP4, NUDT12||RAB9P1, BBX,* and *EDNRB|| POUF4*. Other SNPs shared by ADHD and ASD were located at loci, including *JMJD2A, PTPRF, ST3GAL3, SNX7, SGOL1 ||VENTXP7, EEFSEC||DNAJB8, MAGI1, RSRC1, TNIP2|| SH3BP2, KCTD16||PRELID2, COL19A1, FOXP2, C8orf68, GFRA2, RSU1, FAM76B, PRKD1, NPAS3, TMEM83, MAPT,* and *RPL12P4||CBLN4* (Table 3). All these 63 genes together annotated a few biological processes, cellular component, and molecular functions, but none of these functional annotations and clustering were significant after multiple testing correction (Table S10).

Genetic variants shared by all five disorders

Through a combined analysis of 6,391,075 SNPs available common to all five studies, we identified 713 SNPs at 47 genes in 24 genomic regions (defined as above) shared by all five disorders at genome-wide significance (Figure 2A). We note that no SNPs were associayed with schizophrenia (Figure 2B), Bipolar (Figure 2D) or ASD (Figure 2E); but SNPs *NUDT12||RAB9P* on 5q21.2-21.3 and 10q24-25 have been associated with ADHD (Figure 2C) and MDD (Figure 2E), respectively. SNPs at *NUDT12 ||RAB9P* appear in strong LD in multiple original studies (Figure 2 C, E, F) and SNPs at 10q24-25 were mapped to multiple genes including *BLOC1S2, CHUK, CWF19L1, ERIN1, PKD2L1,* and *SORCS3.* The locus 10q24 has been identified in a previous genome-wide analysis of shared effect by five psychiatric disorders.

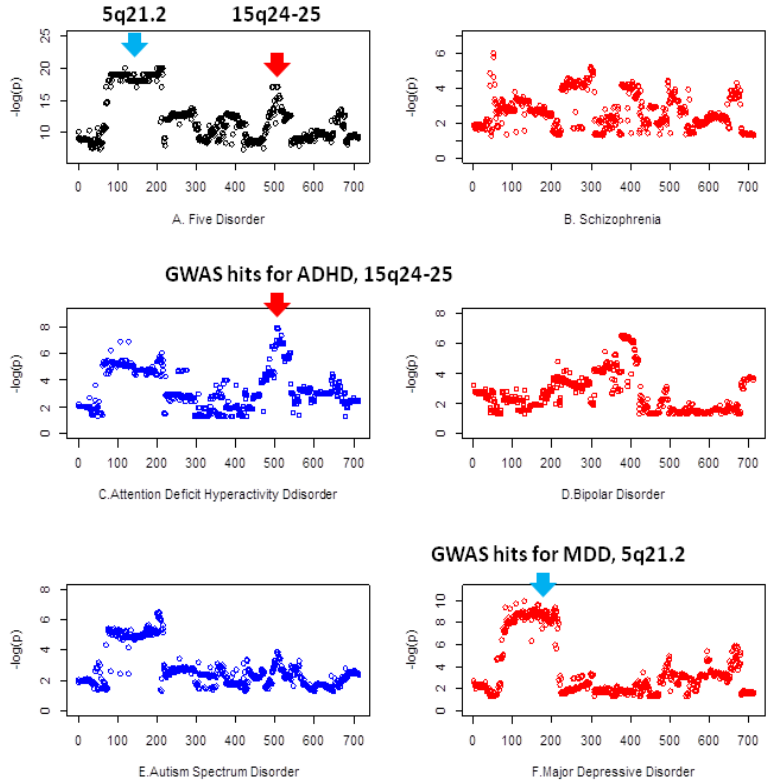

Figure 2. Scatter plots of p values (-log) for 713 SNPs shared by five disorders at genome-wide significance and signals for an individual disorder (X-axis is the SNPs in the chromosomal order). A) Combined signals for SNPs shared by five disorders; B) Signals with schizophrenia; C) Signals association with ADHD, and SNPs at 15q24-25 associated with ADHD at genome-wide significance; D) Signals for bipolar disorder; E) Signals for ASD; F) Signals for MDD, many SNPs at 5q21.2 were associated with MDD.

In the 22 novel genomic regions that did not contain an SNP associated with the particular disorder at genome-wide significance, six SNPs, including rs7945989 at *PKP4*, rs9375138 at *C6orf167*,rs12376855 at *PCSK5*, rs67525828 at *CIZ1*,rs9804545 at *CWF19L2*,rs3099047 at *CASTPER2*, were shared by the five disorders (Table 4). Except for *CACNB2* that has been found shared by five disorders, we found additional 40 gene loci in the remaining regions, including *A2BP1, RIMS1, DFNA5, CNTN4, MPP6, PLC1, RFTN2, SLC30A9, PAQR3, ERLIN1, SORCS3, ZNF584,*

and *ZNF132*. The detailed estimates of flanking SNPs at the total 24 regions are in the supplementary (Table S11).

Of the 24 genomic regions that harbored SNPs shared by the five disorders, all had been reported with CNVs associated with ASD (only 15q15.3 known for schizophrenia) and more than half were identified with expression quantitative trait loci (eQTLs) (Table S12). About two-thirds of the CNVs disrupted genes; for example, the CNV at 3p-26.3 were associated with *CNTN6* and *CNTN4*, 6q13 with *RIMS1*, 7p15.3 with *MPP6* and *DFNA5*, 16p13.3 with *A2-BP1,* and 19q13.43 with *ZNF584*. In addition, we identified SNPs in more than 13 genomic regions as eQTLs in human tissues through *cis*-association analysis of the flanking SNPs or alternatives with mRNA expression of the mapped genes. The eQTLs affected gene expression of almost 20 genes, including *ANKRD44, SF3BL, PLCL1, RFTN-2, SLC30A9, PAQR3, NAA11, MPP6, DFNA5, CIZ1, DMM1, C9orf16, BLOC1S2, SORCS3, CWF19L2, STRCP1, ZN584, RPSS,* and *SLC27A5* (Table 4)*.* Additionally, we found that SNP rs3099047 at *CATSPER2* had *cis*-association with about 19 genes in the same region.

Further, we noted that 387 of 713 SNPs shared by five disorders were discovered neither in the analysis of three adult disorders or ADHDASD. However, they were involved in the majority of the genomic regions where the shared 713 SNPs are located (Table S13). We also noted that 146 SNPs were shared by both the three adult-onset disorders and ADHDASD (Figure S1), and they were located at a 400-kb intergenic region *NUDT12||RAB9P1* on 5-q21.2 and *SORCS3* on 10q24-25. The *SORCS3* encodes a type-I receptor transmembrane protein, a member of the vacuolar protein sorting 10 (VSP10) receptor family, and had a biased expression in the human brain tissues of the Human Protein Atlas dataset.

DISCUSSIONS

We conducted a genome-wide association combined analysis of five major psychiatric disorders with an identification of 336 loci shared by three adult psychiatric disorders, 63 loci by ADHD and ASD, and 47 independent loci in 24 genomic regions associated with the combined all five disorders. The more loci shared among three adult disorders were consistent with a recent study that the overlapping gene expression pattern in human brains that SCZ, BD, and MDD have a moderate to high level of transcriptome correlation, but a low correlation with ASD, indicated[23]. Our study reveal a different genetic architecture, in particular the xMHC region, but some shared common variants between the childhood and adult-onset psychitric disorders.

In the present study, we found a substantial heterogeneity of genetic variants shared by three adult-onset psychiatric disorders and two childhood disorders. The analysis of a large number of SNPs across the genome indicated that the xMHC SNPs shared by three adulthood disorders had stronger associations with SCZ and MDD than bipolar disorder, but the two childhood disorders did not share them. In addition, three adulthood psychiatric disor-ders shared a number of non-xMHC loci including *DR-D2, GRIN2A, GABRA1, GABRG2, KCNB1,* and *CACNAI1*, in particular *CACNA1C, TSNARE1, KLC1*, *MYTIL* that have been found differentially methylated in the human prefrontal cortex between schizophrenia and controls[24], suggesting that these variants may be associated with the risk of the adulthood psychiatric disorders through an epigenetic mechanism. However, none of these genes were found overlapped with that mapped by the SNPs shared by the two childhood disorders. Further, among SNPs at 63 loci shared by ADHD and ASD, only two loci at *NUDT12||RAB9P1* and *SORCS3* were common to the three adult psychiatric disorders. Given that, 80% of SNPs shared by two childhood disorders are located at genomic regions with CNVs known for ASD; it may support that CNVs contribute to the genetic causes of two childhood disorders significantly, at least for ASD[25]. However, this study was not aimed to focus on CNVs or rare mutations, which have been implicated for the biology of autism spectrum disorder[26].

Despite the noted heterogeneity for the loci between adult-onset and childhood disorders, new novel or specific loci were identified for five disorders, and they indicate that neurodevelopmental genes may play a role in the development of a spectrum of major psychiatric disorders. *First*, all of the loci shared by the five disorders were located at genomic regions with CNVs reported for ASD. Previous studies have found that both microdeletions and microduplications greater than 100-kilobases disrupt genes in the neurodevelopmental pathway in schizophrenia[27]. Several genes identified are worthy of mention here. *A2BP1* has been identified as the top candidate gene in one of two network modules revealed through transcriptome analysis of differentially expressed genes between autism and controls in post-mortem human brains [28]. Disruption of *CNTN4* has been shown to result in developmental delay and contributing to the 3p deletion syndrome [29]. *CATSPER2* encodes a protein in the family of cation channel proteins that localize to the flagellum of spermatozoa; defects at this locus cause male infertility, and further a single SNP rs3099047 at *CATSPER2* was shown as a significant *cis*-association with nearly 20 genes in the GTEx dataset. *Moreover*, we noted that 102 SNPs in a 5-Mb region on 10q24.31-25.1 were mapped to multiple genes and they were eQTLs for *BLOC1S2* and *SORCS3* in multiple human tissues; and 2-3 Mb *de novo* deletions within this region have been detected in individuals with mental retardation and multiple congenital anomalies [30]. All these findings support that the genetic variants shared by the five disorders play a role in neurodevelopment and may through this general mechanism contribute to risk for all psychiatric disorders.

Further, the loci shared by five disorders may have implications for common neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD). Of the loci shared by five psychiatric disorders, genetic variants at *SOCRS3* and *CACNB2* have been assoc-

Table 4. Genomic regions and loci annotated by 713 SNPs common to five disorders at genome-wide significance ($P<5x10^{-08}$).

Reg	ID	Chr	SNP1	BP1	SNP2	BP2	#SNP	Annotated genes or loci	eQTL*	CNVs	
1	1	1	rs904307	30473551	rs1874784	30507893	41	*PTPRU||MATN1*		1p35.2	
2	42	1	rs28374258	190949551	rs12139300	191010445	2	*FAM5C||RGS18*		1q31.2	
3	44	2	rs79452989	159452935		159452935	1	*PKP4*		2q24.1	
4	45	2	rs711810	176888760	rs6755092	176906255	5	*KIAA1715||EVX2*	*AC009336.24*	2q31.1	
5	50	2	rs13404366	198522632	rs2033570	198952637	4	*PLCL1,RFTN2*	*ANKRD44, SF3B1, PLCL1, RFTN2*	2q33.1	
6	54	3	rs6778940	2571220	rs75345673	2576606	4	*CNTN4*		3p26.3	
7	58	4	rs1454607	33643360	rs1454608	33643627	2	*RP11-79E3.2*		4p15.1	
8	60	4	rs6846961	42176060	rs11930133	42184374	3	*BEND4, SLC30A9*	*SLC30A9, BEND4*	4p13	
9	63	4	rs13108290	80198876	rs17441732	80221215	8	*LINC01088,PAQR3||ARD1B*	*LINC01008, PAQR3, NAA11(ARD1B)*	4q21.21	
10	71	5	rs7716818	103684787	rs325528	104048590	146	*NUDT12||RAB9P1*	*RP11-6N13.1*	5q21.2	
11	217	6	rs16880300	50384635	rs6930867	50936376	80	*DEFB112, TFAP2D, FTHP1, FPS17L4, TFAP2B*		6p12.3	
12	297	6	rs9360557	73132745	rs9351918	73149101	9	*RIMS1||LOC643067*		6q13	
13	306	6	rs9375138	98518518		98518518	1	*C6orf167||LOC100129158*		6q16.1	
14	307	7	rs2529055	24590331	rs12154649	24832129	114	*CTRB1,DFNA5,OSBPL3, MPP6, NPY*	*MPP6, DFNA5*	7p15.3	
15	421	9	rs12376855	78591275		78591275	1	*PCSK5*		9q21.13	
16	422	9	rs67525828	130953511		130953511	1	*CIZ1*	*CIZ1, DNM1, C9orf16, LCN2, CERCAM*	9q34.11	
17	423	10	rs58703423	18540122	rs7091833	18660333	22	*CACNB2*	*RP11-499P20.2*	10p12.33	
18	445	10	rs12769818	1019155629	rs13854010	107329835	102	*BLOC1S2,CHUK,CWF19L1, ERLIN1, PKD2L1, SORCS3*	*BLOC1S2, SORCS3, PKDZI1*	10q24.31-q25.1	**
19	547	11	rs9804545	107278817		107278817	1	*CWF19L2*	*CWF19L2*	11q22.3	
20	548	15	rs3099047	43926033		43926033	1	*CATSPER2*	*AC011330.5, ADAL, CATSPER2, CATSPER2P1, CCNDBP1, CKMT1A, ELL3, LCMT2, PDIA3, RNU6-554P, SERF2, STRC,STRCP1,TGM5,TGM7,TP53BP1,WDR76,ZSCAN29*	15q15.3	
21	549	16	rs61547418	6345040	rs12448420	6346613	14	*A2BP1*		16q13.3	
22	563	18	rs4890712	38903507	rs72893943	39318793	89	*KC6||NPM1P1,MIR924||KC6*	*RP11-142I20.1*	18q12.3	
23	652	18	rs7505145	50711776	rs8091083	50870391	28	*DCC*	*DCC*	18q21.2	
24	680	19	rs73060258	58902954	rs12981875	58935130	34	*FLJ39005, ZNF584, ZNF132*	*ZFN584, ZNF132, RPS5, SLC27A5, AC016629.3, CHMP2A, CTD-2619J13.14, ZNF324, ZNF446, ZNF497*	19q13.43	

#SNP, the number of SNPs shared by five psychiatric disorders in the genomic region;
*, eQTLs were tested mostly for the flanking SNPs or alternative SNPs in the loci at each region;
**, of this region, 2-3-Mb size of de novo deletions have been detected with mental retardation and multiple congenital anomalies (MCA) in multiple populations;
Highlighted, SNPs associated with the individual disorder at genome-wide significance;
SNP1 and SNP2 are flanking SNPs for individual region; and BP1 and BP2 are corresponding position (hg19).

iated with Alzheimer's disease, and both genes had a biased expression in normal brain tissues of the Human Protein Atlas RNA-Seq data. Knockdown of *SORCS3* in cell culture leads to an increase in amyloid precursor protein (APP) processing, and APP may be as a mediator of the synapse pathology in the AD [31]. Common genetic variants at *CACNB2* have been associated with AD or through a within-locus SNP by SNP interaction [32]. In addition, we identified five SNPs at microtubule-associated protein tau (MAPT) that were shared by ADHD and ASD. Common genetic variants at *MAPT* have been associated with the risk of the late-onset AD, and also of PD in an early large family-based study [33], and shared by AD and PD[34]. Mutations in MAPT have also been detected in the early-onset AD and MAPT-related disorders, a group of neurological disorders including frontotemporal dementia with parkinsonism-17 (FTDP-17), progressive supranuclear palsy (PSP), cortico-basal degeneratetion (CBD), and mild late-onset parkinsonism, dementia with epilepsy**.**

Finally, it is worth highlighting that our study identified multiple shared genes *CACNA1C, TSNARE1, KLC1*, and *MYTIL* for three adult disorders that have been found differentially methylated in the human prefrontal cortex between schizophrenia and controls in a recent study [24]. Findings of genetic loci would provide a clue about what kind of environment would have caused the high methylation at individual genes associated with psychiatric disorders or a biological basis for conducting gene-environment interaction analyses. While the collection of environmental data is essential for a complete study of the etiology for common human disorders in the future, the effect that environmental factors exert on humans has to be through the human genome. It would be a great challenge to measure environmental factors across lifespan compared to measure the variants across the whole genome. What is more, most of the lifestyle and behavioral factors are not exogenous, and genetic variants might influence them. In addition to the further neurobiological study, our study provides a manageable list of anchors from which to investigate epigenetic mechanism or gene-gene interaction on the development of neuropsychiatric disorders.

In summary, we identify a sizeable number of genetic variants shared by three psychiatric disorders diagnosed during adulthood, by two childhood disorders, and by all five disorders. These variants indicate genetic heterogeneity but also point to the genetic etiology of neurodevelopment in five major psychiatric disorders or other neurological disorders. Our study provides new insights into genetic etiology and may have important implications for precision neuropsychiatry or medicine.

SUPPLEMENTARY MATERIALS

Published as e-cintent at the journal's website. (hppts: //www.gcatresearch.com).
Figure S1 and Table S1-S13

CONFILCTS OF INTERESTS

The authors declare no conflict of interest regarding the publication of this paper.

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Article

Consequences of Cervical Cancer Treatment on Sexual Health in Chinese Cancer Survivors: A Qualitative Study

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ABSTRACT

Background: The attitudes and perceptions of cervical cancer survivors (CCS) toward sexual activity subsequent to a diagnosis of cervical cancer and its treatment are unknown. This study describes the experience of CCS in Hunan Chinese in relation to sexuality and sexual function after cervical cancer treatment.

Methods: We used descriptive phenomenology to qualitatively assess these experiences. Purposive sampling was used to recruit 20 CCS. Data were collected through in-depth interviews and analyzed according to Colaizzi's method to explore the essence of the experience in sexuality among CCS after cancer treatment.

Results: Uncertainty, fear, and worry dominated the attitudes and behaviors of CCS-related to sexual activity after treatment. Four themes explain these complex emotional responses: 1) needing information; 2) dealing with sexual changes physiccally and emotionally; 3) communicating with a partner; 4) attribution of fault to her one-lifetime sexual partner.

Conclusion: Sexual life was influenced by the physical changes associated with cervical cancer treatment. These changes in combination with inadequate information and limited communication led to uncertainty, fear and worry about engaging in sexual activity and relationship distress. Chinese CCS need targeted and patient-centered information on the change in sexual life caused by treatment, anticipatory guidance, and support in communication with providers and partners, and strategies to cope with the physical and psycho-sexual sequelae of treatment, all of which must be congruent with their cultural norms.

KEYWORDS

Cervical cancer survivors; sexuality; sexual health; phenomenology; quality of life

INTRODUCTION

Cervical cancer is the second most common cancer in women in less developed countries and is the fifth most common cancer in women worldwide. In 2012 there were nearly 530,000 new cases of cervical cancer patients in the world [1]. There are 60,000 new cases diagnosed with cervical cancer each year in China, which account for about 29% of the world's total new incidence [2]. The screening and early detection initiative through the "National Cervical Cancer Screening Program" survivorship [3]. However, the majority of women in China diagnosed with cervical cancer receive surgery, often combined with radiotherapy and/or chemotherapy. The side effects of multimodality therapy can adversely affect sexuality and sexual function, resulting in emotional and marital relationship distress [4, 5].

Sexuality is an inherent component of a woman's quality of life and sexual health issues after gynecologic cancer treatment which can profoundly affect the quality of life in the ensuing survivorship [6-8]. Cervical cancer survivors (CCS) had a significant decline in sexual functions compared with healthy people [9]. Surgery can alter sexual organs; surgery and chemotherapy can induce premature menopause, and radiotherapy can damage the vaginal mucosa often resulting in stenosis and fibrosis [4]. Cancer treatment-induced menopause produces adverse effects including but not limited to vasomotor symptoms, vaginal atrophy, painful intercourse, decreased libido and arousal and changes in body image. Nearly half of cervical cancer survivors are at risk for sexual dysfunction due to the menopausal symptom profile [8, 10]. The psychological sequelae may include anxiety, fear of performance, guilt, depression, concerns about appearance and worry about cancer recurence.

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Studies on the sexuality of cervical cancer patients in China have predominantly focused on the effects of surgical treatment [11, 12]. The attitudes toward sexuality among the CCS in China and how they adapt to post-treatment changes in sexual function are relatively unknown. Culture is an important consideration in understanding a woman's experience related to sexuality [5]. In general, Chinese women have more conservative attitudes, beliefs, and behaviors toward sexuality compared to women in Western society [13, 14]. Chinese women often are uncomfortable with intimate issues, and there is an accepted silence about discussing sexuality in traditional Chinese families [15]. We have found no qualitative studies that describe the experience of sexual function after cervical cancer treatment from the perspective of Chinese women. The purpose of this present study was to describe and understand the experience of sexual health after cervical cancer treatment in women from Hunan, China.

METHODS

Research design

Descriptive phenomenology is a qualitative method to understand the essence of the life experience for persons who have experienced the phenomenon of interest. Colaizzi's methodological framework was used to guide this study [16]. This framework offers a holistic view of objective and subjective realities and provides direction and guidance to capture the complexity of people's life experiences. The goal is to gain an understanding of the experience through written and verbal description, identifying the distinct and critical components of the phenomenon [17].

Study participants

The participants were recruited from the regional cancer center of Hunan province and with the largest gynecologic oncology department in Hunan, China. The physicians in the Outpatient Department of the Hunan Cancer Hospital assisted in the study by identifying and recommending qualified survivors who returned to the hospital for a follow-up examination. Eligible participants diagnosed with cervical cancer met the following criteria: >18 years old; stage I, II, or III cervical carcinoma with within 3 months to 3 years post-treatment; no severe mental disorder or dementia; no current evidence of cancer recurrence; able to read, write, and speak Chinese; willing to provide informed consent. Purposive sampling was used to identify those women who experience the phenomenon of interest and who had the potential to be rich informants. Demographic characteristics of all participants were shown in Table 1.

Procedure

Semi-structured interviews were used to collect qualitative data from participants. The interview guide consisted of several open-ended questions (Table 2). Researchers recorded the participants' non-verbal reactions during interviews and took notes of critical Information supplied by participants. After each interview, detailed notes of the interview process, including thoughts, feelings, and responses were recorded. All the interviews were conducted by the same investigator [WZ] in a private office of the gynecologic oncology clinic in the regional cancer center of Hunan province where confidentiality and privacy could be maintained. The interviews lasted 30 to 60 min and were audiotaped. Interviews were then transcribed verbatim.

Table 1. Demographic Characteristics of Participants (n=20).

Characteristic	*n*	%
Age		
30–40	7	35
41–50	10	50
51–60	3	15
Marital Status		
Married	17	85
Divorced	3	15
Education		
College	2	10
High school	15	75
Primary school	2	10
Illiterate	1	5
Residence		
Rural resident	18	90
Urban resident	2	10
Occupation		
Housewife	6	30
Freelance	4	20
Village cadre	1	5
Farmer	8	40
Worker-employed	1	5

Ethical considerations

The Institutional Review Board of the regional cancer center and the academic institution approved our study. The informed consent was signed after participants were informed and stated that they fully understood. All participants were voluntary and anonymous, and they could stop the interview at any time without any influence on their care. Participant names were removed and replaced by coded numbers. All data were locked and accessed only by the investigator who had conducted the interviews (WZ).

Data analysis

After each interview, the transcriptions of recordings were reviewed noting any pause, changes of tone, sighing, sobling, laughing and those were marked on the transcribed interviews. Field notes and observations were also integrated throughout the analysis. Data were entered into the qualitative software program Nvivo 10.0 for data management. The analysis was witnessed by instructors as well as transcribed interviews and data analysis was performed according to the seven-step procedure established by Colaizzi (1978) [16]. Those steps included: 1) reading and re-reading in order to obtain a general sense about the whole content, 2) after reviewing the transcripts, significant statements/phrases relating to the experiences under research were extracted, 3) formulation of meanings from these significant statements, 4) sorting, meanings into categories, clusters of themes, and themes, 5) integrating findings into an exhaustive description of the phenomenon under study, 6) describing the fundamental structure of the phenomenon and 7) validating the findings from the research participants to compare the researcher's descriptive

results with their experiences. The transcripts of interviews were returned to participants for verification of authenticity, accuracy, and completeness of information. In subjects' participants acknowledged and verified that the transcript was correct, that the analyses were correct and the participants had accepted that these documents were accurate.

Table 2. Interview Guide.

Please tell me did cervical cancer treatments affect your sexual life? If yes, how?
• Probe 1: How long after the end of cancer treatments, did you resume your sexual life? How did you get the information, which helped you make the decision to resume your sexual life?
• Probe 2: Before cancer treatments, did you consider their impacts on your sexual life? If yes, how?
Did the disease affect your self-perception? If yes, how?
Did the disease affect your body image? If yes, how?
Did cancer treatments affect the communication between you and your partner? If yes, how?
• Probe 3: How did your partner communicate with you about those changes of your sexual life?
Did cancer treatment affect your ability to be intimate sexually? If yes, how?

RESULTS

Themes

The findings of this study suggest that uncertainty, fear, and worry are dominant emotional responses of the CCS-related to inadequate information about sexual health after treatment, physical changes and symptoms and communication with partners, all of which negatively affected the CCS' sexuality, sexual function and willingness to engage in sexual activity and intimacy. Four themes described the essence of the phenomenon of sexuality and sexual function after cervical cancer treatment: needing information, dealing with sexual changes-physically and psychologically, communicating with the partner, and attribution of fault to her one-lifetime sexual partner.

Needing information

A few participants gained information from formal sources, such as health care providers or educational materials.

"I have read some books; I know that appropriate sexual life can improve physical and mental health. At the return visit, a patient inquired the provider, and the provider said that appropriate sex was beneficial to her body and mind." (Age 37, divorced, rural resident, high school education, freelance)

However, informal information channels profoundly influenced many participants as personal experiences and myths perpetuated by friends or family members within their culture. These generated fear, anxiety, and worry and negatively contributed to sexual function decision behaviors.

"My aunt's sister who got uterus surgery at 17 years old, and then she got married. I thought if she got married, she must have a sexual life." (Age 46, married, rural resident, high school education, freelance)

"I heard folk people said that I should not have too much sex, and should not be together with a sexual partner too frequently. People told me that for a long lifetime, my partner should not stay with me, and we should be apart from each other." (Age 41, married, rural resident, high school education, farmer)

"I have a little fear of sex. When I found this disease, my husband and I slept together and the bleeding happened. For this kind of reasons, I am very scared of sex." (Age 45, married, rural resident, high school education, housewife)

Dealing with sexual changes-physical and psychological responses

Participants described many adverse alterations in their sexual health that influenced sexual function, activity, and sexual responsiveness. Sexual desire and arousal were diminished, menopause symptoms were common including vaginal dryness, painful intercourse, and loss of pleasure, satisfaction and orgasmic ability. Further, the sexual and reproductive side effects resulting from treatment for cervical cancer include premature menopause, loss of fertility, and a reduction in vaginal elasticity, often resulting in pain and impaired sexual function.

"After surgery, my abdomen was all cut off, so it is empty inside now. When my husband had sex with me, he said that it's empty. Sometimes, when I walk, hike or step for a long time, I feel really painful, something inside falling down, and uncomfortable inside." (Age 41, married, rural resident, high school education, housewife)

Body image played an important role in CCS sexual health. For example, the surgical scar had a substantial negative impact, created emotional distress and resulted in worry about a negative partner response during sexual activity.

"There is a huge scar in my belly, at least 15 to 25 cm. Frequently, it is itching, and I will scratch. Many times, scratching makes me upset and annoyed, and I will lose my temper to my husband. I would show my belly to my husband, but I was afraid that my husband would see me ugly. Sometimes I would stop him to see the scar. I was afraid that he would avoid me, dislike me." (Age 41, married, rural resident, high school education, housewife)

Fear of cancer recurrence was a universal concern expressed by participants. The majority of Chinese cervical cancer survivors lacked accurate information and believed that engaging in sexual activity could be related to the relapse of cancer or genital infection.

"I heard some women who got this disease said, if my husband and I sleep together, I should be cautious of inflammation. I was scared when heard these. Sometimes I really want sex, but feel scared. If by any chance accident happens, I will die." (Age 44, divorced, rural resident, primary education, farmer)

The physical changes because of cervical cancer treatment resulted in physical and psychological distress and a high degree of sexual dysfunction and relationship strain.

Communicating with partners

Women appeared to have limited communication with partners, little insight into how the partner was coping was offered, and the CCS seemed to make decisions on her own about engaging in sexual activity. We found many couples can cope with the cancer diagnosis and treatment, and were able to draw on support from each other and their families. There was also evidence of the partner's concern for the woman's health as a priority over sexual function.

"He said he would respect my feelings. I asked him what if my conditions would not endure sex. He said he would follow my willing. He said he would respect my choice, and his only wish was that I got a better life—better to my health." (Age 34, married, rural resident, high school education, housewife)

"He is afraid of that if someday I die, he would have no such companion anymore. He is thinking for the sake of daily life, for a company, for reliance. Even there is no talking between us, getting the sight of me is a routine of his life." (Age 45, married, urban resident, high school education, freelance)

To some couples, changes caused by cancer treatment can lead to the end of their relationship. Infertility is a consequence of hysterectomy, which the majority of participants had as well as chemotherapy. For two participants, infertility caused significant distress and regretted for the treatment decision due to divorce.

"While he wanted to divorce, I indeed did not. I think that I would have a better life without this disease. I always feel that if I have no cervical cancer surgery and I still have the ability to give birth, I may want a marriage. But I have no ability now and have this disease with me, I won't consider getting married." (Age 37, divorced, rural resident, college education, freelance, first divorce due to infidelity of an ex-husband and second divorce due to loss of fertility caused by cancer treatment)

We also found that some survivors feared to be unable to meet the partner's sexual needs. Concerns about performance and providing an adequate sexual life and satisfaction for the partner transformed how the participant viewed loyalty in their relationship. Moreover, some even considered the idea of letting the husband go out and find another woman as a buffer to their inability to resume sexual life.

"Sometimes, he bothered me for sex. I was annoyed and repulsed him. I was annoyed but afraid of speaking out. Because I was upset, I got an idea in mind like, 'You go out! You go out to find other women! I will not care.' However, I didn't dare to speak out. I was afraid that he really went out to find other women. Honestly, I didn't want to let him go." (Age 41, married, rural resident, high school education, farmer)

Attribution of fault to her one-lifetime sexual partner

In addition to the relationship strain, some women accused her partner of giving her disease. Generally, those women only had one sexual partner in her whole life. After diagnosis and treatment, they were aware of cervical cancer risk factors, so they accused their only partner as the cause of the disease.

"I am well-behaved. I have no affairs in my life. However, my husband was a truck driver. I had heard of gossips about his affairs. I had fights with him on this issue. Even I showed some evidence I found; he did not admit. I believe the only possible cause of my disease is my husband's unclear behaviors." (Age 46, married, rural resident, high school education, freelance)

Those women developed negative feelings towards their partner which could influence their sexual life after cancer treatments. Those feelings can also be worsened by the side effects of cancer treatments and fears of cancer recurrence.

"Because of the fear and anger from my disease and his behaviors, I feel hard to be intimate with him. Every time we had sex, I recalled his affairs, and I lost feelings. I blamed him that he is the one killing me." (Age 46, married, rural resident, high school education, freelance)

Even some of them considered to divorce, but most of them would not make the decision of divorce, because culture and social norms bound traditional marriage. For those women, divorce not only ends relationships in a family but also affects their social functions in their community.

"In my opinion, if you are married, you have to remain to that family for the rest of your life. It is a great shame for a woman to divorce her husband. So I chose to stay with my husband, although we had bad feelings in a sexual relationship." (Age 47, married, rural resident, high school education, farmer)

DISCUSSIONS

Cancer-related sexual problems have a complicated relationship with biologic, inter-personal, psychological, and social- cultural factors [5]. The findings of this study confirm that those factors are integrally related and result in physical and psychological distress and decreased the quality of life [4, 7, 18]. Sexual health is recognized as vital to the quality of life for gynecologic cancer survivors [7,8]. The themes in this study of inadequate information, dealing with sexual changes, communicating with partner, and attribution of fault to her one partner were associated with emotionally distressful responses related to the decreased quality of life. In contrast to many studies published with cancer survivors in the western world, the influence of Chinese

culture was significant and must be integrated into all interventions designed for Chinese CCS [5].

In contrast to a study with British CCS who reported wanting more discussion and sex information from their providers [19], the Chinese CCS in our study did not initiate such conversations with their providers. In CCS communication and relationship with their physicians, it is very rare to discuss sex topics as an influence of Chinese cultures and norms. Inquiries about sex are communicated obliquely as "Is sleeping together allowed?" and "Will this behavior cause problems?" Myths, reliance on personal experiences, and the Chinese cultural influence related to sexual attitudes were dominant factors in the experience for participants in our study. Traditional held Chinese beliefs about sex suggest that sexual life can damage one's energy and frequent sexual activity may be harmful to health [13, 14]. Fear related to sexual activity was also a common emotional response of participants in our study.

Education supplied by health providers about physical changes and strategies to manage the consequences of those changes is a critical intervention to manage symptoms and to dispel myths associated with the consequence [8, 18]. However, the best way of addressing and discussing sexual issues has not yet been well established in China and most likely are predominated by the traditional environment surrounding discussion of sexual function with patients, especially in the rural regions of China where traditional cultures and norms are highly influential. Changing personal beliefs and community culture could help develop strategies to deal with sexuality issues and create a basis for the rehabilitation professional to provide a supportive and safe environment to assist the CCS for their needs regarding sex after treatment. Once communities understand the meaning and importacne of sexual health to overall health, like sex after cervical cancer treatments, providers will be better able to talk to patients about sex openly.

Partners of gynecologic cancer survivors are deeply affected by the change in their sexual lives and often have strong and conflicting emotions of worry about the woman's health, desire for sexual activity and some, guilt for their desires of sex and intimacy [7]. There was evidence of these responses in our study but also concerns about not meeting the sexual needs of the partner and the combined inadequate knowledge, lack of strategies to manage symptoms and limited partner communication often resulted in the CCS deciding to restrain, avoid or reject sexual activity. In a study conducted in Australia with cervical cancer survivors, couples were able to talk about sexual life openly and frankly [19], in contrast, our study showed limited or lack of partner communication. Consideration of the sexual partner's loyalty was identified in our participants. In a study with gynecological cancer survivors in Hong Kong, 33% of participants reported that their husbands had an affair after a cervical cancer diagnosis, while the remaining participants also expressed their worries about that their husbands may have affairs currently or in the future [20]. Psycho-education interventions including the survivor and the partner can be very successful in stabilizing and maintaining quality partnered relationships and improving the sexuality and intimacy for the couple [4, 7, 8, 18, and 21].

Our study found an instance that some participants accuse their partner's risk behaviors as the cause of their disease. The role of male behavior in cervical carcinogenesis has been reported in many research studies. For women with one lifetime sexual partner, it had reported when husbands had sexual relationships both before and during the marriage, wives' risk of getting cervical cancer increased by 6.9 [22]. Most of our participants are from rural regions which are usually constrained by traditionnal social norms such as arranged marriage, living with husband's family, taking household work, the stigma of divorce, and restrictive moral supervision. Many of them only have a one-lifetime sexual partner—their husband. Meanwhile, those husbands, who have more power and less constraint in the family, usually are the major family economic support. In these traditional Chinese families, disparities of social norms and inequality of labor and power division exist between the two genders, which can increase the risk of transmission of HPV infection from men to women [23].

We found when women had been diagnosed with cervical cancer and learned about its risk factors, some of them would try to trace what was the cause of her case of the disease. For women who have a one-lifetime sexual partner and negative experience of partner's infidelity, they may attribute the fault to their one-lifetime sexual partner, which can worsen their marital relationships and negatively impact their sexual life. Strategies to improve this situation include providing CCS with information about protective sexual behaviors and education in healthy sexual psychology. More importantly, when investigators design sexual health studies in cervical cancer, the social risks for women who participate as study subjects need to be carefully considered.

The purposive sample of 20 participants provided sufficient qualitative data to supply a rich description of their experience. However, it may not have provided an exhaustive description as there were a limited number of women >50 years, only two who reported the impact of infertility, the number of years married was not known, and the majority were of relatively low social status. Also, only cervical cancer survivors were interviewed. Interpretation of our data may have been enhanced by data collected through interviews with gynecologic physicians and survivors' partner.

CONCLUSION

The sexual life of cervical cancer survivors was adversely influenced by the physical changes induced by the cancer treatment and further compromised by the emotional responses, inadequate information, limited or lack of communication, and attribution of fault to her one-lifetime sexual partner. The majority of women in this study were <50 years of age and faced with dealing with cancer, premature menopause, and significant surgical changes.

These women struggle with multiple losses and at the same time are trying to resume a sexual life. In an environment of traditional Chinese culture and the silence of discussing sexuality, it is a medical imperative to develop educational and supportive interventions for Chinese cervical cancer survivors to cope with their sexual life and partner relationships.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review

An Overview of Genetic and Environmental Risk of Autism Spectrum Disorder

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ABSTRACT

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder typically diagnosed in children in the first few years of life. Genetic studies have demonstrated a moderate to high heritability of ASD, but only a limited number of single nucleotide polymorphisms (SNPs) have been identified. Meanwhile, numerous single *de novo* rare variants and copy number variations have been detected in patients with ASD, which are likely caused by environmental factors. Here we provide an overview of genetic and environmental factors that may contribute to the risk of ASD and we recommend that further study should be focused on both genes and environmental factors, as well as their interactions with the expectation that epigenetic studies will lead to understanding the link between the environment and risk of ASD.

KEYWORDS

Genetic association; environmental risk factors; autism spectrum disorder

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by 1) persistent deficits in social communication and social interaction across multiple contexts and 2) restricted, repetitive patterns of behavior, interests, or activities [1]. Symptoms of ASD are present in the early post-natal developmental period, and more than 70% patients with ASD have co-morbdities with other somatic diseases, developmental disorders, or behavioral conditions, such as epilepsy, gastrointestinal problems, intellectual disability, attention-deficit hyperactivity disorder, anxiety, depression, aggressive and self-injurious behaviors, *etc* [2]. Therefore, ASD is considered to be a serious disorder that adversely affects individual health and often times leads to lifelong disability. However, in the absence of any effective pharmacological treatments and the need for special education and training as the major approach to care for the children with ASD, this disorder imposes a heavy burden on the family and society. The lifetime cost of individuals with ASD was estimated at 1.4–2.4 million dollars in the United States and 0.92–1.5 million pounds (the US $1.4–2.2 million) in the United Kingdom [3]. In addition, in regions that lack special education resources, more than half of parental employment is greatly affected by the burden of child-care with the average loss of annual household income associated with having a child with ASD estimated to be RMB 44,077 ($7,226) in China [4].

The prevalence of ASD has increased greatly over the past decades. While expanded diagnostic criteria may be a significant contributor to the rising prevalence in ASD, prevalence has increased from 0.5/1000 in the 1960s to 6.2–26.4/1000 in the 2000s [5–9]. Recent data for autism and developmental disabilities from a monitor-ing network show the estimated prevalence of ASD to be 16.8/ 1000 (one in 59) in the United States [10]. A large-scale study tracking 677,915 Danish children over several decades reported that changes in reporting pra-ctices might account for more than half (60%) of the incr-ease in the observed prevalence of ASD [11]. However, this study did not consider other factors such as changes in envir-onmental exposures, which are likely to contributte to the continuing rising in prevalence [12,13]. In this review, we present recent findings in genetic and environmental studies with discussion of their roles in the pathogenesis of ASD and we provide our perspective of gene-environment interactions that may help to dis-sect the etiology of ASD.

GENETIC VARIANTS ASSOCIATED WITH RISK OF ASD

ASD is a complex disorder with strong genetic components. Twin and population-based studies have demonstrated that the heritability of ASD was high to 50–95% [14–16]. In the past ten years, multiple approaches have been used in the genetic study of ASD, including a genome-wide association study (GWAS) [17], copy number variations (CNVs) analysis [18], whole-exome sequencing (WES) [19] and whole genome sequencing (WGS) [20].

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These studies provide insight into the genetic etiology of ASD and implicate biological processes in the occurrence of ASD [21].

Common variants

GWAS have identified a few common genetic risk factors associated with ASD. The first large-scale GWAS was conducted in a cohort of 780 families (n=3,101 subjects) with ASD children of European ancestry populations and a second cohort of 1,204 ASD patients and 6491 control subjects, and identified six single nucleotide polymorphisms (SNPs) at an intergenic region between cadherin 10 (*CDH10*) and cadherin 9 (*CDH9*) [17]. These two genes encoded neuronal cell-adhesion molecules, suggesting a role of neuronal cell-adhesion molecules in the pathogenesis of ASD. Meanwhile, linkage and association mapping study were conducted with 1031 multiplex autism families (1,553 affected offspring). While the initial analysis did not yield genome-wide significant associations, genotyping of top hits in additional families identified an SNP on chromosome 5p15 (−80 kb upstream of *SEMA5A*, a gene implicated in axonal guidance) associated with autism at genome-wide significance. The expression of *SEMA5A* was reduced in post-mortem human brains from subjects with autism, further implicating axonal guidance in the pathogenesis of ASD [22]. Subsequently, common variants located within *MACROD2, CNTNAP2, CACNA1C*, and *CACNB2* were associated with ASD; however, all these studies were conducted in European ancestry populations [23–25]. Later, the first GWAS in a Han Chinese population identified multiple genes including *TRIM33, BCAS2, AMPD1, DENND2C,* and *NRAS-CSDE1* on 1p13.2 as genetic susceptibility loci to autism, and these findings were replicated in three additional cohorts of family-based samples of European ancestry despite some genetic heterogeneity noted for these loci [26]. Table 1 presents a summary of these findings.

Table 1. Common variants associated with Autism Spectrum Disorder.

Chr	Gene	SNP	Pubmed ID
1	*ACTN2*	rs2297956	19812673
1	*AMPD1*	rs926938, rs761755	24189344
1	*CSDE1*	rs8453, rs11558867, rs10489525	24189344
1	*DENND2C*	rs6537841, rs7539721	24189344
1	*RASSF5*	rs11118968	22843504
1	*SLC22A15*	rs12726299	22843504
1	*TRIM33*	rs6537825, rs1102800,rs383773, rs3827735	24189344
1	*TRIM33*	rs11582563, rs11585926, rs7511633, rs6661053	24189344
2	*DNER*	rs6752370	22843504
2	*ERBB4*	rs1879532	22843504
2	*GALNT14*	rs10205350	22843504
2	*PARD3B*	rs4675502	22843504
3	*YEATS2*	rs263025, rs263030	22843504
4	*ZNF827*	rs12331851	22843504
5	*CTNND2*	rs6891903	22843504
5	*FER*	rs3797817	22935194
5	*FLJ46010*	rs29456	22843504
6	*CDKAL1*	rs7741604	22843504
6	*SLC22A3*	rs12194182	22843504
7	*CNTNAP2*	rs1718101, rs7794745	22843504
7	*RAC1*	rs836474	22843504
7	*SDK1*	rs17134117	22935194
8	*FAM135B*	rs2056412	22935194
10	*PCDH15*	rs1930165	22843504
10	*SORCS1*	rs7910584	22843504
11	*GUCY1A2*	rs11211996	22843504
11	*NELL1*	rs1429793	22935194
11	*PC*	rs7122539	22843504
11	*PICALM*	rs669556, rs618679, rs527162, rs2077815	24189344
11	*ZBTB16*	rs3782000	22843504
12	*TMEM132B*	rs16919315	22843504
14	*PPP2R5C*	rs7142002	20663923
14	*SYNE2*	rs2150291	22843504
16	*TAF1C*	rs4150167	22843504
17	*RPH3AL*	rs7207517	22843504
17	*SLC39A11*	rs9302952	22843504
18	*LAMA1*	rs600695	22843504
18	*LIPG*	rs2000813	22843504
18	*MYOM1*	rs10853291	24189344
20	*MACROD2*	rs4141463, rs14135, rs6110458, rs1475531	20663923
20	*SLC23A2*	rs6053022	22843504
21	*ERG*	rs2836439	22843504

Common variants are very significant as genetic determinants of autism. A recent study using the whole-genome SNPs from a unique population-based family sample in Sweden estimated the narrow-sense heritability to be 52.4%, which is mostly attributed to the common variants. However, no specific variant was found at a genome-wide significance in this study [16]. This implies that many genetic variants remain undetected, possibly due to the limited sample size. Few previous genetic studies have considered thepossible role of environmental factors as well as their interactions with genetic variants. Unlike rare variants that may directly cause biological dysfunction, common genetic variants may affect the risk of ASD through their interaction with environmental factors, influencing the expression of genes and then leading to the development of ASD. Furthermore, the common variants found so far implicate many biological mechanisms including cell-adhesion molecules, axonal guidance, histone acetylation, calcium channel signal and transcription. This range of possible mechanisms certainly warants further studies that incorporate genetic and environmenttal factors simultaneously.

Rare *de novo* variants

The substantial research effort has been invested in atempts to discover rare variants that contribute to autism through copy number variations analysis, whole-exome sequencing and whole genome sequencing. The first significant copy number variations analysis was performed in 111 families with sporadic cases of autism, 47 families with multiple cases of autism and 99 control families. *De novo* CNVs were identified in 10% of patients with sporadic cases of autism, 3% of patients with affected first-degree relatives, and 1% of controls without a diagnosis of autism. This indicates that *de novo* germline mutations account for a proportion of ASD [18]. Subsequently, more studies have searched for *de novo* mutations and found several sites or genes with *de novo* CNVs, located at 15q11-13, 16p11.2, 15q24 or disrupting *NRXN 1, CNTN4, NLGN1, UBE3A, PARK2, RFWD2, FBXO40, SHANK3, NLGN4X, DPP6, DPP10, PCDH9, ANKRD11, DPYD, PTCHD1, SHANK2, SYNGAP1, DLGAP2* and *DDX53-PTCHD* [27–31] (Table 2).

Table 2. Copy number variations detected in individuals with Autism Spectrum Disorder.

Chr	CNV Type	Region	Disrupted Genes	Pubmed ID
1	dup	1q25.1-q25.2	*RFWD2*	19404257
1	del	1p21.3	*DPYD*	18252227
2	del	2p15-p16.1	*PEX13, FANCL*	16963482
2	ins	2p16.3	*NRXN1*	18179900
2	del	2p16.4	*NRXN1*	17322880
2	dup	2q14.1	*DPP10*	18252227
3	del/dup	3p26.3	*CNTN4*	18349135
3	dup	3q26.31	*NLGN1*	20010541
3	dup	3q13.33	*FBXO40*	19404257
6	del	6q25.2-q27,	*PARK2*	19404257
6	del	6p21.32	*SYNGAP1*	19196676
7	del	7q36.2	*DPP6*	18252227
8	dup	8p23.3	*DLGAP2*	17630015
11	del	11q13.3-q13.4	*SHANK2*	20531469
13	dup	13q21.32	*PCDH9*	18252227
15	del/dup	15q13	*FAN1, MTMR10, TRPM1, KLF13, OTUD7A, CHRNA7*	27459725
15	dup	15q11-13	*UBE3A*	19404257
15	del/dup	15q24	More than *50 genes*	21480499
16	del/dup	16p11.2	*ATP10A, GABRB3*	18184952
16	del	16q24.3	*ANKRD11*	18252227
22	del	22q13.3	*SHANK3*	17173049
X	del/dup	X	*NLGN4X*	12669065
X	del	Xp22.11	*PTCHD1*	18252227

A whole exome study of ASD first identified *de novo* single point mutations in 4 out of 20 probands, particularly among more severely affected individuals, in *FOXP1, GRIN2B, SCN1A* and *LAMC3* [19]. Later, more than one hundred mutational variants were detected; for example, at *CHD8, POGZ, NTNG1, KATANAL2, SCN2A, GIGYF2, MYT1L, CUL3, DOCK8*, and *ZNF292* [32–34]. Some rare variants have been reported in multiple studies. Recently, 18 new candidate genes were found in ASD by using a large whole-genome sequencing in more than five thousand ASD families; and there were an average of 73.8 *de novo* single nucleotide variants and 12.6 *de novo* insertions and deletions or copy number variations per ASD subject [35]. Many rare mutations have been identified in at least three independent studies (Table 3).

While contributing little to the heritability, all these studies suggest that *de novo* rare variants play a role in the etiology of ASD. The major pathways implicated for biological mechanisms include synaptic formation and stabilization, the growth of dendrites and axons, neuron branches and migration [36]. A whole-exome sequencing study in more than 2,500 simplex families, each having a single child with ASD, estimated that coding *de novo* mutations contribute to abo-

ut 30% of all simplex diagnoses [32]. A whole-genome sequencing study of 85 family-Quads (*i.e.*, parents with one affected and one unaffected child) found 69.4% of the unaffected siblings carried different ASD-relevant mutations [20]. These studies indicate that rare *de novo* mutations contribute substantially to individual liability and emphasizes that substantial genetic heterogeneity exists in ASD. However, these rare *de novo* variants may be due to environmental exposures.

Table 3. Rare mutations that have been identified by at least three studies of Autism Spectrum Disorder.

Chr	Gene	Pubmed ID
2	*PRKCQ, CELF2, CAMK1D*	29700473, 28965761, 26749308
2	*TCERG1L*	28965761, 25363768, 26749308
2	*ECHS1, ANO9, PHRF1*	28965761, 25363768, 25961944
2	*MS4A4A, VWCE*	28965761, 29700473, 25363768
2	*C2CD3*	28965761, 25961944, 26749308
2	*MMP8*	25363768, 28965761, 26749308
3	*NLRX1, CBL, MCAM*	25961944, 28965761, 25363768
3	*MFRP, GRIK4*	25363768, 26749308, 28965761
3	*MED13L*	26749308, 28965761, 25363768
3	*NCOR2, TMEM132B*	25363768, 28965761, 26749308, 29700473
4	*CARKD, DCUN1D2, GAS6-AS2*	25363768, 28965761, 29700473
4	*CCDC88C, SMEK1, TRIP11*	25961944, 28965761, 25363768
5	*CREBBP, ADCY9, C16orf96*	25961944, 25363768, 26749308
6	*MYO1D, ASIC2, TMEM132E*	25363768, 26749308, 28965761
7	*ANKFN1, MSI2*	29700473, 26749308, 28965761
7	*TRAPPC8, KLHL14*	28965761, 25363768, 25961944
7	*HDHD2*	25363768, 28965761, 29700473
7	*PIK3R2, IFI30, ZNF43*	28965761, 25363768, 29700473
8	*BRSK1, NLRP11*	26749308, 25961944, 28965761
8	*CPSF3, PDIA6*	25961944, 26749308, 28965761
9	*SRBD1, PRKCE*	25363768, 28965761, 29700473
9	*FLJ30838, BCL11A*	26749308, 29700473, 25363768
9	*MBD5*	26749308, 23160955, 25363768
9	*NR4A2, UPP2*	25363768, 29700473, 26749308
12	*CSRNP1, CTNNB1*	29700473, 26749308, 23160955
12	*CACNA1D, CACNA2D3*	25418537, 26749308, 28965761, 29700473
15	*TRIO, FBXL7*	25363768, 28965761, 26749308
21	*FBXO10, CNTNAP3, PIP5K1B*	25363768, 28965761, 26749308
21	*PIP5K1B, APBA1, PTAR1*	26749308, 28965761, 25363768
21	*FBXO10, CNTNAP3, PIP5K1B*	25363768, 28965761, 26749308
22	*DNM1, PTGES*	28965761, 25363768, 25961944

ENVIRONMENTAL RISK FACTORS

The range of environmental risk factors in etiological studies of ASD is very broad and includes not only factors in the physical environment such as air pollution, heavy metals, toxic substances, microorganism, and pharmacyological medications, but also includes physiological, infectious and psychosocial environmental exposures to which parents and/or offspring were exposed before or after birth. For example; advanced parental age, allergic and autoimmune diseases, mental stress and disorders, delivery mode and premature birth have been suggested as risk factors for ASD [37]. A rigorous quantitative estimate of genetic heritability of ASD and shared environment with twin pairs have indicated that a large proportion of the variance in the liability of ASD (58%) can be explained by the shared environment in addition to moderate genetic heritability (38%) [38]. Furthermore, many environmental factors that may interact with genes have been associated with the onset of ASD, and their influence may distribute across a wide range of the early life, from germ cell to the early postnatal development [37,39] (Table 4).

Advanced parental age

Advanced parental age has been suggested as a risk factor for ASD. A Western Australia population-based study comprising 465 cases, 481 siblings of the cases and 1,313 controls has shown that ASD patients tend to be offspring of older parents [40]. Additionally, a meta-analysis of epidemiological studies showed that advanced paternal age is a risk factor for ASD in the offspring [41]. Another review and meta-analysis also supported an association between advancing maternal age and risk of autism [42]. The possible underlying biological mechanism might be that *de novo* genomic aberrations such as CNVs, mutations, or epigenetic alterations are associated with aging [43,44].

Table 4. Main environmental factors associated with Autism Spectrum Disorder.

Main Type	Details
Physical factors	
Air pollution	PM2.5, PM10, NO_2, O3, *etc.*
Heavy metals	Lead, mercury, *etc.*
Toxic substances	Pesticides, herbicides, other endocrine-disrupting chemicals (fragrance, testosterone, *etc.*)
Medication drugs	Exposure to selective serotonin reuptake inhibitor (SSRIs), Benzodiazepines, Valproate, or Antiviral drugs (acyclovir, *etc.*) medication during pregnancy
Physiological and pathological factors	
Advanced age	Paternal and maternal age
Delivery mode	Cesarean delivery
Allergic and autoimmune diseases	Asthma, Atopic Dermatitis, *etc.*
Infection and parasite	Bacterial or viral infections, Toxoplasma gondii during pregnancy
Abortion	Induced and threatened abortion
Premature birth	The gestational week of childbirth <37 weeks
Psychological factors	
Mental stress	Family issues and psychosocial stress during pregnancy
Mental disorders	Major depressive disorder and other psychiatric problems

Gestational conditions

The association between infections in pregnancy and ASD has long been noted. In the 1970s, Stella Chess found a high prevalence of autism in children with congenital rubella syndrome (CRS), which was 200 times that in the general population [45]. Although rubella infections have been greatly reduced in the past few decades, recent evidence linking ASD to rubella are still found [46]. In addition, viral, bacterial and parasitic infections including flu, measles, mumps, chickenpox, polio, pneumonia, sinusitis, tonsillitis, and toxoplasma gondii, have also been associated with ASD in retrospective and prospective studies [47–49]. Recently, in a large prospective study comprising 874 ASD and 874 matched controls from the Finnish national birth cohort (n=1.2 million) and the national psychiatric registries, parasitic infection with toxoplasma gondii was consistently associated with ASD [50]. The hypothesis of maternal immune activation (MIA) may explain the link between diverse maternal infections and ASD [51].

Psychosocial stress during pregnancy may increase the risk of developmental abnormallities in offspring and has been identified as a risk factor for mental disorder in children [52–54]. In the early 1990s, Ward reported that mothers of autistic children were more likely to experience family issues and diagnoses of psychiatric problems than the mothers of normal children [55]. A subsequent study found that mothers of children with ASD were more likely to have suffered from psychosocial stress in pregnancy than mothers of typically developed children. A higher number of maternal psychosocial stressors was observed at 21–32 weeks gestation, with a peak at 25–28 weeks, who gave birth to children with autism [56]. Although genestress interaction might be a potential explanation, empirical studies are relatively scarce [54].

Mothers who used medications during pregnancy might increase the risk of ASD in offspring. A Danish population-based case-control study comprising 473 ASD and 4,712 controls showed that maternal use of medicine during pregnancy may have a 1.5 fold increase in the risk of ASD in offspring. Additionally, a detailed analysis of different types of medications used during pregnancy indicated that the use of psychoactive drugs was associated with ASD [57]. Subsequently, a number of studies reported that maternal exposure to selective serotonin reuptake inhibitors (SSRIs) during pregnancy could increase the risk of ASD in offspring [58–60]. However, the evidence of the association between SSRIs and ASD lacks consistency [61,62]. Additionally, some neuropsychiatric drugs such as benzodiazepines, valproate, and antiviral drugs such as acyclovir are banned for pregnant women due to their severe adverse effect on fetal nervous system development.

Obstetric and perinatal conditions

Threatened abortion has been considered a risk factor for ASD. A meta-analysis of studies showed that threatened abortion could increase the risk of ASD in offspring by 81% [63]. This possible association is confounded by the fact that threatened abortion (a) has been associated with many child developmental disorders and (b) is likely caused by many factors such as fetal genetic defects, the maternal reproductive environment, maternal exposure to a toxic and harmful substance as well as physical and psychological trauma. Therefore the associ-ation between threatened abortion and ASD might be complicated and the two conditions might simply share a number of common risk factors. One population-based study indicated that mothers of children with ASD had a higher prevalence of threatened abortion than mothers of controls, however the cases did not differ from that seen in their siblings [40]. Another population-based study investigated all live singleton births in Western Australia between January 1984 and December 1999 (n= 383,153) and found that threatened abortion was only associated with an increased risk of ASD with intellectual disability, but not for ASD without intellectual disability [64]. Therefore, further studies are needed that account for comprehensive phenotype measurements of children with

ASD, the possible causes of threatened abortion as well as other potential genetic and environmental factors.

Premature birth (<37 weeks) is associated with many developmental disorders due to the immaturity of the nervous system and has been identified as a significant risk factor for ASD [65]. A previous small retrospective study (*n*=91) found twenty-six percent of extremely preterm infants were positive in autism screening [66]. In a subsequent prospective study investigating all births at less than 26 weeks of gestation in the United Kingdom and Ireland in 1995, extremely preterm births had significant impairment in social communication compared to their classmates, and 8% of extremely preterm births were diagnosed with ASD [67]. In a recent report from the Autism and Developmental Disabilities Monitoring (ADDM) Network, the average population attributable fractions of premature delivery were 4.2% for the 1994 cohort (*n* =703) and 2.0% for the 2000 cohort (*n*=1,339) [68]. However, there are still some inconsistencies. For example, the significant association between preterm birth and ASD was not found in a cohort study in Sweden, which was with 408 ASD and 2,040 matched controls [69]. In another cohort study in Denmark with 473 ASD and 4,712 matched controls, the association between preterm birth and ASD was significant in the crude analysis but became less significant when adjusted for mother and fathers age, mothers citizenship, birthweight, Apgar, birth defect and irregular fetal position [57]. It must be noted that premature delivery is not an exogenous variable since it may be affected by many other environmental factors and the maternal genetic background. In some cases, prematurity may indeed be a key mediator of risk for autism. Therefore, one or more studies with rigorous design and sophisticated analyses may be needed to validate or invalidate this association.

Cesarean delivery is common in more developed countries and has increased rapidly along with economic growth in less developed countries such as China [70]. Although cesarean birth can avoid many adverse events of natural childbirth, it may increase the risk of ASD in offspring significantly [71]. Two population-based studies found that ASD patients were more likely to be delivered by an elective or emergency cesarean section [40, 69]. In a recent cohort study of ASD, the average population attributable fractions for cesarean birth were 7.9% for the 1994 cohort and 6.7% for the 2000 cohort [68]. Nevertheless, the association between cesarean birth and ASD in offspring is still inconclusive. A Danish population-based study found that only scheduled cesarean sections were associated with infantile autism, and its significance was influenced by many factors such as parental age, birth weight, birth defects and aberent fetal presentations [57]. Although the mechanism underlying this association is unclear, anesthesia during childbirth, the absence of passage through the birth canal, lack of exposure to the maternal vaginal microbiome, and possibly other factors might be involved.

Environmental pollution

Environmental pollution such as air pollution, pesticides, herbicides, endocrine-disrupting chemicals (EDC), and heavy metals can persistently affect offspring from exposures across a range of developmental stages from the germ cell stage through postnatal early childhood and lead to an increased risk of ASD [37,72–74]. Most studies focus on exposure in one or more of critical windows, including (1) the first to the third trimester of pregnancy, (2) the entire pregnancy, and (3) the postnatal period. Fewer studies have been conducted to examine the effect of exposure throughout all of the critical developmental windows. Flores-Pajot *et al.* reported that exposure to PM2.5 and NO2 during pregnancy or after birth was associated with an increase in the risk of ASD while exposures to O3 was weakly associated with ASD during either the third trimester of pregnancy or the entire pregnancy [75]. These findings imply that the effect of maternal exposure to air pollution on neural development in offsprings may vary with the type of pollution and the period of pregnancy. Although many studies from the United States indicate that exposure to air pollution in early life is associated with ASD in children, European data showed that early-life exposure to low levels of NOx and PM_{10} from road traffic did not appear to increase the risk of ASD [76].

Furthermore, the effect of environmental factors or hormone exposures may have differences by sex of the offspring. First, exposure to fragrances may lead to male bias in the risk of ASD, and the possible mechanism is that men may lack OXYP+ and AVPR+ neurons [77]. Second, maternal exposure to prenatal and neonatal testosterone of fetal origin may be associated with sexual dimorphism in ASD [78]. Finally, some toxic exposures may interfere with conduction of electrical or chemical signals during development and cause neurodevelopmental disorders, whereas other exposures may disturb the endocrine and immune systems and evoke abnormal maternal immune responses which may, in turn, increase the risk of ASD [73].

SUMMARY

While many genetic variants and environmental risk factors have been identified in ASD, more attention should be paid to the interacttion between genes and the environment. To conduct these types of studies, cohorts will need to be well-characterized in terms of genetics and in terms of accurate measurements of maternal exposures to environmental factors. Accounting for both classes of variables (gene and environment) should increase the power to detect genetic variants that may be more robustly associated with ASD. In the MIA hypothesis, maternal immune activation during pregnancy is considered as a general liability of risk of ASD and it may make an individual more vulnerable to the influence of genetic and environmental factors [51]. In terms of epigenetic mechanisms, environmental factors can mod-ify the expression of genes via epigenetic changes which may thereby lead to an increased risk of ASD [79]. All of these hypotheses and potential interactions among them

need to be further tested with more empirical studies. For example, based on data from the Childhood Autism Risks from Genetics and Environment (CHARGE) study which started in 2006 [80], Kim *et al.* reported in 2017 that global copy number variation (duplication) may interact with certain environmental factors (ozone) to increase the susceptibility of ASD [81].

We acknowledge the challenges in conducting such studies, especially when it comes to the interaction between genes and the environment. First, quantitative measures of the exposure to many environmental factors of many types are a challenge to perform and many will need to be carried out over various periods of time in order to adequately document the levels and duration of such exposures and how those attributes relate to various developmental intervals. Not only are those measurements of environmental factors costly and time-consuming, genotyping large numbers of samples also demands substantial research resources.

Study of both genetic and environmental factors associated with ASD can provide new evidence for effective prevention or to identify novel drug targets for developing future therapies for ASD. Given that, the cost of chip-based whole genome genotyping has dropped, it will be possible to conduct one or more large-scale studies on population-based samples. A cohort study with a follow-up of a number of parents, especially for those exposed to specific environmental factors of interest to examine how the environment may cause the development of ASD or other neurodevelopment disorder should be undertaken. Those studies would provide opportunities to identify high-risk sub-populations for prevention or early therapeutic treatment(s). Finally, additional patient cohorts need to be established in order to monitor other comorbidities of ASD children during their critical stages of development.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review

Altered Gut Microbiome in Autism Spectrum Disorder: Potential Mechanism and Implications for Clinical Intervention

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ABSTRACT

Autism spectrum disorder is a heterogeneous neurodevelopmental disorder with an increased prevalence around the world over the past two decades. Remarkably, a large number of individuals with ASD have gastrointestinal disorders. Recent studies demonstrate that the endogenous gut microbiota has a close relationship with ASD according to the analyses of human host intestinal microbial composition and animal model studies. Here, we review the reports of microbial dysbiosis in ASD, and then discuss the recent evidence of biological interactions among microbiota, metabolism, immunity, neurodevelopment, and behaviors. We also describe the role of the gut microbiome in the link between ASD and environmental risk factors. Finally, we suggest adjuvant treatments to consider in attempts to correct autistic behaviors.

KEYWORDS

Gut microbiome; autism spectrum disorder; clinical interventions

INTRODUCTION

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder characterized by persistent deficits in social communication as well as unusually restricted and repetitive patterns of behavior or interests. Partly due to the changes in the diagnostic criteria, the prevalence of ASD has been reported from 0.60 to 2.64% [1–3]. The number of ASD cases was estimated at 62.6 million around the world in 2015, and the costs of the care for ASD children impose an enormous burden on families [4, 5]. In addition, individuals with ASD often experience co-morbid disorders including irritability, aggression, sleeping problems, developmental delay and epilepsy [6–8], which may cause additional medical expenses.

While population-based family studies estimate the heritability approximate 50–90% [9, 10], only a few common variants that contribute to the heritability have been associated with ASD. Previous large-scale genetic studies have identified a number of rare variants such as copy number of variants and single point mutations [11], and the shared environmental influence was 30% [10]. ASD children often have gastrointestinal problems such as abdominal pain, diarrhea, and constipation [12–14], which are associated with the microbiome, in particular, evidence of *Sutterella* species have been found in the patients diagnosed with ASD but not in the control children with GI symptoms [15].

In this paper, we provide an overview of recent evidence about the interrelation among the gut microbiome, metabolism, immunology, and neurobiology. We review the epidemiological studies of gastrointestinal diseases and the composition of the gut microbiome in individuals with ASD, and then discuss gut-immune function, neurodevelopment, metabolism, and other environmental factors. Finally, we review the existing evidence at a molecular level that might suggest some possible therapeutic interventions.

GASTROINTESTINAL DISTURBANCES AND GUT MICROBIOTA

While the prevalence of digestive problems in ASD individuals has been reported varying at 23–70%, there is evidence that individuals with ASD have more likelihood of having gastrointestinal disorders [12]. A study found a strong correlation between gastrointestinal symptoms with autism severity [16], and altered gut microbiota has been found in children with ASD.

Microorganisms in the gut are essential for the host and play a crucial role in digestion [17, 18]. Nondigestible nutrients such as polysaccharides and cellulose can be fermented by microbiota in the intestine to produce energy

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and short chain fatty acids [18]. The short chain fatty acids, including butyrate produced by a single bacteria strains, *Clostridium tyrobutyricum* (CBut), acetate and propionate produced by *Bacteroides thetaiotaomicron* (BEta), can influence the expression of the tight-junction proteins (ZO-1, occludin, claudin-5) which are related to blood-brain barrier permeability and thus may have a secondary impact on the function of the brain [19].

Meanwhile, individuals with ASD have a different composition of microbiota in the gut compared with the neurotypical-developed children (NTD) [20]. *Sutterella* were found in more than 50% of individuals with both ASD and intestinal dysfunction, but not in the NTD children with pure dysfunction of the gut [21,22]; and Kang reported lower levels of *Prevotella, Coprococcus*, and unclassified *Veillonellaceae* in the gut bacterial populations in children with ASD [23]. It has been indicated that changes in a few microbial species may lead to behavioral problems but the mechanism is not defined.

METABOLIC DISTURBANCE AND GUT MICROBIOME

The metabolic disturbance has been observed in individuals with ASD. Compared to their unaffected siblings and age-matched healthy controls, children with ASD children tend to have a distinct profile of metabolic phenotyping in urine [24]. Through a metabolomic analysis, docosahexaenoic acid (DHA) and sphingosine 1-phosphate (S1P) in serum have been found to be lower in individuals with autism [25] and an elevated level of p-cresol in urine has been identified and replicated in an independent study [26]. In the human gastrointestinal tract, symbiotic bacteria play an indispensable role in degrading nondigestible carbohydrates.

Animal studies suggest that those metabolic changes are likely due to an imbalance of the gut microbiome. Germ-free mice show a significant difference in metabolites in luminal contents of the colon compared with ex-germ free mice which were with a gavage of feces from specific pathogen-free mice [27], indicating that the microbiota may profoundly influence the colonic luminal metabolome. The metabolomic analysis also suggested that gut microflora have significant effects on mammalian blood metabolites [28].

When rats were treated with maternal high-fat diet (MHFD) for several weeks, the level of acetate dramatically increased due to the gut microbiota-food interaction. Acetate may increase glucose-stimulated insulin secretion (GSIS) across the activated parasympathetic nervous system [29]. This positive feedback may lead to the development of obesity as a critical element of the metabolic syndrome. Another study in mice shows that a high-fat diet altered the composition of gut microbiota including *Bifidobacterium*, which plays a functional role in barrier-protection [30]. Further, in contrast with mice with a gavage of heatkilled bacteria, mice with a gavage of *Akkermansia muciniphila* showed maintenance of the intestinal barrier and a reversal of high-fat-diet-induced metabolic disorders, including fat-mass gain and insulin resistance [31, 32].

Studies have shown that the gut microbiota modulates ASD-related behaviors and affect the levels of specific metabolites. Gastrointestinal barrier defect and alteration of microbiota have been noted in maternal immune activation (MIA) mouse model associated with ASD [33]. MIA offspring treated with the human commensal *Bacteroides fragilis* had a significant improvement in gut permeability, microbial composition, and ASD-related behaviors. This treatment also modulates the levels of several specific metabolites, suggesting that gut bacterial effect on the host metabolome affect behaviors. These findings support a gut-microbiome-brain connection in a mouse model of ASD.

IMMUNE DYSREGULATION AND GUT MICROBIOME

Immune dysregulation including maternal inflammation or infection may increase the vulnerability of neurodevelopment, such as ASD [34–38]. A study of brain tissues and cerebrospinal fluid (CSF) have shown an activation of microglia and astroglia or a marked increase in a small cytokine monocyte chemoattractant protein 1 (MCP1) in individuals with ASD [39]. A meta-analysis of 19 cytokines in plasma and serum from 17 studies shows that individuals with ASD have a marked elevation of prion-flammatory cytokines, including interleukin (IL)-1, IL-6, IL-8, interferon (IFN)-γ, eotaxin, MCP1 in blood compared with healthy controls [40]. However, consistent data are still lacking for other cytokines, likely due to the difference in the methodology, etiological variability and population heterogeneity [41–43].

The immune system and gut-resident microbes have a pronounced interaction [44]. In an animal model, MIA causes an ASD-like phenotype and the maternal inflammation during critical periods of the embryonic development leads to an alteration of the immune system and further disturbs the fetal environment, which may cause aberrant behaviors in offspring [45]. MIA male mice offspring show a lower rate of ultrasonic vocalizations, fewer harmonic, and more complex voice, which may continue into adulthood. Additionally, MIA offspring display increased repetitive or stereotyped behaviors, a mouse version of the core symptoms of autism [33, 45, 46]. The target region of MIA was located in the primary somatosensory cortex (S1DZ) [47] where there was an increase in neural activities that project to the temporal association cortex (TeA) or striatum in this cortical region, which can in turn result in impaired sociability. Moreover, the inhibition of neural activity is sufficient to suppress the abnormal behaviors [47].

Despite the fact that immune activation contributes to abnormal behaviors, it is not clear how microbes participate in the induction of immune activation. In the germ-free mice, the introduction of gram-positive gut-residing segmented filamentous bacteria (SFB) can cause an autoimmune disorder [48]. SFB increase the number of T helper 17 (T_H17) cells, which differentiate from $CD4^+$T cells induced by the collaboration of TGF-b and IL-6 and secrete IL-17 in promoting inflammatory responses. Maternal colonization of SFB together with

injection of poly (I:C) can stimulate the activity of T_H17 cells and lead to abnormal behaviors in offspring [49]. However, mice injected with poly(I:C) alone show a significant increase in TNFα and IFN-β compared with endotoxin-free phosphate-buffered saline (PBS)-injected mice, but not exhibit the change in the behaviors of offspring. Treatment of pregnant mice with vancomycin reducing SFB and the abnormal behaviors of offspring can be rescued [49]. In contrast to SFB, some other microbes are involved in suppressing the immune response in the host. Mucosal colonization of *Bacteroides fragilis* could suppress T_H17 response in germ-free mice [50]. The underlying mechanism may be that the polysaccharide A on *Bactericides fragilis* promotes immunol-ogic tolerance through increasing the proportion of regulatory T cells by action at the Toll-like receptor 2 [50,51].

Another potential bacterial modulator of MIA is the lipopolysaccharide (LPS) endotoxin located on the surface of gram-negative bacteria. LPS can bind the Toll-like receptor receptors, which that trigger an immune response. Maternal inflammation in mice triggered by LPS (*E. coli* serotype 0111:B4) can cause a proliferation of neural stem and progenitor cells in offspring, which also exhibited autism-associated behaviors including reduced ultrasonic vocalizations [52–54]. A study showed that offspring of rats with maternal immune activation by LPS had an alteration of specific gene expression profiles of interneuron migration and oxidative stress without a triggering a fetal immune response and exhibited reduced social and exploratory behaviors [55].

GUT MICROBIOME AND NEURODEVELOPMENT

The microbial composition and function in the intestine affect the development of nerve cells from the embryo to adulthood. These effects include the differentiation of neural stem cells, migration, and projection of immature neurons to their destinations, the growth of axons and dendrites, and the formation of synapses. While germ-free mice exhibited an increase in adult neurogenesis in the dorsal hippocampus, which plays a role in spatial learning and memory, the recolonization of microbiota in germ-free adult mice cannot change this tendency, suggesting a critical part of microbiota in early developpment life. This is consistent with the past demonstration that stress or long-term antibiotic treatment may potentially inhibit neurogenesis in the hippocampus [56].

Microglia are tissue macrophage cells located in the brain that participate in immune responses including removal of dead cells and pathogens. Several studies found that marked activation and augmented number of microglia in different regions of the brain are pathophysiologic characteristics of ASD [39, 57, 58]. The complexities of host microbiota can contribute to maturation and function of microglia. Germ-free mice exhibited global defects in microglia with altered cell proportions and an immature phenotype, which show impaired innate immune responses to LPS. The full repertoire of microbes or SCFA is necessary and sufficient to restore impairment of microglia stability [59].

Administration of antibiotics to the pregnant mice not only influence gut microbiome composition but also affect the behavior of the offspring in locomotor activity and anxiety. It has been shown that the behaviors of the offspring from antibiotic-treatment pregnant-mice can be rescued at week 4 when normal pregnant mice fostered them from the post-natal day one [60]. Another study found that female rats exposed to a diet containing 1% succinyl sulfa thiazole, a non-absorbable antibiotic, during periconceptional period, can alter the offspring behaviors without maternal infection, also likely due to the alteration of maternal gut microbiota [61].

GUT MICROBIOME LINKS BETWEEN ASD AND ENVIRONMENTAL RISK FACTORS

Neurons are highly sensitive and vulnerable to environmental factors such as maternal stress in early development in humans, especially in the first three years. The method of delivery, breastfeeding, maternal separation and infection all may have an impact. The mode of obstetrical delivery has a significant influence on the composition of the intestinal microbiota at the very beginning of human life [62, 63]. Epidemiological studies report that cesarean section (C-section) delivery is associated with the non-communicable chronic diseases including immune and metabolic disorders [64]. Children born by C-section including elective and emergency C-section are more likely to have ASD, although this tendency does not appears in sibling controls [65, 66]. Children delivered through C-section show a different pattern of gut microbiome compared to infants born via vaginal delivery. This difference seen in C-section infants can be restored to that seen in vaginally delivered infants by exposure to maternal vaginal fluids [67].

Maternal stress is another environmental factor that may affect major physiological systems including neurodevelopmental disorders. Maternal stress increases the proinflammatory state of the fetal central nervous system, and such neuroimmune inflammation has been implicated in ASD [68]. The microbiome of the maternal vagina can be disturbed by the early-life stress and in turn influence the gut microbiome of offspring. For example, the decrease of *Lactobacillus* in the maternal vagina of the mouse model may lead to a reduction in the transmission of this bacterium to offspring, which influence the offspring microbiota composition and metabolic processes that are essential for normal neurodevelopment [69]. In addition, prenatal stress induces impairment of novel object recognition memory in a rat model, which shows a significantly lower memory index measured by novel object recognition test (NORT) [70].

Maternal infection with different types of agents (bacterial, viral, parasitic, and fungal) and site of infection (genitourinary infections and skin infections) during pregnancy is another risk factor that can increase the prevalence of abnormal behaviors associated with autism [71, 72]. Maternal infection affects the maternal immune responses and the fetal neurodevelopmental environment at a critical time. In utero exposure to valproic acid (VPA), a

drug used to treat epilepsy and bipolar disorder, causes a change in the composition of gut microbiota and further influences metabolism in a murine model of ASD [73].

Maternal exposure to pesticides and certain fungicides such as pyraclostrobin, trifloxystrobin, famoxadone and fenamidone in the environment or the food, is associated with the neurodevelopmental disorders including ASD [74, 75]. All these chemicals produce transcriptional changes *in vitro* that are similar to those observed in brain samples from individuals with autism including stimulation of free radical production and disruption of microtubules in neurons. These effects can be reduced by pretreating with a microtubule stabilizer, an antioxidant, or with sulforaphane [76].

THE DIRECTION OF POTENTIAL INTERVENTIONS

So far, several potential mechanisms including immune, metabolic and neurological pathways have been implicated by the use of animal and cellular models, and each of these pathways can be considered as potential therapeutic targets for one or more phenotypes of ASD. First, psychotropic drugs including risperidone and aripiprazole are often used to improve behavioral symptoms of ASD. The atypical antipsychotic risperidone was the first drug approved by the FDA for treating repetitive behavior and hyperactivity, and it acts via modulation of the 5-HT and dopamine neuronal systems, which are related to ASD [77–81]. Second, sulforaphane or broccoli extracts can reduce free radical production and protect microtubules in neurons by inducing expression of Nrf2, which is a transcription factor that regulates the expression of antioxidant proteins and protect cells against oxidative damage [74, 82, 83]. ASD children taking oral sulforaphane for 18 weeks had improvement of phenotypes including social interaction and communication after discontinueing treatment about four weeks [76]. Third, oxytocin treatment or activating oxytocin neurons can also restore the ASD-like social behaviors in Cntnap2 knockout mice, which shows a reduced number of oxytocin neurons [84]. Detailed discussions of the mechanism relating oxytocin and neuromodulation in ASD as well as potential medical treatments have been presented in the literature in the past [85, 86].

Further, evidence shows the relationship between the intestinal microbiome and the function of the nervous system. It has become plausible to consider using commensal probiotics to treat neurodevelopment diseases such as ASD. For example, MIA mice offspring treated with a single organism, *Bacteroides fragilis*, show improved ASD-related defects in the core symptoms of communicative, stereotypic, anxiety-like and sensorimotor behaviors [33, 45]. In another study in mice, *Lactobacillus reuteri*as, a single probiotic and a commensal strain, corrected the social deficits in the MHFD offspring by inducing increased oxytocin [87].

Finally, fecal microbiota transplantation (FMT) has already been used to alleviate intestinal diseases such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), presumably by correcting the composition of the gut microbiota [88–90]. A recent report indicates that Microbiota Transfer Therapy (MTT) and then an extended fecal microbiota transplant (FMT) can improve autism symptoms and alter the gut ecosystem [91]. In this open-label study, autistic individuals with moderate to severe gastrointestinal problems were treated with antibiotics for two weeks and then used a dose of standardized human gut microbiota for 7–8 weeks. ASD-related symptoms showed a significant improvement.

Although the prospects are promising, potential negative aspects of treatment on the subject must be considered. Children treated with risperidone do not improve core behaviors associated with autism, and improved symptoms will return to the original state when the drug is discontinued. Moreover, children showed a significant gain in weight due to an increased daily intake of food, especially carbohydrates that may have implications for risk of insulin resistance and metabolic syndrome [92]. Microbiota transplantation may also disturb the balance of the intestinal microbial composition. Individuals with recurrent *Clostridium difficile* infection who received FMT had adverse events such as diarrhea, abdominal discomfort, and even one death occurred because of aspiration during sedation for FMT administered via colonoscopy in the treatment of *Clostridium difficile* infection [93–96]. Whether diverse microbiota does benefit or harm to the homeostasis of the intestine are still unknown at present, and it is also not yet understood what microbes play vital roles in the healthy functioning of the gut. Therefore, in-depth characterization of defined communities or even various individual microbial species that may ameliorate ASD symptoms should be a focus in the future.

CONCLUSION

In summary, environmental risk factors contribute to the development of ASD and the role of the gut microbi-ome in host homeostasis is now well established. The gut microbiome influences metabolism, immunity, and neurophysiology and these converge to influence development processes in the brain and following behaviors (Figure 1). We have reviewed recent evidence for neurodevelopment and behavior changes in both animal models and humans as influenced by gut microbiome status for ASD-associated behaviors. However, the possible mechanism depends upon exposure to specific microbes, beneficial or pathogenic, induction of certain phenotypes in the immune system, the metabolic system, the central nervous system, or the enteric nervous system.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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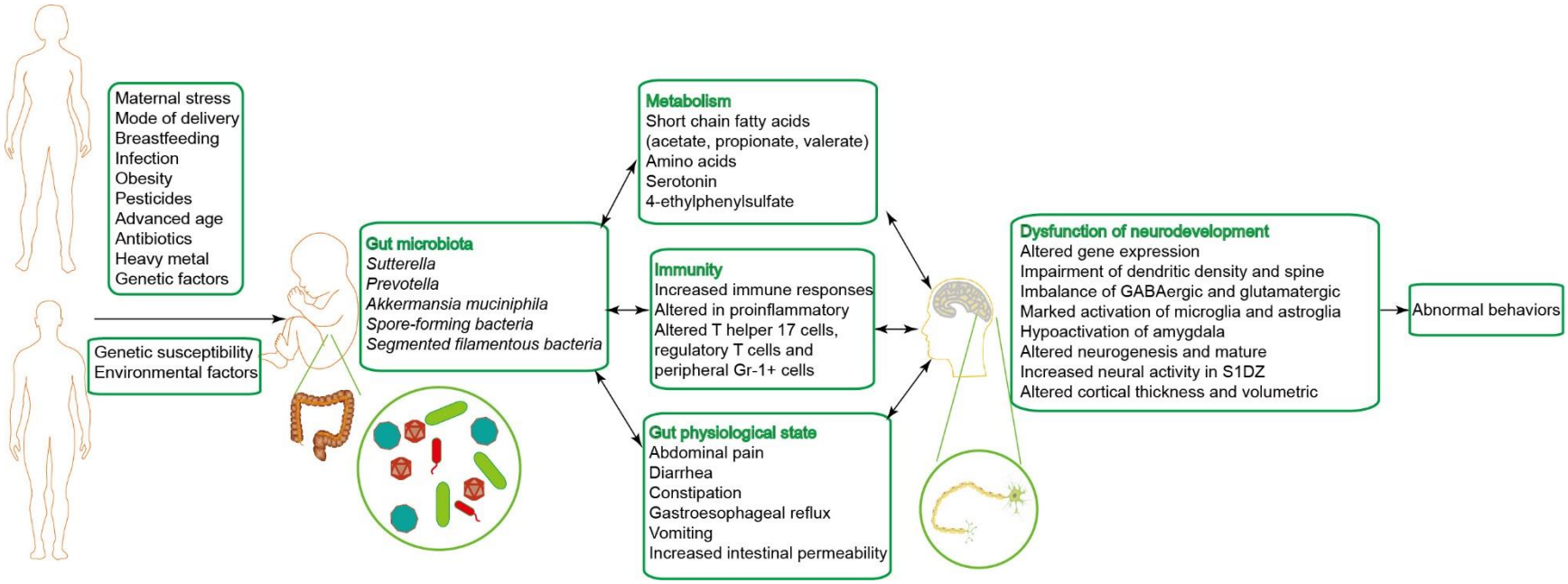

Figure 1. Schematic diagram illustrating the hypothesized role of the gut microbiome in autism spectrum disorder (ASD). Environmental exposures and genetic factors directly or indirectly affect microbiotic exposures. The microbiota interacts with gut immune physiology, metabolism and the brain, and these alterations elicit the abnormal behaviors of ASD.

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Short Report

Childhood Adversity and Depression among Older Adults: Results from a Longitudinal Survey in China

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ABSTRACT

This study examined the association of childhood adversity with depression or severity of depressive symptoms among Chinese older adults, using data from the China Health and Retirement Longitudinal Study (CHARLS). The data is from a nationally representative sample of Chinese residents aged 45 or older and surveys of the sample population were conducted in 2011 and 2013; and individuals aged at 60 years or older, and interviewed for depressive symptom were included in this study. Multiple logistic regression analyses showed that the likelihood of depression was significantly associated with poor parental mental status, physical abuse, and emotional abuse during childhood. Our study adds to research in the area of adverse childhood events and its effect on adult psychological and physical well-being.

KEYWORDS

Depression; childhood adversity; population aging

INTRODUCTION

Depression is a common and serious mental health problem in the general population and affects about 4–15% of older adults [1]. According to the estimates of the World Health Organization in 2017, depression is more prevalent in the general population of North America. The United States 2012–2013 National Survey estimated that the 12-month and lifetime prevalence of depression was 10.45% and 20.6%, respectively [2]. While the prevalence of depression is estimated to be lower in Asian countries, the lifetime prevalence of suicide attempts in patients with depression could be as high as 40% [3]. Meanwhile, depression symptoms in older adults are often mistaken for other symptoms of normal aging and so do not draw attention clinically, and patients do not get treatment.

Studies have indicated that childhood adversity experience is not only associated with risk of suicide [4] and risky behaviors such as alcohol misuse, risky sexual practices and criminal activities which persist into adulthood [5], but also with geriatric depression in later life [6]. Greater psycho-social adversity in childhood has been associated with poorer physical capability in people of middle age in Western societies [7]. Recently, a cohort study has indicated that low childhood socioeconomic status is associated with the onset of depression in Japanese older adults [8]; childhood abuse has been associated with depression in later life in older persons in The Netherlands [9]. In this Dutch study, the investigators suggested that older adults with a history of childhood abuse may be more negatively affected by stress or stressful events, and presented data which suggested the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [9]. In addition, the causes of depression in older adults are not clear, and study of childhood adversity may help elucidate the etiology of depression in older adults. Since no study has been conducted in the Chinese populations, we performed a study to examine the effect of childhood adversity on depression symptoms and depression score among the elderly.

DATA AND METHODS

This study used the datasets from surveys conducted in 2011 and 2013 as a part of the China Health and Retirement Longitudinal Study (CHARLS) [10]. The sample included Chinese residents aged 60 years or older who were interviewed as part of a nationally representative sample of Chinese residents; and individuals who participated in the interviews conducted in both 2011 and 2013 were included for this study.

The depression outcome was measured using the Center for Epidemiologic Studies Depression 10-item (CES-D10) scale, a short self-report scale designed to measure depressive symptomatology in the general population [11]. Depression was defined when the total CES-D10 score was greater than 10 and the total score of depression

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was obtained. The childhood adversities were measured by five domains: physical abuse, emotional abuse, emotional neglect, the worse mental status of parents or guardian, and a negative relationship of parents. Because the sample was with a repeated- measure in both 2011 and 2013, we performed, after a descriptive analysis of depression by the childhood adversity, a multiple logistic regression analysis and a multiple linear regression with random-effect model to examine the association of the risk for depression and depressive symptom score with the childhood adversity, respectively, in individuals who were interviewed in the surveys at both years.

RESULTS

The baseline statistics of individual child hood adversity measurement and coding are presented and score was summarized by individual domains (Table 1). Depression and the mean depression score were increased with multiple domains of childhood adversity experienced. Based on data from 2011, the rate of depression was significantly elevated in individual older adults who experienced poor mental status of parents or guardians, such as guardians who often felt nervous and anxious (47.64%), guardians who often got upset easily or feel panicky (50.97%), guardians who showed sadness or depression lasting over two weeks (46.35%) compared with those who did not have these respective experiences (P<0.0001). Individuals who experienced physical abuse such as being beaten by the guardian (40.8%) and emotional abuse such as the guardian being too strict (40.5%) in childhood also had a significantly elevated rate of depression in later adult life compared those who did not have such experiences (P<0.0001) (Table 2).

While the rate of depression was a little lower, the findings were consistently present in the follow-up survey conducted in 2013. Individuals who experienced a childhood adversity were more likely to have depression in 2013 when the follow-up survey was conducted. These associations of childhood adversity with depression in later life were also consistent when the total score of depression was analyzed in both 2011 and 2013.

After adjusting for covariates such as age, sexes, as well as other variables that measure physical condition, multiple domains of childhood adversity experience were associated with the rate of depression and total score of depression in the combined analysis of the two surveys together. Multiple logistic regression analysis of depression in both surveys (2011 and 2013) found that the likelihood of depression was significantly associated with poor parental mental status (OR=1.44; 95% CL: 1.31–1.57), physical abuse (OR=1.35; 95% CL: 1.12–1.65), and emotional abuse (OR=1.23; 95% CL: 1.04–1.46) (Table 3). In addition, we found that the poor parental mental health status during childhood was associated with depression symptom scores in later life (Beta =1.14; 95% CL: 0.95–1.33); and it was still significant even after adjusting for the confounding factors (Beta=0.87; 95% CL: 0.71–1.04). Further, the number of total adverse events experienced in childhood significantly increased the likelihood; and this may suggest a "dose-response" relationship between childhood adversity and depression in later life.

Table 1. Descriptive statistics and coding for the independent variables at the baseline in 2011 (n = 3,436).

Variable	Code	%	Mean (SD)
Poor mental status of parents or guardian	Sum score		0.55 (0.90)
Guardians often felt nervous and anxious	1, most, often or always 0, No or selfdom	18.51	
Guardians often got upset easily or feel panicky	1, most, often or always 0, No or selfdom	15.02	
Guardians' sadness or depression lasting over 2 weeks	1, Yes 0, No	21.54	
Physical abuse	Sum score		0.26 (0.44)
Beaten by guardians	1 = often or sometime 0 = seldom or never	25.87	
Emotional abuse	Sum score		0.33 (0.50)
Guardians were too strict	1, strict or too strict 0, no or little strict	33.41	
Guardians treat siblings better than you	1, Yes 0, no	1.46	
Emotional neglect	Sum score		0.44 (0.68)
Female guardian often gave love and affection	1, No or selfdom 0, Sometimes or often	21.21	
Female guardian often into watching over you	1, No or selfdom 0, Sometimes or often	23.52	
Poor parental relationship	Sum score		0.28 (0.60)
Parents often quarrel	1, Sometime or often 0, few or never	19.70	
Your father often beat up your mother	1, Sometime or often 0, few or never	7.67	
Your mother often beat up your father	1, Sometime or often 0, few or never	1.17	

Table 2. Distribution of childhood adversity and depression among older adults in China (*n* = 3,436).

Variables	Code	Depression as binary(1 =Yes, 0 =No)				Depression as continuous score			
		2011		2013		2011		2013	
		%	*P*	%	*P*	Mean	*P*	Mean	*P*
Poor mental status of parents or guardian									
Guardians often felt nervous and anxious	0 = No	32.96		25.10		8.40		7.53	
	1 = Yes	47.64	<0.0001	40.30	<0.0001	10.76	<0.0001	10.03	<0.0001
Guardians often got upset easily or feel panicky	0 = No	32.98		25.20		8.42		7.58	
	1 = Yes	50.97	<0.0001	43.40	<0.0001	11.22	<0.0001	10.31	<0.0001
Guardians felt sadness or depressed over 2 weeks	0 = No	32.75		24.40		8.34		7.49	
	1 = Yes	46.35	<0.0001	40.70	<0.0001	10.65	<0.0001	9.82	<0.0001
Physical abuse									
Beaten by guardians	0 = No	33.88		26.40		8.62		7.88	
	1 = Yes	40.83	<0.0001	32.10	0.0010	9.48	<0.0001	8.31	0.0640
Emotional abuse									
Guardians were too strict	0 = No	33.26		26.20		8.55		7.82	
	1 = Yes	40.51	<0.0001	31.30	0.0020	9.42	<0.0001	8.35	0.0130
Guardians treat siblings better than you	0 = No	35.50		27.50		8.81		7.97	
	1 = Yes	48.00	0.0670	37.50	0.1350	10.94	0.0190	9.76	0.0330
Emotional neglect									
Female guardian often gave you love and affection	0 = No	35.26		27.80		8.77		7.99	
	1 = Yes	39.46	0.1100	29.00	0.6280	9.46	0.0460	8.03	0.8900
Female guardian often into watching over you	0 = No	35.33		27.30		8.77		7.92	
	1 = Yes	40.00	0.1300	35.70	0.0040	9.76	0.0160	9.04	0.0030
Poor parental relationship									
Parents often quarrel	0 = No	34.68		26.70		8.71		7.82	
	1 = Yes	40.15	0.0080	33.20	0.0010	9.40	0.0120	8.73	<0.0001
Your father often beat up your mother	0 = No	34.92		26.90		8.71		7.86	
	1 = Yes	44.27	0.0020	40.00	<0.0001	10.21	<0.0001	9.71	<0.0001
Your mother often beat up your father	0 = No	35.70		27.80		8.83		7.97	
	1 = Yes	37.50	0.8100	35.90	0.2620	9.90	0.2900	9.93	0.0370

Table 3. Multiple regression with a random-effect model estimates of childhood adversity on depression in older adults (age ≥ 60 years).

	Depression as a binary						Depressive score					
	Model 1			Model 2			Model 1			Model 2		
	OR	95% CL		OR	95% CL		Beta	95% CL		Beta	95% CL	
Physical abuse	1.43	(1.16,1.77)	***	1.35	(1.12,1.65)	**	0.44	(0.03,0.84)	*	0.24	(−0.11,0.58)	
Emotional abuse	1.32	(1.11,1.58)	**	1.23	(1.04,1.46)	*	0.44	(0.09,0.78)	*	0.27	(−0.03,0.57)	
Emotional neglect	1.21	(1.01,1.46)	*	1.08	(0.90,1.30)		0.44	(0.08,0.81)	*	0.25	(−0.06,0.57)	
Poor parental mental status	1.59	(1.43,1.75)	***	1.44	(1.31,1.57)	***	1.14	(0.95,1.33)	***	0.87	(0.71,1.04)	***
Poor parental relationship	1.15	(0.99,1.34)		1.09	(0.95,1.26)		0.33	(0.03,0.62)	*	0.21	(−0.04,0.46)	

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$; Model 1, Controlling for age, sex, marital status, resident; Model 2, Adjusting for additional physical status, such as living with children, Instrumental activities of daily living (IADL), number of chronic illness, cognitive status, memory, sleep, type of medical insurance, community condition, education and previous occupation before retirement.

DISCUSSIONS

Our findings provide novel evidence that childhood adverse events affect the likelihood of depression and levels of depressive symptom scores in later life in the Chinese population which is consistent with previous studies in older adults [8, 9] in other global regions. Previous studies have indicated that childhood adversity interacted with past year stressful life events on the 12-month prevalence of major depression, post-traumatic stress disorder and anxiety disorder [12] in general populations based on the National Epidemiological Survey of Alcohol and Relate Conditions. Individuals with 3 or more childhood adverse events may have a 2-fold increase in the risk of developing depression when experiencing stressful events during the past year compared to those without suffering from the childhood adverse events. In addition, a large meta-analysis of 18 case-control studies, 10 prospective studies and 8 population-based studies demonstrated a consistent association with moderate to high effect size of childhood adversity with psychosis [13].

In summary, using a population-based longitudinal survey in older adults, we found evidence that childhood adversity significantly increased the likelihood of depression in later life in residents of China. Mental health is essential not only for child development but also for health in later life. One limitation of our study is that we did not have objective data on these childhood adverse events but relied on recall of people in the survey, and it is likely that people currently experiencing depression will be more likely to remember adverse childhood events than people who are not depressed. Even with this potential bias, our study adds to research in the area of adverse childhood events and its effect on adult psychological and physical well-being. Further research is needed to find interventions that can be translated into real life moderation of childhood adverse effects on subsequent adult well-being.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Introduction to the editors

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