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Fetal alcohol spectrum disorders: Genetic and epigenetic mechanisms

Nina Kaminen-Ahola

Environmental Epigenetics Laboratory, Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland

Correspondence
Nina Kaminen-Ahola, Department of Medical and Clinical Genetics, Biomedicum Helsinki, PO Box 63, FI-00014 University of Helsinki, Finland.
Email: nina.kaminen@helsinki.fi

Abstract

Fetal alcohol spectrum disorders (FASD) are a consequence of prenatal alcohol exposure (PAE). The etiology of the complex FASD phenotype with growth deficit, birth defects, and neurodevelopmental impairments is under extensive research. Both genetic and environmental factors contribute to the wide phenotype: chromosomal rearrangements, risk and protective alleles, environmental-induced epigenetic alterations as well as gene-environment interactions are all involved. Understanding the molecular mechanisms of PAE can provide tools for prevention or intervention of the alcohol-induced developmental disorders in the future. By revealing the alcohol-induced genetic and epigenetic alterations which associate with the variable FASD phenotypes, it is possible to identify biomarkers for the disorder. This would enable early diagnoses and personalized support for development of the affected child.

1 | INTRODUCTION

Prenatal alcohol exposure (PAE) is a leading cause of preventable mental disability and birth defects in the Western world. PAE can produce fetal alcohol spectrum disorders (FASD), which is an umbrella term for all alcohol-related neurodevelopmental disorders and birth defects. This continuum of disabilities consists of growth deficits, physical abnormalities, neurocognitive and behavioral deficits, and increased vulnerability to mental health problems and other comorbidities.[1-3] There are four diagnostics categories within FASD. The most severe category is fetal alcohol syndrome (FAS) with diagnosed pre- and post-natal growth retardation—especially in the head—as well as characteristic facial dysmorphology, and central nervous system alterations. In addition to FAS, there is partial fetal alcohol syndrome, alcohol-related neuronal disorders, and alcohol-related birth defects.[4] There are several factors contributing to the complex phenotype of the alcohol-induced disorders, such as genetic susceptibility, drinking pattern, timing of drinking, amount of alcohol as well as maternal metabolism and tolerance for alcohol[5-8] (Figure 1). The estimated prevalence of FASD ranges from 3% to 5% in Europe and North America to over 10% in South Africa,[9] but owing to the complex, highly variable phenotype, and lack of proper diagnostic tools, FASD is severely underdiagnosed. In a recent study among first-graders in the United States, 222 children were diagnosed with FASD and only two of them had previous diagnosis.[10]

Currently, the assignment of an FASD diagnosis is a demanding medical diagnostic process and requires a multidisciplinary team of experts in pediatrician, clinical genetics, clinical dysmorphology and neuropsychology with complementary experience, qualifications, and skills.[4] Owing to the challenges in current diagnostics, a solid diagnostic method is needed to separate the categories from each other as well as to understand the wide variety of subphenotypes. Also, the severity of defects in each category should be determined: it is already known that FASD individuals without the full criteria of FAS can be severely impaired in brain function.[11] Furthermore, a method to discern similar phenotypes like attention deficit hyperactivity disorder and autistic spectrum disorders from FASD is needed.

The complexity and broadness of the PAE-induced phenotypes can be perceived by analyzing comorbidities in individuals with FASD diagnosis. They are at risk of multiple comorbidities, like conduct disorder, receptive and expressive language disorders, and abnormal results of function studies of peripheral nervous system, and special

senses.[3] For example, the pooled prevalence of hearing loss (for both conductive and sensorineural), psychological disorders, and chronic serous otitis media are 126, 97, and 77 times higher, respectively, among individuals with FAS compared to general population. According to a recent study, 428 comorbid conditions are co-occurring in individuals with FASD, spanning across 18 of 22 chapters of the International Classification of Diseases (ICD-10).[3]

What's already known about this topic?

- Several factors contribute to the complex phenotype of fetal alcohol spectrum disorders

What does this study add?

- Current knowledge about genetics and epigenetics in the etiology of FASD
- A potential method for early diagnosis or even prevention of FASD

2 | THE GENETIC BACKGROUND OF FASD

Alcohol is a teratogen that rearranges chromosomes and kills cells. The toxic effect of alcohol is partly caused by its oxidation product acetaldehyde, which is highly reactive toward DNA, consequently damaging chromosomes and mutating stem cells.[12,13] Owing to this, it is not surprising that in recent studies 9% to 14% of children diagnosed with FASD had chromosomal deletions or duplications that could explain at least part of the features.[14-16] In addition to chromosomal rearrangements, twin studies have suggested that genetic factors—susceptibility or resistance alleles—could affect the alcohol-induced phenotype. Monozygotic twins with identical genomes have been observed to be 100% concordant for diagnosis, while dizygotic twins who share only 50% of their DNA, were only 64% concordant.[17] Also, a recent research where both twins and siblings were studied supported that conclusion: The prevalence of pairwise discordance in FASD diagnosis increased from 0% in monozygotic twin pairs to 44% in dizygotic twin pairs and continued to 59% in full-sibling pairs and 78% in half-sibling pairs.[18] It is known that the genetic relatedness between these four groups decreases from 100% to 50% to 50% to 25%, respectively, and this strongly supports the role of a genetic component in the etiology of alcohol-induced developmental disorders. However, it is essential to separate chromosomal rearrangements from risk or protective alleles: Instead of suggested risk alleles, an 18q12.3-q21.1 microdeletion was detected in an affected twin in a prenatally alcohol-exposed dizygotic twin pair with discordant FAS phenotype.[19] This microdeletion resides in a known 18q deletion syndrome region, which has been associated with growth restriction, developmental delay or intellectual deficiency, and abnormal facial features in previous studies,[20-22] and thus it likely explains the phenotypic discordancy between the twins.

The most interesting candidate genes in the etiology of FASD are involved in alcohol metabolism: alcohol dehydrogenase (ADH) converts alcohol to acetaldehyde and aldehyde dehydrogenase subsequently converts acetaldehyde to less harmful acetate. The human

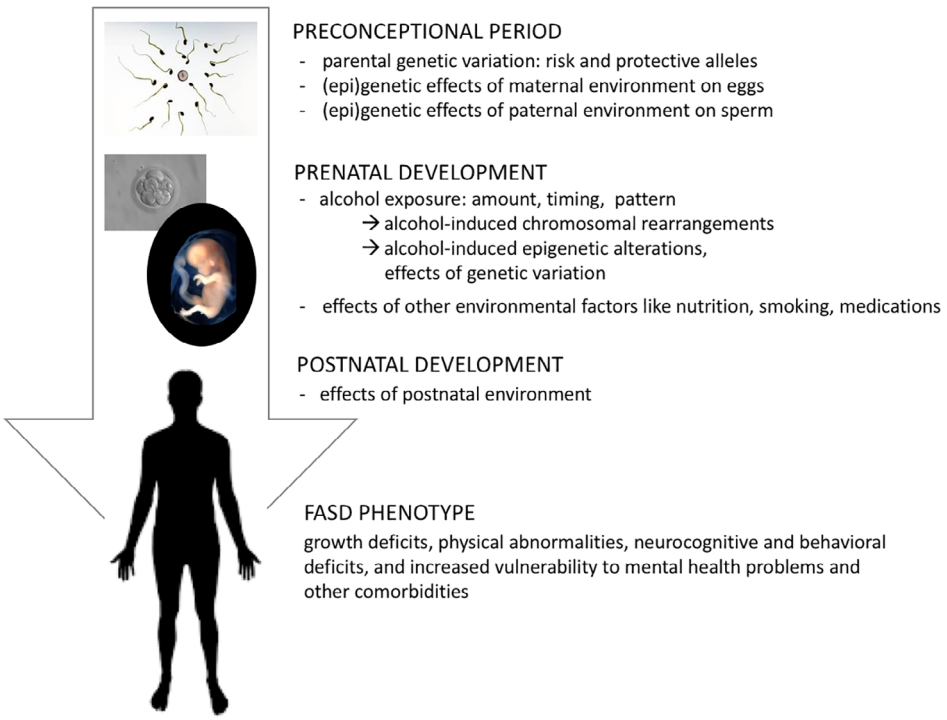

FIGURE 1 Several genetic and environmental factors during developmental periods are contributing to the complex FASD phenotype

FASD studies have focused on three ADH loci and their alleles. According to these previous studies, maternal alleles that are associated with efficient metabolism of alcohol are underrepresented in FASD individuals.[23-25] However, the association between ADH alleles and FASD seems to be more complex: Some of the ADH alleles have been associated to alcohol addiction in previous studies,[26] which means that mothers with a certain genotype could consume more alcohol.

3 | EPIGENETIC EFFECTS OF PRENATAL ALCOHOL EXPOSURE

There is an increasing amount of evidence supporting that PAE could affect the regulation of gene expression without DNA base pair changes. Effects of PAE have been detected in epigenome, which is a regulator of the genome and is needed for the normal development of a multicellular organism. Epigenetic marks include DNA methylation and histone modifications, which regulate gene expression prior to transcription through their effects on chromatin structure. Also, noncoding RNAs, such as microRNAs that inhibit the translation of messenger RNAs into proteins, are a part of the epigenetic regulation. Epigenetic variation induced in utero is a strong candidate mediator of environmental effects and indeed, increasing amount of associations between adverse gestational exposures and permanent changes in offspring's DNA methylation profiles have been observed.[27]

3.1 | A candidate gene study: PAE-associated alterations in IGF2/H19 locus

Several cell, animal, and human studies have revealed associations between PAE and alterations in DNA methylation, histone modifications, and microRNAs.[28] Many studies have focused on specific loci in the genome, often on *Insulin-like growth factor 2 (IGF2)/H19*, which is known to be crucial for embryonic and placental growth. This locus is imprinted and the genes are expressed through epigenetic mechanisms in parent-of-origin manner: *IGF2*, a major driver of growth, is expressed from the paternal allele[29] and noncoding, negative growth controller *H19* from the maternal allele.[30] The locus is regulated by allele-specific DNA methylation at the *H19* imprinting control region (*H19* ICR), which contains seven binding sites for methylation-sensitive zinc-finger proteins, CCCTC-binding factors (CTCFs). These binding factors organize chromatin contacts and have a critical role in the establishment and maintenance of imprinting.[31]

Both decreased and increased DNA methylation levels at the *H19* ICR have been associated with PAE in previous mouse and human studies.[32-34] We recently introduced a genetic factor, which associated with genotype-specific effects of PAE on placental DNA methylation, gene expression, and phenotype of alcohol-exposed newborns.[35] A polymorphism rs10732516 at the *H19* ICR, in the binding site of the CTCF regulatory protein, associates with genotype-specific changes in DNA methylation in a parental-origin manner. Interestingly, we observed similar genotype-specific decreased methylation levels in in vitro fertilized placentas,[36] suggesting genotype-specific sensitivity for environmental effects in this locus.

Functional studies will reveal potential genotype-specific-environmental-induced alterations in the binding of CTCF protein, but the genotype-specific phenomenon itself is not novel: It is already known that individual genotypes at specific methylation quantitative trait loci can result in different DNA methylation patterns across extended genomic regions.[37] The underlying mechanism can be associated directly with genetic polymorphism or with a genotype-environment interaction. Gene-environment interaction should be considered in methylation studies: A strong environmental factor, such as smoking, can have effects on DNA methylation alone, but the genotypic effects in addition to the exposure can provide more information of the variation in DNA methylation profiles.[38]

3.2 | Genome-wide DNA methylation analyses

Epigenome-wide association studies (EWAS) have provided a more inclusive comprehension of PAE-induced alterations in the methylome, and long-lasting changes, both decreased and increased methylation levels, have been observed in mouse studies.[33,39] Only a few EWAS for human cohorts have been performed so far and although the altered DNA methylation levels associate with several genes presumably involved in FASD—such as imprinted and neurodevelopmental genes—the results are inconsistent. A meta-analysis of six cohorts (1147 exposed and 1928 unexposed newborns) did not reveal significant effects of light-to-moderate maternal alcohol consumption on DNA methylation in cord blood.[40] A signature of 658 FASD-associated differentially methylated CpG sites in buccal epithelial cells have been found in a study with 110 FASD and 96 control children[34] but only 161 CpG sites of these 658 were replicated in a study of 24 FASD and 24 control children's buccal cell samples.[41] Also, associations between FASD—even between one of the subphenotypes—and altered DNA methylation levels in blood samples have been discovered in a study with 39 children with FASD diagnosis and 64 controls, as well as in a replication study with 7 FASD and 28 control children.[42] However, the observed methylation changes were mainly inconsistent with the previous studies and any solid biomarkers for FASD have not yet been revealed.

The inconsistency of the EWAS studies could be explained by several limitations: only low-to-moderate alcohol exposure, cellular heterogeneity, different age of the affected individuals, variability in timing and amount of exposures, limitations of the methods, or differences in genetic background, socioeconomic status and culture. A strong environmental factor, such as maternal smoking, can be a confounding factor in the analysis. Often women who drink also smoke, and the separate effects of these two harmful factors or their interactions are still unclear. Interestingly, prenatal exposure to maternal smoking is associated with a larger magnitude of alterations compared to PAE and with systematically replicated changes in DNA methylation, including xenobiotic-related pathways,[43-45] unlike in the PAE EWAS studies. Furthermore, all the previous EWAS studies for

PAE have been performed by microarrays, in which 450 000 selected CpG sites for DNA methylation groups were analyzed (450 K microarray Illumina). This is only approximately 1.6% of all the CpG sites in the human genome and thus majority of the methylation information is missing from the results.

3.3 | Epigenetic effects of early prenatal alcohol exposure

Recent studies have shown that the beginning of pregnancy appears to be particularly vulnerable to the effects of environmental factors and disruption of these processes can have long-term effects on development.[46-48] This is a dynamic period of cell divisions, DNA replication, and epigenetic reprogramming.[49] The reprogramming is fundamental for normal development: It removes epigenetic marks and returns totipotency for the first cells of the new individual. In the end of this early programming the cell-specific epigenetic profiles for adequate gene function will be formed again. We have shown for the first time that alcohol could affect adult phenotype by altering the epigenotype of the early mouse embryo. Early PAE is capable of changing the DNA methylation level at the epigenetically sensitive *Agouti viable yellow* (A^{vy}) allele and consequently the coat color of alcohol-exposed offspring.[50] A^{vy} is a dominant mutation of the *Agouti* locus in mouse, caused by the insertion of a retrotransposon. Retrotransposons are transposable elements, "jumping genes," which by affecting gene regulation can contribute to development and disease.[51,52] Interestingly, the offspring phenotype in this mouse model was reminiscent of human FASD with craniofacial dysmorphology, postnatal growth restriction,[50] and structural[53] as well as functional[54] changes in central nervous system.

Instead of killing the cells, maternal alcohol consumption could alter the establishment of epigenetic marks in the epigenetic programming period of early embryo, change the gene regulation and consequently bring forth the wide FASD phenotype. Several stem cell and mouse embryo studies have revealed associations between early alcohol exposure and alterations in DNA methylation[39,55,56] and histone modifications.[57] Although causal molecular mechanisms of early alcohol-induced epigenetic alterations and adult phenotype have not yet been revealed, this will be possible due to the development of cost-effective sequencing methods. Instead of small fraction of selected CpG sites on arrays, they will enable comprehensive and systematic examination of the whole epigenome including repetitive elements. Owing to the significant role of TEs in normal development as well as disorders, also their potential role in alcohol-induced developmental disorders should be explored in future studies.

Although early PAE-induced molecular changes can be relatively subtle and will not terminate the development, they can be significant for the development of brain. If they occur very early in the development prior to the differentiation of the three germ layers, they would be expected to be present in all tissue types. In our previous mouse study, early PAE was associated with similar changes in gene expression in ectodermal hippocampus and main olfactory epithelium as well as mesodermal bone marrow of infant mice, which supports our hypothesis of early epigenetic origin of alcohol-induced disorders.[53] The changes in gene regulation may have already taken place in embryonic stem cells—in hematopoietic stem cells as well as in stem cells of central and peripheral nervous system. These changes can be replicated to daughter cells along cell divisions, and can be seen in the different tissue types of an adult organism. This could prove significant for the challenging work of diagnosing alcohol-related damage.

4 | MOLECULAR MECHANISMS OF PAE-INDUCED EPIGENETIC ALTERATIONS

4.1 | Alcohol's effects on metionine cycle

Although the significance of prenatal environment in adult phenotype seems to be indisputable, the molecular mechanisms underlying these associations or causality of the observed alterations are poorly understood. Alcohol consumption causes oxidative stress, which in addition to chromosomal abnormalities also provoke enzymatic malfunction. The reduced activity of *DNA methyltransferase 1*, the enzyme which is essential for maintaining of DNA methylation profiles, has been associated with decreased global methylation level in embryos exposed to alcohol.[58] Furthermore, it has been observed that alcohol exposure in liver inhibits the enzymatic activity in methionine cycle.[59] This one-carbon pathway, which is found in all tissues, links folate, methyl group transfers and homocysteine metabolism, and is essential for global DNA and histone methylation levels (Figure 2). Methionine adenosyltransferase enzyme converts methionine to S-adenosylmethionine, which is a methyl donor substrate for methyltransferase-catalyzed reactions. In addition to methyl groups, also S-adenosylhomocysteine is formed, which is hydrolyzed to homocysteine. Homocysteine is remethylated back to methionine by methionine synthase enzyme (MS) using dietary folate or by betaine-homocysteine methyltransferase reaction using choline's precursor betaine.[60] Vitamins B6 and B12 are important cofactors in the cycle: B12 in the remethylation of homocysteine to methionine and B6 in the removal of homocysteine by transsulfuration pathway.

Alcohol consumption decreases the amount of folate (B9 vitamin)[61-63] and according to a recent study, folic acid transport

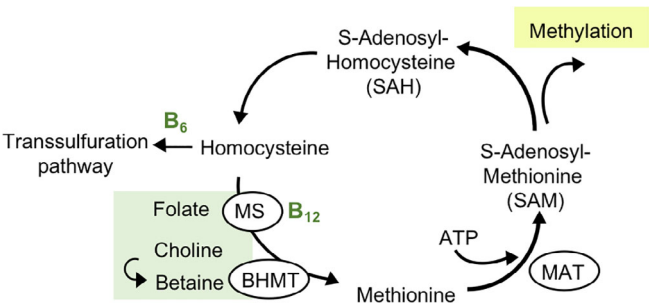

FIGURE 2 The methionine cycle [Colour figure can be viewed at wileyonlinelibrary.com]

to the human fetus is decreased in pregnancies with chronic alcohol exposure.[64] Also, the absorption of B6 and B12 vitamins has been associated with chronic alcohol consumption, but the results have not always been consistent.[65] Together the previous studies support the hypothesis that alcohol exposure could affect the methionine cycle and consequently methylation level of the epigenome in the cells of a developing embryo.

Interestingly, based on animal studies, PAE-induced epigenetic changes and adverse effects on the phenotype could be returned by methyl donors.[66-69] Despite of a small sample size—35 alcohol-exposed and 35 alcohol- and choline-exposed newborns—the results of a preliminary human clinical trial about maternal choline supplementation from mid-pregnancy are encouraging: Considerable catch-up growth in weight and head circumference as well as better visual recognition memory were detected in infants born to choline-treated alcoholic mothers compared to a placebo group.[70] However, owing to the unknown molecular mechanisms of choline supplements, it is not clear whether the supplementation specifically targets alcohol-related impairment or improves development in all infants.

4.2 | Alcohol's effect on histone acetylation

Alcohol metabolism in the liver is a source of acetate in the body.[71] Interestingly, a recent mouse study reported a direct link between alcohol consumption and changes in histone acetylation in the brain (Figure 3).[72] Acetyl groups from alcohol were rapidly incorporated into histone acetylation in the hippocampus and prefrontal cortex by acetyl-CoA synthase 2 (ACSS2) dependent way, which consequently altered transcription of the genes. Behavioral tests also suggested that this activation of memory- and learning-related genes associates with an addictive phenotype. The observed alcohol-related associative learning was abolished in ACSS2-knockout mice, demonstrating that ACSS2 is required for the phenotype. In addition to the adult mice, these dynamic histone acetylation changes were detected also in fetal brain affected by maternal alcohol consumption.[72] The effects of alcohol-induced histone acetylation on brain development and the role of acetylation in the molecular mechanisms of FASD should be explored in future studies.

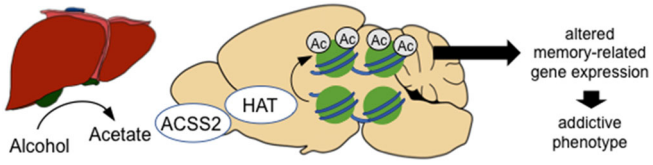

FIGURE 3 Alcohol is metabolized into acetate in the liver, which increases the acetate level in blood. The acetate is activated by acetyl-coA synthetase 2 (ACSS2) in both adult and fetal mouse brain. Histone acetyltransferases use the activated acetate to acetylate histone proteins. Histone acetylation alters the expression of learning- and memory-related genes and facilitates alcohol-related associative learning in adult mice[72] [Colour figure can be viewed at wileyonlinelibrary.com]

5 | BIOMARKERS FOR FASD

Owing to the challenges in diagnostics of FASD, particularly with the subphenotypes in the spectrum, there is an urgent need for solid biomarkers. At the moment early intervention is crucial for a positive outcome in the affected child. By revealing the molecular alterations and their associations with the phenotypes, it is possible to elucidate early biomarkers for FASD. Early, widespread PAE-induced epigenetic alterations could enable FASD diagnosis from a blood sample or simply buccal cells from the inside of a newborn's cheek. It has been shown by blood[42] and buccal cell samples,[34,41] that children with FASD could have unique DNA methylation defects. Both buccal cells from mouth epithelium and brain tissue are of ectodermal origin and therefore, in theory, early epigenetic alterations in brain development could also be seen in the buccal cells. Those changes might be used as biomarkers for alcohol-induced neuronal disorders in the future. This hypothesis is supported by a recent rat study, where correlation in alcohol-associated DNA methylation alterations between peripheral tissue and hypothalamus was observed.[73] However, despite of the recently observed high correlation for DNA methylation of CpG sites in human buccal and brain tissue across 21 individuals ($r = 0.85$, $P < 2.2 \times 10^{-16}$), for site-specific DNA methylation within one individual it has been reported rather low: only 17.4% of all CpG sites were correlated at a nominal level of significance ($P < .05$).[74]

6 | FUTURE STUDIES

Although FASD research has been mainly focused on the gestational period and maternal environmental factors during the pregnancy, also the impact of preconceptional—specifically paternal environment before fertilization—has been recognized. Paternal alcohol consumption can decrease the methylation level in human sperm[75] and paternal alcohol exposure has been associated with offspring's restricted growth, congenital anomalies and cognitive impairment in animal studies.[76-79] The mechanism how paternal environmental exposures can affect the offspring's development is not yet known, but it seems that micro RNAs could explain how the alcohol-induced effects can be transferred to the next generation.[80,81]

By revealing the solid molecular biomarkers for FASD, it is also possible to understand both mitotic and meiotic epigenetic inheritance of PAE. Owing to the several confounding factors like genetic heterogeneity and diverse environmental exposures, transgenerational inheritance of environmental-induced epigenetic alterations are challenging to explore in human. Also, the length of human generation hampers the studies: Maternal transmission of transgenerational epigenetic alteration needs to be observed at least in three affected generations due to the primordial germ cells inside the both, mother and fetus—otherwise changes could be caused by direct environmental effects. Interestingly, there are already some rat and mouse studies, which support the transgenerational inheritance of alcohol-induced epigenetic alterations.[82,83] In the future, solid biomarkers could enable to track inherited PAE-induced alterations also in human.

Despite the dispersed information gathered so far, any clear causality between a molecular mechanism of PAE and its consequences on offspring's phenotype has not been identified. In attempts to understand the mechanisms and causality, it will be pivotal to clarify the role of genetic (eg, chromosomal rearrangements, risk alleles) and environmental factors (eg, alcohol and smoking exposure, nutrition) in the FASD phenotype, and to explore their interactions. Furthermore, the effects of alcohol on different developmental periods should be examined. This all is possible by systematic research with modern methods and adequate models. Also, invaluable human cohorts with biological samples and developmental information will be needed. In future studies, by understanding the mechanism and consequences of molecular alterations caused by PAE, it is possible to clarify the phenotypic categories and also their relation to comorbidities. This would also provide tools for diagnosis and prognostic markers, and even establish methods to prevent or intervene in the alcohol-induced developmental disorders.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Nina Kaminen-Ahola https://orcid.org/0000-0003-1255-4716

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