Investigating the role of Huntingtin in development and disease using the zebrafish model organism

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Declaration

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Amanda Lumsden

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Abbreviations

aa:	amino acid
ALAS:	5-aminolevulinic acid synthase (as in ALAS1 and ALAS2)
AP:	alkaline phosphatase
ATP:	adenosine triphosphate
BCIP:	5-bromo-4-chloro-3-indolyl phosphate
bp:	base pairs
BSA:	bovine serum albumin
c-aconitase:	cytosolic aconitase
cDNA:	complementary DNA
CHAPS:	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
*c*MO:	control MO
DAB:	3,3-diaminobenzidine
Dcytb:	duodenal cytochrome B
DIC:	(Nomarski) differential interference contrast
DMSO:	dimethyl sulfoxide
DMT1:	divalent metal transporter 1
DNA:	deoxyribonucleic acid
dNTP:	deoxynucleoside triphosphate
dpf:	days post fertilisation
DRPLA:	dentatorubral pallidoluysian atrophy
dUTP:	deoxyuridine triphosphate
EDTA:	ethylenediaminetetraacetic acid
EGFP:	enhanced green fluorescent protein
ELT:	Expand Long Template
ES:	embryonic stem (as in ES cells)
Fe:	iron
GABA:	γ-aminobutyric acid
Hb:	haemoglobin
HD:	Huntington's disease
*hd*MO:	MO antisense to *hd* mRNA (as in *hd*MO1 and *hd*MO2)
hpf:	hours post fertilisation
HPLC:	high performance liquid chromatography
Htt:	Huntingtin
ICM:	intermediate cell mass

IPTG:	isopropyl-β-D-thiogalactopyranoside
IRE:	iron responsive element
IRES:	internal ribosomal entry site
IRP:	Iron responsive protein (as in IRP1 and IRP2)
ISC:	Iron-sulfur cluster
kB:	kilobase pairs
kDa:	kilodalton
LB:	luria broth
m-aconitase:	mitochondrial aconitase
*mc*MO1:	mispair control MO
mL:	millilitre
mM:	millimolar
MO:	morpholino oligonucleotide
MQ:	MilliQ
mRNA:	messenger RNA
NBT:	nitro blue tetrazolium chloride
NEB:	New England Biolabs
ng:	nanogram
nL:	nanolitre
NMDA:	*N*-methyl-D-aspartate
ORF:	open reading frame
pA:	polyadenylation
PBS:	phosphate buffered saline
PBST:	phosphate buffered saline plus 0.1% Tween 20
PCR:	Polymerase Chain Reaction
PNRC:	perinuclear recycling compartment
POD:	peroxidase
qPCR:	quantitative PCR
RNA:	ribonucleic acid
rpm:	revolutions per minute
SAP:	shrimp alkaline phosphatase
SBMA:	Spinobulbar muscular atrophy
SCA:	Spinocerebellar ataxia (as in SCA1, SCA2 etc.)
SOC:	Super Optimal Broth plus glucose (originally for catabolite repression)
SSC:	NaCl (salt)/sodium citrate buffer

TBE:	Tris/boric acid/EDTA buffer
TdT:	Terminal deoxynucleotide transferase
Tf:	Transferrin
TfR:	Transferrin receptor
TMR:	Tetramethylrhodamine (as in TMR red)
TUNEL:	Terminal deoxynucleotide transferase (TdT)-mediated dUTP nick-end labelling
UTR:	untranslated region
UV:	ultraviolet light
YE:	yolk extension
X-Gal:	X-galactoside (5-bromo-4-chloro-3-indolyl -β-D-galactoside; BCIG)

Abstract

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder of typically mid-life onset, for which there is currently no cure. HD is one of nine neurological disorders caused by the expansion of a CAG trinucleotide repeat that encodes an extended polyglutamine tract within the respective disease proteins (which, in the case of HD, is Huntingtin). Curiously, despite these proteins having mostly widespread patterns of expression in the brain, a specific subset of neurons is preferentially affected in each disease, whilst other neurons also expressing the mutant protein are relatively unaffected. Furthermore, although the expression patterns of these disease proteins often overlap in distribution within the brain, the population of neurons that is most vulnerable differs from one disease to the next. Knowledge of what determines the specificity of neuronal vulnerability is likely to provide insight into the molecular mechanism(s) underlying the pathology in these diseases.

The aim of this work was to use the zebrafish model organism to investigate two factors hypothesised to contribute to the specificity of neuronal vulnerability in HD: 1) region-specific somatic expansion of the disease allele, and 2) disruption of normal Huntingtin (Htt) protein function. The most significant findings of this study resulted from the investigation into the normal function of Htt. Antisense morpholino oligonucleotides were used to specifically knock down Htt expression in early zebrafish development, resulting in a wide variety of developmental defects. Most notably, Htt-deficient zebrafish had pale blood due to a decrease in haemoglobin production, despite the presence of (apparently unavailable) iron within these cells. Provision of additional iron in a bio-available form to the cytoplasm restored haemoglobin production in Htt-deficient embryos. Since blood cells acquire iron via receptor-mediated endocytosis of transferrin, these results suggest a role for Htt in the release of iron from endocytic compartments into the cytosol.

Iron is required for the function of many cellular proteins and enzymes that play key roles in oxidative energy production. Disrupted iron homeostasis and decreased energy metabolism are features of HD pathogenesis that correlate to the major sites of degeneration in the HD brain. The findings of this study raise the possibility that perturbation of normal Htt function (by polyglutamine expansion) may contribute to these defects, thereby providing a novel link between Htt function and specificity of neuronal vulnerability in HD.