Making a case for the role of DNA repair in autism

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Abstract
Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by behavioral and social impairments and genetic predisposition. The genetic heterogeneity of autism and the complexity of the disease have made it difficult to delineate one mechanism or diagnostic biomarker that influences ASD risk. Mounting evidence points towards exposure to DNA damaging environmental pollutants as a major ASD risk factor. Studies have also identified genomic instability, defective DNA repair and greater oxidative DNA damage using ASD animal models and patient samples. This review highlights the salient findings that connect defective DNA repair with ASD risk and makes a case for the need of functional DNA repair assays characterizing the role of DNA repair mechanisms in autism etiology.

Keywords: autism, ASD, DNA damage, DNA repair, environmental pollutants, polycyclic aromatic hydrocarbons

Introduction

Autism spectrum disorder (ASD) is characterized by complex neurodevelopmental and behavioral dysfunction along with social and communication impairment [1]. While ASD is diagnosed by clinical, psychological and behavioral observation, there are no currently available biomarkers or genetic tests used for detection or confirmation of diagnosis [2]. Although a genetic basis of the disease has been widely accepted, the complexity of the disease poses a challenge in delineating the genetic components that contribute towards autism initiation and severity [3].

Several studies using linkage analysis, bioinformatics and high throughput genomic strategies have attempted to identify the neurobiological basis of this disease [4-6]. Although a majority of these early studies focused on genetic determinants associated with central nervous system defects, current research is veering focus towards factors such as exposure to environmental toxins and systemic genetic defects that may influence response to these toxins but may not necessarily be directly involved in CNS development [7-12]. This increased association between autism and exposure to environmental pollutants bears striking resemblance to the influence of exposure to carcinogens on elevated cancer risk [13-16]. Moreover, a lot of these environmental pollutants such as cigarette smoke, exhaust fumes and oxidative stress elicit their cellular effects through DNA damage, which is also a mechanism of carcinogenesis [12, 15-19]. In the present article, I highlight studies that provide evidence for the association between DNA repair and ASD risk. This review aims to summarize the potential correlation between genome protective DNA repair mechanisms with the neurological and genetic observations made in ASD patients and to discuss further strategies in delineating this association for diagnostic or therapeutic purposes.

DNA repair in the brain

The structural effects of DNA damage are highly diverse and a single repair process cannot cope with the multiple types of DNA lesions [20, 21]. Hence, specific recognition and repair

mechanisms operate based on the type of DNA damage. For instance, bulky adducts formed by a common class of environmental pollutants called polycyclic hydrocarbons is repaired by the nucleotide excision repair pathway (NER). Homologous recombination (HR) and non-homologous end-joining (NHEJ) are the two main pathways involved in repairing double-stranded DNA damage and act differentially depending on cell cycle phase [22-26]. The MMR pathway plays an essential role in the correction of base-base mismatches that result from the misincorporation of nucleotides and BER targets small chemical alterations of the DNA bases, which generate miscoding lesions possibly resulting from cell metabolic events or single-strand breaks [21]. From the context of DNA repair mechanisms involved in neural development, the early cortical progenitors are hypersensitive to replication-associated DNA damage making mechanisms such as HR and MMR critical in repair during this stage [27, 28]. In the mature brain, on the other hand, cells are more susceptible to oxidative damage and may hence employ the BER or NER pathways [29-31]. The neurodevelopmental changes in autism are thought to correspond to early embryogenesis or prenatal environmental influences [32].

DNA repair defects, environmental pollutants and ASD risk

ASDs and intellectual disability often overlap: it is estimated that 70% of individuals with autism also have an ID. Both ASDs and ID are defined behaviorally and their onset is associated with neurological development. Additionally, both ASD and ID are associated with de novo copy number variants (CNV) a form of genetic instability that was recently associated with error-prone DNA repair mechanisms. In 2009, Dorus et al concluded that autosomal genes involved in DNA repair (FANCA, FANCC, NBS1, and XRCC8), demonstrated significantly elevated frequencies of positive Darwinian selection compared to other categories in ID [33]. Most recently, Maria Karayiorgou's group at Columbia University used a family-based cluster detection approach designed to localize significant rare disease-risk variants clusters within the chromosome 15q13.3. Unlike other previously used methods which identify variants only within a specific gene, Karayiorgou's group used a scan statistic approach which identifies association and clustering of variants in a small window of a larger genetic region by analyzing the findings from two independent whole exome sequencing data sets. Using this rigorous methodology, they implicated a region mapping to a 20kb window of the *Fanconi-associated nuclease 1 gene* (FAN1) in autism susceptibility. FAN1 functions within the Fanconi Anemia pathway that is involved in DNA repair of interstrand crosslinks and replicative DNA damage. This latest finding is in line with a previous study by Peprah et al. which reported a significant down regulation of DNA damage/repair pathway transcripts in the expression profiles of autistic males due to a Fragile X mutation. Since Fragile X syndrome is currently the leading genetic cause of autism, this study was a significant indication of the relevance of DNA repair pathways in the genetics of autism. In 2011, Kinney et al discussed the puzzling association between high autism rates and hypomelanotic skin disorders suggesting that UV-B protection and vitamin D deficiency may play a large role in influencing autism risk [34]. While this review briefly mentioned the role of vitamin D in DNA repair, much remains to be understood about the precise pathway by which vitamin D in regulates DNA repair or nucleotide excision repair in particular since it is the primary pathway involved in repairing of UV-B induced DNA damage [35, 36]. This is especially important in the context of autism because of accumulating evidence that supports the association between vitamin D deficiency and ASD risk as well as at least one report of an autistic patient with an XPC splice site mutation [37, 38]. XPC is required for genomic nucleotide excision repair and a long-term deficiency in XPC reportedly is also associated with suppressed double stranded break repair in combination with

other genetic defects. A hallmark study published in *Nature* reported that that inhibition of topoisomerases adversely affects the expression of long genes, including numerous long genes associated with synaptic function and ASD [39]. These findings make it imperative to evaluate two alternative hypotheses 1) environmental toxins result in mutations within DNA repair genes such as topoisomerases causing widespread genome instability that influences specific genetic hotspots or 2) inherent repair deficiencies in autistic individuals makes them more susceptible to the DNA damage inflicted by environmental pollutants resulting in the downstream genetic mutations.

An analysis of 100 million US medical records recently revealed that autism and intellectual disability (ID) rates are correlated with an increased incidence of genital malformations in newborn males which is also an indicator of possible congenital exposure to harmful environmental factors [40]. Although DNA defects vary for ASD cases, Courchesne et al, using brain tissue samples from ASD samples discovered a shared set of overlapping gene pathways that included DNA damage detection and repair as one of the major mechanisms influencing dysregulation of early brain development [41]. This finding is further supported by Jeggo et al. where the authors theorize that high levels of oxidative DNA damage during neuronal development would be consistent with roles for HR, ATR-signaling and NHEJ proteins in protecting proliferating cells from damage [42]. In an excellent review published in Nature Neuroscience, Dr. Peter McKinnon makes the case for the role of various DNA repair pathways in maintaining neural homeostasis especially during early embryonic development when neurogenesis is driven by proliferation and cells are most susceptible to replicative or environmental DNA damage [27].

Perspective

Despite mounting evidence for the role of aberrant DNA repair mechanisms in autism, the general consensus within the field remains that autism is a highly complex disorder that may involve several gene loci, low penetrance alleles and gene-environment interactions that define its etiology thus complicating diagnosis. While the focus of current studies remains on the means to identify single genetic biomarkers or cytogenetic characteristics for diagnosis, there are no reports about the impact of DNA repair defects and exposure to environmental toxins using high-throughput functional assays on DNA repair capacity during neuronal development associated with ASD risk. Several genes are involved in the maintenance of genome integrity in response to environmental pollutants, and there is great variation amongst individuals with respect to common polymorphisms that impact on the activity of these enzymes. Hence there is a need for molecular diagnostics that can a) identify the aberrant DNA repair mechanism and b) identify the stage of neurodevelopment where the repair deficiency is most critical in influencing ASD risk. These functional repair assays can examine pre/peri or gestational exposure to DNA damaging environmental pollutants in place of biomarkers of these toxins which have thus far resulted in inconsistent findings [43]. While Fenech et al demonstrated that autistic individuals are not abnormally susceptible to oxidative stress compared to their non-autistic siblings, the authors did acknowledge that there was a need for more DNA repair markers and assays to test the differential effects of DNA damage in vivo on autistic neural cells [44].

It is also tempting to speculate if reduced maternal DNA repair capacities combined with environmental factors can increase autism risk. Studies using brain tissue or neurons obtained from patients can be subjected to high-throughput DNA repair assays that are currently being used in the cancer genetics field to assess repair defects that contribute towards cancer risk or therapeutic outcome [45-51]. Similarly, the impact of vitamin D or nutritional element

on repair efficiency or protein kinetics is worth examining in cellular models of autism. The advent of somatic cell reprogramming now makes it possible to study ASD at a cellular level using pluripotent patient-derived cells [52-54]. This model offers an excellent tool to study the effects of various forms of DNA damage and the DNA repair capacity of cells derived from ASD versus normal cohorts. If a repair deficiency is identified, this cell model can also enable screening of phytochemicals or genes that can correct this defect.

Summary

In conclusion, the pattern of DNA repair pathways and defects emerging repeatedly in assessing autism susceptibility is highly suggestive of a role for DNA repair mechanisms in autism risk. The biological connection between DNA repair and autism deserves further analysis considering the now well-established link between environmental DNA damage, down-regulated DNA repair pathways and ASD risk in available literature and recent reports. Since dysfunctional cellular mechanisms of genomic stability during early embryogenesis and neural development can result in a plethora of genetic mutations and brain abnormalities, it is important to evaluate whether the link between environmental pollution and ASD is driven by DNA damage and exacerbated by reduced DNA repair capacity.

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