MOLECULAR GENETICS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS LINKED TO THE HUNTINGTON DISEASE LOCUS

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ABSTRACT

New Zealand families segregating the Huntington disease (HD) phenotype were investigated for linkage of the HD locus to the anonymous DNA locus G8, from chromosome 4p16.3. Linkage of the two loci was indicated in the largest family assessed. The results from this family together with those from 10 other smaller families were consistent with the existence of a single locus (that is, genetic homogeneity for HD). One crossover between the HD locus and the G8 marker locus was detected. Overall, the results of the linkage analysis indicate a distance of 4cM separating HD from G8.

The usefulness of G8 as a marker in predictive testing for HD was examined in the New Zealand families. In agreement with overseas findings, G8 and DNA contiguous with G8 are useful for predictive testing in some families. The main limitation for predictive testing is the family structure which often results in the unavailability of key individuals for testing and thus prevents prediction in others.

Sequence analysis of three sites which produce restriction fragment length polymorphisms (RFLPs) detected by G8 revealed that single point mutations were responsible for the presence or absence of the polymorphic sites *H1, *H2 and *E. The sequences were highly conserved between individuals in the regions investigated. The conservation of sequence provided the potential for the use of the polymerase chain reaction (PCR) to amplify each polymorphic region for more rapid assessment of these RFLPs.

The three regions were amplified successfully from genomic DNA. In addition, amplification of the three regions was possible with template DNA which was degraded or crude or isolated from tissue which had been fixed in formalin. However, the results of the subsequent analyses of the amplified products by restriction enzyme digestion showed that there are problems that can render the PCR unreliable. The presence of non-target sequences hindered detection of the genotype in the *H1 and *H2 regions and masked the true genotype of a person in the *E region. Thus, the potential of the PCR for presymptomatic diagnosis of HD remains to be realised. However, when the problems are overcome a very rapid analysis of RFLPs linked with HD using the PCR will be possible, as will be retrospective analyses.

ABBREVIATIONS

ABM	aminobenzyloxymethyl
Ap	ampicillin
ASO	allele specific oligonucleotide
ATP	adenosine triphosphate
BCIG	5-bromo-4-chloro-3-indolyl-β-galactopyranoside
bp	base pair (s)
BMD	Beckers muscular dystrophy
BRL	Bethesda Research Laboratories
BSA	bovine serum albumin
°C	degrees Celsius
CF	cystic fibrosis
CGD	chronic granulamatosis disease
Cm	chloramphenicol
cM	centimorgan (1cM is a map distance over which 1%recombination occurs and which equates approximately with a physical distance of 1×10^6 bp)
cpm	counts per minute
CsCl	caesium chloride
CTP	cytidine 5'-triphosphate
CTD	calf thymus DNA
CVS	chorionic villus sample
dATP	2'-deoxyadenosine 5'-triphosphate
ddCTP	dideoxycytidine 5'-triphosphate
ddGTP	dideoxyguanosine 5'-triphosphate
ddNTP	dideoxyribonucleoside triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
dpm	decays per minute
ds	double stranded
DTT	dithiothreitol
dTTP	deoxythymidine 5'-triphosphate
EDTA	ethylene diamine tetra-acetic acid (disodium salt)
EtBr	ethidium bromide
GF/C	glass fibre filter
GTP	guanosine 5'-triphosphate
HD	Huntington disease
hfl	high-frequency lysogeny
HGM	human gene mapping
HPRT	hypoxanthine guanine phosphoribosyl transferase
HVR	hypervariable region
IPTG	isopropyl-β-D-thio-galactopyranoside
Kb	1000 base pairs
KF	Klenow fragment
Km	kanamycin
LB	Luria broth
LOD	log of the odds
M	moles per litre
mA	milliampere
mcs	multiple cloning site
mg	milligram
ml	millilitre
mM	millimoles per litre
MMD	myotonic muscular dystrophy

NEB	New England Biolabs
NEN	New England Nuclear
ng	nanogram
nM	nanomoles per litre
OD	optical density (absorbance)
OR	oligonucleotide restriction
ori	orientation
OTC	ornithine transcarbamylase
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFGE	pulsed field gel electrophoresis
pfu	plaque forming unit
PIC	polymorphism information content
PKU	phenylketonuria
PMSF	phenyl methane sulphonyl fluoride
PVP	polyvinyl pyrrolidine
RF	replicative form
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rp	reverse primer
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
Sm	streptamycin
ss	single stranded
SSC	sodium chloride, sodium citrate
Tc	tetracycline
TCA	trichloroacetic acid
Td	dissociation temperature
TE	10mM Tris-pH 8, 1mM EDTA
TLC	thin layer chromatography
Tm	melting temperature
Tris	Tris (hydroxymethyl) aminomethane
TTP	thymidine 5'-triphosphate
µCi	microcurie
µg	microgram
µl	microlitre
µM	micromoles per litre
UTP	uridine 5'-triphosphate
UV	ultra-violet
V	volt
VNTR	variable number of tandem repeats
YT	yeast tryptone broth
17mer	universal primer, 17 bases