IHG pharmaco

Clarity, accuracy and simplicity in genetic testing

IHG pharmaco

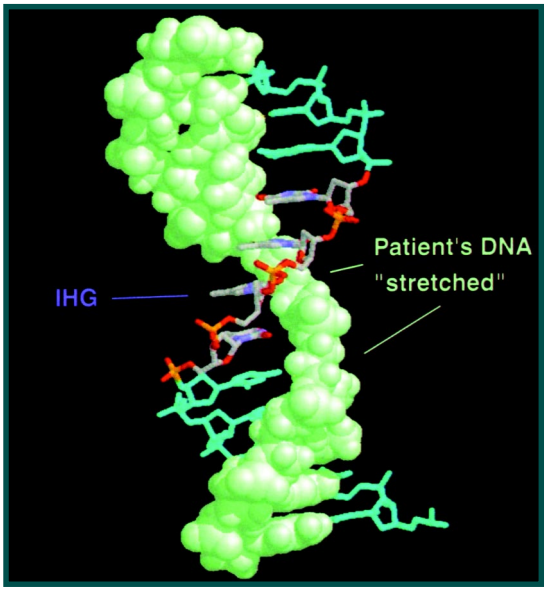
IHG Pharmaco Limited is a spin out from the University of Bristol, with 10 years of research behind it, and two SMART awards (1999 and 2002). The company was formed in 1999, and well over 60 diagnostic kits have been developed, many of which have been proven in National Health Service laboratories. The research and development is based in Bristol University School of Medical Sciences with production in a nearby centre.

Genetic medicine and the application of rapid diagnostic techniques

Genetic medicine embraces key disciplines such as:

- Diagnosis of genetic disease
- Determination of genetic risk factors
- Forensic medicine
- Pharmacogenetics – where the genetic configuration of an individual determines how that individual responds to a particular compound. This is of particular importance in cancer therapy, where a patient's genetic configuration profoundly influences the success of a particular treatment, or treatment regime.

To take advantage of the progress made in these disciplines, there is a requirement for accurate, rapid and easy to use diagnostic methodology. IHG Pharmaco has developed a patented technology using induced heteroduplex generators (IHG) to screen for SNPs. The basic principle of the technology is shown in the illustration on the right, which shows a model of a gene with a mutation specific for an inherited genetic disease.
One of the two strands of the patient's DNA (shown in green) has been replaced with a "mimic" strand of DNA – an induced heteroduplex generator (IHG). This stretches the conformation of the Patient's DNA in the region where the genetic defect is located. The 4 bases used to induce heteroduplexes in the helix are shown in the middle with individual atoms multicoloured.

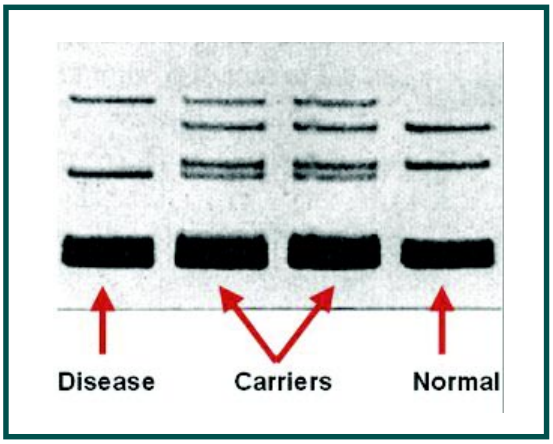


Using IHGs for genetic diagnosis

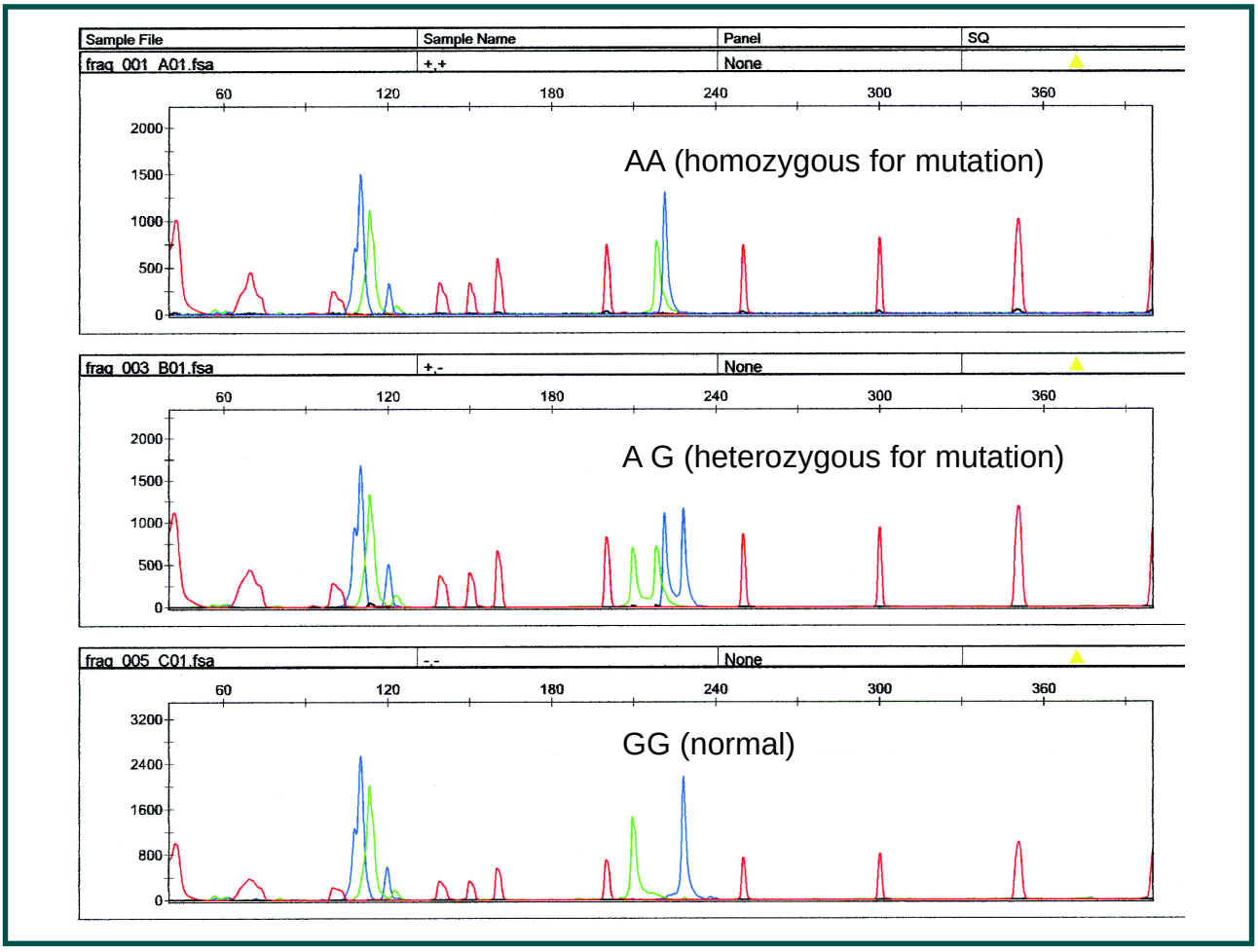
The effect of stretching the patient's DNA is to change its structural conformation, which allows accurate identification of:

- Normal individuals unaffected by the disease
- Those who are carriers of the disease, but who themselves are unaffected
- Those who have inherited, and will develop, the genetic disease.

The picture shows the results of IHG diagnosis of a common blood clotting disorder (Factor V Leiden) displayed in both PAGE (polyacrylamide gel electrophoesis) minigel and in CE (capillary electrophoresis) readouts.



PAGE Minigel readout



Capillary Electrophoresis (CE) readout

Single nucleotide polymorphisms (SNPs) and mutations

- Single nucleotide polymorphisms (SNPs) are the most frequent type of genetic variation in the human genome. They account for more than 90% of all differences between individuals. It is likely that these variable patterns of SNPs will account for many of the complex phenotypic characteristics found in humans. SNP analysis has the potential to predict susceptibility to a variety of clinical conditions including cancer, cardiovascular disease and mental illness and in making targeted drug therapy a reality. Millions of SNPs have already been identified within the human genome. SNPs are considered to be important genetic markers.

  This is due to their abundance within the human genome and association they may have with many genetic traits and disease susceptibility. SNPs may be situated close together within a region of DNA and can be inherited in different combinations or haplotypes.
- Mutations are a class of SNP which create aberrant proteins or differences in protein expression associated with classical inherited metabolic or other diseases. Examples include: Cystic Fibrosis, Phenylketonuria, Sickle Cell Disease and Von Willebrand's Disease.

How is IHG technology relevant to SNP and mutation detection?

Unlike many currently used DNA diagnostics, a single Induced Heteroduplex Generator (IHG) is capable of identifying single or multiple SNPs, and haplotypes. This is possible because of the unique design of the IHG. For many applications, this permits a substantial reduction in the number of tests required. Haplotyping is a great advantage because it is increasingly apparent that haplotypes, not genotypes, may be more relevant in disease and protein expression studies. IHG-based haplotyping results provide unequivocal evidence of the physical linkage of SNPs.

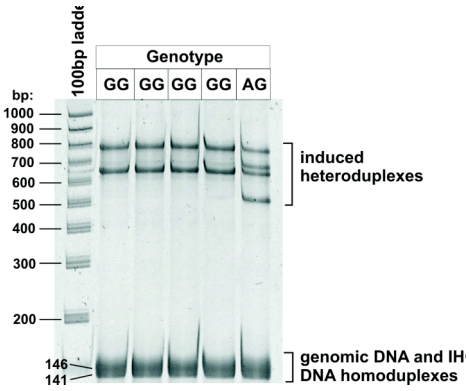
How does the IHG reagent work?

An IHG reagent mimics regions of DNA within the genes but differs from them due to the possession of one or more 'identifiers'. These identifiers are found adjacent to the SNPs within the DNA.

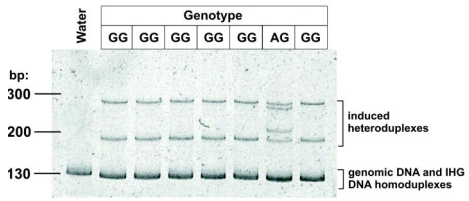
When human DNA (containing the SNPs in question) is mixed with IHG reagent (containing the identifiers) under certain experimental conditions, it undergoes a conformational change -the DNA bends and becomes more rigid- in a manner which is specific to the SNP or mutation causing the disease.

A single IHG reagent possessing a combination of identifiers can cause unique conformational changes to occur for single or clustered SNPs and haplotypes. These changes are induced by heteroduplex formation and can be detected on various instrument platforms.

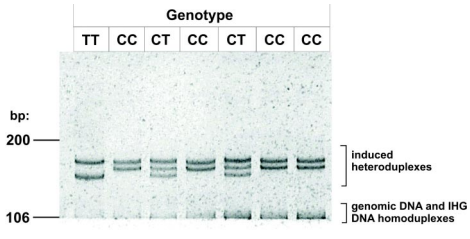
Clinical minigels



Factor V Leiden PAGE minigel showing induced hetroduplexes



Prothrombin PAGE minigel showing induced hetroduplexes



MTHFR PAGE minigel showing induced hetroduplexes

What are the advantages of IHG technology?

High Degree of Accuracy

Means no need to retest

Simplicity

Does not require highly trained staff or prolonged training

Single tube PCR for both genomic and IHG DNAs followed by short mix-heat-cool step. No need for restriction enzymes, probes, hybridization or washing steps, multiple primer pairs

Flexible Platforms

From low tech gel to high tech microcapillary and WAVE

No need to be tied to one platform

Suits labs of different sophistication and funding

Flexible Throughput

No need to run big batches

Means results produced more rapidly for patient

Linkage

Uniquely, a single IHG can identify single, multiple SNPs AND haplotypes. Can multiplex.

Labour and time saving with accuracy retained

Control DNAs for all known alleles

Allows identification of new alleles to give new insight into disease diagnosis

Ability to develop new tests in weeks

Allows rapid response to customer needs and developing markets

How IHG technology compares

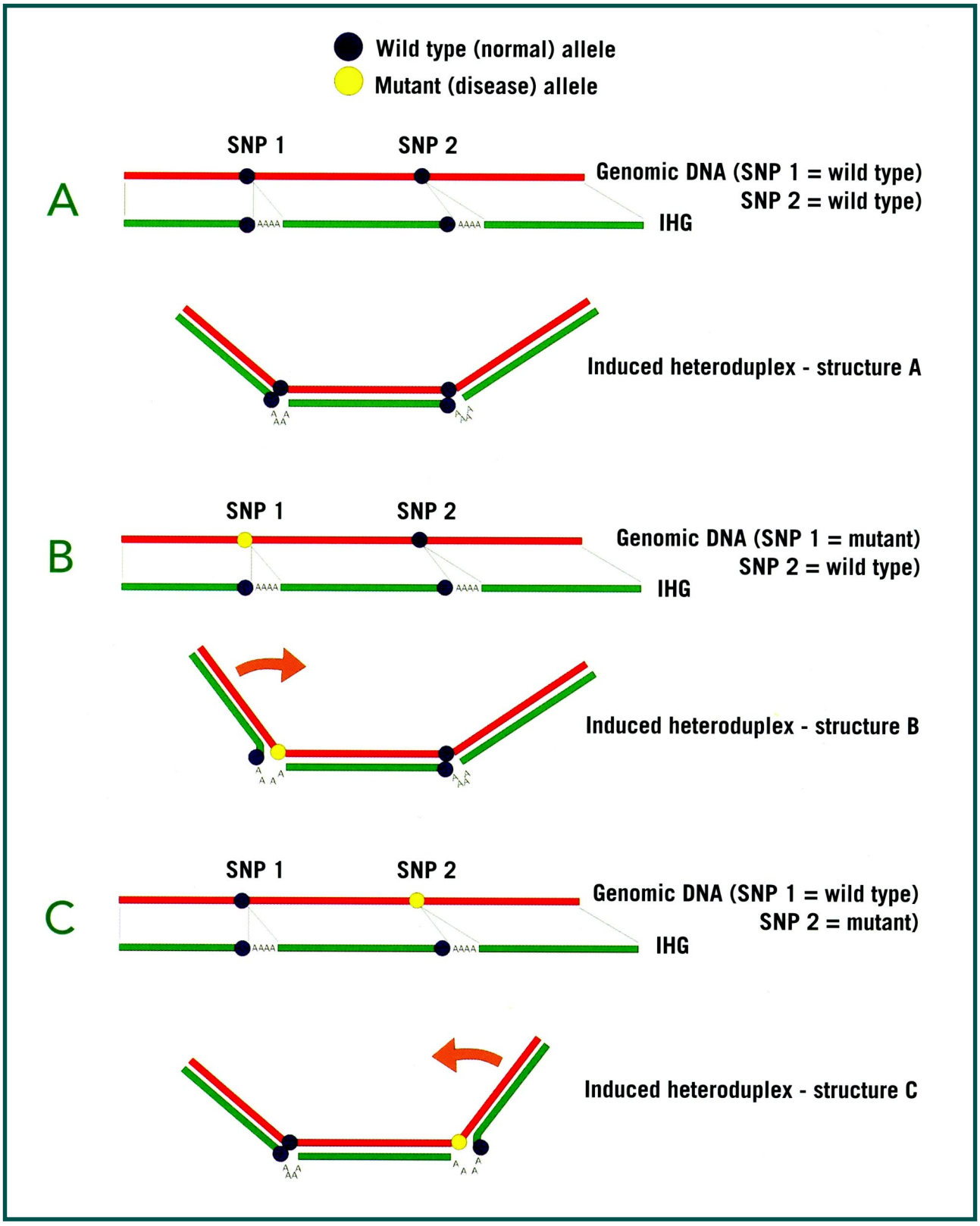
Method	IHG	SSO dot	SSO reverse dot	SSP (ARMS)	RFLP	SSCP	Sequencing	RSCA	Gene Chip mircoarray
Identifies haplotypes without cloning?	✓	✗	✗	✗	some	some	✗	some	✗
All heterozygotes unequivocal?	✓	✗	✗	✗	✗	some	✗	some	✗
Single reagent for multiple SNPs?	✓	✗	✗	✗	✗	n/a	n/a	n/a	✗
Multiple probes/enzymes not required?	✓	✗	✗	✗	✗	✓	✓	✗	✗
Simple post-PCR manipulation?	✓	✗	✗	✓	✗	✓	✗	✓	✗
Flexible instrument/gel platform?	✓	✓	n/a	✓	✓	✗	✗	✗	✗
Identifies new mutations?	✓	✗	✗	✗	✗	<80%	✓	some	✗
Rapid?	✓	✗	✓	✓	✗	✓	✗	✗	✗
All allelic controls provided?	✓	✗	✗	✗	✗	✗	n/a	✗	✗

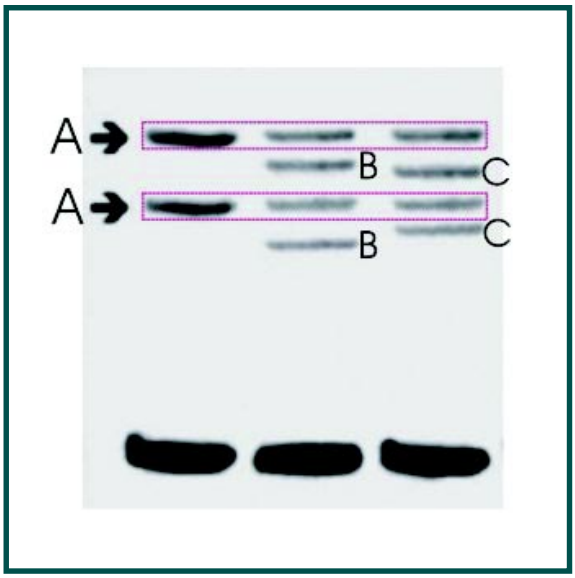
Current methods for the detection of SNPs include the following:

1. SSO (Sequence Specific Oligonucleotide) Dot
2. SSO (Sequence Specific Oligonucleotide) Reverse Dot
3. SSP (ARMS): Sequence-Specific Primers (Amplification)
4. RFLP (Restriction Fragment Length Polymorphism)
5. SSCP (Single Strand Conformation Polymorphism)
6. Direct Sequencing
7. Reference Strand Conformational Analysis
8. Gene Chip Microarray/Microarray Sequencing

The above methods are often intensive, expensive, time-consuming and imprecise. They are also frequently dictated by the availability of existing equipment platforms. More fundamentally extant tests are unable to determine what haplotypes an individual possesses (see above).

How can a single IHG reagent identify 2 or more individual SNPs and haplotypes?





In the example on the previous page, a single IHG can detect 2 SNPs present within the same amplified region of DNA (shown in red). The wild type (normal) sequence at each of the SNPs is shown in blue, the mutant (disease) sequence is shown in yellow (see panels B and C). The 3-dimensional conformation of the heteroduplexes formed with the IHG differ (shown by different angles of the “arms”) according to whether a wild type or mutant sequence is present at one or other SNP sites. These differences in conformations can be detected by, for example, polyacrylamide gel or minigel electrophoresis (see panel above). In the left hand column, the individual has two normal chromosomes (conformation A), which gives rise to two characteristic (arrowed) bands as shown.

In the middle column, the individual has one normal chromosome and one mutated at SNP 1 (patterns A and B respectively). In the right hand column, the individual has one normal chromosome and one mutated at SNP 2 (patterns A and C respectively). In practice, up to 6 or more mutations can be detected in a single region of DNA with a single IHG reagent, by extension of the principle to include, for example, more poly (A) insertions at appropriate places in the IHG.

IHGs can also detect different combinations (haplotypes) of SNPs (not shown). For example, a different pattern of bands would be observed if the individual had mutations at both SNP 1 and SNP 2 sites.

IHG reagent kits currently available

Coagulation Disorders

Factor V Leiden (FVL)

FVL is the most common inherited coagulation disorder. Between 3 and 8 percent of the Caucasian (white) U.S. and European populations carry one copy of the FVL mutation, and about 1 in 5,000 people have two copies of the mutation. The mutation is less common in other populations. FVL increases the risk of venous thrombosis 3-8 fold for heterozygous (one bad gene inherited) and substantially more, 30-140 fold, for homozygous (two bad gene inherited) individuals.

Prothrombin (PTR)

The prothrombin G20210A mutation is the second most common inherited clotting abnormality with heterozygotes having a 3-6 fold greater risk of thrombosis. PTR is only a mild risk factor for clots but together with other risk factors or combined with other clotting disorders the risk of clotting increases dramatically.

Methylenetetrahydrofolate reductase (MTHFR)

5,10-methylenetetrahydrofolate reductase is the metabolic enzyme involved in the conversion of homocysteine to methionine. Reduced levels of MTHFR activity (such as those caused by the C677T mutation) lead to elevated levels of homocysteine. This is referred to as hyperhomocysteinemia and is a risk factor or both arterial and venous thrombosis. Possession of the MTHFR mutation can lead to a 5 fold increased risk of clotting.

The three coagulation disorders and associated mutations described above can be a significant risk factor of particular relevance in the following circumstances:

Birth control pills

All women have a risk (about 4 fold) of having a blood clot while taking the contraceptive pill. For a woman with one FVL mutation, this risk increases 30 to 35 times. If a woman inherits one PTR mutation, the risk is 3 fold higher. For women with more than one mutation, the risk is 100 times higher.

Hormone replacement therapy (HRT)

All women have a risk (about 2 to 4 fold) of having a blood clot while taking HRT. For a woman with one FVL mutation, the risk increases to 13 to 15 fold. For women with two mutations, the risk is even higher.

Pregnancy

Blood clots are the major cause of maternal death during pregnancy. All pregnant women have a risk (about 5 to 6 fold) of having a blood clot. For a woman with one FVL mutation, this risk increases 7 to 16 fold. For women with two mutations, the risk is 40 fold.

Surgery

There is always a risk of having a blood clot during surgery or recovery from surgery. For a person with a FVL mutation the risk increases about 20 fold.

Long haul flights or other trips

A person with an inherited coagulation disorder has a greater risk of developing a blood clot during long distance travel (3 hours or more), perhaps up to 100 fold.

	Relative Risk with 1 mutation	Relative Risk with > 1 mutation
Birth Control Pills	30 - 35	100
HRT	13 - 15	Higher
Pregnancy	7 - 16	40
Surgery	20	Higher
Long Trips	10	Higher

The presence of two mutations increases the risk 3 fold above the risk of a single mutation. With prophylaxis, appropriate awareness and management, these risks can be significantly reduced and in some cases removed completely.

Cancer

The risk of venous thrombosis in cancer patients is 7 fold higher than that of healthy patients. Patients with leukaemia have a 28 fold risk of thrombosis. Carriers of FVL and PTR have a 12 fold higher risk of thrombosis than those individuals with this mutation who do not have cancer. It has been recommended to consider prophylactic coagulant therapy for patients with cancer who have an increased risk of venous thrombosis.

Blom, J. W., Doggen, C. J., Osanto, S., Rosendaal, F. R. (2005). Old and new risk factors for upper extremity deep venous thrombosis. Journal of Thrombosis and Haemostasis. 3 (11): 2471-8.

IHG reagent kits available in the future

- **Phenylketonuria: PAH gene**
- **Sickle-cell disease: b-globin gene**
- **Haemochromatosis C282Y and H63D**
- **β-thalassaemia: HBB gene**
- **Cystic fibrosis: CFTR gene**
- **Von Willebrand's disease: vWF gene, 2A, 2B and 2N variants**
- **Mannan-binding protein deficiency: MBL gene**
- **TPMT* *(IHG-RT-PCR)***
- **ApoE 2/3/4***
- **ApoA IV**
- **Stromelysin-1 5T/6T**
- **BChE**
- **Factor XIII**
- **HLA-A, B, C, DRB***
- **N-Ras**
- **Cytokine gene promoter polymorphisms:IL10*, TNFSF2*, IL6, TGFB*, IL1B**

***** = *haplotyping IHGs*

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