DNA damage to human genetic disorders with neurodevelopmental defects

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Although some mutations are beneficial and are the driving force behind evolution, it is important to maintain DNA integrity and stability because it contains genetic information. However, in the oxygen-rich environment we live in, the DNA molecule is under constant threat from endogenous or exogenous insults. DNA damage could trigger the DNA damage response (DDR), which involves DNA repair, the regulation of cell cycle checkpoints, and the induction of programmed cell death or senescence. Dysregulation of these physiological responses to DNA damage causes developmental defects, neurological defects, premature aging, infertility, immune system defects, and tumors in humans. Some human syndromes are characterized by unique neurological phenotypes including microcephaly, mental retardation, ataxia, neurodegeneration, and neuropathy, suggesting a direct link between genomic instability resulting from defective DDR and neuropathology. In this review, rare human genetic disorders related to abnormal DDR and damage repair with neural defects will be discussed.

Key words: DNA damage, DNA repair, Single-stranded DNA breaks, Double-stranded DNA breaks, Central nervous system diseases.

Introduction

Since Kelner [1,2] reported in late 1940s that damaged DNA could be corrected, our knowledge about DNA damage and repair has increased exponentially [3]. DNA damage can be divided into 2 broad categories: base damage and strand breaks. When bases are chemically modified or damaged, they can be removed and corrected by the base excision repair (BER) pathway. If there are bulky helix-distortion lesions, most likely due to ultraviolet (UV) exposure, nucleotide excision repair (NER) mechanisms are activated. The DNA mismatch repair (MMR) pathway recognizes and repairs erroneous insertion, deletion and mis-incorporation of bases [3,4]. In 2015, the Nobel Prize in Chemistry was awarded to 3 DNA damage repair

researchers: Tomas Lindahl (Emeritus group leader at Francis Crick Institute and Emeritus director of Cancer Research at Clare Hall Laboratory, UK., for BER), Aziz Sancar (Sarah Graham Kenan Professor of Biochemistry and Biophysics, University of North Carolina School of Medicine, USA., for NER) and Paul Modrich (James B. Duke Professor of Biochemistry at Duke University School of Medicine, USA., for MMR) for their research into mapping the mechanisms that underlie DNA damage repair at a detailed molecular level. On the other hand, DNA strand breaks could happen to one or both strands. Organisms have developed sophisticated mechanisms to deal with either DNA single strand breaks (SSBs) or DNA double strand breaks (DSBs) [3-6]. DNA DSB repair mechanisms are further divided into 2 major distinct pathways: homologous recombination repair (HRR) and non-

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homologous end joining (NHEJ) [3,4,6].

The ability to maintain genomic integrity by controlling the balance between DNA damage and repair is a fundamental feature of all living organisms. DNA damage triggers the DNA damage response (DDR) which activates not only DNA damage repair but also cell cycle checkpoints/arrest, programmed cell death and cellular senescence [3,6,7]. However, when defects in DDR happen in living multicellular organisms, particularly in human beings, they lead to rare genetic diseases with complex consequences such as developmental delay/defects, premature aging, infertility and immune system defects [3]. Additionally genomic instability resulting from DNA damage is one of the hallmarks of tumorigenesis [4]. A certain group of human syndromes are characterized by unique neurological manifestations including microcephaly, mental retardation, ataxia with cerebellar defects, neurodegeneration, and neuropathy [5,6,8,9]. These neurological phenotypes are more evident in genetic disorders with faulty DNA strand break detection or repair mechanisms. In this review, we discuss the current state of knowledge regarding human syndromes with neurological symptoms due to defects in the DNA strand break repair pathway.

Pathways of DDR and DNA Strand Break Repair

DNA strand breaks could happen to either one or both strands. Oxidative stress is the most common source for DNA SSB. SSBs can also be caused by the abortive activity of DNA topoisomerase I (TOP1) whereby TOP1 is covalently connected to the 3'-end of DNA strand breaks [5,6,10]. Several proteins are involved in the detection and end processing of SSB repair. Poly(ADP-ribose) polymerase 1 (PARP1) is one of the first proteins recruited to DNA break sites, then X-ray repair cross-complementing protein 1 (XRCC1) is recruited and brings with it multiple proteins, including DNA polymerase β (POL β), polynucleotide kinase phosphatase (PNKP), aprataxin (APTX), DNA ligase III (LIG3), and tyrosyl-DNA phosphodiesterase 1 (TDP1). These proteins are essential for the processing of broken DNA ends and for the restoration of the 3'-hydroxyl and 5'-phosphate moieties (Fig. 1) [5,6,10].

There are two major pathways to repair DSBs: HRR, which is restricted to the late S to G2 phases of cell cycle as it requires sister chromatids as a template to accurately copy the intact DNA sequence and repair the damage; and NHEJ, which is active throughout the cell cycle and in fully differentiated cells such as

Fig. 1. DNA single strand break (SSB) repair. DNA SSBs could result from oxidative attacks by reactive oxygen species (ROS) or from abnormal topoisomerase I (TOP1) activity. Poly(ADP-ribose) polymerase I (PARP1) is one of first proteins recruited to sites of SSB. Poly(ADP-ribosyl)ation is also controlled by Poly(ADP-ribose) glycohydrolase (PARG). After the damage detection step, X-ray repair cross-complementing protein 1 (XRCC1) recruits several key proteins needed for broken DNA end processing and for DNA ligation including DNA polymerase β (POL β), polynucleotide kinase phosphatase (PNKP), aprataxin (APTX), tyrosyl-DNA phosphodiesterase 1 (TDP1), and DNA ligase III (LIG3) to the sites of damage. Depending on the size of gap, either DNA ligase I (LIG1) or LIG3 is required for gap filling.

neurons. HRR and NHEJ require distinct groups of proteins for their pathways [3,11]. The HRR pathway starts with a resection of the broken DNA ends by the MRE11-RAD50-NBS1 (MRN) complex and CtBP-interacting protein (CtIP) to produce a single stranded DNA which the replication protein A (RPA) trimers bind to with high affinity. The breast cancer 2 (BRCA2) protein mediates displacement of RPA and binding of RAD51 to the single-stranded DNA. RAD51, together with other proteins, invades the homologous DNA strands to find and copy intact DNA sequences (Fig. 2) [12-14]. In contrast, the NHEJ process is initiated by the binding of the KU70/80 heterodimer to both broken ends of the DNA strands resulting in the recruitment of DNA dependent protein kinase catalytic subunit (DNA-PKcs) to the sites of DSBs. Additional factors including ARTEMIS and DNA polymerases process the broken DNA ends to create compatible DNA ends for ligation. Finally the protein complex composed of

DNA ligase IV (LIG4), XRCC4 and XRCC4 like factor (XLF) ligates the 2 processed DNA ends (Fig. 2) [3,11,15,16].

Though our current understanding of DDR dynamics, particularly for the recognition of DNA DSB, is quite advanced, the entire picture of DDR is still not complete. Once DNA DSB takes place, the MRN complex immediately moves to the sites of DSB and recruits ataxia telangiectasia mutated (ATM) for its activation. Consequently ATM phosphorylates numerous downstream proteins including histone H2AX that provides a binding platform for the mediator of DNA damage checkpoint protein 1 (MDC1), which is another ATM kinase substrate [4,17-20]. After the ubiquitination steps by ring finger protein (RNF) 8 and RNF168, the tumor protein p53 binding protein 1 (53BP1) protein recognizes several histone modifications including H2AK13ub (ubiquitination), H2AK15ub, H2K79me (methylation), H4K20me, and H4K20me2 to promote NHEJ. The recruitment of BRCA1 to

Fig. 2. DNA double strand break (DSB) repair. DNA DSBs can be repaired by either homologous recombination repair (HRR) or non-homologous end-joining (NHEJ) depending on the availability of a sister chromatid. Ku70/80 heterodimers bind to DSB, recruit DNA-PKcs and initiate NHEJ. After broken DNA end processing is done (e.g., help of ARTEMIS), the breaks are ligated by DNA ligase IV (LIG4), which interacts directly with X-ray repair cross-complementing protein 4 (XRCC4) and indirectly with XRCC4-like factor (XLF). During HRR, the resection results in a single-stranded DNA that is stabilized by replication protein A (RPA) and, later replaced by RAD51 with help from BRCA1, BRCA2, partner and localizer of BRCA2 (PALB2), and Fanconi anemia (FANC) proteins. After strand invasion and copy, the Holliday junction can be resolved by either the non-crossover or crossover mechanisms.

Fig. 3. Detection of DNA double strand breaks (DSBs). (A) The immediate response to DNA DSBs. Upon DNA DSBs, the MRN (MRE11-RAD50-NBS1) complex recognizes and binds to the break sites to trigger DNA damage response (DDR). NBS1 recruits ataxia telangiectasia mutated (ATM) to the break site. Once ATM is activated by auto-phosphorylation, ATM phosphorylates many proteins including H2AX (γ -H2AX—phosphorylated H2AX), and the mediator of DNA damage checkpoint protein 1 (MDC1). Phosphorylation (P) of MDC1 induces sequential ubiquitination (Ub) of several target proteins such as histone H2A/H2AX by RNF8 and RNF168. It seems that the recognition of poly-ubiquitinated histones by BRCA1 and its associated proteins promotes homologous recombination repair (HRR), while mono-ubiquitination and methylation of histones are recognized by 53BP1 for the promotion of non-homologous end joining (NHEJ) (H, histone; Me, methylation). (B) Similar to ATM, ataxia telangiectasia and Rad3 related (ATR) is also activated by DNA damage such as replication stress, and then phosphorylates numerous proteins some of which are also ATM phosphorylation targets. However, CHK2 and CHK1 are phosphorylated by ATM and ATR respectively upon DNA damage to regulate cell cycle checkpoints.

sites of DSB boosted by CtIP phosphorylation, and displacement of 53BP1 by BRCA1 initiate the resection process for HRR during the S and G2 phase (Fig. 3) [21-24].

Another protein kinase, ataxia telangiectasia and Rad3 related (ATR), responds to single-stranded DNA that occurs at stalled replication forks [25]. Similar to ATM, ATR has a protein kinase function, and shares common substrates (such as p53) with ATM. However, some proteins are unique to either kinase, e.g., ATM phosphorylates CHK2, whereas CHK1 is phosphorylated by ATR to regulate cell cycle checkpoints upon DNA damage (Fig. 3) [26].

Faulty DDR in Neurodevelopmental Defects

1. Ataxia-telangiectasia mutated (ATM)

As one of the early responders to DNA strand breaks, ATM (a member of the phosphatidylinositol 3-kinase family) phosphorylates a plethora of proteins immediately upon DNA damage including H2AX, NBS1, CHEK2, p53, MDM2, BRCA1, CTIP, and RAD9, which regulate programmed cell death and cell cycle checkpoints [7,27,28]. It is well known that mutations in the ATM gene cause the human genetic disease, ataxia-telangiectasia (A-T, also known as Louis-Bar syndrome) [29,30]. Clinical manifestations of A-T include telangiectasia, immune system defects, chronic infections, infertility and an increased risk of leukemia and lymphoma. The prominent clinical phenotypes of A-T are related to the nervous system such as progressive ataxia due to neurodegeneration in the cerebellum, chorea, neuropathy, and leukodystrophy [27-30]. A-T patients in Korea were rarely reported. The Korean patients recently reported showed limb and truncal ataxia with cerebellar atrophy, oculomotor apraxia with gaze-evoked nystagmus and choreoathetosis of the bilateral extremities [31]. These Korean A-T patients carry a known missense mutation (c.G8546C, p.Arg2849Pro substitution) and a novel intronic variant of intron 17 causing exon 18 skipping.

2. Nibrin (NBN, NBS1)

The MRN complex is critical for initiating DDR as well as DNA recombination. In the MRN complex; MRE11 has an endo/exonuclease activity, RAD50 is required for binding to DNA broken ends, and NBN modulates DDR by interacting with ATM, ATR, and possibly DNA-PK [12,20]. NBN is also a major player for the intra-S phase checkpoint in an ATM dependent manner [32,33]. Several mutations in the NBN gene were reported to cause Nijmegen breakage syndrome (NBS), including the five nucleotide deletion in exon 6 (657del5), the

p.Ile171Val mutation, and the p.Arg215Trp substitution [19,34]. NBS patients have a characteristic facial appearance with a combination of a receding forehead, a receding mandible and a prominent midface, often referred to as a 'bird-like face'. They also show growth retardation, short stature, immunodeficiency, microcephaly, and mental retardation [19,34]. Interestingly Japanese patients with the Ile171Val substitution in the NBN gene did not show any signs of NBS, but developed aplastic anemia [35].

3. Meiotic recombination 11 homolog A (*S. Cerevisiae*) (MRE11A)

The endo- and exonuclease functions of MRE11 act on asymmetric openings of DNA hairpin loops and processes broken DNA ends to facilitate DNA strand break repair [12,19]. MRE11 is also involved in HRR and telomere length maintenance [12]. There are more than 10 different known homozygous or compound heterozygous mutations, which result in ataxia-telangiectasia-like disorder 1 (ATLD1), including the p.Arg633Ter premature truncation mutation, the p.Asp113Gly missense mutation, and the compound heterozygous mutation of a p.Asn117Ser missense and a p.Arg572Ter premature termination [36]. Only very detailed linkage analyses are able to distinguish these two syndromes as the ATM gene is located at chromosome 11q22-23 and the MRE11A gene is located at chromosome 11q21. The clinical features of ATLD1 include progressive cerebellar ataxia resulting from neurodegeneration, oculomotor apraxia, and increased susceptibility to radiation, but ATLD1 has a milder clinical course than A-T [12,19,36]. Unlike in A-T, ATLD1 patients do not generally show signs of telangiectasia and immune deficiency.

4. Proliferating cell nuclear antigen (PCNA)

Baple et al. [37] reported that a homozygous missense (c.G683T, p.Ser228Ile) mutation in the PCNA gene is compatible with viability in humans, however it results in a rare syndrome characterized by neurological abnormalities called, ataxia-telangiectasia like disorder 2 (ATLD2). PCNA is an essential DNA replication protein and plays a key role in several DNA damage repair mechanisms that occur during the final stages of the repair process [38,39]. Interestingly, the p.Ser228Ile mutation did not influence the protein level of PCNA during DNA replication, yet mutant cells derived from patients were sensitive to UV irradiation and had defective NER mechanisms. The authors [37] found four patients from one family that displayed developmental retardation, learning difficulties, hearing loss,

progressive ataxia, progressive muscle weakness, dysphagia, dysarthria, telangiectasia and photosensitivity. All patients also showed dwarfism.

5. Ataxia–telangiectasia and Rad3–related (ATR)

As a member of the phosphatidylinositol 3-kinase family, ATR is an early and essential regulator of DNA damage repair, cell cycle checkpoints, and DNA replication/stability [25]. Similar to ATM, ATR is a protein kinase that is activated by a single-stranded DNA, and, once activated, phosphorylates numerous proteins including BRCA1, CHEK1, MCM2, RAD17, RPA2, SMC1 and p53 [7,25]. Mutations in the ATR gene cause 1 of 2 distinct syndromes: Seckel syndrome (SCKL) 1 or cutaneous telangiectasia and cancer syndrome, familial (FCTCS). SCKL1 is characterized by intrauterine growth retardation, dwarfism, microcephaly, mental retardation and a 'bird-headed' facial appearance (a beak-like protrusion of the nose, narrow face and receding lower jaw) which is also common in NBS and LIG4 syndromes [34,40–42]. SCKL1 is caused by ATR mutations such as compound heterozygous mutations of a p.Met1159Ile substitution and a c.C6897+464G, Val2300GlyfsTer75 premature termination, or the p.Asp1897Tyr substitution and one 540-kilobase deletion [43,44].

However, the missense mutation of c.A6431G, p.Gln2144Arg substitution leads to a new syndrome called FCTCS with telangiectasia that appears during infancy in both sun-exposed and sun-protected sites. Thinning eyebrows, patchy alopecia, thin dental enamel and dental caries, developmental anomalies of hair, nails, and a predisposition to cancer, predominantly oropharyngeal cancer are all characteristics of FCTCS [45].

6. Retinoblastoma-binding protein 8 (RBBP8), also known as CtBP-interacting protein (CtIP)

Clinically similar to SCKL1, SCKL2 (also known as microcephalic primordial dwarfism 2) occurs due to mutations in the RBBP8 gene, such as the c.T2347+53G resulting in an alternatively spliced transcript and a truncated protein or the c.C298T, p.Arg100Trp substitution [46,47]. SCKL2 patients displayed growth retardation, microcephaly, mental retardation, a characteristic 'bird-headed' facial appearance, and sensitivity to DNA damaging reagents [46–48]. The RBBP8 protein binds directly to retinoblastoma protein (Rb) and BRCA1 to regulate cell proliferation and transcription and to control cell cycle checkpoints. It has an endonuclease function for DNA damage repair, particularly microhomology-mediated alternative end joining and DNA end processing [13,49].

A homozygous 2-base pair deletion in exon 11 which causes a frameshift mutation and a premature termination were found in seven members of a consanguineous Pakistani family who showed microcephaly, sharply sloping foreheads, mental retardation, anonychia congenita, digital malformation (polydactyly and synpolydactyly), and white spots on the skin of the hands and feet [48,50]. This syndrome is called Jawad syndrome (JWDS; also known as Kelly syndrome, or microcephaly with mental retardation and digital anomalies).

Five more genetically distinct SCKLs, numbered from 4 to 8, are currently found. All SCKL syndromes share similar clinical features such as pre- and postnatal growth retardation, microcephaly with mental retardation, and specific dysmorphic features (receding chin, high forehead; a 'bird headed' facial appearance). All responsible genes for SCKL4 to SCKL7 are involved in the maintenance and function of the centrosome: SCKL4–CENPJ (centromeric protein J, also known as centrosomal p4.1-associated protein [CPAP]), SCKL5–CEP152 (centrosomal protein, 152-KD), SCKL6–CEP63 (centrosomal protein, 63-KD), and SCKL7–NIN (Ninein, also known as GSK3 β -interacting protein) [51–54]. Interestingly, it has been found that two members from consanguineous marriages carry a homozygous mutation for a 1-base pair deletion (c.3372+6delC) in intron 20 of the DNA2 gene resulting in a truncation of one transcript and abnormal slicing of another 2-transcripts. This mutation in the DNA2 gene (responsible for SCKL8) causes short stature, 'bird headed' facial features, microcephaly and mental retardation [47,55]. However, patients with mutations in the DNA2 gene (p.Arg284His, p.Lys313Glu, or Val723Ile substitution) generally display muscle weakness, mainly affecting the lower limbs, abnormal gait with hyperlordosis, external ophthalmoplegia, exercise intolerance, and mitochondrial DNA deletion [56]. This symptom is called progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant 6 (PEOA6).

7. Ring finger protein 168 (RNF168)

As one of the E3 ubiquitin ligases involved in DDR, RNF168 acts with E2 ubiquitin-conjugating enzyme (UBC13) to amplify the RNF8-dependent ubiquitination of histones upon DNA damage, particularly in DNA DSBs. This catalyzes the formation of Lys63 linked ubiquitin conjugate and promotes the ubiquitination of H2A and H2AX [18]. When the RNF168 gene is mutated, e.g., by the compound heterozygous mutation of 1-base pair duplication (397dupG) and a 4-base pair deletion (1323delACAA) which results in a frameshift mutation and a premature protein truncation, or a p.Arg131Ter substitution resulting

in the complete loss of the protein, RIDDLE syndrome occurs [57,58]. RIDDLE stands for radiosensitivity, immunodeficiency, dysmorphic features, and learning difficulties. Some patients also showed microcephaly, impaired motor control, ataxia and ocular telangiectasia [57,58].

Defective DNA SSB Repair in Abnormal Neurodevelopment

1. Tyrosyl-DNA phosphodiesterase 1 (TDP1)

During replication, topoisomerases relieve the topological tension on the DNA double-helical structure. These enzymes also create a covalent protein-DNA intermediate, which is hydrolyzed by tyrosyl-DNA phosphodiesterases (TDPs) [59]. TDP1 is particularly involved in the catalysis of stalled TOPI and DNA complexes. Several members of a Saudi Arabian family were affected with peripheral axonal motor and sensory neuropathy, muscular atrophy, cerebellar ataxia, and seizure due to a homozygous mutation in the TDP1 gene (c.A1478G, p.His493Arg) [60]. The patients of Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy (SCAN1) did not show a predisposition to neoplasia or dysfunctional proliferation of tissues, rather they had defective DNA SSB repair in terminally differentiated, non-dividing neuronal cells [61].

2. TRAF- and TNF receptor-associated protein (TTRAP), also known as tyrosyl-DNA phosphodiesterase 2 (TDP2)

TDP2 was originally identified as a protein that interacted with CD40 and TRAFs in order to regulate nuclear factor kappa B (NF- κ B) signaling pathways [62]. Cortes Ledesma et al. [63] then discovered that the protein also cleaved 5'-phosphotyrosyl bonds between topoisomerase II and DNA, thus restoring the 5'-phosphate terminus for proper DNA ligation. Recently it has been reported that 3 brothers in a consanguineous Irish family and one additional unrelated patient developed progressive ataxia, intellectual disability (IQ 30-40), and symptomatic generalized epilepsy [64,65]. Exome and Sanger sequencing analysis revealed 2 mutations in the TDP2 gene; one was a missense variant (c.T919C, p.Ile307Val) which is most likely benign, and the other was the putative splice-donor mutation (c.G425+1A) which results in the insertion of a premature stop codon within the N-terminal half of the encoded protein [64]. This syndrome is currently unnamed.

3. Aprataxin (APTX)

APTX, a member of the histidine triad (HIT) superfamily,

interacts with DNA damage repair proteins including XRCC1, PARP1 and XRCC4 [5,66,67]. The main function of APTX is to resolve aberrant DNA ligation intermediates such as adenylate groups linked to the 5'-phosphate ends of broken DNA, 5'-monophosphoramidate, and diadenosine tetraphosphate [68]. Mutations in the APTX gene cause a genetic disease called ataxia, early-onset, with oculomotor apraxia and hypoalbuminemia (EAOH, also known as ataxia-oculomotor apraxia 1 [AOA1]) [5,69,70]. Clinical manifestations include oculomotor apraxia, progressive ataxia, peripheral neuropathy and choreoathetosis [5,8,71]. Even though the neurological symptoms are quite similar to those of A-T, the onset of symptoms tends to be later and lacking in extra-neurologic features [71]. Several mutations in the APTX gene are reported to cause this syndrome. The most common mutation is the insertion of a T after nucleotide 167 and the p.Pro206Leu mutation [69]. AOA1 patients with complete deletion of the APTX gene were also found [72,73].

4. Senataxin (SETX)

Similar to AOA1, Spinocerebellar ataxia, autosomal recessive 1 (SCAR1, also known as ataxia-oculomotor apraxia 2 [AOA2]) is a hereditary disorder with neurodegenerative features, including progressive cerebellar ataxia, cerebellar atrophy, axonal sensorimotor peripheral neuropathy, tremor, and oculomotor apraxia [8,74]. The gene responsible for this syndrome is SETX which has a DNA/RNA helicase domain suggesting that it might be involved in DNA and RNA processing [74]. AOA2 cells were very sensitive to oxidative DNA stress but not to ionizing radiation (IR) indicating that SETX could contribute to a certain type of DDR [75]. Several mutations of the SETX gene have been found including the p.Arg1363Ter premature termination mutation and the p.Gln868Ter protein truncation mutation [74]. Four individuals from one family displayed AOA2 characteristics with the p.Pro629Ser substitution in the PIK3R5 (phosphatidylinositol 3-kinase, regulatory subunit 5; called ataxia-oculomotor apraxia 3 [AOA3]) gene and no mutations in the SETX gene [76]. PIK3R5 does not have an exclusive role in DNA damage repair.

5. Polynucleotide kinase 3-prime phosphatase (PNKP)

For DNA strand break repair, the ends of broken strands must have a 5'-phosphate and a 3'-hydroxyl group for proper ligation. PNKP has a dual function (5'-kinase and 3'-phosphatase) as it can process both damaged DNA ends [77]. Certain mutations in the PNKP gene, such as the p.Glu326Lys substitution, a

homozygous 17-base pair duplication resulting in a frameshift and premature termination, or a compound heterozygous 17-base pair deletion and duplication, cause microcephaly, seizures, and developmental delay (MCSZ; also known as epileptic encephalopathy, early infantile, 10 [EIEE10]) [5,8,78].

Other complex mutations in the PNKP gene include the p.Gly375Trp substitution, a 5-base pair insertion (c.1322_1323insAGCCG), a 3-base pair deletion (c.1221_1223del), or an 8-base pair insertion (c.1549_1550ins). These mutations lead to Ataxia-oculomotor apraxia 4 (AOA4) which is characterized by ataxia, cerebellar atrophy, oculomotor apraxia, peripheral neuropathy and a prominent dystonia that attenuates with age [79].

Defective DNA DSB Repair in Abnormal Neurodevelopment

1. Protein kinase, DNA-activated, catalytic subunit (PRKDC), also known as DNA protein kinase (DNA-PK)

DNA-PKcs, encoded by the PRKDC gene, is a serine/threonine-protein kinase required for NHEJ and for V(D)J recombination during the immune response. The holoenzyme is composed of DNA-PKcs and the KU70/80 heterodimer [80]. The research group led by Penny Jeggo found a patient with the compound heterozygous mutation of a p.Ala3574Val substitution and a missing exon 16 [81]. This patient displayed typical signs of severe combined immunodeficiency (SCID) such as an absence of circulating B and T cells and normal NK cells. However, the patient also had growth retardation, persistent oral and perineal candidiasis, and dysmorphic features including a prominent forehead, a wide nasal bridge, a long philtrum and a small chin. The neurological features observed in this patient were microcephaly, simplified gyral pattern, pachygyria, thin corpus callosum, small hippocampus, hypomyelination, and seizures.

2. Non-homologous end-joining factor 1 (NHEJ1), also known as XRCC4-like factor (XLF) or Cernunnos

NHEJ1 interacts with the LIG4-XRCC4 complex to stimulate it, yet accumulation of NHEJ1 at the damaged site is KU dependent and XRCC4 independent [16]. This protein is expressed in human embryonic brain including the telencephalic ventricular and subventricular zones and in the adult cerebral cortex and cerebellum [82]. Buck et al. [15] and Dutrannoy et al. [83] identified five SCID patients with microcephaly, growth retardation, the occasional facial dysmorphic feature, and sensitivity to IR, due to NHEJ1 mutations including

the p.Arg178Ter truncation, the p.Asp166ArgfsTer20, the p.Arg176Ter or the compound mutation of a p.Arg57Gly and a p.Cys123Arg, called NHEJ1 syndrome.

3. Ligase IV, DNA, ATP-dependent (LIG4) and X-ray repair, complementing defective, in chinese hamster (XRCC4)

The LIG4-XRCC4 complex is responsible for the ligation of the two restored broken DNA ends during the final step in NHEJ. This complex is also an essential component of V(D)J recombination during the immune response [84]. LIG4 mutations result in LIG4 syndrome with immunodeficiency, developmental growth delay including unusual facial features, and pancytopenia that are quite similar to NBS [41,42,85]. One patient with 2 heterozygous single-nucleotide deletions (c.613DelT and c.1904delA) which resulted in a frameshift and a premature stop codon displayed lymphopenia, extreme radiosensitivity, primordial dwarfism, and neurological abnormalities including corpus callosum agenesis and colpocephaly [86].

On the other hand, a short stature, microcephaly, and endocrine dysfunction (SSMED) syndrome results from XRCC4 mutations such as the p.Trp43Arg substitution or premature termination codons [47,87]. Endocrine disorders seen in affected individuals included hypogonadism, goiter, diabetes mellitus and hypothyroidism [88,89]. Interestingly, one SSMED patient with microcephaly and progressive ataxia did not show any clinical immunodeficiency [89].

Conclusion

As summarized in Table 1, we have described here several rare human genetic diseases with genomic instability due to faulty DDR or damage repair defects. We have focused on genetic diseases with characteristic neurological phenotypes such as ataxia due to neurodegeneration in the cerebellum, neuropathy, microcephaly and mental retardation. As most of the proteins involved in DDR or damage repair are fundamental during development, lethality *in utero* is the expected outcome, when the machinery for DDR or damage repair is completely inactivated. Therefore, most of genetic diseases described herein are due to hypomorphic mutations.

It is becoming increasingly clear how abnormal DDR and damage repair result in neural manifestations. However, there is still much work to be done in order to gain the complete understanding of the molecular mechanisms and the development of new therapeutic strategies. One certainty is that DNA damage happens naturally during neurogenesis.

Table 1. Summary of human genetic diseases resulting from defects in DNA damage repair

	Syndrome	Responsible gene	Clinical phenotypes
DNA damage response defects	Ataxia telangiectasia (A-T)	ATM (ataxia telangiectasia mutated)	Progressive ataxia Neuropathy Immune system defects Prone to tumors
	Nijmegen breakage syndrome (NBS)	NBN (Nibrin)	Microcephaly/mental retardation Growth retardation Unique craniofacial features Immune system defects
	Ataxia telangiectasia like disorder 1 (ATLD1)	MRE11 (meiotic recombination 11)	Progressive ataxia Neuropathy
	Ataxia telangiectasia like disorder 2 (ATLD2)	PCNA (Proliferating cell nuclear antigen)	Progressive ataxia Learning difficulties Growth retardation
	Seckel syndrome 1 (SCKL1)	ATR (ataxia telangiectasia and Rad3 related)	Microcephaly/mental retardation Growth retardation Unique craniofacial features
	Seckel syndrome 2 (SCKL2)	RBBP8 (retinoblastoma binding protein 8)	Microcephaly/mental retardation Growth retardation Unique craniofacial features
	Radiosensitivity, immunodeficiency, dysmorphic features, learning difficulties (RIDDLE) syndrome	RNF168 (ring finger protein 168)	Progressive ataxia Microcephaly/mental retardation Learning difficulties Growth retardation
DNA single strand break repair defects	Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy (SCAN1)	TDP1 (tyrosyl-DNA phosphodiesterase 1)	Progressive ataxia Neuropathy Seizure
	Unnamed	TDP2 (tyrosyl-DNA phosphodiesterase 2)	Progressive ataxia Mental retardation Seizure
	Ataxia oculomotor apraxia 1 (AOA1)	APTX (aprataxin)	Progressive ataxia Neuropathy Oculomotor apraxia
	Ataxia oculomotor apraxia 2 (AOA2)	SETX (senataxin)	Progressive ataxia Neuropathy Tremor Oculomotor apraxia
	Microcephaly, seizures, and developmental delay (MCSZ)	PNKP (polynucleotide kinase phosphatase)	Microcephaly Seizure Growth retardation
	Severe combined immunodeficiency (SCID)	PRKDC (protein kinase, DNA activated, catalytic subunit)	Microcephaly Seizure Growth retardation Unique craniofacial features Immune system defects
DNA double strand break repair defects	SCID, also NHEJ1 syndrome	NHEJ1 (non-homologous end joining factor 1)	Microcephaly Growth retardation Unique craniofacial features Immune system defects
	LIG4 syndrome	LIG4 (DNA ligase IV)	Growth retardation Unique craniofacial features Immune system defects
	Short stature, microcephaly, and endocrine dysfunction (SSMED)	XRCC4 (X-Ray repair, complementing defective, in Chinese Hamster)	Microcephaly Endocrine dysfunction

However, the nature of this DNA damage is unknown. Several fundamental questions need to be answered: what is the origin of DNA damage in the developing brain, what type of DNA

damage occurs during neurogenesis, and why some genetic diseases that arise due to genomic instability only affect the nervous system, even though every cell in the entire body carries

the same mutations.

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