

Familial Hypercholesterolaemia

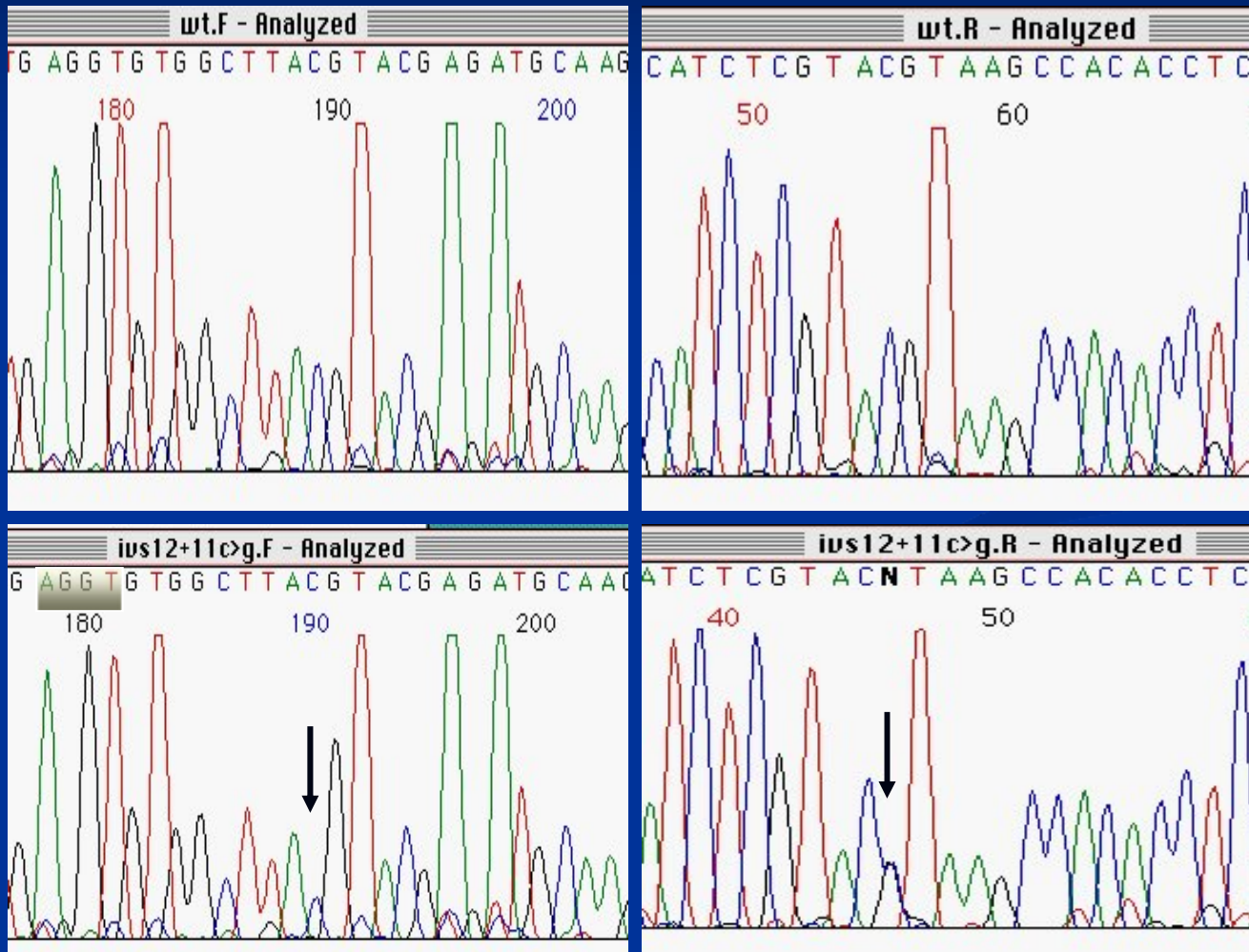
Splicing defects account for around
15% of mutations in LDLR gene

Reference	Population	No. of different point mutations	No. of splice defects	%
<i>Hobbs et al. 1992</i> ¹⁹	<i>Mixed</i>	<i>105</i>	<i>3</i>	<i>3</i>
<i>Day et al. 1997</i> ²⁰	<i>UK</i>	<i>51</i>	<i>1</i>	<i>2</i>
<i>Graham et al. 1999</i> ⁵	<i>N Ireland</i>	<i>23</i>	<i>1</i>	<i>4</i>
<i>Lombardi et al. 2000</i> ¹¹	<i>Dutch</i>	<i>51</i>	<i>8</i>	<i>16</i>
<i>Ansellem et al [in press]</i> ¹²	<i>France</i>	<i>54</i>	<i>13</i>	<i>24</i>
<i>Graham et al 2005</i>	<i>N Ireland & N England</i>	<i>44</i>	<i>6</i>	<i>14</i>

Splicing defects in LDLR causing Familial Hypercholesterolaemia

- 15/97 families with defined mutations causing FH have splicing defects in LDLR
- 6 separate mutations
 - c.313+1g>a
 - c.313+2t>c
 - c.621c>t
 - c.1587-1g>a
 - c.1706-1g>a – 4 families
 - c.1845+11c>g – 7 families

Sequence screen ex12 / intron12



LDLR i vs 12+11C>G

LDLR c.1845+11C>G

⬆ Creates cryptic splice site

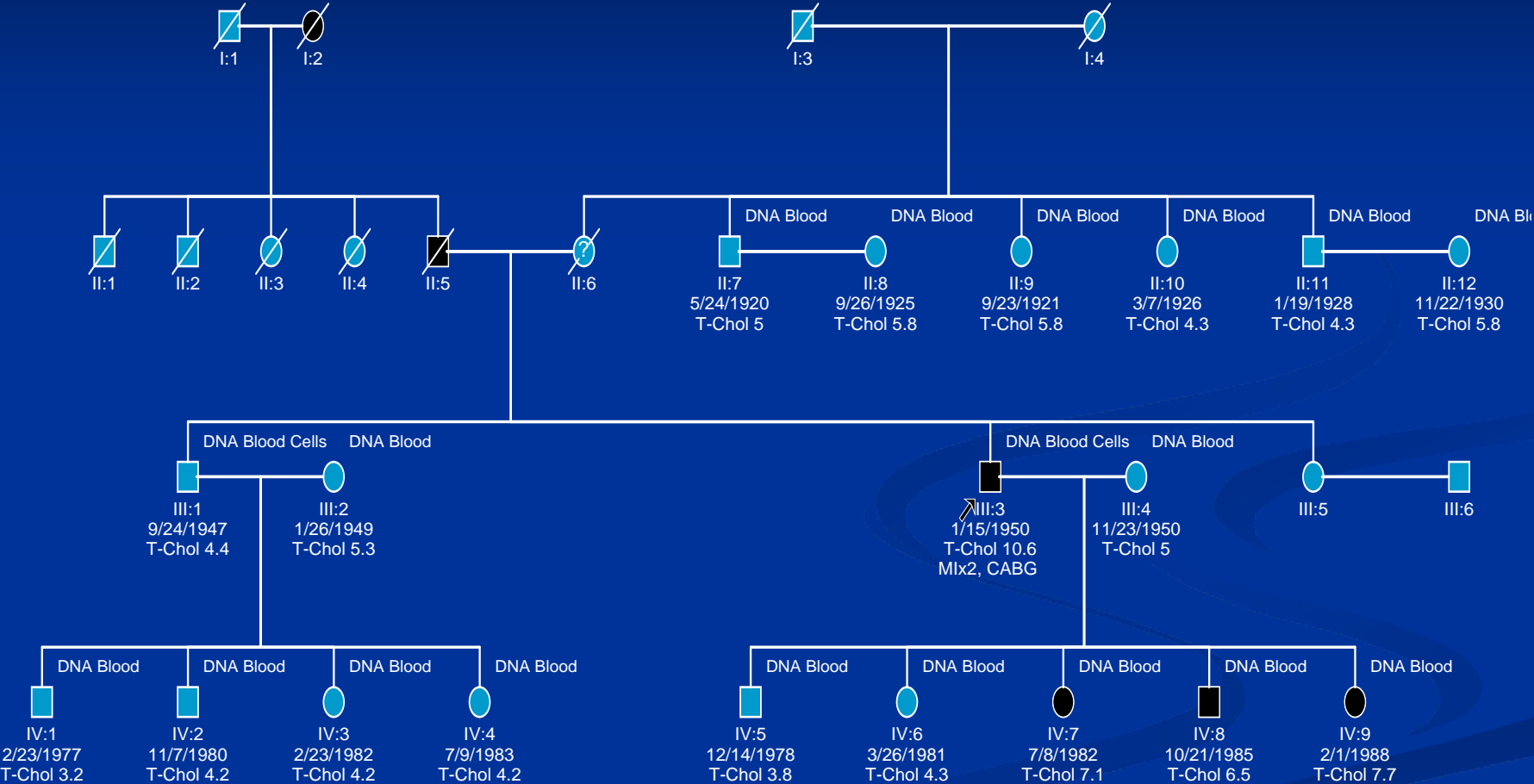
Exon --- A G | G T A A G T consensus donor splice site
% 64 73 100 100 62 68 84 63

Exon 12 --- A G | G T G T G G C T T A C / G G T A

c.1845 +11 --- A G | G T A C G A

- ⬆ Not in 200 normal chromosomes
- ⬆ Not found in any unaffected family members

Family with LDLR 1845+11c>g splice mutation.



LDLR exon 12/intron 12 sequence

¹CTCTGGGACT GGCATCAGCA CGTGACCTCT CCTTATCCAC TTGTGTGTCT AGATCTCC TC⁶⁰
⁶¹AGTGGCCGCC TCTACTGGGT TGA CTCCAAA CTTCACTCCA TCTCAAGCAT CGATGTCAAC^{*120}
¹²¹GGGGGCAACC GGAAGACCAT CTTGGAGGAT GAAAAGAGGC TGGCCCACCC CTTCTCCTTG¹⁸⁰
¹⁸¹GCCGTCTTTG AGGTGTGGC T TAC/G[#]GTACGAG ATGCAAGCAC TTAGGTGGCG GATAGACACA²⁴⁰
²⁴¹GACTATAGAT CACTCAAGCC AAGATGAAC²⁶⁹ c. 1845 + 11 c>g

(A)

¹ACTCCAAACT TCACTCCATC TCAAGCATCG ATGTCAAC GG GGGCAACCGG AAGACCATCT⁶⁰
⁶¹TGGAGGATGA AAAGAGGCTG GCCCACCCCT TCTCCTTGGC CGTCTTTGAG
 GTGTGGCTTAC/G^{#121} GACAAAGTA TTTTGGACAG A¹⁴¹

Intron 12 (11 bp)

(B)

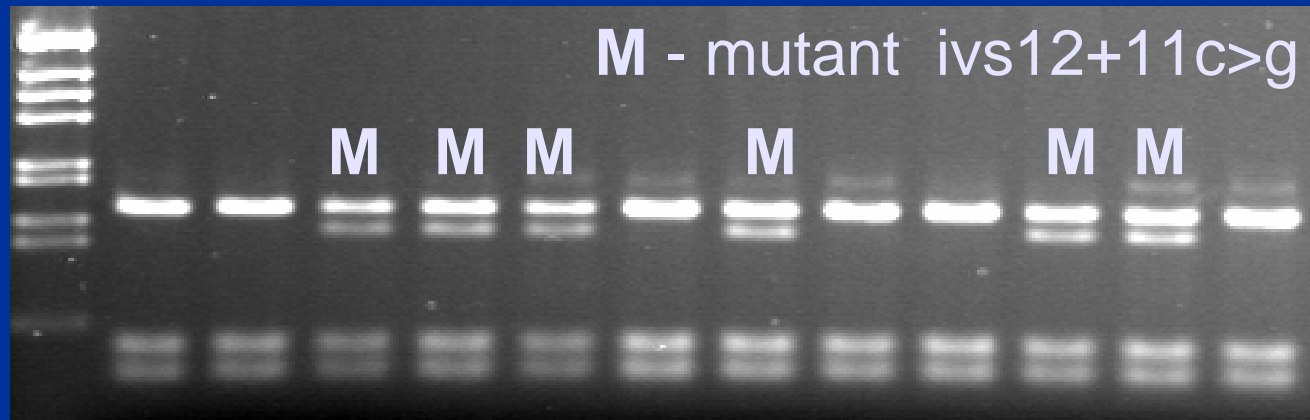
1773 c primer
¹TCTCAAGCAT CGATGTCAAC GGGGGCAACC GGAAGACCAT CTTGGAGGAT GAAAAGAGGC⁶⁰
⁶¹TGGCCCACCC CTTCTCCTTG GCCGTCTTTG AG GTGTGGCT TA C/G[#]GACAAAG TATTTGGAC AGA¹²³

Exon 12 **Intron 12 (11 bp)** **Exon 13**

(C)

Restriction enzyme assay for *ivs12+11c>g* mutation.

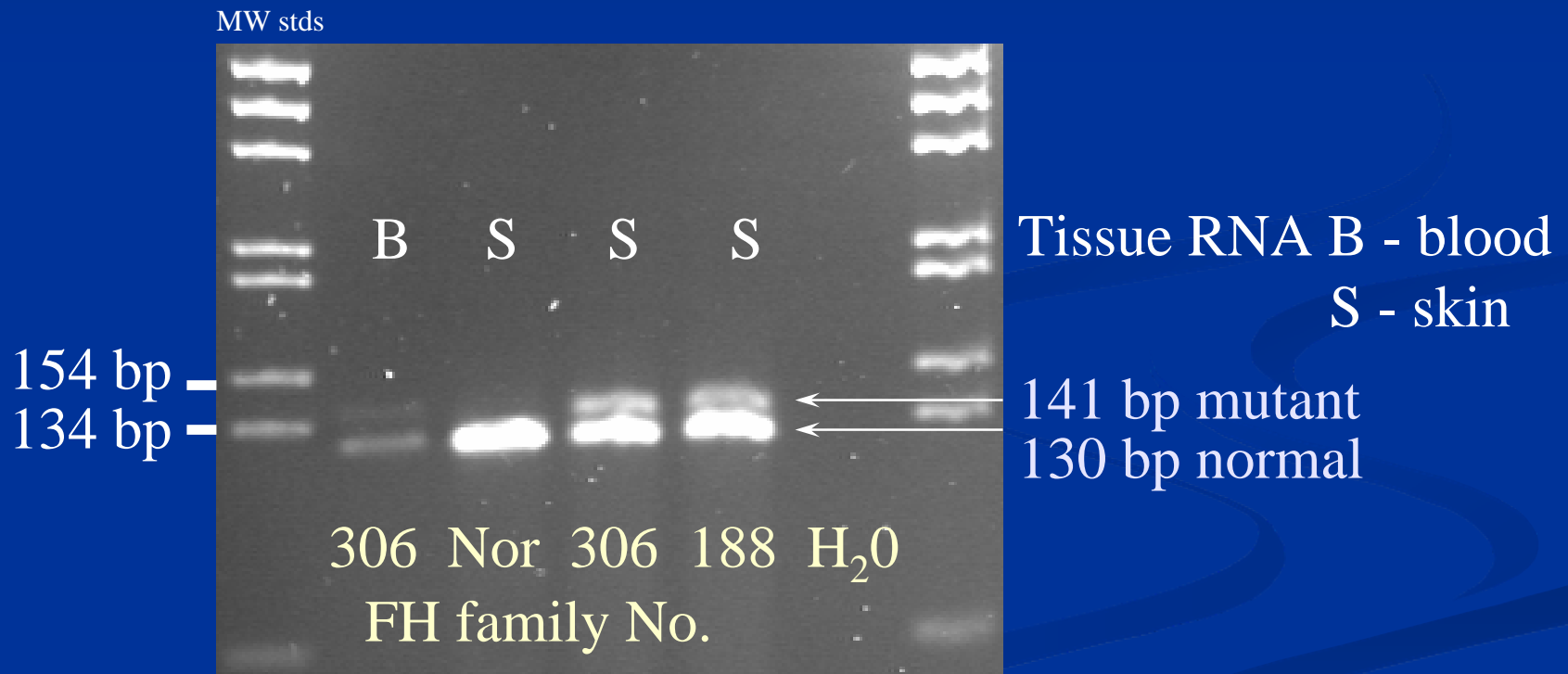
LDLR exon 12 PCR product digested with Dde.I



2% Nusieve + 1% BRL agarose.

Assay A

RT-PCR assay specific for LDLR exon 12 splice site on Blood and Skin Fibroblast RNA



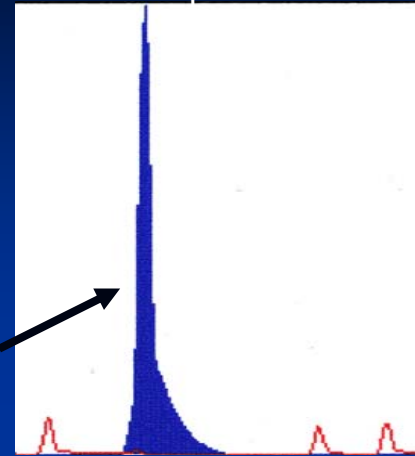
Assay B

Allele specific fluorescent RT-PCR

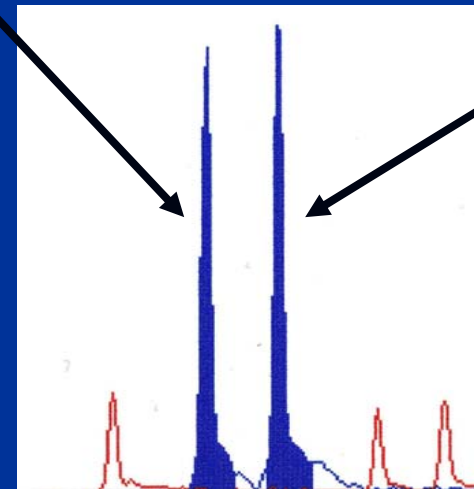
Assay C

Atherosclerosis 182 (2005) 331-340

Normal patient



Normal
splice
site product
112 bp



Cryptic
splice
site product
123 bp

LDLR c.1845+11c>g
patient

c.1845+11c>g splice mutation creates a premature stop in exon 13 and a truncated LDLR protein is predicted.

ldlr.ivs12+11g					
10	20	30	40	50	60
ttcatgtact	ggactgactg	gggaactccc	gccaaagatca	agaaaggggg	cctgaatggg
PheMetTyrT	rpThrAspTr	pGlyThrPro	AlaLysIleL	ysLysGlyGl	yLeuAsnGly
70	80	90	100	110	120
gtggacatct	actcgctggg	gactgaaaac	attcagtggc	ccaatggcat	caccctagat
ValAspIleT	yrSerLeuVa	lThrGluAsn	IleGlnTrpP	roAsnGlyIl	eThrLeuAsp
130	140	150	160	170	180
ctcctcagtg	gccgcctcta	ctgggttgac	tccaaacttc	actccatctc	aagcatcgat
LeuLeuSerG	lyArgLeuTy	rTrpValAsp	SerLysLeuH	isSerIleSe	rSerIleAsp
190	200	210	220	230	240
gtcaatgggg	gcaaccggaa	gaccatcttg	gaggatgaaa	agaggctggc	ccacccttc
ValAsnGlyG	lyAsnArgLy	sThrIleLeu	GluAspGluL	ysArgLeuAl	aHisProPhe
250	260	270	280	290	300
tccttggcog	tctttgaggt	gtggcttagg	acaaagtatt	tttgaca	
SerLeuAlaV	a lPheGlu	a lTrpLeuArg	ThrLysTyr	he<*>	

11 bp insertion in RNA

Stop codon TGA in exon 13