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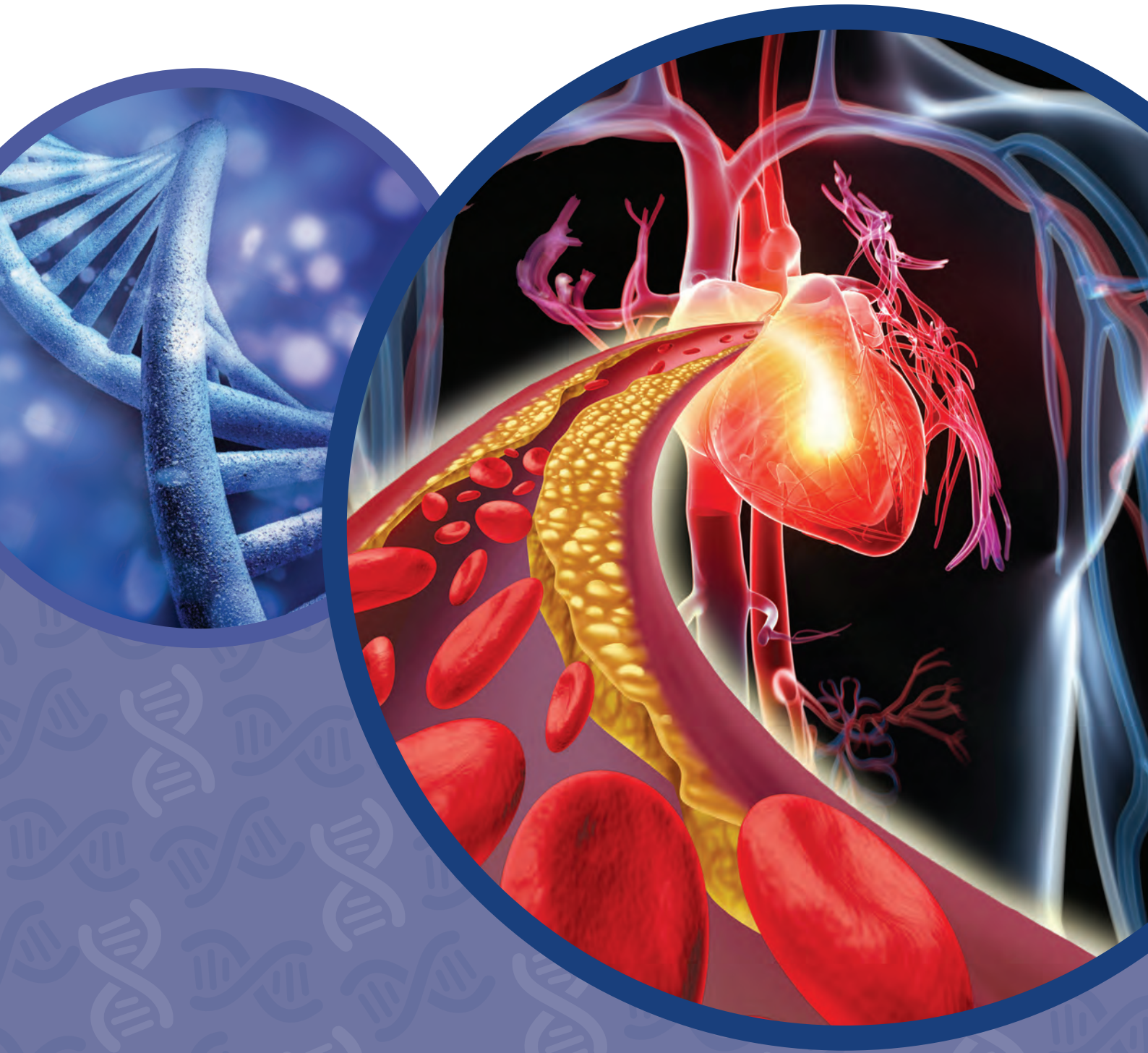
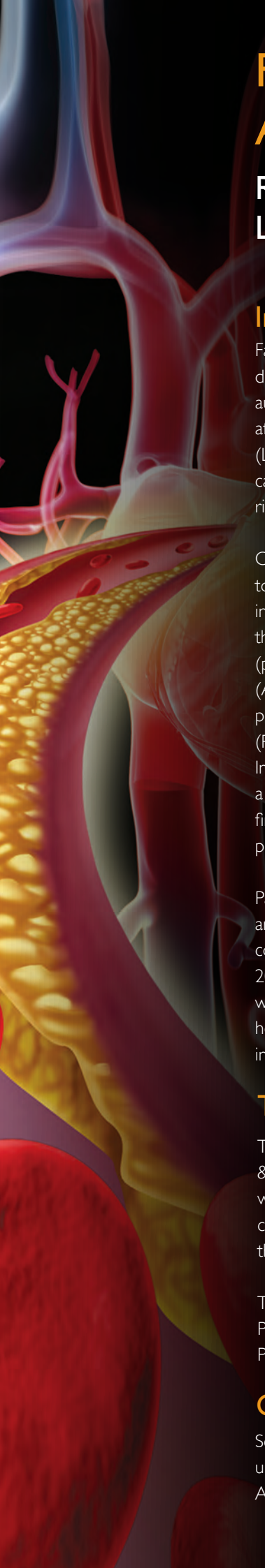
BIOSCIENCES
Discovery | Drug Development | Diagnostics

Familial Hypercholesterolaemia (FH) Arrays

Rapid and reliable genetic assessment of patients with suspected FH





Familial Hypercholesterolaemia (FH) Arrays I & II

Rapid, simultaneous detection of 40 mutations within the LDLR, ApoB and PCSK9 genes

Introduction

Familial Hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism. It is a common autosomal dominant, or inherited, disease which affects the plasma clearance of LDL-cholesterol (LDL-C), resulting in premature onset of cardiovascular disease (CVD) and a higher mortality risk.[1-3]

Common genetic defects in FH are attributed to mutations in three genes encoding proteins involved in the uptake of LDL-C from the plasma: the low density lipoprotein receptor (LDLR) gene (prevalence of 1 in 250), the apo-lipoprotein B (ApoB) gene (prevalence of 1 in 1000) and the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene (prevalence of less than 1 in 2500).[1, 3] Interestingly whilst previous research has suggested[9] a FH prevalence of 1:500, more recent research findings indicates the occurence is more likely 1:250, presenting a more significant health problem.[5]

Patients who have one abnormal gene mutation are known as heterozygous. Heterozygous FH is a common genetic disorder, occurring in 1 person in 200-500 in most countries. Homozygous FH occurs when the patient has two abnormal gene mutations, however this is much rarer, with an occurrence of 1 in a million.[4-5]

Early diagnosis of FH is crucial as by the time the heterozygous FH sufferer enters early adulthood they will have accumulated >20 years of continuous exposure to build up of fatty or lipid masses in arterial walls and are at a hundred-fold greater risk of a heart attack than other young people. Patients with homozygous FH are at such high risk that they may not live beyond childhood into early adulthood.[4]

The UK National Institute for Health and Clinical Excellence (NICE) guidelines published in 2008 recommend that all FH patients be offered a DNA test to confirm the diagnosis and that identified mutations should be used as the basis for cascade testing of first-degree relatives of index cases. Patients newly identified by such screening can then be offered treatment to reduce the risk of premature cardiac events.[1]

Only a few countries currently have national genetic screening programs for FH despite evidence demonstrating that implementing such a program is highly cost-effective; particularly for cascade testing of known index cases as roughly 50% will have inherited the mutation.[6]

The Familial Hypercholesterolemia (FH) Arrays I & II

The Familial Hypercholesterolemia (FH) Arrays I & II are rapid, simple and accurate diagnostic tests which enable simultaneous detection of 40 FH-causing mutations (20 mutations per array) within the LDLR, ApoB and PCSK9 genes.

The assay is based on a combination of multiplex PCR and biochip array hybridisation. Innovative PCR priming technology permits high discrimination between multiple targets. A unique primer set is designed for each target which will hybridise to a complementary oligo-nucleotide probe spotted on a biochip discrete test region (DTR). This combination of priming and spatially organised biochip array technology enables enhanced specificity of the assay. Analysis can be completed from template DNA through PCR to data readout in ~3 hours.

Clinical data

Several validation studies were completed using FH samples, assessing several hundred blinded and unblinded samples. Total correlation of 98% was observed when using the Familial Hypercholesterolemia Arrays I & II.

Familial Hypercholesterolemia (FH) Arrays I & II

FH Array I mutation coverage

Analyte Name		Mutation	Protein
APOB	FH1	c.10580G>A	p.(Arg3527Gln)
LDLR	FH2	c.2292delA	p.(Ile764Metfs*2)
	FH3	c.1444G>A	p.(Asp482Asn)
	FH4	c.551G>A	p.(Cys184Tyr)
	FH5	c.1845+11C>G	p.(=)
	FH6	c.693C>A	p.(Cys231*)
	FH7	c.933delA	p.(Glu312Serfs*58)
	FH8	c.301G>A	p.(Glu101Lys)
	FH9	c.313+1G>A	p.(=)
	FH10	c.1706-1G>A	p.(=)
	FH11	c.2029T>C	p.(Cys677Arg)
	FH12	c.2054C>T	p.(Pro685Leu)
	FH13	c.1447T>C	p.(Trp483Arg)
	FH14	c.1432G>A	p.(Gly478Arg)
	FH15	c.214delG	p.(Asp72Thrfs*134)
	FH16	c.259T>G	p.(Trp87Gly)
	FH17	c.1897C>T	p.(Arg633Cys)
	FH18	c.681C>G	p.(Asp227Glu)
	FH19	c.2061dup	p.(Asn688Glnfs*29)
PCSK9	FH25	c.1120G>T	p.(Asp374Tyr)

FH Array II mutation coverage

Analyte Name		Mutation	Protein
LDLR	FH20	c.1285G>A	p.(Val429Met)
	FH21	c.680_681delAC	p.(Asp227Glyfs*12)
	FH22	c.1187-10G>A	p.(=)
	FH23	c.1048C>T	p.(Arg350*)
	FH40	c.118delA	p.(Ile40Serfs*166)
	FH24	c.1168A>T	p.(Lys390*)
	FH26	c.232C>T	p.(Arg78Cys)
	FH27	c.1587-1G>A	p.(=)
	FH28	c.1706-10G>A	p.(=)
	FH29	c.1796T>C	p.(Leu599Ser)
	FH30	c.1436T>C	p.(Leu479Pro)
	FH31	c.1474G>A	p.(Asp492Asn)
	FH41	c.501C>A	p.(Cys167*)
	FH33	c.662A>G	p.(Asp221Giy)
	FH34	c.682G>T	p.(Glu228*)
	FH42	c.1150C>T	p.(Gln384*)
	FH36	c.938G>A	p.(Cys313Tyr)
	FH37	c.136T>G	p.(Cys46Gly)
	FH38	c.2042G>C	p.(Cys681Ser)
	FH39	c.1618G>A	p.(Ala540Thr)

FH Arrays I & II protocol

1 Extraction
Genomic DNA extracted from blood

2 Amplification
Multiplex PCR reaction

3 Hybridisation
Amplicon hybridisation/ conjugation to biochip array

4 Detection
Imaging and results processing by Evidence Investigator analyser

~3 HOURS

Benefits of the Randox Familial Hypercholesterolaemia (FH) Arrays I & II

Product features

- Rapid turnaround time of ~3 hours from extracted genomic DNA to result
- Samples can be assessed in small batches (as few as 3 samples) with only 20ng of genomic DNA required per array

Benefits to the laboratory

- Developed with leading experts to test for 40 specific FH-causing mutations with ~78% coverage, providing a targeted, cost-effective assay for FH testing. Rapid turnaround time allows results to be reported within days, compared to lengthy comprehensive genetic analysis (CGA) which can take weeks or months to report results
- The FH40 panel consists of 2 mutation arrays, allowing for rapid cascade screening of known mutations offering further laboratory cost savings

Benefits to the patient

- Mutational status can be determined rapidly from a single test, with a reduced need for confirmatory testing with NGS
- Genetic analysis for FH mutations allows for more accurate diagnosis compared to lipid profiling

PRECISE DIAGNOSTICS FOR TARGETED THERAPY

Understanding drivers of disease is vital in delivering effective patient care. Through the unravelling of the genetic code, healthcare practitioners are able to predict and prevent disease and prescribe appropriate targeted treatments to specific subgroups, for optimal patient outcomes.

Randox Molecular offers a range of assay formats including SNP genotyping, gene expression, pathogen detection and mutation detection across infectious diseases, cardiovascular disease and oncology. Utilising innovative Biochip Array Technology (BAT) for multi-analyte screening of biological samples, our assays provide a comprehensive patient profile from a single sample for rapid, accurate result reporting.

References

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