Huntington disease: new insights into molecular pathogenesis and therapeutic opportunities

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Huntington disease (HD) is a neurodegenerative disease caused by CAG repeat expansion in the *HTT* gene and involves a complex web of pathogenic mechanisms. Mutant HTT disrupts transcription, interferes with immune and mitochondrial function, and is aberrantly modified post-translationally. Evidence suggests that the mHTT RNA is toxic, and at the DNA level, somatic CAG repeat expansion in vulnerable cells influences disease course. Genome-wide association studies have identified DNA repair pathways as modifiers of somatic instability and disease course in HD and other repeat expansion diseases. In animal models of HD, nucleocytoplasmic transport is disrupted and its restoration is neuroprotective. Novel cerebrospinal fluid (CSF) and plasma biomarkers are amongst the earliest detectable changes in individuals with premanifest HD, and have the sensitivity to detect therapeutic benefit. Therapeutically, the first human trial of a *HTT*-lowering antisense oligonucleotide successfully, and safely, reduced CSF concentration of mHTT in individuals with HD. A larger trial, powered to detect clinical efficacy, is underway, along with trials of other *HTT*-lowering approaches. In this Review, we discuss new insights into the molecular pathogenesis of HD and future therapeutic strategies, including the modulation of DNA repair and targeting the DNA mutation itself.

[H1] Introduction

Huntington disease (HD) is caused by a dominantly inherited CAG repeat expansion in exon 1 of the Huntingtin gene (*HTT*), and is characterised by progressive involuntary choreiform movements **[G]** , behavioural and psychiatric disturbances, and dementia[1]. HD is one of over 40 diseases that are caused by expansion of simple repeats, most of which, for unknown reasons, primarily affect the nervous system[2]. CAG encodes the amino acid glutamine and a sequence of several glutamine units is referred to as a polyglutamine tract; HD is the most common of the nine polyglutamine diseases[2]. HD occurs worldwide and has a prevalence of ~12 per 100,000 individuals in populations of European descent[3]. Onset of the motor symptoms of HD, known as motor onset, can occur from childhood to old age, with a mean onset around 45 years, and is followed by inexorable disease progression[4,5]. Repeats of 36 or more CAG units are pathogenic, with longer repeats typically causing earlier onset[1]. Repeats of between 36 and 39 CAG units confer reduced penetrance[1], and individuals carrying these reduced penetrance alleles are likely to be carriers of HD with disease onset beyond the normal lifespan.

Huntingtin (HTT) is a large, ubiquitously expressed protein, the evolution of which can be traced back over millions of years[6]. The polyglutamine tract first appeared in the sea urchin and increased in length throughout the evolution of vertebrates; humans have the longest tract[7]. HTT contains both nuclear export and nuclear localisation signals, so the protein shuttles between nucleus and cytoplasm via active transport [8-10]. HTT is involved in CNS development, including neural tube formation and neuroblast migration, and *HTT* knockout mice die before birth, shortly after the formation of the nervous system[11,12]. HTT is also involved in axonal transport, synaptic function and cell survival[13].

The mutant huntingtin protein (mHTT) that results from CAG repeat expansion affects many cellular functions, leading to cell death, and establishing which of these effects are primary or secondary pathogenic processes is difficult. Striatal medium spiny neurons are most vulnerable to the presence of mHTT, although substantial neuronal dysfunction and death also occurs in the cerebral cortex[14-18]. Polyglutamine tract length affects the post-translational modification of HTT, which in turn influences the subcellular distribution, stability, cleavage and function of the protein[19]. HTT also binds and interacts with DNA in many genes, and the presence of an expanded polyglutamine tract in HTT results

in transcriptional dysregulation[20]. Transcription is substantially disrupted in the brains of individuals with HD compared with healthy controls[21]. This disruption results in upregulation of the immune response and mRNA processing, and downregulation of metabolic processes and synaptic function. The anatomical distribution of transcriptional disruption correlates with areas of cell death, being most marked in the caudate nucleus[21]. Transcriptional dysregulation also occurs in the peripheral tissues of individuals with HD, such as muscle and blood, and the sets of genes that are dysregulated significantly overlap with those that are dysregulated in the caudate[20].

Animal models of HD have had a key role in increasing our understanding of pathogenesis and testing therapeutic compounds; genetic models are produced by introducing all or part of human *mHTT*in a transgene, or inserting an expanded CAG repeat into the endogenous *HTT* gene, which is known as a 'knock in' strategy [22]. Invertebrate models of HD, such as C. *elegans* and drosophila, show progressive neurodegeneration, motor abnormalities and reduced survival [23]. Rodent models of HD are the most commonly used, and show HTT aggregation, somatic instability, motor, cognitive and behavioural abnormalities, and reduced lifespan[24]. Large animal models, including sheep, pigs and non-human primates, are genetically more similar to humans, but use of these models has been limited by expense and the lag time to symptom onset. In this Review, we discuss the latest developments in our understanding of the pathogenesis of HD, and discuss new CSF and plasma biomarkers. We also review ground-breaking clinical trials of HTT-lowering therapies and discuss future therapeutic strategies that target the DNA mutation itself.

[H1] Pathogenesis of HD

In this section, we summarise the current understanding of the molecular mechanisms underlying HD, before introducing the latest developments in our understanding of disease pathogenesis in the sections that follow. In individuals with HD, the expanded polyglutamine tract causes mHTT to fold abnormally, which causes soluble monomers of HTT protein to combine, forming oligomers. These oligomers then act as seeds for the formation of mHTT fibrils and large inclusions in both the cytoplasm and nucleus[25-27]. Large mHTT inclusions were previously thought to be pathogenic [28,29], but inclusions can occur without cell death, and vice versa[30-32]. More recent evidence suggests that N-terminal mHTT oligomers

are toxic[33-38], and that the subsequent formation of inclusions might even be protective[31,34]. This topic is discussed in more detail below (Toxic exon1 protein). Endoplasmic reticulum stress precedes, and then improves on mHTT aggregation, suggesting the toxicity of oligomers is mitigated by their aggregation into larger inclusions[39,40]. Small mHTT oligomers and fibrils, which are precursors of large inclusions, have been observed in the brains of individuals with HD[41,42]. In mouse and drosophila models of HD, the formation of mHTT oligomers and fibrils occured before the onset of symptoms, and levels increased as the disease progressed[42]. Polyglutamine-containing N-terminal fragments of mHTT, which can be produced either by proteolytic cleavage[26] or abnormal splicing[43], aggregate in the brains of individuals with HD[44] more rapidly than the full length protein does[45-47].

Evidence also suggests that mHTT can transfer between cells. For example, synthetic polyglutamine peptides can be taken up by cells in culture[48,49], and in co-culture experiments, fluorescently tagged mHTT can transfer between neighbouring cells[50,51], including through tunnelling nanotubes. Furthermore, in Drosophila, mHTT can be released from synaptic terminals and taken up by neighbouring neurons by endocytosis[52], and mHTT taken up phagocytically by Drosophila glia, can act as a seed for aggregation of wild-type HTT, which is properly folded and would not usually aggregate[53]. In one study, mHTT spread between neurons via functional synapses in three models, including from human HD iPSC-derived neurons to wild-type mouse brain slices, from HD mouse cortical neurons to medium spiny neurons in a wild-type mouse corticostriatal brain slice, and following injection of a mHTT fragment into wild-type mouse cortex[54]. This contiguous propagation is distinct from truly 'prion-like' behaviour, which involves the infectious prion protein inducing the misfolding of the normal form and has not been demonstrated in HD[55]. Evidence for cell-to-cell spread of mHTT in humans is more limited; postmortems of individuals who had received fetal striatal transplants showed inclusions in the extracellular matrix of the graft, suggesting that mHTT is released by neurons, although no inclusions were found within cells[56].

The two main protein degradation systems of the cell are the ubiquitin–proteasome system, which clears damaged proteins, and autophagy, which degrades protein complexes and damaged organelles. Evidence from human tissue and animal models suggests that these systems are compromised in

HD[57,58]. Furthermore, inducing autophagy increases mHTT clearance and improves the phenotype in animal models of the disease[59]. CNS inflammation has been implicated in several neurodegenerative diseases, including Alzheimer disease, Parkinson disease, multiple sclerosis, prion disease and amyotrophic lateral sclerosis[20,60,61], although whether this inflammation is a primary pathogenic process or a response to other pathologies remains unclear. The levels of reactive microglia and proinflammatory mediators in the brain are higher in individuals with HD than in healthy controls[62,63], and immune activation is also observed in the peripheral blood of individuals with the disease[61].

Mitochondria were implicated in HD pathogenesis after mitochondrial toxins, such as 3-nitropropionic acid, were found to cause selective death of striatal medium spiny neurons[64]. Mitochondrial ATP production, which is essential for the survival of neurons, is lower in postmortem brain samples from individuals with HD than in control samples[65]; this observation is supported by evidence from animal and cell models of HD[47,66,67]. Mitochondrial ultrastructure is disrupted in the brains of individuals with HD[68], and the number of mitochondria[69] and the activity of enzyme complexes[70-72] is lower than in controls. Furthermore, mitochondrial membrane potential is lower in lymphoblasts derived from individuals with HD than in lymphoblasts from controls[73,74]. Brain imaging studies showed that, in some brain regions, individuals with HD had lower levels of glucose metabolism and higher lactate concentration than healthy individuals[75-78], which could be a result of mitochondrial alterations. In animal models, mHTT disrupted anterograde and retrograde motility of mitochondria[79-81], resulting in the accumulation of mitochondria in the soma[82]. In addition, the expression of PGC1α, which regulates mitochondrial biogenesis, is lower in cell and animal models of HD than in controls[70,83]. mHTT interacts with the mitochondrial outer membrane, thus triggering calcium release that could cause cell death[84,85], and also interacts with the inner mitochondrial membrane, thus disrupting the import of mitochondrial proteins[86,87].

Although a substantial body of evidence suggests that the mHTT protein is toxic, neurodegeneration was observed in animal models that express untranslated CAG repeat-containing transcripts, suggesting that mHTT RNA can also contribute to cell death[88]. RNA foci **[G]** were also toxic in animal models with CAG repeats in *ATXN3* or *GFP*[89-91]. Unconventional translation initiation, or repeat-associated

non-ATG translation **[G]**, occurs in the brains of individuals with HD in a CAG length-dependent manner and produces monopeptides that aggregate, particularly in the striatum, but the toxicity of these monopeptides has not yet been established[92,93]. Indeed, a very recent study has shown that HD knock-in mice lack repeat-associated non-ATG translation-mediated toxicity, suggesting that the role of this form of translation in HD pathogenesis is debatable[94].

The *HTT* CAG repeat is somatically and meiotically unstable, progressively lengthens throughout life and tends to expand between generations[95-97]. In studies that analysed samples of blood and post-mortem cortex from individuals with HD, greater CAG expansion was associated with an earlier age of disease onset[97,98], suggesting that somatic instability **[G]** of the CAG repeat has a role in pathogenesis. The degree of somatic instability varies among tissues, with expansion particularly prominent in neurons from brain regions that show marked pathology such as the striatum and cortex[99-101], in which repeats of over 1,000 CAG have been observed post-mortem[102]. In other tissues, such as cerebellum and blood, the CAG repeat was relatively stable, either not changing with age or increasing by only a few CAG in a small proportion of cells[103]. In one study, a mathematical model fitted to data on repeat length and phenotype in individuals with HD[104] indicated that motor onset occurs when the repeat expands beyond a threshold of around 115 CAG units in a sufficient number of vulnerable cells[105]. In postmortem brain tissue from individuals with HD and animal models, the anatomical distribution of somatic CAG repeat instability often overlaps with areas of HD neuropathology, suggesting that somatic CAG expansion might underlie the selective vulnerability of striatal medium spiny neurons[106].

[H2] Genetic modifiers

Pure CAG repeat length is the main determinant of the course of HD[107] and accounts for around 50–70% of variation in age at onset[98,108], but up to half of the remaining variability is also heritable and therefore results from differences elsewhere in the genome[109]. Large patient cohorts are now available in which to carry out unbiased, genome-wide searches for disease course-modifying genetic variation. The Genetic Modifiers of Huntington's Disease (GeM-HD [110]consortium's genome-wide association

study (GWAS) of 4,082 individuals with HD identified two loci, one on chromosome 8 and the other on chromosome 15, that were associated with age at onset[107]. Two independent signals identified on chromosome 15 were likely to correspond to the gene encoding FAN1, which is a DNA endonuclease and exonuclease that is involved in interstrand crosslink repair and replication fork recovery[111]. One of these chromosome 15 signals was associated with disease onset >6 years earlier than would be expected from CAG length alone, and the other was associated with disease onset 1.4 years later than expected. Knockout or short hairpin RNA-mediated lowering of *FAN1* increased somatic expansion of the *HTT* CAG repeat in a human osteosarcoma cell line, patient-derived iPSCs and differentiated neurons[112]. Although the known functions of FAN1 all involve nuclease activity, inactivation of the FAN1 nuclease domain did not influence the rate of CAG expansion. This observation suggests that an unknown function of FAN1, such as an interaction with other DNA repair components, is protective against CAG repeat instability. Knockout of *FAN1* in a mouse model of Fragile X syndrome increased the somatic expansion of a CGG repeat, indicating that *FAN1* also is also involved in other repeat expansion diseases[113]. Curiously, *FAN1* knockout did not alter intergenerational CGG repeat expansion, suggesting that the mechanisms underlying somatic and meiotic instability could be distinct. The chromosome 8 signal observed in the GeM-HD GWA study was associated with disease onset 1.6 years earlier than expected from CAG repeat length and could correspond to *RRM2B*, which is involved in nucleotide synthesis, or *UBR5*, a ubiquitin ligase which might have a role in HTT aggregation[114,115].

In another study, the disease onset-modifying variants identified by the GeM-HD [110] were genotyped in an independent cohort of 3,314 individuals from the European Huntington's Disease Network and the signals on chromosome 8 and 15 were again associated with age at disease onset[98]. In addition, a locus at *MLH1* on chromosome 3, that was not identified in the GeM-HD GWAS, was associated with a 0.7 year delay in disease onset. MLH1, part of the mismatch repair MutL endonuclease complexes, which cut DNA, is required for somatic instability in HD mice[116] and directly interacts with FAN1[112].

In a study by Hensman Moss, et al. [117] a disease progression measure based on longitudinal motor, cognitive and imaging data was used to conduct a GWAS in 216 participants from the TRACK-HD study and 1,773 participants from the REGISTRY study. Variation at a chromosome 5 locus, which

corresponds to *MSH3* or *DHFR*, was associated with slower disease progression, as well as reduced *MSH3* expression in blood and fibroblasts. MSH3 identifies mis-paired bases or loop-outs and initiates DNA mismatch repair[118]; knockout of *MSH3* in a mouse model of HD prevented somatic expansion and decreased mHTT aggregation in striatal neurons[119,120]. DHFR is an enzyme involved in nucleotide and amino acid synthesis[121]. Another study showed that the chromosome 5 signal was driven by a 9 bp tandem repeat variant in exon 1 of *MSH3*[122] . In individuals with HD, this variant was associated with reduced *MSH3* expression in blood and brain[122], decreased somatic CAG expansion, delayed disease onset and slower progression[122] In individuals with myotonic dystrophy type 1 (DM1), which is caused by a CTG repeat expansion in *DMPK*, the same *MSH3* variant was associated with less somatic expansion and delayed disease onset[122]. *MSH3* and *DHFR* share a bidirectional promoter, but increased expression of *MSH3* was associated with more repeat expansion and earlier onset of HD, whereas increased expression of *DHFR* was not[122]. The GeM-HD GWAS[110] was recently extended to include a total of 9,064 individuals with HD[98]. This extended study replicated the findings of the original GeM-HD GWAS and also identified new HD onset-associated loci that correspond to the DNA repair genes *PMS1*, *MSH3*, *PMS2* and *LIG1*, as well as *HTT*, *TCERG1* and *CCDC82*. *TCERG1* is a nuclear regulator of transcriptional elongation and splicing, and was proposed as a potential HD modifier due to its interaction with HTT[123,124], whereas CCDC82 is a relatively unknown coiled-coil domain protein that is phosphorylated in response to oxidative stress[125]. The *HTT* signal resulted from sequence variation within the CAG repeat. At the very 3' end of the CAG tract there is a CAACAG motif, which encodes an extra two glutamines. In individuals lacking this CAA interruption the onset of HD occurred an average of 12.7 years earlier than would be expected from CAG repeat length, and in individuals with a duplication of the CAACAG motif, onset was delayed by an average of 5.7 years, despite the duplication increasing the total number of glutamines. Loss of the CAA interruption is also associated with increased somatic *HTT* CAG expansion in blood and sperm[107]. Such interruptions, which can have different sequences, limit expansion in many repeat disorders, including spinocerebellar ataxia (SCA) type 1, 2, 3 and 17; fragile X syndrome; Friedreich's ataxia and DM1[126]. *HTT* CAG repeat length predicted the age of HD onset more accurately than the number of glutamines in the protein, suggesting that altered DNA repair, acting through somatic expansion, is the main modifier of pathogenesis[98,107].

Therefore, introducing interruptions into the *HTT* CAG could be a strategy for the treatment of HD. The occurrence of *HTT* CAG sequence variation, although rare, means PCR fragment-sizing assays, which assume that a single CAACAG motif is present, might overestimate or underestimate pure CAG repeat length, and could contribute to the variable penetrance of alleles sized at 35–39 repeats [107].

On chromosome 5, the extended GeM-HD GWAS[98] replicated the findings from the Hensman Moss, et al. [117] study by identifying a locus corresponding to *MSH3* or *DHFR* that was associated with 0.6 year delayed onset of HD[81]. Two additional, independent signals were also identified at *MSH3* or *DHFR,* one associated with an 0.8-year earlier onset and the other associated with a 6.1-year delay in onset. The onset-hastening variant was associated with higher expression of *MSH3* and increased CAG expansion in blood. In *LIG1*, which encodes a DNA ligase that seals DNA to complete replication and repair[127], two signals were identified, one associated with a <1 year delay in onset and the other associated with <1 year earlier onset. In a transcriptome-wide association study, the onset-hastening variant was associated with higher *LIG1* expression in cortex[98], which is consistent with the increase in CAG instability that was observed when *LIG1* was overexpressed in human cells *in vitro* [128], as well as the reduced expansion and increased CTG repeat contraction seen in DM1 mice with a mutation that impairs *Lig1* activity[129]. A third, rare variant in *LIG1* that was predicted to impair protein function was associated with a 7.7-year delay in onset of HD.

MLH1 heterodimerises with PMS2, PMS1 or MLH3 to form the MutLα, MutLβ or MutLγ mismatch repair endonuclease complexes, respectively. Variation in *PMS2* was associated with 0.8-year delayed onset, and *PMS1* with 0.8-year earlier onset [98]. MLH3 was associated with age at disease onset in a gene-wide association analysis[98], and is a component of DNA repair pathways that were also associated with disease onset. Interestingly, knockout of *Pms2* and *Mlh3*, but not *Pms1*, reduced somatic instability in HD mice[116,130]. In a transcriptome-wide association study, increased expression of *FAN1* and *PMS1*, and decreased expression of *MSH3*, in cortex were associated with later onset of HD[81]. Taken together, these results suggest that MutLα and MutLγ promote HD pathogenesis, and that MutLβ inhibits HD pathogenesis.

Interestingly, one study showed that some of the variants identified as HD modifiers in the GeM-HD GWAS[110], including *FAN1* and *RRM2B*, also influenced the age of onset of other polyglutamine diseases[131]. This observation suggests that DNA repair, probably acting through somatic expansion, is a common contributor to pathogenesis in CAG expansion diseases. Genetic association studies[132 118], as well as studies using mouse models[118], human cell lines[133-139], or patient-derived cells[134,140,141], have also implicated MutSβ (MSH2 and MSH3), MutSα (MSH2 and MSH6), MutLα and MutLγ in DM1, Friedreich's ataxia and fragile X repeat instability.

[H3] Implications for HD pathogenesis

The results of these genetic association studies indicate that DNA repair activity is central to the pathogenesis of HD, with variants in repair proteins likely to influence the rate of somatic expansion in tissues that are vulnerable to repeat instability and neurodegeneration[126]. The proposed models of CAG repeat instability all involve DNA slippage, with displacement of single stranded DNA at repeated sequences leading to mispairing of the complementary bases[142]. MutSβ identifies DNA loop-outs in the CAG tract and targets them for repair by MutLα or Mutlγ; incorrect repair of the loop-outs could introduce short incremental expansions[143] (Fig. 1). MutSα does not seem to be involved in *HTT* CAG instability, which is likely to be because it recognises small DNA loop outs of 1–2 bases, rather than the longer loop outs targeted by MutSβ[144]. In individuals with DM1, clusters of slipped DNA structures are found in tissues with the highest levels of repeat instability, including heart and cortex, but not in the cerebellum, which shows little or no instability[142]. A study of DNA oligonucleotides showed that the stability of these DNA loop-outs at CAG, CTG and CGG repeats is correlated with the threshold for repeat expansion and the expansion rate[145]. CAG·CTG repeat expansion occurs in post-mitotic neurons[112,146] and continues when the cell cycle is arrested[147], suggesting that expansion occurs during DNA repair or transcription. However, evidence also exists for replication-associated trinucleotide repeat instability[148]. The result of this kind of instability depends on the direction of DNA replication, with expansion of CAG and CTG repeats occurring when CAG is on the lagging strand **[G]**, as is the case in HD, SCA7 and DM1[149], and contraction occurring when CTG is on the lagging strand. This

direction-dependence might be because CAG and CTG repeats have different propensities to form slipped structures, or are processed differently by repair machinery.

Excitingly, most of the HD-modifying variants and pathways converge on specific DNA repair mechanisms, particularly mismatch repair, and influence somatic instability[98,110,112,117,122]. These observations suggest that downregulation of MSH3, MutLα, MutLγ and LIG1, the inhibition of interactions between them, or the upregulation of FAN1 and PMS1, could reduce somatic CAG expansion and improve the course of HD (Acknowledgements

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Author contributions

M.F and C.A.R researched data for the article, made substantial contributions to the discussion of the content of the article, wrote the article, and reviewed and edited the manuscript before submission. S.J.T. made a substantial contribution to the discussion of the content of the article, wrote the article, and reviewed and edited the manuscript before submission. E.W. made a substantial contribution to the discussion of the content of the article, and reviewed and edited the manuscript before submission.

Competing interests

In the past two years S.J.T has undertaken consultancy services, including advisory boards, with F. Hoffmann-La Roche Ltd, Ixitech Technologies, Takeda Pharmaceuticals International and Triplet therapeutics. All honoraria for these consultancies were paid to University College London, S.J.T's employer. Through the offices of UCL Consultants Ltd, a wholly owned subsidiary of University College London, S.J.T. has undertaken consultancy services for Alnylam Pharmaceuticals Inc., F. Hoffmann-La Roche Ltd, GSK, Heptares Therapeutics, LoQus therapeutics, Takeda Pharmaceuticals Ltd, TEVA Pharmaceuticals, Triplet therapeutics, UCB Pharma S.A., University College Irvine and Vertex Pharmaceuticals Incorporated. S.J.T. receives grant funding for her research from Takeda Pharmaceuticals and Cantervale Limited. C.A.R. is chair of the Research Advisory Board of the Huntington Study Group. Within the past two years, C.A.R. has consulted for Annexon, Roche, Sage and uniQure. Through UCL Consultants Ltd., a wholly owned subsidiary of University College London, E.J.W. has served on scientific advisory boards for F. Hoffmann–La Roche, Ionis, Mitoconix, Novartis, PTC Therapeutics, Shire, Takeda Pharmaceuticals and Wave Life Sciences. M.F. declares no competing interests. C.A.R. receives funding for HD research from Hoffman La Roche.

Publisher's note

Key points

- Proteins involved in DNA repair, particularly mismatch repair, can modify the age of onset and rate of progression of HD, likely by altering the rate of somatic expansion of CAG repeats in the Huntingtin gene.
- The modulation of DNA repair factors, such as MSH3, FAN1, PMS2 or LIG1, has therapeutic potential in HD and other repeat expansion diseases.

- Nucleocytoplasmic transport is disrupted in HD by sequestration of nuclear pore components in Huntingtin (HTT) aggregates; modulation of nucleocytoplasmic transport is neuroprotective and might provide a novel therapeutic opportunity.

- Changes in cerebrospinal fluid and serum biomarkers, including neurofilament light chain and mHTT, are amongst the earliest detectable changes in HD and can predict disease onset and track progression.

- Intrathecally-delivered non-allele selective antisense oligonucleotides (ASOs) have successfully lowered HTT concentration in the central nervous system of individuals with HD, and trials of allele-specific ASOs are under way.

- Gene editing strategies for HTT lowering, including zinc finger proteins, transcription activator-like effector nucleases and CRISPR-Cas9, are currently in preclinical development, but need to be delivered via the injection of viral vectors, which can be challenging.

Fig. *1*). Although variants in some mismatch repair components such as *MLH1*, *MSH2*, *MSH6* and *PMS2* are associated with cancer, which indicates the need for caution[150,151], the activity of these proteins can vary over a wide range in the general population without adverse effects and none of the modifiers of HD onset or progression have been identified as risk factors in GWA studies of cancer predisposition[98,152]. Importantly, *MSH3* and *LIG1* are tolerant of loss of function mutations[153], making them appealing targets for knockdown, which human genetic data suggest will be protective against HD[98]. Therefore, the modulation of DNA repair has great therapeutic potential in HD, as well as other repeat expansion diseases.

[H2] New findings in molecular pathogenesis

Despite the decades that have passed since the discovery of the pathogenic HTT mutation in 1993[154], the normal function of HTT and the primary pathogenic mechanism(s) of the mutation remain unclear. As our ability to intervene at the DNA, RNA and protein level improves, we need to understand the pathogenesis of HD to enable the identification of new therapeutic targets and understand the effects of modulating these targets. In this section we discuss key developments in our understanding of HD

pathogenic mechanisms that have occurred in the last 5 years, including the toxicity of HTT fragments, dysfunction of the nuclear pore and insights into the structure of the HTT protein.

[H3] Toxic exon 1 protein

Two alternatively spliced transcripts arise from *HTT*. These transcripts differ in the length of their 3' untranslated region (UTR) by 3 kb, but give rise to the same HTT protein[155]. The longer transcript is predominantly expressed in the brain, whereas the shorter version is more widespread[155]. However, highly toxic N-terminal mHTT fragments also exist. Initially, these N-terminal fragments were attributed to proteolytic cleavage of mHTT by caspases and calpains[156], but *mHTT* can also be mis-spliced to generate a short mRNA, which is translated into a highly toxic N-terminal fragment that contains exon 1[43]. This short exon 1 transcript was observed in mouse models of HD and in post-mortem brain samples from individuals with the disease; levels were highest in the brains of individuals with juvenile-onset HD[43,157]. The generation of exon 1 mRNA is thought to result from splicing factors binding to the CAG repeat and allowing read-through into intron 1, which contains a stop codon [43]. The aberrant splicing seems to be CAG length-dependent and is only seen in mutant alleles[43]. Mice expressing N-terminal huntingtin fragments develop a severe phenotype much earlier than those with a similar number of repeats in full-length *mHTT*[158]. The extent to which the mis-splicing of *HTT* exon 1 contributes towards neuropathology in humans remains to be seen.

[H3] Nuclear pore complex disruption

The nuclear pore complex (NPC) is the main conduit by which proteins and RNA are actively transported between nucleus and cytoplasm, and consists of complexes of protein subunits called nucleoporins (NUP) that span the nuclear envelope (Fig. 2) [159]. Interestingly, recessive mutations in the gene encoding nucleoporin NUP62, which is located in the central channel of the NPC, cause infantile bilateral striatal necrosis[160], suggesting a role for NPC dysfunction in the tissue specificity of HD pathology. Ran, which is a small protein involved in nuclear transport, is converted from its GDP-bound form (Ran-GDP) to its GTP-bound form (Ran-GTP) by RCC1 inside the nucleus, and is converted back to Ran-GDP through interaction with RanGAP1, which is located on the cytoplasmic filaments of the

NPC (Fig. 2a). Ran can diffuse freely within the cell, but because RCC1 is located in the nucleus and RanGAP1 is located in the cytoplasm, a concentration gradient of Ran forms is established, with more Ran-GTP in the nucleus and more Ran-GDP in the cytoplasm[161]. This gradient acts as a signal for cellular processes[161]. During nuclear import, cargo proteins are released into the nucleus when their transporter molecule, known as a karyopherin, interacts with Ran-GTP. Conversely, in nuclear export, cargo proteins are released into the cytoplasm when Ran-GTP is hydrolysed to Ran-GDP by RanGAP1 (Fig. 2a). The nuclear to cytoplasmic Ran gradient generated by RanGAP1 is critical, and its loss rapidly results in cell death[162].

Interestingly, mHTT binds to RanGAP1 with greater affinity than the wild-type HTT protein does[163]. In one study, immunofluorescent detection of NPC proteins in brain tissue from mouse models of HD showed that RanGAP1 and the nucleoporins NUP62 and NUP88 are sequestered in mHTT aggregates, which grow with age and are most prominent in the striatum[164]. More RanGAP1 was sequestered as the disease progressed. Intrastriatal microRNA **[G]** (miRNA)-mediated knockdown of the small ubiquitin-like modifier (SUMO) ligase PIAS reduced mHTT aggregation[153], and thereby restored RanGAP1 levels. In post-mortem brain samples from individuals with HD, mitochondrial, RanGAP1 and NUP62 were displaced from their normal perinuclear location into aggregates, the cytoplasm or the nucleus, consistent with disruption of nuclear transport[164]. Immunofluorescent detection of Ran showed that, compared with cells from healthy individuals, iPSC-derived neurons from individuals with HD had a disrupted Ran gradient, with more Ran-GDP in the cytoplasm and less Ran-GTP in the nucleus, which suggests a failure of active transport[164]. MAP2 is usually too large to cross the NPC by passive transport, but levels of nuclear MAP2 were higher in iPSC-derived neurons from individuals with HD than in cells from healthy individuals, suggesting that in HD the NPC is compromised and leaky. In mouse primary cortical neurons transfected with human HTT containing a wild-type 22 CAG repeat or an expanded 82 CAG repeat, a reporter bearing both nuclear import and export signals was observed mostly in the cytoplasm, suggesting nuclear import is particularly deficient. Interestingly, repeat-associated non-ATG translation HTT dipeptides also disturbed active and passive nuclear transport[164]. In a mouse line with a hexanucleotide GGGGCC repeat expansion in *C9orf72*, which causes

amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in humans, repeat-associated non-ATG translation dipeptides sequestered NUPs in aggregates[165], and in a human cell line these dipeptides blocked the nuclear pore[166].

Overexpression of RanGAP1 in mouse primary cortical neurons reduced the amount of cell death caused by the expression of mHTT[164]. In Drosophila, overexpression of Ran rescued the neurodegeneration caused by expression of an N-terminal mHTT fragment, whereas overexpression of a dominant negative form of Ran exacerbated neurodegeneration[164]. O-GlcNAcylation, a post-translational modification in which an uncharged acetylated glucosamine (O-GlcNAc) is attached to a serine or threonine residue, is vital for the localisation and function of nucleoporins[167]. A study that used immunofluorescent techniques to visualise O-GlcNAc residues in brain sections found that O-GlcNAc levels in cortical cells were lower in a mouse model of HD than in wild-type mice[164,127]. O-GlcNAcase removes O-GlcNAc modifications, and inhibition of O-GlcNAcase with Thiamet-G protected against mHTT-related cytotoxicity and restored nucleocytoplasmic transport in primary cortical neurons from a rodent model of HD[164]. Furthermore, inhibition of nuclear export with KPT-350 was neuroprotective in a mouse model of demyelination[168]. A similar molecule, which also blocks nuclear export, reduced neurodegeneration in the eye of a drosophila model that expresses 30 GGGGCC repeats in *C9orf72*[169] and restored nucleocytoplasmic transport in rodent primary neurons that overexpress TDP43[170]. These observations suggest that inhibition of nuclear export could compensate for the disruption of nuclear import that occurs in HD.

[H3] HTT protein structure

Some aspects of HTT protein structure were recently determined using cryo-electron microscopy (EM)[171]. This new information could provide greater insight into the normal cellular functions of HTT, and the pathogenesis of HD[171]. The purification of HTT required co-expression and co-isolation with HAP-40 (Huntingtin-Associated Protein of 40 KDa), which binds tightly to HTT[172]. HAP-40 has roles in endosome function[173], which is consistent with the role of HTT in vesicle transport. The cryo-EM structure showed that HTT consists mainly of supercoiled alpha-helical structures termed “HEAT Repeats”, which had been suggested by the results of previous computational, biochemical, electron

423 microscopy and mass spectrometry studies[6,174-176]. The full-length HTT protein bound to HAP-40 has a
424 compact shape, with three domains — an N-terminal domain, a bridge domain, and a C-terminal domain
425 — wrapped tightly around HAP-40. Unfortunately, several key domains of HTT were not resolved in
426 the cryo-EM structure. These unresolved domains include an N-terminal domain that is approximately
427 the length of exon-1 and contains the poly-glutamine repeat, and a number of loops that are thought to
428 contain unstructured proteolytically sensitive regions. These loops contain many sites of post-
429 translational modification[13,177], which can modulate the toxicity of mHTT, possibly by regulating HTT
430 proteolysis and the interaction of HTT with other proteins[178]. Thus, further studies of HTT structure and
431 biochemistry could provide more information on the normal function and pathogenic interactions of the
432 protein.

433 **[H1] New biofluid biomarkers**

434 Biomarkers are measurable indicators of the severity of a disease and can enable the measurement or
435 prediction of clinical progression, as well as the detection of therapeutically-induced improvement.
436 However, before a biomarker can be considered as a surrogate marker of a clinical endpoint, it must be
437 well understood in terms of disease pathobiology, and must meet strict requirements, including those
438 relating to measurability, accuracy, specificity and reproducibility[179-181]. mHTT is thought to be released
439 from damaged neurons[182] and the concentration of mHTT in CSF samples can be reliably quantified
440 with ultra-sensitive immunoassays that have been validated for use in clinical trials[183,184]. The
441 concentration of mHTT in the CSF of individuals with HD correlates with disease stage and severity,
442 which is determined by age at onset, disease burden score, and Unified Huntington's Disease Rating
443 Scale (UHDRS) motor score[183-185]. CSF mHTT concentration was also the key pharmacodynamic
444 biomarker used in the first clinical trial to demonstrate dose-dependent mHTT-lowering with an
445 antisense oligonucleotide (ASO) in individuals with HD[186].

446 Neurofilament light protein (NfL) is found principally in axons and is released by neuronal damage, for
447 example, in one study serum NfL concentration rose within two weeks of head trauma, compared with
448 uninjured participants, and normalised after 3 months[187]. In several studies, CSF NfL concentration was
449 higher in individuals with HD than in healthy individuals, increased with disease progression and

predicted the rate of progression in individuals with HD[188-192]. A strong correlation between CSF and plasma NfL levels was observed, which suggests that NfL originates in the CSF[191]. In a mouse model of HD, both CSF and plasma levels of NfL were correlated with the degree of brain atrophy and the severity of disease, as determined by motor function and body weight[193]. Plasma NfL levels were also higher in individuals with HD than controls, increased with disease severity and predicted the degree of progressive brain atrophy[191,194]. In premanifest HD carriers, plasma NfL levels predicted the likelihood of clinical onset within the next three years and the rate of subsequent disease progression, as measured by cognitive, functional, and brain atrophy measures[191,194]. When compared with CSF NfL, plasma NfL was a better predictor of the rate of clinical progression, but CSF NfL was more strongly associated with brain volume measures than plasma NfL was. Rising concentrations of mHTT and NfL in biofluids seem to be the earliest detectable changes occurring in individuals with HD, and are followed by changes in brain imaging measures (for example, caudate atrophy), motor scores and then cognitive tests[185]. Plasma and CSF NfL were more strongly associated with clinical measures than CSF mHTT was, perhaps reflecting the direct link between brain atrophy and clinical manifestations of HD, or the complex contributions to the CSF mHTT assay signal, which is likely to be influenced by polyglutamine tract length, protein turnover and neuronal damage[184].

In cross-sectional studies, CSF levels of the microglia-derived inflammatory mediator YKL40, the immune-cell derived enzyme chitotriosidase, and the proinflammatory cytokine IL-6 were higher in HD carriers than in healthy controls[192,195]. CSF levels of YKL40 also increased with disease progression[192,195]. These findings suggest a role for microglial activation and inflammation in HD and support the use of these biomarkers to study relevant pathways.

The concentration of tau was also robustly increased in the CSF of individuals with HD compared with healthy controls[196], and tau aggregation was observed in post-mortem brain tissue from individuals with HD[162,197-199]. Increased phosphorylation and abnormal splicing of tau were observed in the striata of individuals with HD compared with controls[200,201], and mHTT has been found to interact with tau in cell and animal models of the disease[202]. However, whether tau pathology is involved in HD

pathogenesis, is a general feature of neurodegeneration, or is an unrelated part of the aging process is unclear[203].

It will be some time before any biomarker attains official regulatory approval for use as a surrogate endpoint in studies of HD. However, biomarkers such as CSF and plasma NfL, and CSF mHTT, have been used to interpret the effects of HTT-lowering therapies and are included in ongoing and planned trials of similar agents[204-206], which indicates that these markers are becoming increasingly useful and informative.

[H1] Therapeutic opportunities

Currently, treatments for HD focus on the relief of symptoms like chorea, dystonia, and psychiatric and behavioural disturbances[207]. No disease-modifying treatments have been found, despite some candidate drugs showing positive results in preclinical studies[208]. Drugs for which efficacy trials have failed to meet their endpoints include the dopamine stabiliser Pridopidine[209], phosphodiesterase 10A inhibitors[210-212], coenzyme Q10[213,214], creatine[215], cysteamine[216], the sirtuin-1 inhibitor Selisistat[217,218], hydroxyquinoline[219], and the immunomodulators Sativex[220] and Laquinimod[221]. Limited evidence supports the use of human foetal striatal tissue transplants or autologous stem cell transplants to treat individuals with HD[222-224], but much more work is needed to determine the efficacy of these cell replacement therapies. The failure of so many efficacy trials might be owing, in part, to the insensitivity of the selected endpoints, such as functional capacity and motor score, to subtle changes in disease course. A more likely explanation is that, because the pathogenic events that occur downstream from mHTT form a complex web, pharmacological targeting of individual pathways is either too difficult to achieve cleanly, or is insufficient to modify disease course.

Following these failed efficacy trials, the focus of research into HD therapeutics has shifted towards targeting the causative mutation at the RNA and DNA level[225,226]. HD is thought to be caused by toxic properties of mHTT[5,227] and lowering expression of mHTT inhibits pathogenesis in cell and animal models of the disease[186,226,228-231]. However, loss of normal wild-type HTT might also contribute to pathogenesis[13,232], and HTT-lowering therapies could exacerbate this potential haploinsufficiency. *Htt*

knockout is embryonically lethal in mice [11,12,233] and conditional deletion of *Htt* in the forebrain shortly after birth leads to a progressive degenerative neurological phenotype[234]. Evidence suggests that, in adult mice, HTT has several roles, including as a scaffold protein[235,236], in intracellular trafficking[237-241], transcriptional regulation[242-244] and synaptic connectivity[245-247]. The phosphorylation of HTT in response to DNA damage suggests that the protein has a role in the DNA damage response[248]. Partial knockdown of HTT in adult animals is well tolerated in multiple species, including non-human primates[225,249-252]. Deletion of *Htt* in 4-month-old and 8-month-old mice caused no pathological or motor effects during 5 months of observation[253]. Individuals with heterozygous inactivation of *HTT* have no detectable symptoms[254].

The approaches used to reduce *HTT* expression, a process known as "HTT lowering", include RNA interference (RNAi), ASOs and small molecule modulators of RNA processing (Fig. 3). The suppression of mHTT expression without affecting wild-type HTT expression, known as "allele-selective HTT lowering", by targeting the CAG tract[255-257] or variants inherited along with the *HTT* CAG expansion[258-260], is desirable, but challenging. Such allele-selective agents could have off-target effects, for example, at other CAG repeat-containing regions[261]. Therapies that target *HTT* CAG expansion-linked variants would only be effective in individuals with the linked variant, and as no one variant is present in all individuals with expanded HD alleles, at least three such therapies would be needed to treat up to 80% of individuals with HD[262-264]. The assigning, or 'phasing', of variants to the mutant and wild-type alleles is critical, otherwise there could be a risk of lowering the wild-type allele. Additionally, the need to target specific variants, as opposed to the whole gene or transcript, restricts the choice of sequences, which might limit the potency and selectivity of the resulting therapy[225]. Currently, both allele-selective and non-allele-selective methods are under development.

[H2] RNA-targeting approaches

[H3] RNAi

RNAi is an endogenous cellular process that degrades mature, spliced mRNAs[265]. During this process, non-coding miRNAs form hairpin structures, and the antisense guide strand of these structures guides the RNA-induced silencing complex (RISC) to bind to a complimentary mRNA target, leading to

mRNA cleavage and translational repression[266]. Small interfering RNAs (siRNAs) are similar to miRNAs, but are derived from longer double-stranded RNA, do not form hairpins and are more target-specific[267]. The main challenge facing the development of RNAi therapeutics for HD is introducing synthetic siRNAs and/or miRNAs into cells most vulnerable to the disease, such as the striatum. The lowering of *HTT* expression with siRNAs improved phenotype and neuropathology in mouse models of HD[249,268-275].

Delivering RNAi-inducing therapies into brain cells is challenging[226]. Most commonly, viral transduction of siRNAs or miRNAs is required for stable induction of RNAi and permanent suppression of *HTT* translation, although cellular entry has been improved with chemical modifications, liposomes and nanoparticles[276]. Recombinant adeno-associated viruses (AAV) and lentiviruses are non-pathogenic, minimally immunogenic and cannot replicate[277]. AAVs provide stable expression of a construct in non-dividing cells from nuclear episomes, which are extra-chromosomal genetic material, as opposed to integrating into the host genome, as in the case of lentiviruses[277]. Viral vectors typically need to be injected into the target brain regions such as the striatum, as they cannot cross the blood–brain barrier. However, this route of administration carries additional risk and tissue distribution might be limited[278].Viruses that are designed to be administered by peripheral intravenous injection, cross the blood brain barrier, and transduce neurons and glia are currently under development, and include AAV9[279] and AAV-PHP.B[280,281]. The challenges involved in developing RNAi-inducing therapies include the risks of off-target knockdown[282], overwhelming the RNAi degradation pathway[283,284], immunogenicity[285] and the presence of virus-neutralising antibodies[286]. Regardless, a phase II trial of intracerebrally injected, AAV2-encapsulated nerve growth factor RNA in individuals with Alzheimer disease has shown that virally-delivered gene therapy can be safe and well-tolerated[287].

Patisiran, an siRNA designed to treat hereditary transthyretin (TTR)-mediated amyloidosis, is the first FDA approved therapy that uses lipid nanoparticle delivery[288,289]. The lipid nanoparticles containing the siRNA are administered intravenously and are delivered to the liver, which is the primary site of TTR production, although studies have shown that lipid nanoparticles can also convey RNAi therapy to the CNS[290-293].

In January 2019, UniQure received FDA approval to begin the first trial of a *HTT*-lowering gene therapy in individuals with HD. The therapy being tested in this trial is AMT-130 (uniQure), an AAV5-delivered, non-allele selective *HTT* miRNA[294]. In rodent models of HD, bilateral striatal injection of AMT-130 reduced striatal levels of HTT and improved neuropathology compared with saline injection[231]. Similarly, in a minipig model of HD, AMT-130 produced a sustained, dose-dependent reduction in HTT in the striatum 3–6 months post-administration, as well as smaller reductions in other brain regions[295]. Spark Therapeutics and Voyager Therapeutics are developing AAV1-delivered non-allele selective *HTT* miRNA therapies. Striatal injection of an miRNA developed by Spark Therapeutics improved neuropathology and motor phenotype in rodent models of HD compared with injection of an empty vector[250], and safely lowered HTT in wild-type non-human primates[251]. Striatal injection of the miRNA developed by Voyager Therapeutics, VY-HTT01, lowered HTT levels in a mouse model of HD[275], and in a preliminary study of combined putaminal and thalamic injection of VY-HTT01 in primates the treatment produced well-tolerated, sustained knockdown of mHTT RNA in the striatum, with a smaller reduction in cortex[296,297].

[H3] ASOs

ASOs are synthetic, single-stranded, modified DNA molecules that bind to complimentary stretches of mRNA, thus inducing degradation of this mRNA by RNAse H[298]. ASOs act further upstream than RNAi approaches, binding pre-mRNA as opposed to mature transcripts. This pre-mRNA binding means that ASOs can bind intronic as well as exonic regions, providing more potential binding targets[299]. ASOs diffuse well through the CNS and are taken up by neuronal and glial cells, which means viral vectors are not needed for delivery. One benefit of not requiring viral vectors is that the effects of ASOs on gene expression are reversible and titratable[228,299,300]. However, ASOs are not suitable for oral administration and do not cross the blood brain barrier, so they must be injected intrathecally, intraventricularly or intraparanchymally, all of which result in limited spatial distribution of the ASO in the brain[225,226,299]. Following intrathecal delivery, ASO levels are highest in brain regions that are adjacent to the CSF spaces[301], although in post-mortem studies in individuals treated with intrathecal Nusinersen (Spinraza; Biogen), an ASO that modulates splicing of survival motor neuron protein 2

583 (SMN2), the ASO was observed in both cortical and brainstem neurons and glia[302]. In a conditional
584 mouse model of HD that expresses mHTT in either the striatum or cortex, lowering HTT expression in
585 the cortex was more beneficial than striatal HTT lowering, but simultaneously lowering HTT levels in
586 both brain regions resulted in the greatest reduction in motor and behavioural deficits and brain
587 atrophy[303]. Intrathecal delivery of ASOs to treat HD would require repeated lumbar puncture, which
588 could be avoided by the use of medical devices such as implantable pumps, or by chemical modification
589 of the ASOs to enable peripheral administration and CNS penetration, although such compounds are
590 still in development and are not yet ready for clinical translation[299,300,304,305].

591 ASOs have shown efficacy in other neurodegenerative diseases; Nusinersen, which is delivered by
592 intrathecal boluses, dramatically improved motor function and survival in infants with spinal muscular
593 atrophy type 1[306] and has been approved by the FDA. IONIS pharmaceuticals have developed an
594 intrathecally delivered ASO that targets superoxide dismutase 1 (SOD1) and was well tolerated by
595 individuals with ALS-causing SOD1 mutations[307]. Furthermore, in conjunction with Biogen, IONIS
596 have begun a phase I–IIa trial[308] of a more potent SOD1 ASO, Toferson (IONIS-$SOD1_{Rx}$; Biogen/Ionis).

597 In mouse models of HD, intraventricular infusion of a non-allele-selective *HTT* ASO reduced the
598 expression both wild-type and mutant HTT mRNA and protein, leading to reduced transcriptional
599 dysregulation, improved motor phenotype and increased survival compared with saline
600 infusion[186,228,230]. These effects were particularly marked when the ASO was administered earlier in the
601 disease course. Suppression of HTT mRNA and protein levels was sustained for 12 weeks after
602 administration of the ASO and phenotypic improvement outlasted this knockdown by at least 4 weeks.
603 In another study that used a mouse model of HD, an ASO-mediated ~50%–70% reduction in total HTT
604 improved motor and cognitive deficits to a similar degree as a ~50%–70% reduction in mHTT only [309].
605 Although this evidence supports ongoing clinical trials of non-allele selective *HTT* ASOs, allele-
606 selective strategies remain of interest as they are theoretically less likely to cause the long-term side
607 effects that are associated with the reduction of the wild-type protein. Reductions of mHTT by 50% or
608 more are consistently associated with phenotypic improvement in animal models of HD[226]. In wild-type
609 non-human primates, a 21 day lumbar intrathecal infusion of a non-allele specific *HTT* ASO produced

610 a sustained reduction in HTT for at least 3 months, relative to vehicle-treated control animals, and was
611 well-tolerated[186,228].

612 The results of a phase I–IIa trial of IONIS pharmaceutical's non-allele selective ASO HTT_{Rx}
613 (RG6042/tominersen; Ionis/Roche) were published in 2019[186]. In this trial, adults with early-stage HD
614 received a total of four administrations of HTT_{Rx}, one administration every 4 weeks as an intrathecal
615 bolus injection, via lumbar puncture. Of the 46 participants that were enrolled in the trial, 34 were
616 randomly assigned to receive HTT_{Rx} and 12 were randomly assigned to receive placebo. The individuals
617 receiving HTT_{Rx} were divided into five cohorts that each received a different dose of the treatment from
618 10–120 mg. HTT_{Rx} was well-tolerated, with all participants completing the trial and only mild, lumbar
619 puncture-related adverse effects, such as transient headache, being reported. Importantly, the groups of
620 participants who received the ASO showed dose-dependent reductions in CSF mHTT concentration
621 compared with the participants who received placebo (Fig. 4a), which is clear evidence of target
622 engagement. This mHTT lowering began by the first timepoint, which was 28 days after the first
623 administration, and the downward trend continued even between the final two administrations of the
624 ASO, suggesting that mHTT levels would fall further with continued treatment. In the groups receiving
625 the two highest HTT_{Rx} doses, CSF mHTT was 40-60% lower than in the group receiving placebo. This
626 reduction exceeds the degree of mHTT lowering that produced clinical benefit in animal models
627 [186,228,309]. Pharmacokinetic modelling predicted that this 40–60% reduction in CSF mHTT would
628 correspond to a 55-85% reduction in mHTT in the cortex and a 20-50% reduction in mHTT in the
629 caudate. Ventricular volume was larger in the groups of participants receiving the two highest doses of
630 ASO than in the group of participants receiving placebo, but no concomitant decreases in whole-brain
631 volume were observed. This increase in ventricular volume might reflect local parenchymal
632 pseudoatrophy resulting from the resolution of inflammation or gliosis.

633 At the final timepoint, which was between 16 and 20 weeks after the first administration, CSF NfL
634 concentration also showed a small dose-dependent increase in the groups of participants receiving
635 HTT_{Rx} compared with the group receiving placebo; this increase had resolved 7–27 months later[186,185].
636 After the HTT_{Rx} trial, all participants were enrolled in a 15-month open-label extension study in which

they received the 120 mg of the ASO every 4 or 8 weeks. In the extension study, CSF NfL concentrations increased between baseline and ~5 months, and then returned to baseline levels by ~9 months despite continued ASO dosing[310]. These observations are as yet unexplained, and it remains to be seen whether NfL levels will fall below baseline (or below the expected level after disease progression is taken into account) with continued treatment. However, the resolution of this increase in CSF NfL concentration despite continued treatment argues against a long-term adverse effect of total huntingtin-lowering[311].

Although this first-in-human trial was not powered to detect clinical change, HTT lowering was associated with improvements in a novel clinical rating score, the composite Unified Huntington's Disease Rating Scale (cUHDRS) (Fig. 4b). This rating scale combines four assessments: Total Functional Capacity, Total Motor Score (TMS), Symbol Digit Modalities Test (SDMT) and Stroop Word Reading. These assessments were selected, using data from large cohort studies, for their sensitivity to clinical progression, correlation with brain atrophy, and coverage of motor and cognitive domains[312,313]. Independent improvements in the TMS and SDMT components of the cUHDRS were also seen with HTT lowering. Roche is now performing a phase III trial[206] to investigate the clinical efficacy of HTT_{Rx}, with cUHDRS and total functional capacity as primary endpoints.

HTT_{Rx} targets mutant and wild-type *HTT* mRNA equally; however, Wave Life Sciences is currently performing phase Ib–IIa clinical trials of two allele-selective *HTT* ASOs that target SNPs inherited with the mutant allele [204,205,314]. Biomarin have another allele-specific *HTT* ASO in preclinical development, that targets the expanded CAG repeat itself, although this strategy risks off-target knockdown of other CAG repeat-containing genes[315]. Other potential non-allele selective ASO strategies for HTT lowering include binding the AUG translation initiation site, or targeting intron-exon boundaries to modulate splicing[299].

Alternative toxic species of HTT present a challenge to some HTT lowering therapies. A *HTT* exon 1 protein might not be affected by the RNAi and ASOs currently being trialled, but those binding exon 1 mRNA itself should be effective. Repeat-associated non-ATG translation of HTT dipeptides might not

be fully prevented by RNAi, which acts on mature mRNA, but is expected to be inhibited by ASOs as they target pre-mRNA[226,316].

Whether total HTT lowering or allele-selective mHTT lowering is the optimal approach is unclear, but the results of ongoing clinical trials will hopefully provide answers. Encouragingly, an expression-lowering variant in the *HTT* promoter was associated with a delay in disease onset of 9.3 years when on the expanded CAG allele, or 3.9 years when on the normal CAG allele, suggesting that total HTT lowering is beneficial in HD[317]. Total HTT lowering approaches have several advantages over allele-specific approaches, as they permit the targeting of any HTT region and mean a single agent can be used in everyone with HD. Current total HTT lowering approaches aim for partial knockdown and are initiated in adulthood, thus avoiding potential adverse effects on development.

[H3] Small molecule approaches

Given the challenges of delivering RNAi and ASO therapies to the brain, small molecules that reduce HTT expression and can be taken orally are highly desirable. PTC Therapeutics have identified orally-delivered compounds that can alter pre-mRNA splicing of *HTT* and reduce levels of the protein in the brains of HD mice[318]; however, owing to a lack of binding specificity, these compounds carry a higher risk of off-target effects than targeted RNAi and ASOs. A similar approach has been developed for the treatment of SMA; the orally available splicing modulator RG7800 (PTC Therapeutics/Roche) was used to alter SMN2 splicing to include exon 7, which is the only difference between SMN1 and SMN2 proteins. Administration of RG7800 reduced the disease phenotype in a mouse model of SMA, relative to vehicle-treated controls, by compensating for the lack of SMN1[319]. A phase Ib–IIa trial of RG7800 was terminated because ocular complications of the treatment were observed in non-human primates[320]. However, a phase I study of Risdiplam (RG7916; PTC Therapuetics/ Roche), which increases SMN protein levels, was completed in 2016[321], and phase II trials are now underway [322-324]. A different approach, being taken by Nuredis, is to design small molecles that bind to transcription elongation cofactors, which are required for transcription through expanded CAG repeats[325,326].

688 **[H2] DNA-targeting approaches**

689 DNA-targeting approaches aim to modify the *HTT* genetic sequence or its transcription, and typically
690 combine a specific DNA-binding element with an effector, such as a nuclease. The three main DNA-
691 targeting approaches are zinc-finger nucleases (ZFNs)[327], transcription activator-like effector nucleases
692 (TALENs)[328], and CRISPR-Cas9[329]. The ZFN DNA-binding element consists of an array of zinc-finger
693 peptides, each of which binds a sequence of 3–5 nucleotides. Zinc-finger proteins (ZFPs) alone, or
694 containing an active repressor, can selectively target the expanded CAG repeat and reduce its
695 transcription[257]. In one study, several allele-specific ZFP transcriptional repressors were identified from
696 a series of ZFPs designed to target CAG repeats in different frames [273]. AAV-mediated delivery of one
697 of these ZFPs selectively reduced mHTT expression in stem-cell derived neurons from individuals with
698 HD. Furthermore, in three different mouse models of HD, striatal injection of the ZFP reduced the
699 amount of neuropathology and improved some behavioural phenotypes, compared with injection of a
700 GFP-only vector. This improvement was observed despite limited tissue distribution of the ZFP. Off-
701 target knockdown of several other CAG repeat-containing genes was observed, although this
702 knockdown was not associated with toxicity *in vivo*. As an alternative to ZFPs with transcriptional
703 repressors, genome editing with ZFNs could be used to disrupt or correct the CAG expansion[330].

704 TALENs contain a series of peptide repeats that each bind to a specific DNA nucleotide[330]. TALENs
705 have the potential to be more efficient and specific than ZFNs, and have been used to shorten the
706 expanded CAG repeat[331] and suppress *HTT* transcription[332] *in vitro*. However, TALENs require a
707 thymine base to be present at the end of the target sequence, which means they have fewer potential
708 targets than ZFNs[330].

709 CRISPR-Cas9 is a naturally occurring bacterial adaptive immune response to viruses[329]. A single-guide
710 RNA (sgRNA) binds its complementary target sequence, such as the DNA of an invading viral
711 pathogen; this binding requires the presence of a 3' protospacer-adjacent motif sequence. Cas9 is a
712 RNA-guided DNA nuclease that is recruited to the site of sgRNA binding and cleaves the DNA[330]. In
713 cell and animal models of HD, CRISPR-Cas9 has been used to lower HTT levels via several different

714 effectors, for example blocking *HTT* transcription[333], excising CAG repeats[334], or selectively
715 inactivating expanded CAG alleles by targeting associated SNPs[259,260].

716 These three DNA-targeting approaches could provide long-term treatment for HD from a single
717 administration, and could prevent all of the pathogenic events that occur downstream of *mHTT*,
718 including RNA-mediated toxicity, alternative splicing and repeat-associated non-ATG translation.
719 Additionally, correction of the *HTT* mutation would eliminate intergenerational transmission of HD[335].
720 However, these approaches require viral delivery, reach only limited brain regions and are usually
721 irreversible. In addition, DNA-targeting raises concerns about potential off-target effects elsewhere in
722 the genome[336], insertional mutagenesis and immunogenicity[337].

723 **[H1] Conclusions**

724 Substantial progress has been made in our understanding of the pathogenesis of HD, while
725 developments in genetic technology and the availability of large cohorts of individuals with HD have
726 led to the identification of new genetic modifiers of the disease. Somatic instability of the CAG repeat
727 occurs in the tissues that are most vulnerable to HD pathology, particularly the striatum, and the degree
728 of instability negatively correlates with age at disease onset. Genetic association studies have shown
729 that DNA repair components, particularly those involved in mismatch repair, modify somatic instability
730 and disease course. The process underlying this instability is likely to involve DNA loop-outs in the
731 CAG tract, which are targeted by MutSβ, leading to attempted repair that might introduce incremental
732 expansions. Reducing the levels of the pro-instability factors MSH3, PMS2 or LIG1, or inhibiting their
733 function, is expected to reduce somatic instability and be well tolerated. Increased FAN1 expression
734 decreases somatic instability and delays disease onset, suggesting its upregulation would be protective
735 against HD. Excitingly, modulation of these DNA repair components can also reduce the instability of
736 other pathogenic repeat sequences, suggesting that these potential therapeutic opportunities might also
737 be effective in other repeat expansion diseases. mHTT sequesters components of the NPC in aggregates,
738 disrupting nucleocytoplasmic transport. Modulation of nuclear transport pathways was protective in
739 cell models of HD, which could open up new possibilities for therapeutic intervention.

CSF can be readily sampled throughout a clinical trial, and offers more direct access to CNS proteins than other biofluids. NfL is released into CSF, then into plasma, following neuronal damage. CSF and plasma concentrations of NfL strongly correlate with disease progression, and could be used as biomarkers and surrogate endpoints for clinical trials. mHTT is also likely to be released from damaged neurons, and an increase in CSF mHTT is the earliest detectable change in premanifest HD.

After decades of disappointing clinical trial results, we finally seem to be seeing encouraging results from trials of rationally-designed disease-modifying therapies for HD. The first trial of an ASO has reported successful mHTT lowering, with good safety and tolerability[186]. A larger trial aimed at assessing the efficacy of this ASO is underway, as well as trials of mutant allele-specific ASOs[204,205,314]. These early trials are focussing on early manifest disease, looking to see whether we can preserve function. The next step will be to try and push back disease onset in premanifest HD carriers, although this approach presents its own challenges, and will require the development of a battery of clinical, biochemical and imaging biomarkers to demonstrate efficacy. Ultimately, the aim is to find treatments that offer lifelong, safe, sustained benefit from a single administration; this goal is still a long way off, but might eventually be achieved by gene editing strategies that remove CAG repeats, introduce interruptions or inactivate the mutant allele.

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Author contributions

M.F and C.A.R researched data for the article, made substantial contributions to the discussion of the content of the article, wrote the article, and reviewed and edited the manuscript before submission. S.J.T. made a substantial contribution to the discussion of the content of the article, wrote the article, and reviewed and edited the manuscript before submission. E.W. made a substantial contribution to the discussion of the content of the article, and reviewed and edited the manuscript before submission.

Competing interests

In the past two years S.J.T has undertaken consultancy services, including advisory boards, with F. Hoffmann-La Roche Ltd, Ixitech Technologies, Takeda Pharmaceuticals International and Triplet therapeutics. All honoraria for these consultancies were paid to University College London, S.J.T's employer. Through the offices of UCL Consultants Ltd, a wholly owned subsidiary of University College London, S.J.T. has undertaken consultancy services for Alnylam Pharmaceuticals Inc., F. Hoffmann-La Roche Ltd, GSK, Heptares Therapeutics, LoQus therapeutics, Takeda Pharmaceuticals Ltd, TEVA Pharmaceuticals, Triplet therapeutics, UCB Pharma S.A., University College Irvine and Vertex Pharmaceuticals Incorporated. S.J.T. receives grant funding for her research from Takeda Pharmaceuticals and Cantervale Limited. C.A.R. is chair of the Research Advisory Board of the Huntington Study Group. Within the past two years, C.A.R. has consulted for Annexon, Roche, Sage and uniQure. Through UCL Consultants Ltd., a wholly owned subsidiary of University College London, E.J.W. has served on scientific advisory boards for F. Hoffmann–La Roche, Ionis, Mitoconix, Novartis, PTC Therapeutics, Shire, Takeda Pharmaceuticals and Wave Life Sciences. M.F. declares no competing interests. C.A.R. receives funding for HD research from Hoffman La Roche.

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Key points

- Proteins involved in DNA repair, particularly mismatch repair, can modify the age of onset and rate of progression of HD, likely by altering the rate of somatic expansion of CAG repeats in the Huntingtin gene.
- The modulation of DNA repair factors, such as MSH3, FAN1, PMS2 or LIG1, has therapeutic potential in HD and other repeat expansion diseases.
- Nucleocytoplasmic transport is disrupted in HD by sequestration of nuclear pore components in Huntingtin (HTT) aggregates; modulation of nucleocytoplasmic transport is neuroprotective and might provide a novel therapeutic opportunity.
- Changes in cerebrospinal fluid and serum biomarkers, including neurofilament light chain and mHTT, are amongst the earliest detectable changes in HD and can predict disease onset and track progression.
- Intrathecally-delivered non-allele selective antisense oligonucleotides (ASOs) have successfully lowered HTT concentration in the central nervous system of individuals with HD, and trials of allele-specific ASOs are under way.
- Gene editing strategies for HTT lowering, including zinc finger proteins, transcription activator-like effector nucleases and CRISPR-Cas9, are currently in preclinical development, but need to be delivered via the injection of viral vectors, which can be challenging.

Fig. 1 | The potential roles of DNA repair Huntington disease modifiers in somatic instability. a | DNA loop-outs form in the CAG·CTG repeat tract (red). Loop-outs of 1–15 bases are identified by MutSβ, which is a heterodimer of the DNA mismatch repair proteins MSH2 and MSH3[118]. **b. |** The MutSβ complex moves along DNA like a sliding clamp, inducing cleavage of the DNA by endonuclease complexes such as MutLα (a heterodimer of MLH1 and PMS2) or Mutlγ (a heterodimer of MLH1 and MLH3). FAN1, a DNA endonuclease and exonuclease, stabilises repeat tracts. The mechanism

underlying this stabilisation by FAN1 is not yet clear, but it might involve sequestration of MutLα, blocking MutSβ access to the loop out, or direct loop-out repair[112]. **c. |** The cut DNA strand is resysnthesised by a DNA polymerase, and repair is completed by DNA ligase 1 (LIG1). This repair process can induce incremental expansion, represented by the longer repeat tract in part c than in part a. Increased expression of MSH3, MutLα, MutLγ and LIG1 promotes somatic instability and accelerates onset of Huntington disease (HD), whereas FAN1 and the MutLβ heterodimer (MLH1 and PMS1) protect against somatic instability and delay onset of HD..

Fig. 2 | The nuclear transport cycle is disrupted by sequestration of RanGAP1 and nucleoporins in mutant huntingtin aggregates. a | During nuclear import, cargos (purple) with nuclear localisation signals (NLS) are released into the nucleoplasm when their karyopherin (transport factor or importin; grey) interacts with Ran-GTP. Conversely, during export, cargoes with a nuclear export signal (NES), are released into the cytoplasm when Ran-GTP is hydrolysed to Ran-GDP by RanGAP1, located on the cytoplasmic filaments of the nuclear pore complex (blue). This establishes a gradient of Ran forms, with more Ran-GTP in the nucleus and more Ran-GDP in the cytoplasm **b |** In Huntington disease (HD), RanGAP1 and nucleoporins, including NUP62 and NUP88, are sequestered in mutant Huntingtin (mHTT) aggregates. This sequestration results in a loss of the Ran gradient, and a failure of nucleocytoplasmic transport.

Fig. 3 | Therapeutic methods for lowering huntingtin expression. The red sections of DNA, RNA, and protein represent the pathogenic expanded CAG tract and its polyglutamine product. The orange boxes are therapeutic approaches. ASO, antisense oligonucleotide; mHTT, mutant huntingtin; RISC, RNA-induced silencing complex; RNAi, RNA interference; RNase, ribonuclease; TALEN, transcription activator-like effector nuclease; ZFP, zinc-finger protein.

Fig. 4 | Phase I–IIa clinical trial of the HTT_{Rx} antisense oligonucleotide. HTT_{Rx} was administered to adults with early-stage HD every 4 weeks as an intrathecal bolus, via lumbar puncture. Of 46 participants, 34 were randomly assigned to receive HTT_{Rx} and 12 received placebo. The individuals receiving HTT_{Rx} were divided into five cohorts that each received a different dose of the ASO, from

10–120 mg. **a |** Percentage change in the concentration of mutant Huntingtin (mHTT) in the cerebrospinal fluid (CSF) of groups of participants who received one of five different doses of HTTRx or placebo, from baseline (dotted line) to the last available time point, which was 28 days after the last dose and 85–113 days after baseline measurement. Circles indicate individual participants, and horizontal lines indicate group means; 95% confidence intervals are also shown for the groups of participants receiving HTTRx. **b |** Relationship between CSF mHTT reduction at Study Day 85 and composite Unified Huntington's Disease Rating Scale (cUHDRS). The 95% confidence intervals have not been adjusted for multiplicity and should be treated as exploratory. Direction of benefit is shown to the left of the plot. Scale properties (range; clinically meaningful change) are -8-24; 2. Reproduced with permission from Tabrizi, et al. [186].

Glossary:

Choreiform movements: Repetitive and rapid, jerky, involuntary movements.

RNA foci: Expanded RNA repeats that are retained in the nucleus, adopt unusual secondary structures, sequester RNA binding proteins, and can become toxic to the cell.

Repeat-associated non-ATG translation: A repeat-length-dependent process that enables translation initiation at noncanonical codons either within or adjacent to the expanded repeat tract.

Somatic instability: Expansion or contraction of repeat units within a repetitive DNA tract, the rate of which is tissue specific.

microRNA: A small non-coding RNA molecule that functions in RNA silencing and post-transcriptional regulation of gene expression

Lagging strand: The strand of nascent DNA that is synthesised in the opposite direction to the direction of the growing replication fork.

Loop-outs: Formed when one DNA strand is extruded from a CAG CTG repeat region; intrastrand links then lead to the formation of a hairpin, with A-A or T-T base mispairing when the CAG or CTG strand is extruded, respectively.

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