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Recommendations for the clinical interpretation of genetic variants and presentation of results to patients with inherited bleeding disorders. A UK Haemophilia Centre Doctors' Organisation Good Practice Paper

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Abstract

This paper sets out good practice for clinicians involved in interpreting variant reports for patients with inherited bleeding disorders. It is aimed primarily at doctors, nurses and allied healthcare professionals who may not have had specific training in genetic testing methodology or reporting. It deals with uncertainty in classification of variant pathogenicity and the handling of incidental findings.

KEYWORDS

clinical genetic testing, consent for genetic testing, genetic interpretation, genetic reporting, inherited bleeding disorders, sequence variant terminology

1 | INTRODUCTION

Genetic diagnosis for patients with haemophilia has been a routine part of clinical practice in the United Kingdom for two decades. For patients with other inherited bleeding disorders, testing has previously been variably implemented because of reduced availability. This has changed in the last few years with the incorporation of next-generation sequencing (NGS) technology into clinical care for patients with bleeding disorders. Currently, genetic diagnosis can be offered to all patients in the United Kingdom with a bleeding disorder for which the genetic basis is known. Even in those disorders that are suspected to be inherited but for which the genes responsible have not yet been identified, testing has been offered through gene discovery programs such as Genomics England's 100 000 Genomes Project. In the future, the expectation will be that selected individuals suspected to have a bleeding or platelet disorder not amenable to standard coagulation and genetic profiling will undergo large NGS panel or exome-based sequencing as part of their investigation.

For haemostasis genes with a well-established link between the gene involved and the clinical presentation (the genotype-phenotype relationship), the reporting and clinical interpretation of variants is relatively straightforward.[1] However, for many of the genes assayed by NGS, the phenotypic consequences of genetic variants have been studied in relatively small numbers of pedigrees, and so prediction of the clinical effects of a variant is more difficult. This creates challenges for those reporting the variants and for the clinicians explaining the results to patients. There has been extensive work that has generated tools and guidance for the laboratory reporting of variants[2-4] but there has been relatively little guidance for clinicians.

TABLE 1 Variant classification according to American College of Medical Genetics and Genomics guidelines

Variant class definition	Evidence needed for variant classification (simplified)	How can this variant be used in the clinic?
5—pathogenic or clearly pathogenic	• A well-established disease-causing variant with consensus on pathogenicity. • Typically cosegregation with the phenotype has been established in several pedigrees. • Functional studies provide detailed knowledge of the pathogenic effect. • The variant is not seen in normal controls or the frequency is very much lower than in the affected population • Probability of being pathogenic, $P > 0.99$	• This variant can be used to predict phenotype independently or in conjunction with other laboratory data • To screen all relatives that might have inherited the variant • Can be used to direct clinical management (eg, which patients are eligible for prenatal diagnosis or at increased risk of developing inhibitors)
4—likely pathogenic	• Some, but not all of the evidence for a class 5 pathogenic variant. • Many disease-causing but novel or private variants, particularly in genes that have not been extensively studied, will be class 4 • Probability of being pathogenic, $0.9 < P < 0.99$	• Should not be used for predictive testing or prenatal diagnosis without discussion with a Clinical Genetics Unit. • Consider what investigations might be available to clarify the diagnosis, for example, platelet function studies, cosegregation analysis. • Could be cautiously used for clinical management pending ongoing review of variant status bearing in mind the possibility of reclassification
3—variant of uncertain significance (VUS)	• Variant has some characteristics of being disease-causing, but there is conflicting evidence indicating that it may be benign (eg, unexpectedly high frequency in the normal population) OR • There is simply insufficient evidence to place in one of the other categories • Probability of being pathogenic, $0.1 < P < 0.9$	• Should not be used in clinical decision making • In disorders with informative phenotype assays, discuss with the reporting laboratory whether cosegregation studies might enable reclassification into class 4 or 2. It should be made clear that testing of relatives in this situation is not the same as screening for the disease • The accumulation of more data over time might lead to reclassification and so the result should be reviewed periodically.
2—likely benign	• Variant found more commonly in the general population than expected for the known frequency of the disorder • Probability of being pathogenic, $0.001 < P < 0.1$	• Normally not reported as no clinical consequences
1—benign	• The variant does not segregate with the disease (in families with two or more affected individuals) • Functional studies demonstrate no significant effect • Probability of being pathogenic, $P < 0.001$	• Normally not reported as no clinical consequences

Probability of variant being pathogenic from Tavtigian et al.[10]

2 | TERMINOLOGY

Previously, the terms "mutation" and "polymorphism" have been used to distinguish variants based upon the rarity or commonness of the variant in the general population (allele frequency) and whether they are considered to be disease-causing or not. However, there are many variants with variable phenotypic effects and allele frequencies in different populations and so these terms that were designed to be mutually exclusive are often not. This leads to confusion when both terms are used to describe the same variant and so this distinction should no longer be used in the clinic. Even for variants that seem to clearly fall into one of these categories, patients may draw more negative inferences with "mutation" while "polymorphism" may be inaccurately perceived to be more benign.[8] Neutral terms, such as "variant," without this ambiguity should be used when describing sequence differences.[2,9]

In order to reduce ambiguity when referring to the exact location of a variant, the Human Genome Variation Society (HGVS) produced guidance on the nomenclature to be used,[9] with any subsequent nomenclature updates issued and explained on the HGVS website (https://varnomen.hgvs.org/). The numbering of genetic or protein sequences is now uniform, but many historical references use different numbering for coagulation genes and proteins using the conventions that were in place at the time. This can lead to misidentification of a variant when referring to the published literature or misdiagnosis when a patient's result is compared with a historical report from a relative. To reduce these risks, specialist coagulation laboratories that also provide genetic testing should report using the HGVS nomenclature and version number alongside previous (legacy) numbering systems. As genetic testing becomes increasingly centralized, the knowledge of legacy numbering systems is gradually lost and so clinicians need to be aware of this potential pitfall when

comparing new reports with the published literature or previous reports on other pedigree members.

The American College of Medical Genetics and Genomics (ACMG) published guidelines in 2015 setting out how to standardize the classification and reporting of sequence variants.[2] They published a five-tier classification system (Table 1) that is now the standard used in the United Kingdom and globally.[4] It is essential that clinicians undertaking genetic analysis have an understanding of this classification and how to convey the information in reports using it to the patient and family members. The probability of a variant in a specific class being pathogenic should be borne in mind when using a result to influence clinical management. Of note, the probability of a class 4 "likely pathogenic" variant actually being pathogenic is $0.9 < P < 0.99$.[10]

***Recommendations*:**

1. Neutral terms such as "variant," "change" or "alteration" should be used instead of "mutation" or "polymorphism" when describing differences between a patient's sequence and the reference sequence.
2. Clinical records should describe a variant using Human Genome Variation Society (HGVS) nomenclature and numbering. If helpful for the understanding of the phenotypic effect, legacy numbering should also be included alongside the HGVS numbering.
3. Classification of variant pathogenicity should follow the 5-tier system described in the 2015 American College of Medical Genetics and Genomics guidelines.

3 | DEALING WITH UNCERTAINTY

Once the results of genetic testing are available, it may be apparent that there is a definite genetic variant which fully explains the phenotype and any family history. This would be designated "pathogenic" or class 5 in the classification given above. However, it is not infrequent that some uncertainty remains. This may be because a "likely pathogenic variant" or "variant of uncertain significance" has been identified, or because no potentially causative variant has been identified. These situations may arise from a number of factors described below. Because of the potential for misinterpretation, it is recommended that results are always reported to appropriately trained clinicians. Note that variants of uncertain significance may be re-categorized over time and will require regular review as more evidence of their status becomes available.

3.1 | Limitations of the techniques used

Until recently, genetic analysis relied on the identification of a candidate gene, or genes, which were then analysed by Sanger sequencing following PCR amplification of the coding sections of the gene. The amplicons typically would include the splice junctions and immediate upstream/downstream regulatory elements but often not any long-range transcription enhancers or repressors. Thus, causative variants outside the regions sequenced may be missed. Even though NGS allows greater access to these regions, repetitive elements and pseudogenes can still make some sequences relatively inaccessible.

Sanger sequencing is poor at detecting copy number variants so that large heterozygous deletions (bigger than an exon) are not detected, unless they have a boundary within one of the sequenced exons. Partial deletions or duplications can be more readily detected by NGS techniques by a change in read depth. Large deletions and duplications can be detected by multiplex ligation-dependent probe amplification (MLPA). Thus, the possibility of a large deletion or duplication should always be considered and should be detected by currently available techniques.

Complex rearrangements are rare in most genes, but there are some notable exceptions such as the inversions in *F8* which are the commonest cause of severe haemophilia A. These are not detectable by standard sequencing techniques or NGS but are detected by specific PCR techniques. These inversions were discovered through techniques such as southern blot which are now not available in most UK laboratories. Novel complex rearrangements are therefore not detectable by the techniques normally used in standard clinical genetic testing in the United Kingdom today.

3.2 | Mosaicism, lyonization and chromosomal anomalies

For simplicity, genetic testing is invariably performed on peripheral blood samples. This is often not the tissue affected by the disorder and may also not be representative of all the patient's germ cells which produce sperm or ova. Disparities between these may arise from somatic mosaicism, where a new DNA variant is present in only a proportion of cells in a patient, having arisen after they started to form as a very early embryo. Again, the advent of NGS has improved our ability to detect small populations of variant-carrying cells in minor degrees of mosaicism.[11] In practice, it may be difficult to distinguish mosaicism affecting a proportion of cells in a parent, from a de novo variant which affects a single sperm or egg, particularly because the mosaicism may be limited to the gonads (germinal mosaicism). Nevertheless, this distinction is important because the implications for other offspring are quite different.

In a Swedish study of 45 "sporadic" cases of haemophilia, 17 were found not to be present in the mother. Five of these cases were analysed in more detail, and 1/5 found to be a mosaic with 7% of peripheral blood cells carrying the variant.[12] Leuer et al[13] found mosaicism in 13% (confidence interval 0.2%-25%) of 61 families with sporadic haemophilia due to single nucleotide variations. The possibility of mosaicism in a parent of an apparent de novo case of an X-linked or dominantly inherited condition can never be excluded, and the parents of a child with an apparent de novo variant should be made aware that their other children could inherit the same condition even if their own genetic testing is negative.

For variant identification in a female, the phenomenon of X chromosome inactivation should not cause problems. However, this can confound phenotype predictions in females with X-linked

conditions, such as factor VIII or IX levels, and whether or not a carrier will be affected by bleeding symptoms. Extreme lyonization with inactivation of the unaffected X chromosome will result in a more severe phenotype in a carrier female than expected.[14-16] This results in a similar effect to that seen if a variant on one X chromosome is present in a female who has a chromosomal anomaly which is affecting what would otherwise be the normal allele. In females where the phenotype is different from that predicted, karyotype analysis may be informative.

3.3 | Variants of uncertain significance

In a significant proportion of variants, it is not possible to say with certainty whether they are benign or pathogenic. These class 3 "variants of uncertain significance" (VUS) represent 18% of reported variants in ThromboGenomics (an NGS panel of about 100 coagulation genes) and 14% in BRIDGE BPD (an NGS whole-genome study, personal communication from Dr Kate Downes, Cambridge). This percentage will undoubtedly rise as analysis is increasingly applied to haemostatic genes in which the effects of variation have not been well studied. Uncertainty in this context does not exclude the possibility of a causal or pathogenic state, but pathogenicity cannot be confirmed or excluded due to lack of evidence. The probability of a VUS being pathogenic ranges from $0.1 < P < 0.9$.[10] Clinicians should note that VUS can be upgraded to pathogenic or downgraded to benign as more data become available and so a variant's classification should be periodically reviewed. Because this will have to be conveyed to the family members, it is essential to give a clear account of VUS, and the possibility for change, when the initial report becomes available. VUS should not be treated as a positive or negative result and should not influence clinical decision making. A number of factors can be adduced to help clarify the pathogenicity of a variant, some of which are considered in the ACMG categorization above:

1. Frequency of the variant in reference databases compared with the expected incidence of the disease/phenotype. A common variant is unlikely to cause a very significant phenotype. It is essential to use the correct ethnic group for comparison.
2. Cosegregation studies in the family may help to establish pathogenicity.[17] This requires testing of unaffected individuals as well as affected. Thus, while a VUS should not be used for screening of family members for clinical decision making, it is nevertheless useful to genotype relatives, particularly in pedigrees with a mixture of affected and unaffected individuals.
3. Conservation of the residue across species. Variants of poorly conserved residues are less likely to be pathogenic.
4. In silico prediction algorithms. These may indicate the impact of a missense or potential splice variant but it should be noted that the predictions are based upon generalized variant effects on, for example, protein structure. These should not be relied on alone to assign pathogenicity but, in combination with knowledge of the key structural features that alter function of a particular protein or how expression of a particular gene is regulated, they can provide additional evidence.
5. Functional studies may provide demonstrable evidence of pathogenic effect.

These strategies are mostly applicable to coding variants and the classification of non-coding variants in deep intronic and distant regulatory regions is particularly challenging. These variants may not appear in control data sets, as the regions are still relatively inaccessible even with NGS, and so the minor allele frequency may not be known. Ongoing research into the epigenome, coupled with bioinformatic tools aimed at predicting the effects of distant regulatory elements, may assist in classification of these variants in the future. For the moment clinicians should await further data on these variants accumulated through large-scale, collaborative research.

***Recommendations*:**

1. Exercise caution when dealing with apparent "de novo" variants and inform parents that there is still a small chance of other affected children due to the possibility of mosaicism.
2. When dealing with a reported Variant of Uncertain Significance (VUS)
   a Do not rely on the VUS to direct clinical management.
   b In disorders with informative phenotype assays, discuss with the reporting laboratory whether cosegregation studies might enable reclassification into class 4 or 2. It should be made clear that testing of relatives in this situation is not the same as screening for the disease for diagnostic purposes.
   c Check the status of class 4 (likely pathogenic) and class 3 (VUS) during routine clinical reviews to establish whether the variant has been reclassified. When a variant's classification changes, the reporting laboratory should issue a revised report.

4 | INCIDENTAL FINDINGS

Any genetic test involving many genes, particularly whole-genome analysis, will detect at least some of the 3 million differences in DNA (0.1% of DNA sequence) which exist between any two people.[18] Most of these are part of normal variation and of no consequence, but some have a clear association with disease. If this is unrelated to the clinical problem being investigated, these are referred to as "incidental findings".[19] The likelihood for generating incidental findings depends on the type and clinical focus of the DNA test. Essentially, the more genes that are included in a test, the higher the chance of uncovering an incidental finding.

An incidental finding would usually be one of:

1. A variant which is not involved in the direct cause of the phenotype under investigation, nor modifies the severity of that phenotype, but might have other clinical consequences for that person or their relatives.

TABLE 2 Likelihood of incidental findings with different testing strategies

Test type	Indication	Test benefit	Likelihood of incidental findings	Example scenario
Specific variant	Relative of affected patient where pathogenic variant is known	Genetic risk-assessment and choice for at-risk relative	Unlikely	See Cases 1 & 2 in Appendix 1 Where relevant to the particular test, the potential for revealing a contradiction to a stated biological relationship between family members should be included in the consent process.
Single gene	Confirm clinical diagnosis	To find family-specific variant–enabling genetic testing in wider family, and/or reproductive options	Very unlikely	See Case 3 in Appendix 1
Multiple gene panel	Targeted to clinical presentation category	Assists diagnosis and establishes exact genetic basis	Possible	See Case 4 in Appendix 1
Whole-exome/genome	Clinical phenotype does not entirely fit an established gene panel; OR: underlying gene causing the clinical presentation remains unknown	At present most likely to be within research context aimed at gene discovery (eg, Genomics England's 100 000 Genomes project)	Very likely	See Case 5 in Appendix 1

2. A finding which contradicts stated biological relationships between family members.
3. A finding that may challenge a person's assumed identity in relation to a genetically mediated characteristic such as ethnicity or gender.

Case scenario examples for the different types of incidental finding are to be found in the Appendix 1 to this document (Table 2).

The possibility of incidental findings arising should be discussed as part of the consent process. If a patient expressly states that they wish to avoid the possibility of incidental findings, then the feasibility of testing using a more limited strategy, such as a single gene analysis as opposed to whole-genome sequencing, should be discussed. It should be borne in mind that sequencing platforms, such as 100 000 Genomes, may have filters applied to include or exclude specific genes or variants from analysis although this cannot be tailored to individual cases. Whether or not to report an incidental finding should depend on the predicted clinical effect and how strong the evidence is for potential pathogenicity.[19] In practice, this depends on whether it is an "actionable" finding for potential patient benefit which is dependent on how severe the associated phenotype is and whether there is an established impact on clinical management. For example, knowledge of the incidental finding could enable the patient and/or family members to: improve their future health prospects or take better informed lifestyle choices or have better informed reproductive choice. Specific genetic disorders that satisfy these criteria are listed in the 100 000 Genomes Project Protocol and include conditions such as sickle-cell disease, thalassaemia and severe haemophilia but not milder bleeding disorders or thrombotic disorders.[20]

***Recommendations*:**

1. Any clinician requesting a DNA test should know what degree of likelihood there is that the test may reveal an incidental finding, in relation to the broad categories of clinical impact involved, and discuss this with the patient prior to testing in order to enable informed consent.
2. The clinician should assess the clinical or potential emotional importance of any incidental finding and discuss this with the patient when explaining their result.

5 | CORRELATING GENOTYPE AND PHENOTYPE IN THE CLINIC

When conveying information about a genetic report in a patient-clinician consultation, the key question is: "Can the variant(s) identified explain the phenotype?" Subsidiary questions include: "How much of the phenotype is due to this variant?" To assist variant classification, requestors should provide full details of the laboratory and clinical phenotype. For example, in a new case with a suspected abnormality of fibrinogen, providing the reporting laboratory with activity and antigen values as well as details of bleeding

and thrombosis history will result in a more accurate pathogenicity classification. If it is felt that the variant does not fully explain the phenotype, the physician should consider whether an additional disorder (such as a platelet disorder in combination with a confirmed *VWF* variant) may need to be sought. Some genes, such as *VWF* and *F7*, frequently contain multiple variants. Typically, there might be a combination of one rare variant with one or more common variants. While the rarer variant is generally more likely to be pathogenic, the common variants might also affect the phenotype. For example, compound heterozygosity for a variant causing type 1 von Willebrand disease (VWD) in trans with a variant causing type 2 N VWD might well result in an overall clinical classification of just type 2 N in the index case. However, correct assignment of the phenotypic effects will result in better prediction of the disease in the offspring who may exhibit a type 1 VWD phenotype if they inherit the type 1 variant or be asymptomatic if they inherit the type 2 N variant with a normal allele from the other parent. In many cases of multiple variants, classification of pathogenicity will be difficult and appropriate caution should be exercised when interpreting these results.

For many of the inherited coagulation disorders, inheritance occurs through a classical Mendelian pattern and a family tree or pedigree will allow calculation of the probability that a person with a known affected relative carries the abnormal allele. For haemophilia and other X-linked disorders, this may require the additional use of Bayesian calculation methods to allow for the number of unaffected sons or brothers of a potential carrier.[21,22] In the clinic, it is fairly common to be faced with a young woman who has a family history of haemophilia and who wishes to know her likelihood of being a carrier. In cases where a pathogenic (class 4 or 5) variant has been identified, testing for any relative that might have inherited the variant should be offered. This includes obligate carriers because of the various mechanisms that can lead to unexpected results. The maintenance of full and up-to-date pedigrees is vital in ensuring that all relatives that might have inherited the variant are identified. However, if no variant has been identified and there are no affected individuals or obligate carriers available for testing, being able to discuss the probability of carriership with a patient is helpful in managing expectations.[23] In these circumstances, if it is possible to determine that the predicted risk of a particular female relative being a carrier is very low, then genetic screening without an identified variant is of little or no benefit. This is because a negative result (the most likely outcome) will not exclude carrier status, and assigning pathogenicity for any variants identified will be difficult because an association has not been established with the phenotype in the pedigree.

In the example pedigree shown in Figure 1, there are no affected individuals or obligate carriers available for testing. A few studies have investigated the origin of the variant in such cases of sporadic haemophilia A[12,24,25] and B.[26] These observational studies have found that 62%-82% of mothers (II.2) have the same variant as the index case (III.1) but only 2%-11% of maternal grandmothers (I.1). The maternal carriership rate is affected by the relative mutation rates in male and female gametes and is in keeping with the knowledge that variants causing severe haemophilia mostly arise during spermatogenesis, and mainly in the unaffected maternal grandfather (I.2), or in an earlier unaffected male ancestor of the grand-maternal line. However, the figures above derive from observation across many families and therefore only represent the figure for an "averaged" family structure in relation to the number of unaffected male sibs in each generation. In practice, each family structure is individual, and the carrier risk of the maternal grandmother will be considerably higher (approaching half of the mother's risk) where there is no unaffected male sib in the mother's or grandmother's generation. A maternal aunt (II.3) would be predicted to have half the risk of her mother, that is, 1%-6% for an "averaged" family, but up to 15%-20% (up to 1/4 of her sister's risk) if neither she nor their mother have any unaffected brothers, and she has no sons herself. Where the aunt has a low probability of carriership, genetic screening for an unknown variant is of very limited value and simply advising the consultand of her probability is likely to be more useful. Where the risk is higher (eg, 15%-20%), a combination of factor level and DNA analysis should be considered.

Evaluating the correlation between genotype and phenotype is key, especially for variants classified as types 3 or 4. Close collaboration between the laboratory scientists who will be able to provide information about the effects of a variant on gene function, and the clinicians who can interpret the clinical phenotype, in association with any known family information, has never been more important. In the era of rapidly increasing genetic information, the sharing of data to develop an understanding of genotype-phenotype relationships more rapidly and with greater certainty is needed.[27] DECIPHER (https://decipher.sanger.ac.uk) is a database which can be used to evaluate both the evidence for a variant's pathogenicity using the ACMG criteria and to assist with the combined variant and clinical-level interpretation.[4,28] There are various databases and registries of human variation. Some cover multiple diseases, such as Online Mendelian Inheritance in Man (OMIM) and Orphanet, while others are locus specific, such as the EAHAD Coagulation Factor Variant Databases (www.eahad-db.org). The type and amount of phenotypic and clinical data captured in these resources varies. Some capture multiple reports of the same variant while others only report a single, usually the first, occurrence. Multiple reports are obviously advantageous when predicting the range of phenotypic effects due to a particular variant. This raises the possibility of the same variant being classified inconsistently depending on what sources of evidence are used. The Clinical Genome Resource is an international collaboration to create expert panels in specific disease categories with the aim of improving and harmonizing variant classification.[29]

***Recommendations*:**

1. Clinicians requesting genetic analysis should enter on the request form all the laboratory and clinical data that might be used in disease classification.

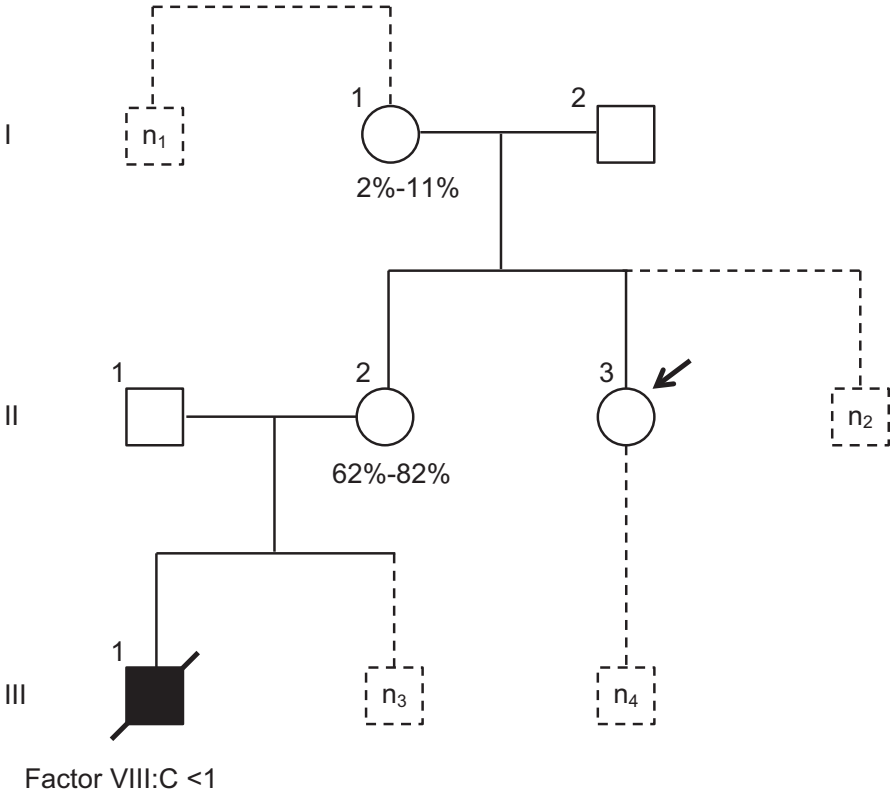

FIGURE 1 Pedigree of a sporadic case of haemophilia in which genetic testing has not yet been carried out. The maternal aunt (arrowed) is the consultand. The chance of female ancestors carrying the pathogenic variant from observational studies averaged over different family structures is shown as a percentage. The chance in any specific family is dependent also on the actual number of unaffected males in each generation (n_1, n_2, n_3, n_4, etc)

2. A detailed pedigree showing the laboratory and clinical phenotype of family members should be obtained when the test is requested.

6 | CONSENT

Written, informed consent is recommended prior to testing individuals for heritable bleeding conditions[7] and should be taken only after comprehensive, non-directive genetic counselling. There is guidance in the UK on how consent should be sought for genetic testing within clinical practice.[30] However, these predate the routine use of high-throughput gene testing methods and updated guidance is in development.[31]

In principle, counselling and subsequent consent for genetic testing should cover several areas. These are set out in Table 3 and are broadly applicable to all types of genetic testing from single gene analysis to whole-genome sequencing. Until fairly recently, genetic testing for heritable bleeding conditions has been undertaken to confirm the presence of a variant in a single gene, thereby providing supportive information for a condition that was already suspected or known; for example confirming the presence of a *F8* variant in a male with a factor VIII level of 0.01 iu/mL. This information has then been used (if shared by the patient) to test at-risk members of their extended family. Working through the consenting process for testing in these cases has been fairly straightforward, since the test is most commonly looking for a pathogenic variant and is undertaken, at least for the proband, when an abnormal laboratory result is already known.

However, as we move from single gene analysis to multiple gene panels or genomewide analysis, the consent process must evolve to take account of the issues discussed above. Much more consideration should be paid to ensuring that the patient understands the breadth of genetic testing being offered and that there may be uncertainty regarding the effects of variants in some genes. There should be specific discussion regarding VUS and the subsequent action that might need to be taken such as further testing of the patient and/or relatives with the aim of reducing this uncertainty. The possibility of incidental findings, and whether or not these are specifically sought, should be discussed. In addition, a patient should know that the test may include haemostasis genes that can have clinical effects in other organs or systems. Examples include pulmonary fibrosis in some of the genes that cause Hermansky-Pudlak Syndrome, renal failure associated with *MYH9* or leukaemia associated with *RUNX1*, *GATA1* and *ETV6*. The consent process should cover the chances of finding such results and the action that should be taken if such a variant was reported. If the patient does not wish to learn about variants with effects outside blood coagulation, then the test should not go ahead, or an alternative more simple test should be considered. Health Education England Genomics Education Programme has developed a course "Preparing for the consent conversation," a comprehensive online learning tool which explores many of these issues at https://www.genomicseducation.hee.nhs.uk/courses/. The probability of a negative result and the limitations of the testing strategy used should also be made clear.

Genetic testing for inherited bleeding disorders is sometimes conducted during childhood and informed consent is taken from a

TABLE 3 Points to consider and discuss during counselling and consenting

Clinical areas that should be covered and implications of testing for the patient: • Personal and family bleeding history • Pedigree • Inheritance of condition being considered • Chance of patient having an abnormal gene • How the genetic result might affect clinical management • What a negative result means • Assess the patient's beliefs and expectations of the test • Limitations of the test and what the patient would like to be told about variants of uncertain significance, potential phenotypic consequences and incidental findings • Sharing of data through anonymized and non-anonymized confidential databases • The use of their result for testing family members	Implications of testing the patient on the wider family: • Clear discussion about who may be affected or carry the abnormal gene in the wider family • How to contact family members • Maintaining confidentiality of individuals involved • Awareness that non-paternity may become evident through family testing
Practical considerations around testing: • How the test is taken • What happens to the sample and whether it is stored • How the results will be conveyed • How long the results may take • Potential need for further testing (particularly relevant with VUS results) • Mechanisms for ensuring that data is entered into databases and can be updated or removed as required	Prior to completing consent: • Ensure the patient has a clear understanding of the reasons for testing, including the wider implications, and has had time to have all questions answered • Ensure the patient is aware that consent is voluntary and can be withdrawn

person with parental responsibility prior to sampling. It is then particularly important to be quite clear what the purpose of genetic testing is. It might be for aiding diagnosis in a symptomatic child, or it might guide clinical management if, for example, it leads to identification of a variant with a high risk of inhibitor formation in a boy with haemophilia A. In other situations, variant identification might not be expected to affect clinical management of the index child but might be required for diagnosis in a relative. The consent process for this raises ethical issues that are currently being reconsidered by the Joint Committee for Genomics in Medicine who are in the process of updating their guidance. However, genetic heterozygote carrier testing per se in an asymptomatic child who is deemed not yet to have the capacity to give their own informed consent would not normally be acceptable. An example of this would be to inform carrier status for haemophilia in an asymptomatic preadolescent girl. Children may not remember having the test and the authors recommend, in particular bearing in mind the increasing complexities of genetic testing, that when children transition to adulthood re-consenting should be considered.

Finally, it is important to make sure that the potential to share the data is discussed. Not only is genetic information of significance to family members, but also large numbers of patients providing linked genotype-phenotype data becomes a powerful tool for developing understanding of pathogenesis of disease and future treatments at a research level, particularly for rare diseases.

***Recommendations*:**

1. Consent should be taken prior to genetic testing for all inherited bleeding disorders and should cover: breadth of testing, implications of results for patient and extended family, VUS and incidental findings, data sharing and non-haemostatic effects of some variants.
2. If genetic testing is carried out on a child with parental consent, the child should be re-consented when he/she reaches adulthood and/or has capacity.

DISCLAIMER

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METHODOLOGY

This Good Practice Paper was written by the Genetics Working Party of the UKHCDO according to the British Society for Haematology (BSH) process set out at https://b-s-h.org.uk/. The UKHCDO produces Good Practice Papers to guide health care professionals in areas where there is a limited evidence base but for which a degree of consensus or uniformity is likely to be beneficial to patient care.

LITERATURE REVIEW DETAILS

The PubMed database was searched for all article types in March 2018 using the following keywords and phrases: clinical genetic testing, genetic reporting, sequence variant terminology, variant

reporting using the Boolean operator AND with bleeding disorders. This generated 31 results which were analysed in full. A further search was done using genetic interpretation filtered with guideline and English language. This generated 45 results of which seven were considered relevant. Publications not identified by these searches but within the personal knowledge of the working party and considered to be relevant were also included.

REVIEW OF THE MANUSCRIPT

Review of the manuscript was performed by the Advisory Committee of the UKHCDO and the BSH Haemostasis and Thrombosis Task Force. It was also on the members section of the BSH website for comment. It has been reviewed by the Joint Committee on Genomics in Medicine and Genetic Alliance UK. These organizations do not necessarily approve or endorse the contents. Members of the writing group will inform the writing group chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) current guidelines website if it becomes obsolete.

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CONFLICT OF INTEREST

The UKHCDO paid the expenses incurred during the writing of this Good Practice Paper. The authors have no conflicts of interest to declare.

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APPENDIX 1

CASE SCENARIO EXAMPLES FOR INCIDENTAL FINDINGS FOLLOWED BY RECOMMENDED ACTIONS IN ITALICS

CASE 1

A 25-year-old woman is 10 weeks into her first pregnancy. Her mother's brother has "mild" haemophilia A with a known *F8* variant associated with mild disease. The woman proves on DNA testing not to be a carrier for this, but to be a carrier of a different and coincidental *F8* variant associated with severe disease, such that any son is unexpectedly at risk of severe, not mild, haemophilia.

Consider the evidence for pathogenicity of the incidental F8 variant and whether testing of other relatives might be useful in confirming the phenotype. Discuss the conclusions with the consultand.

CASE 2

A 17-year-old schoolgirl studying biology A-level understands that she must be a carrier for haemophilia B as this affects her father, and requests testing for both of them to find out the exact gene change involved. She proves not to have inherited her affected father's *F9* variant. Possible explanations are that he is not her biological father or that the father is a mosaic, with variable presence of the haemophilia-associated variant in his somatic and gonadal cells.

The possibility of the father's variant not being found in the daughter should have been raised when taking consent for the test. There should be an established process for dealing with this scenario so that the consultand can be informed of the finding with appropriate support.

CASE 3

The variant in a 23-year-old man with haemophilia A is found to be a translocation between the X chromosome (through the *F8* gene) and an autosome, conferring unexpected additional fertility or reproductive implications.

Do inform and refer for genetic counselling.

CASE 4

A 12-gene coagulation factors panel test is used to investigate a 38-year-old Irish-American man with possible von Willebrand disease. The result finds not only a pathogenic *VWF* gene variant, but also a variant in the *FGA* gene (coding for the fibrinogen α chain), which has been reported in two families of similar ethnicity, and does not affect clotting but predicts a high likelihood for him to develop nephrotic syndrome due to renal amyloidosis.

The small possibility of revealing additional unexpected health information should be raised when taking consent. Review evidence for variant pathogenicity and predictive value of this information. Do inform, and consider involvement of other specialists eg Clinical Geneticist.

CASE 5

A 31-year-old woman with a suspected platelet function disorder, but no likely causative variant on a 6-gene panel test, undergoes whole-exome sequencing. In addition to finding a likely pathogenic variant and a VUS in two different genes associated with platelet function, she proves coincidentally to be heterozygous for the factor V Leiden variant.

Discussion of the possibility of such findings should be integral to the consent process. This case is included to inform clinicians about reporting practices in the UK and the UKHCDO does not make any recommendations about which variants should or should not be reported. Both the likely pathogenic variant and the VUS in the genes affecting platelet function would be reported but factor V Leiden is an incidental finding that would not be reported. This is because its effect is not severe enough to be considered actionable under current guidance.

APPENDIX 2

GLOSSARY

Amplicon—A short sequence of DNA amplified during a PCR reaction. The amplicon size is the length of the DNA from the start of the forward primer to the end of the reverse primer.

Cosegregation—The combination of phenotyping and genotyping in multiple family members (both affected and unaffected) to establish whether the variant under investigation coincides with the expected phenotype

Exome—The part of the genome that is transcribed for translation into protein (exons) and the intronic flanking regions that are mostly responsible for regulation of expression or might have an effect on splicing. In humans this is about 2% of the genome.

Genome—The entirety of a species' DNA sequence. For analysis purposes, it should be noted that some parts of the genome are not easily analysed such as repeat elements.

Heterozygote— A person whose two copies of a particular DNA sequence are different. The term is often used with reference to someone having one pathogenic and one non-pathogenic copy of a gene, such as a female carrier for haemophilia.

MLPA—Multiplex ligation-dependent probe amplification. A probe-based method for amplifying specific regions of DNA, usually an exon. The amplified products can be quantified allowing detection of deletions or duplications which are collectively referred to as copy number variants.

Next-generation sequencing—Techniques such as massively parallel sequencing that allow access to vast amounts of DNA sequence in a single assay. Also referred to as high-throughput sequencing

Sanger sequencing—A well-established technique for sequencing DNA developed by Frederick Sanger and colleagues in the 1970s. The current approach involves PCR amplification of the target sequence to create a template which is then subjected to stepwise incorporation of terminating dideoxynucleotides which are labelled with discrete fluorescent dyes. This allows the DNA sequence to be read using an automated analyser.

Southern blot—A technique developed by Edwin Southern in 1975. The initial step is cutting of the target DNA by restriction endonucleases. The restriction fragments are then separated by electrophoresis, transferred or "blotted" onto a membrane and detected by probes labelled with either a radioactive or a non-radioactive reporter system. Fragment size is altered by gross genetic defects such as inversions or deletions.