

The Molecular Genetics of the
GRHPR gene in Primary
Hyperoxaluria Type II

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Primary Hyperoxaluria

Type I

Type II

Incidence

1×10^5 to 1×10^6

1×10^6 to 1×10^7

Diagnosis

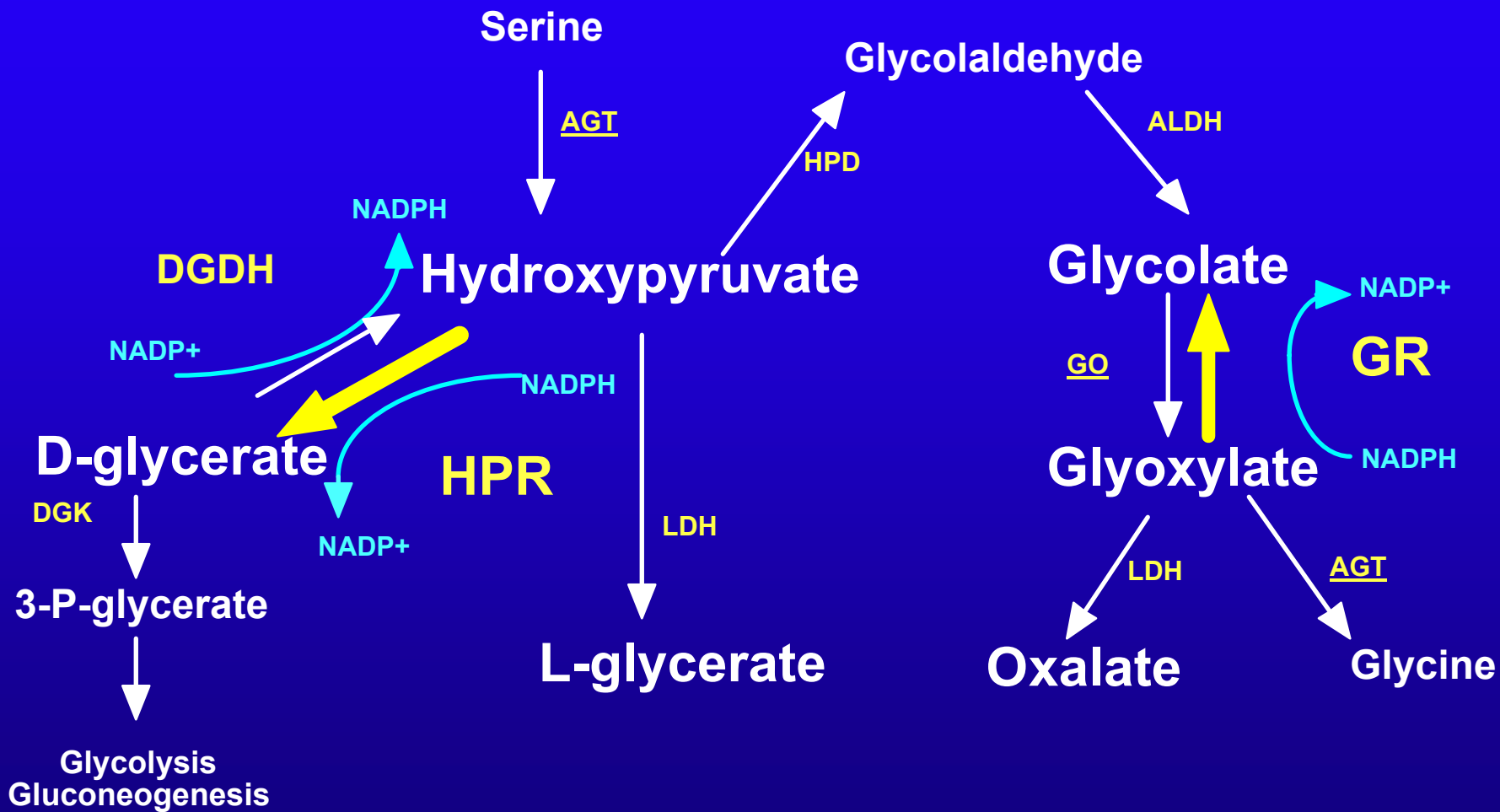
Elevated Urinary
Oxalate, Glycolate

Elevated Urinary
Oxalate, L-Glycerate

Enzyme Deficiency

Alanine-Glyoxylate
Amino Transferase
Gene cloned in 1990

Glyoxylate Reductase
Hydroxypyruvate Reductase
D-Glycerate Dehydrogenase
Gene Cloned in 1999



H. sapiens 1MRPVR..... LMKVVFVTRRIPAEGRVALARAADCEVEQWDSDEPIP..AKELERGVAGAHGLLCLLS
P. horikoshii 1 malsilflsiasltlssslidhyfhlsflrnPlksqsgggkmpKVFiTReIpeVg..ikmledefEVEvWgdekeIP..reiLlkkVkevdaLvtmLS
E. coli 1meRsmk..... psViLykaIpddl..lqrLqehftVhQvanlsPqt..veqnaaifAeAeGGLGsn.
H. methylov. 1mskk..... iliTwpIpeaa..mararesyDViahgdDpkIt..idEmietaksvdaLLitLn
C. maxima 1manrvqvevwnpngk..... yrVvsTkpmPgtrwinLlieqDCrVEictekktIlsvedivaligdkcdGviggLT
S. pombe 1mtlsgk..... paallVgtlkhahkewaAlgkyaElktySDgtred.flakcktefgnvkaicrtyn
CONSENSUSs.k.....p-v--t-.lp---.l.---eve.--d-.i.---.---vdgl-.l-

H. sapiens 61DHVDKRILDAAG.ANLKVISTMSVGIIDLALDEIKKRGIRVGYTPDVLTDTTAELAVSLLLTCRRLPEAIEEVKNGGWTS...WKPLWLCGYG
P. horikoshii 97eridKevfenA..pkLrivanyaVGyDnidieEatKRGiyVtnTPDVLTDaAdLafALLLaTaRhvvkgdrfVrsGeWkkrgvaWhPkWfIGyd
E. coli 58enVnaalLekm..pkLratSTiSVGyDndfdvDaltaRkIllmhTPTVLTeTvAdtImaLvLsTaRRvvEvaErVKAgeWtas...igPdWyygt.d
H. methylov. 55ekcrKevidrip.eNiKcISTySiGfDhidLDacKaRGiKVGnaPhgvTvaTAEiAmlLLLgSaRRagEgekmirtrsrWpg...WePlelvGek
C. maxima 72edwgevlfsAlsrAgrKafSnMaVGynnVdvnaanKyGIaVGnTPVLTTeTTAELAAaSLsLaaaRRivEAdEfmraGrydg...WlPnlfvGnl
S. pombe 62 skfymgIfDKeIiDnlp.psvKfIchlgaGyetVdvaactaRGiQvshvPkavdDaTAdvgifLmLgaLRgfnngIfElhknnWna.....nckpshd
CONSENSUSe--dke--d.-.p.lk.-st--vGydn-dvda t.rgI.vgntP.vltdttAdla--LlL-taRr-veg-e.vr-g-w-....w.p-w--g.d

H. sapiens 151 LTQSTVGIIGLGRIGQAIARR.LKPFQVQRFLYtGRQPR..PEEAAEFQAEFVS.....TPELAAQSDFIVVACSLTPATEGLCNKDFQKM
P. horikoshii 190 vvgkTiGIIGLGRIGQAIAR.aKGFnmr.iLYysRtrk..eEverElnAEfKp.....ledLlreSDFvVlAvpLTreTyhLiNeerlklm
E. coli 147 vhhkTLGIvGmGRIGaAlaQrahfgFnmp.iLYnaRrhh..kEaerFnArycd.....ldtLlqeSDFvclilpLTdeThhLfgaeqFakm
H. methylov. 145 LdnkTLGIyGfGsIGQALAKr.aggFdmDidyEdthras..ssdeAsyQAtFhds.....ldsLlsvSqFfslnapsTpeTryffnKatiksl
C. maxima 163 LkgqTVGvIGaGRIGsAyARmmvegFkmnliyfdlyQstrlekvftaygefIkangevptwrrassmdEvlreaDvIsIshpvLdktTfhLvNKeslkam
S. pombe 154 pegkTLGIIGLGGIGktnAKr.araFdmk.ivYhnRtPl...pEeeaeGAEFVS.....fddLlAKSDvlslnlpLnahTrhiinKpeFqkm
CONSENSUS l.gkTLGIIGLGRIGqa-Akr.a-gF-m..ily-.r.....e-e--.aef.....ld-ll-esdf-sl.-plt.eT.hl-nk-.fkkm

H. sapiens 235 KETAVFINISRGDVVNODDLYQALASGKIAAAGLDVTSPEPLPTNHPLLTLKNCVILPHIGSATHRTRNTMSLLAANNLLAGLRGEPMP.....S
P. horikoshii 273 KkTailINiARgkVVdtnaLvkaLkeGwIAGAGLDVfeePyyn.eeLfkLdNvVltPHIGSAsfgaRegMaeLvAkNLIaFkRGEipP.....t
E. coli 231 KssAiFINagRGpVVdenaLiaALqkGeIhaAGLDVfedePLsvdsPLLsmaNvVavPHIGSATHeTRygMaacAvdNLIaALqGkveknvphvatkp
H. methylov. 230 pggAivvNtaRGDLVdnelvvaALeaGrlayAGLDVfagEpnin.egyydLpNtflfPHIGSAAatqaRedMahqAndlidalfGadMs.....y
C. maxima 273 KkdAilINcSRGpVideaaLvehLkenpmfrvGLDVfedePyMK.pgLadmKNaiIvPHIaSaSkwTRegMatLAAINvLgkikqyPvw.....a
S. pombe 236 KrgiVivNtaRGaVmdeaaLveALdeGivysAGLDVfeeEPkih.pgLLenekvillPHIGtnsleTgykMecavLmNvkngivndsIP.....n
CONSENSUS k--ai-in-ARg.vvde-alv.aL.eg.ia.aGLDVfeEP.....ll-l.nvvl-PHIGsas-.tregMa.laa-nl-a.--g--p.....-

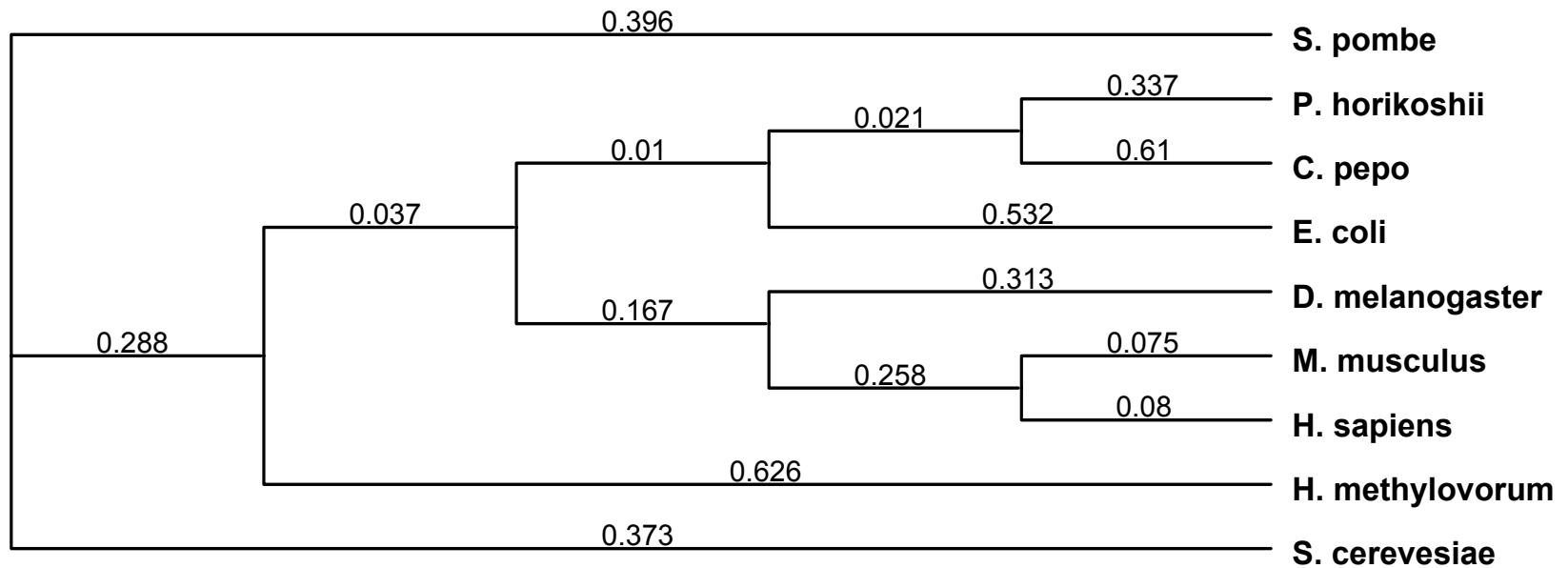
H. sapiens 325 ELKL.....
P. horikoshii 362 lvnrevikirkpgfe.....
E. coli 331 rLrrvkdnrsgfysllgcsnccspglikrlmlcr
H. methylov. 319 aLa.....
C. maxima 362 dpnrvepFldenappaaspsivnakalelpvskl
S. pombe 325 lvpeqrgdie.....
CONSENSUS .l.r....-

Evolutionary Relationships between “Predicted” GRHPR Proteins

Method: Neighbor Joining; Best Tree; tie breaking = Random

Distance: Poisson-correction

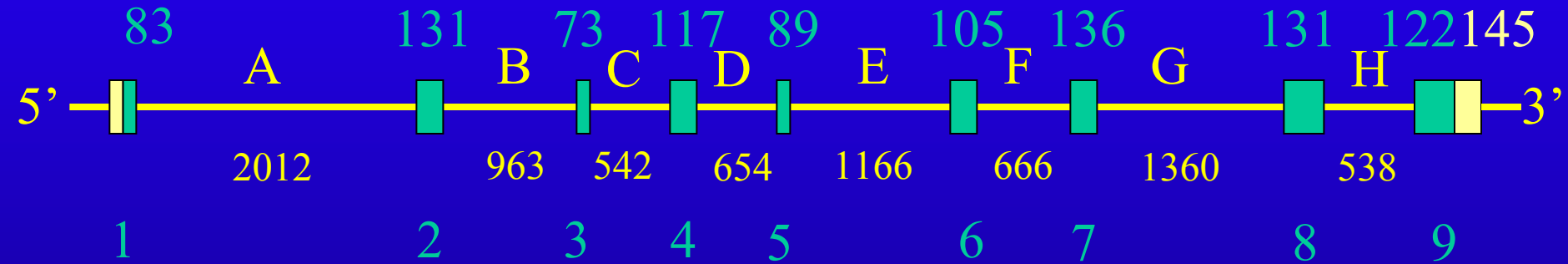
Gaps distributed proportionally



GRHPR Genomic Structure

9q11/p11

← $\cong 9.1$ Kb →



9 Exons

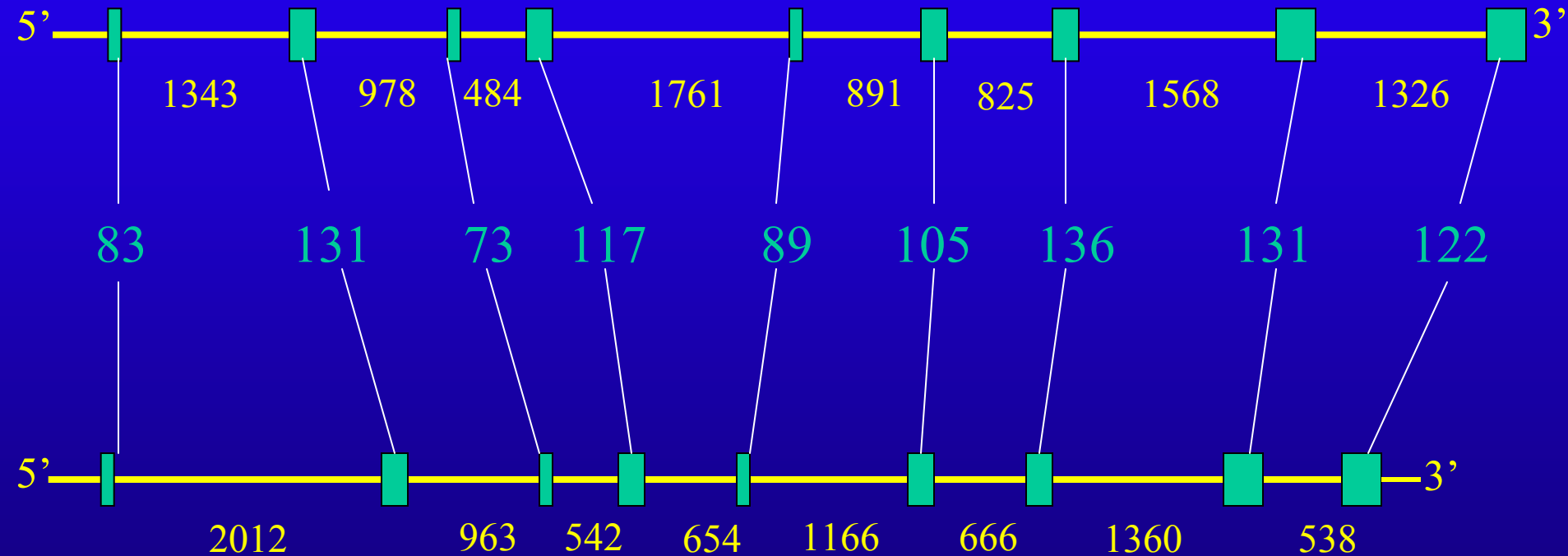
■ = Protein coding

8 Introns

■ = Untranslated

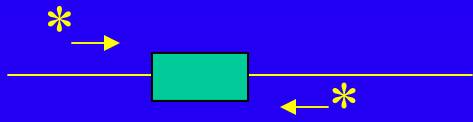
mGRHPR Genomic Structure

Mouse (10.1 kb)



Human (9.1 kb)

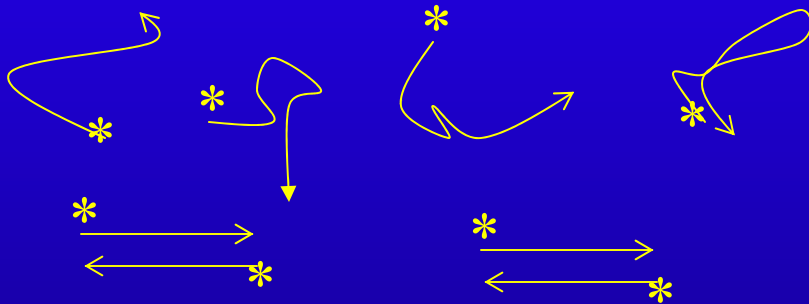
PCR-SSCP Strategy



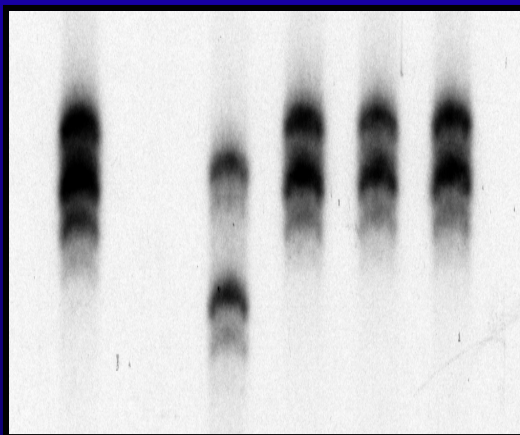
PCR Amplify Exon and Boundaries
with radioactive primers



Double-stranded radioactive product
is denatured and cooled



Single-stranded products separated
on acrylamide gel under non-denaturing
conditions



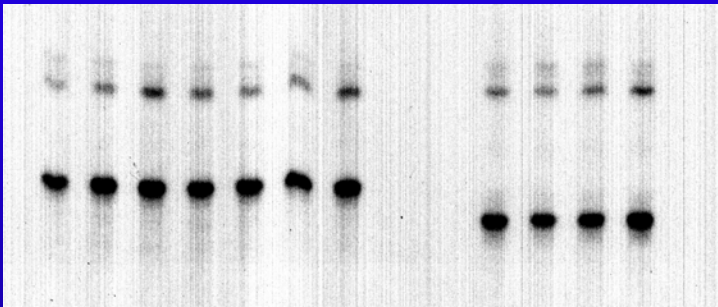
Look for differences in secondary
structure by banding pattern

Exon 2

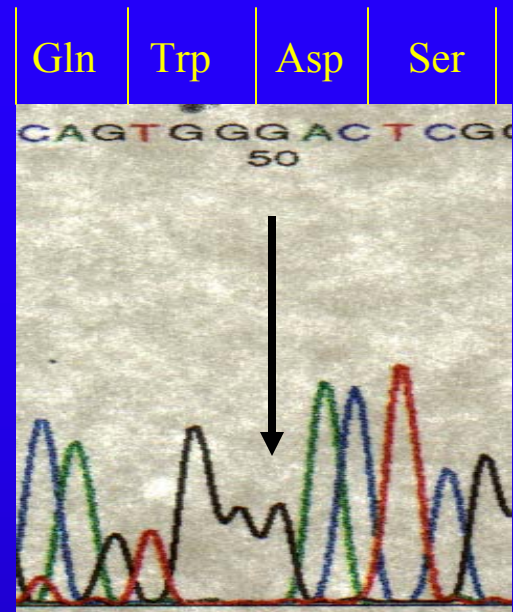
Controls

Patients

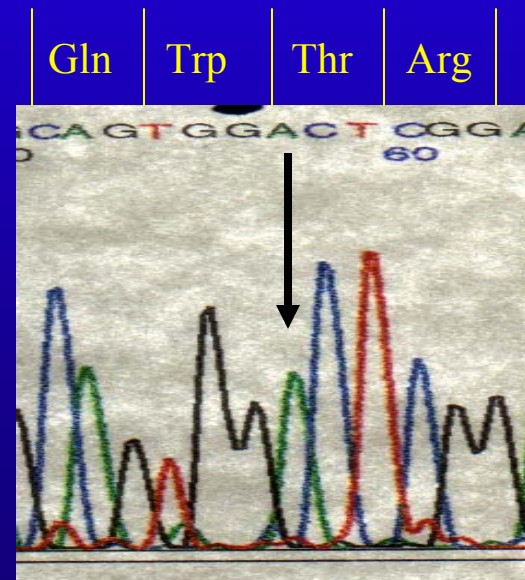
1 2 3 4



Deletion in Codon 35 results
in frame-shift.
Stop codon at 44

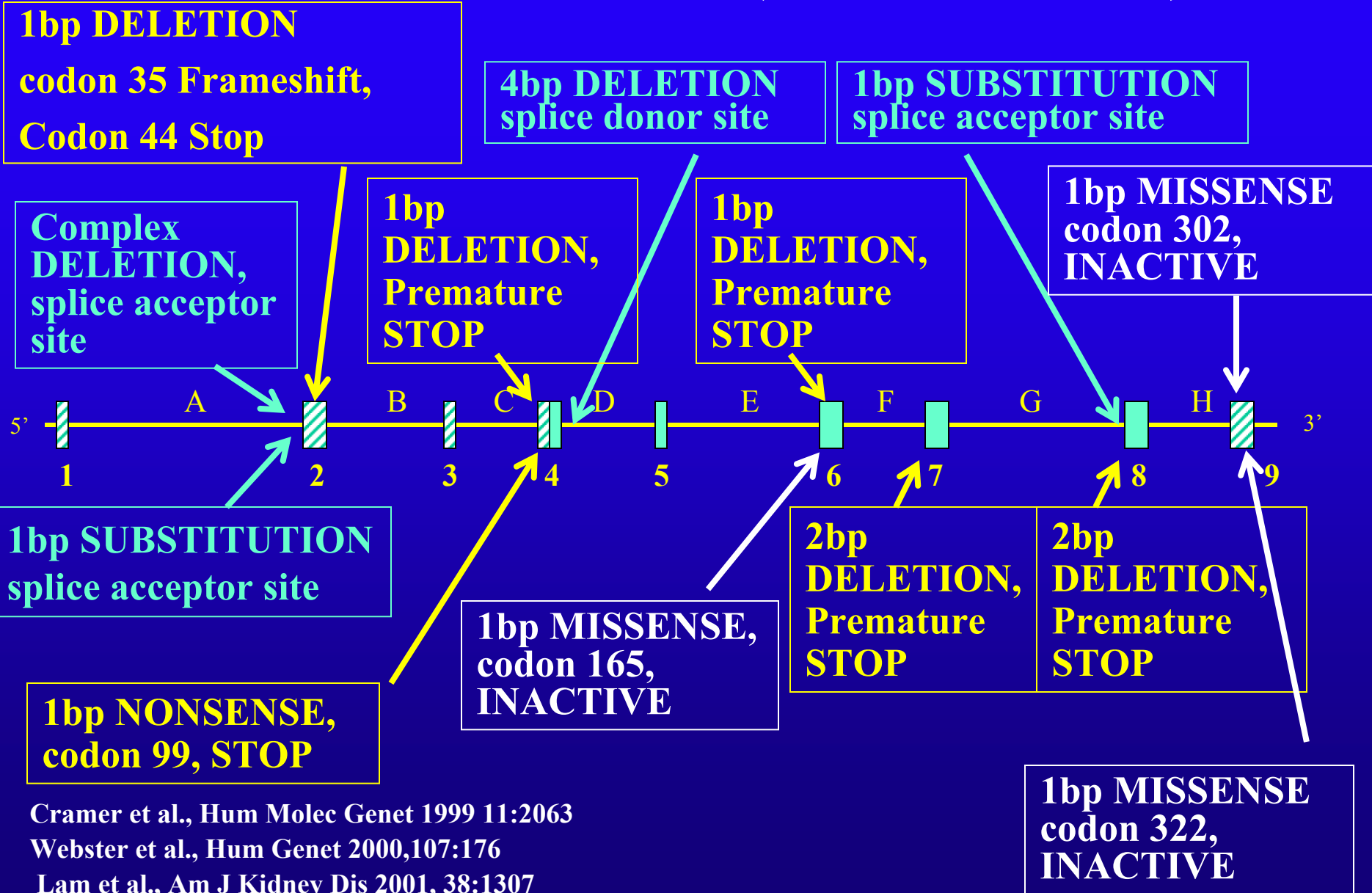


Control Exon 2



Patients 1-4 Exon2

13 Described Mutations (31 PH2 Patients)



Cramer et al., Hum Molec Genet 1999 11:2063

Webster et al., Hum Genet 2000,107:176

Lam et al., Am J Kidney Dis 2001, 38:1307

Hum Mutation, Creegan et al, In Press

13 Described Mutations (31 PH2 Patients)

1bp DELETION

**codon 35 Frameshift,
Codon 44 Stop**

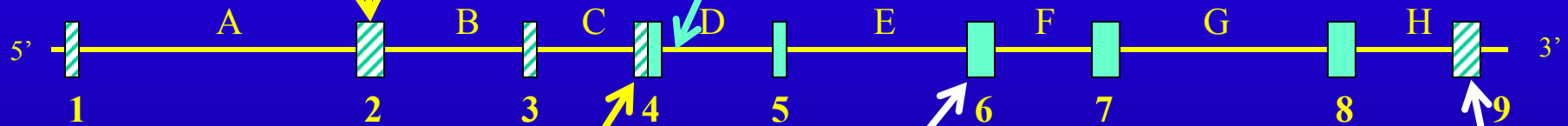
26/62-42%

**Northern European
BsmF1 RFLP**

**4bp DELETION
splice donor site**

9/62-14.5%

**Asian and Caucasian
SSCP or direct sequence**



6/62-9.7%

Bam HI RFLP

**1bp MISSENSE,
codon 165,
INACTIVE**

6/62-9.7%

Xba I RFLP

**1bp NONSENSE,
codon 99, STOP**

Cramer et al., Hum Molec Genet 1999 11:2063

Webster et al., Hum Genet 2000,107:176

Lam et al., Am J Kidney Dis 2001, 38:1307

Hum Mutation, Creegan et al, In Press

2/62-1.6%

**African American
Stu I**

**1bp MISSENSE
codon 322,
INACTIVE**

GRHPR Genetics Summary

All mutants described result in a loss of enzyme expression or function

Genetics is unlikely to help with disease management
But may help with genetic counseling

$\Delta 103G$ is most common mutation (Northern European founder likely)

Presence of numerous rare mutation and population-specific mutations will make genetic screening difficult
(The limited data set limits the utility)

GRHPR Human Tissue Expression Profile

Tissue/Cell	Northern ¹	EST ²	Tissue/Cell	Northern ¹	EST ²
Adrenal		X	Liver	X	X
Aorta		X	Lung	X	X
Blood		X	Ovary		X
Brain	X	X	Pancreas	X	
Breast		X	Parathyroid		X
CNS ³		X	Placenta	X	X
Colon		X	Prostate		X
Eye		X	Skeletal Muscle	X	
Germ Cell		X	Synovial Membrane		X
Heart	X	X	Testes		X
Kidney	X	X	Tonsil		X
			Whole Embryo		X

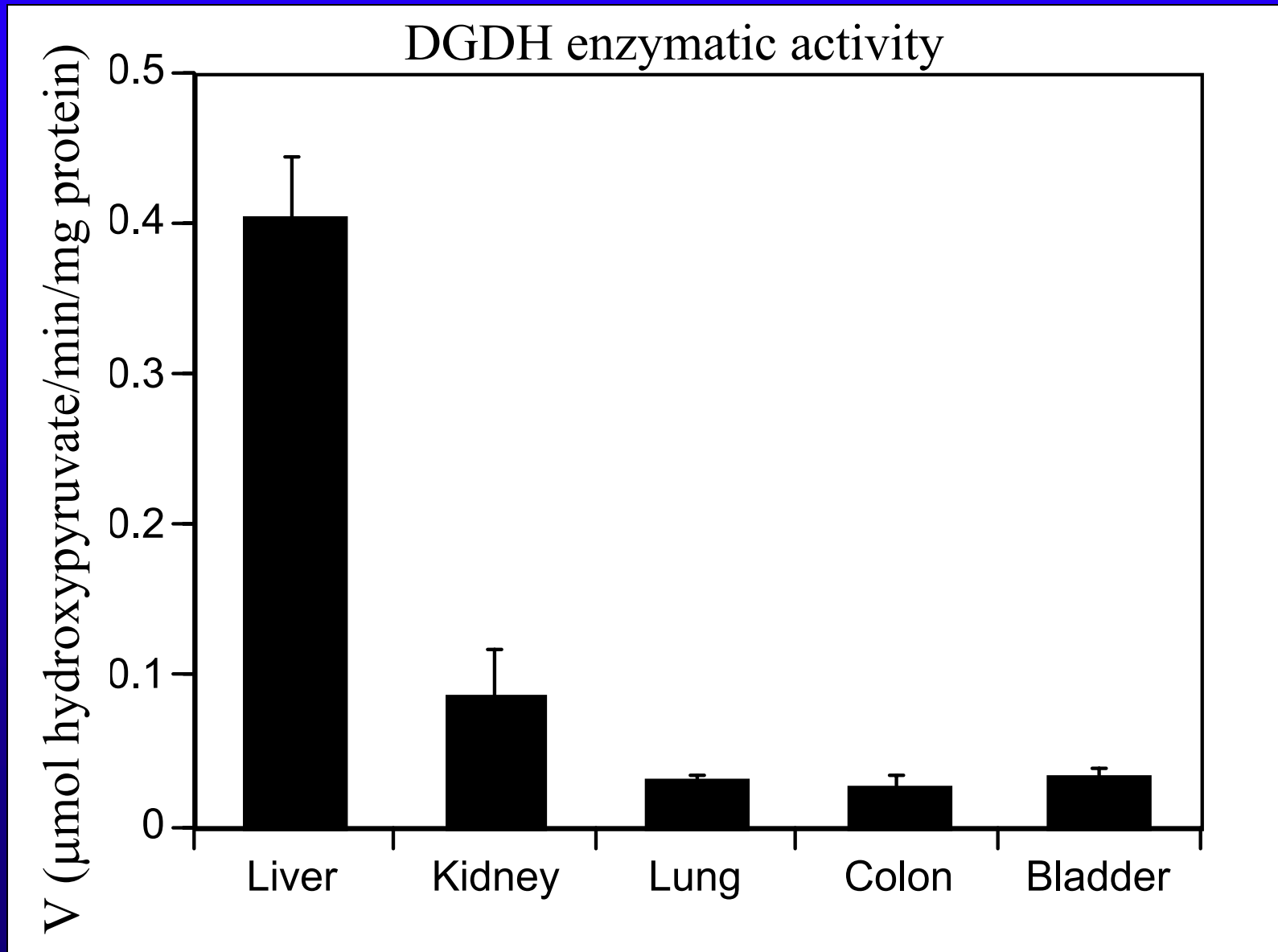
1) Data are from Huang et al., BBRC 2000, 268:298-301.

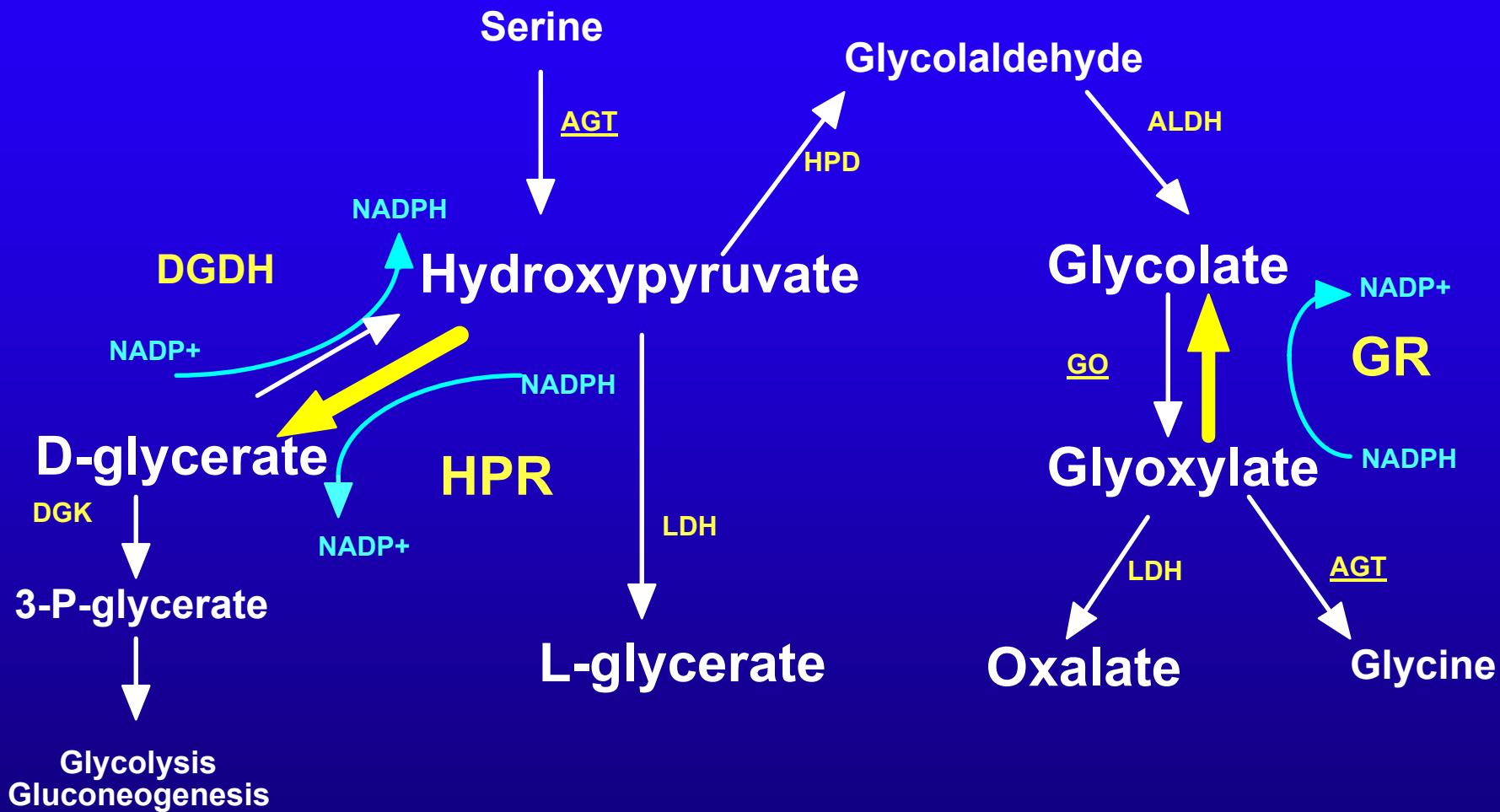
2) EST, Expressed Sequence Tag, Blast Search performed using the human *GRHPR* cDNA

(Accession number AF146018) against the human EST database at Genbank.

3) Central Nervous System.

GRHPR is expressed in many adult Human tissues







Acknowledgements

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Martha Kennedy

April Coan

Mayo Clinic

Dawn Milliner

Northwestern University

Craig Langman

Heather Price

PH1 and PH2 Patients and Families

\$ Oxalosis Hyperoxaluria Foundation

NIDDK