REVIEW

DNA analysis: what and when to request?

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Over the last 15 years genetic testing by DNA analysis has expanded enormously both in volume and range due to advances in scientific knowledge and analytical technology. This type of analysis has the potential to provide rapid, cost effective, and accurate diagnostic information but also has its limitations. Some of the changes detected may be of ambiguous consequence and as the knowledge base expands so too does the recognition that other factors can influence the clinical picture. In many cases outcomes may be predicted only on a statistical basis rather than individually. Careful attention should therefore be given to the clinical question that is being addressed before such testing is requested.

in the potential of genomic profiling to revolutionise personalised healthcare and disease prevention. However, further epidemiological evidence and clinical evaluation of recommended interventions will be required before the true potential of this approach becomes clear.¹

Requests for genetic tests should be made by a clinician with a good understanding of the potential outcomes, future implications for the individual, and consequences for other relatives. Some tests are of a more sensitive nature such as that for Huntington's disease and some require complementary investigations, such as karyotype analysis for fragile X syndrome where the number of sex chromosomes influences the interpretation of the DNA analysis. Clinical geneticists are available to provide counselling to families.

The number of requests for DNA analysis in paediatric health care has increased significantly, in part simply because more tests have become available following the characterisation of many disease linked genes. Around 500 different gene tests are listed on the directory of UKGTN, the UK Genetic Testing Network (www.geneticstestingnetwork.org.uk). These tests can be cheaper and more definitive than alternative methods of analysis such as enzyme assays. DNA tests may also be used for carrier testing and prenatal diagnosis. The sample requirements for the tests are often minimal and relatively flexible, making it easier to offer such analysis. There has also been an expansion of neonatal screening programmes that may include mutation analysis. In addition, for some new treatments such as gene therapy and enzyme replacement therapy, characterisation of the mutation may be a requirement.

There are many advantages of using DNA analysis for clinical diagnosis, but there are also some limitations. The interpretation of data may be ambiguous since some changes can be partially penetrant, and without further functional studies it may not be possible to distinguish between a rare variant and a pathogenic change. Furthermore, analytical sensitivity is rarely 100% and some diseases may be caused by defects in more than one gene, for some of which tests may not be available. DNA analysis may not be capable of delivering a definitive diagnosis when used in isolation and may therefore not be cost effective. The costs of mutation analysis vary considerably ranging from around £50 for a p.Phe508del cystic fibrosis mutation test to £3000 for a full mutation screen in large genes such as for COL1A1 and COL1A2 in osteogenesis imperfecta. There is great interest

TYPES OF ANALYSIS

DNA analysis is approached in a number of ways according to the circumstances. Direct mutation analysis is used to detect a known or defined mutation, such as in the case of fragile X syndrome by sizing the CGG trinucleotide repeat expansion site or in the case of cystic fibrosis by testing for a panel of 33 commonly occurring mutations. Such tests generally have a known sensitivity and limited number of possible outcomes, and are used when there is just one or a limited number of possible changes. Mutation screening involves searching the gene(s) of interest for unknown change. A mutation scan using various techniques may be used as a preliminary step to target the region for direct sequencing. The technique used determines the sensitivity, but in general these analyses do not make any prior assumptions about the type of mutation and may also identify changes of ambiguous significance. These tests tend to take longer and are more expensive than the direct mutation tests.

Marker analysis is a form of indirect mutation analysis and is used in a number of ways including linkage, identity by descent, and identity testing. Linkage analysis is used to track the segregation of a genetic marker linked to the disease in a family with a known disorder. This remains a useful tool where it has not been possible to identify the underlying mutation, where samples are not available from affected individuals, or for consistency checking, particularly if a change detected by mutation screening is of uncertain significance. The main limitation is the need for samples from several family members of known clinical status in order to determine the phase of the association and find informative markers. The reliability of these tests depends on the location of markers with respect

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to the gene of interest and also requires that family relationships are as they appear to be. Linkage analysis is still commonly used in carrier and prenatal or neonatal testing for Duchenne muscular dystrophy, in cases where it has not been possible to identify the mutation because of lack of sample availability from the index case, or where there may not be time to complete a mutation screen. By using flanking markers, error due to recombination may be reduced so that the reliability is very high. A panel of markers across a gene locus may also be used to investigate risk of autosomal recessive disease in consanguineous families to determine if a child appears to have inherited identical haplotypes from each parent (identity by descent). Identity testing may be used to determine if twins are identical or not, with possible implications for recurrence risks. Methylation analysis may be used for disorders associated with imprinting errors such as Prader Willi or for determining X inactivation status that may be useful in detecting carriers of X-linked disorders by demonstrating preferential inactivation of the mutant X. For example, demonstration of skewed X inactivation in T cells of females with X-linked severe combined immunodeficiency provides molecular evidence of the diagnosis in cases where it has not been possible to identify a mutation.

SAMPLE REQUIREMENTS

DNA may be extracted from a wide variety of samples by different techniques that yield differing qualities and quantities of DNA. The sample required is determined by the technique to be used for analysis and any tissue variation in expression of the mutation. The standard sample requirement is 2–5 ml blood collected into potassium EDTA anticoagulant. Samples may be stored at room temperature prior to extraction and sent in appropriate approved packaging by regular post. DNA extracted from blood spot (Guthrie cards) as collected for newborn screening programmes and buccal scrapes may be suitable for some PCR based analyses.

For genomic Southern based analysis such as that used for congenital myotonic dystrophy, a larger amount of high molecular weight DNA is required. It may be possible to culture cells from a small sample, but this will take around 2 weeks. The technique of whole genome amplification may also be used, but this technique has yet to be fully validated for clinical practice.

For some disorders it may be necessary to extract DNA from a particular tissue to detect an anomaly with variable expressivity. For example, in the investigation of some mitochondrial disorders a muscle biopsy is recommended. In the case of transfused patients, the suitability of the sample for analysis will depend on the extent of the transfusion and whether it involved nucleated cells. In these cases, a buccal scrape may provide an alternative sample source. DNA extracted from blood samples from patients who have undergone bone marrow transplant will represent the DNA of the donor rather than recipient. For this reason it is important to identify clearly if the sample is pre- or post-transplant.

REPORTING ISSUES

The results of DNA analysis are reported with reference to the clinical question being addressed. The type of analysis will determine the sensitivity and specificity, although this may also be dependent on other factors such as the ethnic origin of the patient. Any family history that indicates a subject is an obligate carrier and any mutations already identified in the family are relevant to the interpretation of the DNA results. For some conditions the result of DNA analysis will indicate that the individual carries a mutation that has variable expressivity or penetrance. For example, female

carriers of ornithine transcarbamylase deficiency may develop the full range of symptoms or appear normal.

If the analysis involves testing a panel of known mutations then the report is likely to be clear; the mutation(s) are present or absent. Mutation screening may reveal ambiguous sequence changes such as novel changes of uncertain pathogenicity. In these cases a number of considerations and possible further studies may be needed to assess the relative likelihood that the change is significant. This usually takes the form of looking at the predicted effect on the protein and conservation across species. In some cases it may not be possible to be certain, but provided there is no genetic heterogeneity for the disease in question, it may still be possible to use the identified variant as a marker for the disease. In some cases, due to limited assay sensitivity, a risk calculation is used to determine the relative likelihood of a subject being a carrier or affected. This usually involves a bayesian approach to take into account all the available information. In this context it is important to consider the way in which the risk information is presented and how people perceive high and low risk.²

UNUSUAL SCENARIOS: NON-TECHNICAL SOURCES OF ERROR

The interpretation of DNA analysis often relies on a number of assumptions that may not always hold true. Uniparental disomy occurs when both alleles for a genetic locus have been inherited from the same parent. This may give rise to an erroneous interpretation of non-paternity or non-maternity. Rare cases of chimerism have been described in which different tissues within an individual have markedly different genotypes. This may be mimicked by a bone marrow transplant. Less unusual is the observation of mosaicism, where different cell lineages may carry or lack a particular mutation.

PURPOSE OF DNA ANALYSIS

The reasons for analysis fall into several categories. The referral may be made to exclude or test for a number of disorders as part of a differential diagnosis. For example, a baby presenting as a floppy neonate may be referred for testing for Prader Willi syndrome, myotonic dystrophy, spinal muscular atrophy, and mitochondrial disease. This bundle of tests tends to be used as a screen in which the sensitivity and specificity of the individual DNA tests is high, but the likelihood of any one of these diseases is generally low.³ In other cases DNA analysis may be requested to confirm a diagnosis made on clinical or other criteria such as enzymology or immunology. In such cases, testing is usually extended to cover as many mutations as possible. For example, in the case of cystic fibrosis where the clinical diagnosis has been confirmed by a positive sweat test, extended analysis is recommended if the molecular confirmation has not been made on initial screening for the most common mutations. The reasons for needing molecular confirmation vary. Access to some expensive treatments or clinical trials such as those involving enzyme replacement therapy or gene therapy often require molecular confirmation since the type of mutation detected may be helpful in determining the likely efficacy of treatment. Parents may on occasion refuse to accept a clinical diagnosis. Unfortunately this can cause difficulties if limited analytical sensitivity or inconclusive results of DNA analysis mean that it is not possible to provide molecular confirmation. Characterisation of the mutation allows other relatives to undergo more reliable carrier testing and prenatal diagnosis.

Carrier testing may also be important to help interpret the DNA results in the affected relative by confirming in the case of recessive disorders that the detected mutations are on

opposite parental alleles. Cases of double mutant alleles have been reported in a number of disorders.^{4,5} Due to the possibility of double mutant alleles and non-paternity, carrier testing of parents is always recommended prior to offering prenatal diagnosis, even where they appear to be obligate carriers. Carrier testing of parents will also help determine if the mutation has arisen *de novo*. For some disorders, such as ornithine transcarbamylase deficiency, the incidence of new mutations is high. But even if the parent does not carry the mutation in their lymphocyte DNA, there is still the possibility that they may carry the mutation in their germline. Germline mosaicism has been reported for a number of dominant and X-linked disorders.^{6,7} The risk of recurrence is then difficult to predict as it depends on the level of mosaicism. For this reason, prenatal diagnosis is usually still offered to the couple. For some disorders that are genetically heterogeneous or have variable expressivity, it may be important to confirm that different affected individuals within a family do indeed have the same disorder. Likewise where transplants are being considered it is good practice to confirm that the donor relative is not also affected.

The use of the specific mutation data derived from DNA analysis to customise treatment and determine prognosis is an area of expanding knowledge. However, the clinical use in individual cases is still limited.

STORAGE

Sometimes the type of analysis required may not be clear or even available, or the child may be terminally ill or undergoing a bone marrow transplant or other procedure that provides a unique opportunity to collect a sample. In these circumstances it can be very valuable to collect, extract, and store DNA so that it can be used when analysis becomes available or the family are ready to proceed with testing. Current practice in most UK diagnostic laboratories is generally to store all DNA samples indefinitely, but some time limit may eventually be imposed as storage facilities become limited.

CONSENT AND DISCLOSURE OF INFORMATION

Informed consent must be obtained for all sample collection and analysis. There are many professional and statutory guidelines governing these processes and relating to use of material for both diagnostic and research use. In the UK, the Human Tissue Act comes into force during 2006 and is designed to provide a legislative framework for issues relating to the taking, storage, and use of human organs and tissue.⁸ Most guidelines require that patients should agree to a sample being collected, to specified investigations, and to storage of the sample for future use and should have determined whether the results may be shared with other relatives affected by this information. Consent should be informed so that the individuals understand the potential consequences for insurance and future employment and implications for their relatives.

SPECIAL CONSIDERATIONS APPLYING TO GENETIC TESTS

Genetic tests may be predictive of future clinical status and often have implications for other relatives. Consideration therefore needs to be given to how the results from the test will be used. In the case of testing children, while it is appropriate to use the tests to make a clinical diagnosis that will benefit the management of the child, it is generally not appropriate to undertake carrier testing where carrier status itself is not associated with any clinical features, or to undertake predictive testing for adult onset disorders. In these situations, it is generally advised that testing should be delayed until the age of 16. Occasionally special circumstances may arise such as in the cases of major

parental anxiety, or where young people are acting as transplant donors, going for adoption, or are pregnant. In these situations referral to a clinical geneticist is recommended. It should be noted that these concerns are not specific to DNA analysis but apply equally to any inherited condition.

SCREENING PROGRAMMES

A number of general population or sub-population screening programmes include DNA analysis. Such programmes are provided where a set of recognised criteria are met that relate to the severity of the condition in question, the performance of the test, the treatment options and their effectiveness, the acceptability of the screening programme, and the overall benefit to the population. Examples of common screening programmes where DNA is used (though often as the secondary level of testing) include phenylketonuria, cystic fibrosis, haemoglobinopathies, medium chain acyl-CoA dehydrogenase deficiency, and deafness. The number of newborn screening programmes has increased in many countries including the UK,⁹ so paediatricians should bear in mind that for some disorders tests may have already been performed at an earlier date. However, it is possible that individual cases may have escaped testing or that a diagnosis may be missed due to limited assay sensitivity. Samples from these earlier tests may also be available if needed for further analysis.

Sub-population screening

These tests may be requested where a high sensitivity test is available for a disorder for which an early diagnosis has a benefit and the risk of being affected is greater than that in the general population. Examples include (i) testing of specific mutations associated with Tay Sachs, Gaucher, Canavans disease, familial dystonia, glycogen storage disease type 1a, mucopolidosis IV, Niemann Pick disease, Fanconi anaemia, and Blooms syndrome in the Ashkenazi Jewish population, (ii) screening of MYH7 and other selected genes associated with hypertrophic cardiomyopathy in sudden cardiac death of the young, and (iii) testing to define the monogenic subtypes of maturity onset diabetes of the young that may change treatment. This is a rapidly changing area of medicine and there are several websites that provide an up to date comprehensive list of disorders for which testing may be available along with background information. Particularly useful sites are ORPHANET (EU based; <http://www.orpha.net/international/Orphanet-uk-1.htm>) and GeneTests (US based; <http://www.geneclinics.org/>).

CHECK LIST OF INFORMATION REQUIRED FOR DNA BASED ANALYSIS

- Identification of patient (name, date of birth, address, sex)
- Family information (pedigree)
- Ethnicity
- Consent to investigation, storage, research use
- Identification of sample (date of collection, pre or post transplant, etc)
- Purpose of the analysis (molecular confirmation, carrier testing, etc)
- Nature and priority of tests
- Results from other relevant investigations (biochemistry, immunology, etc)
- Urgency
- Sample source (tissue source)
- Minimum sample quantity obtained
- Appropriate collection medium (potassium EDTA antic-oagulant, etc)

CONCLUSION

The use of diagnostic DNA analysis is determined by the analytical validity, clinical validity, clinical usefulness, and ethical, legal, and social considerations. At present most DNA analysis is limited to single gene disorders that are individually rare but collectively account for a significant percentage of childhood disease. The current limitations of DNA analyses are set by our limited understanding of gene-gene interactions, gene-environment interactions, and the level of normal variation throughout the genome.¹⁰ In the near future there is likely to be greater use of DNA analysis for optimising drug treatment, for whole genome analysis (as traditional cytogenetic analysis is superseded by DNA based analysis), and for the diagnosis of complex but common disorders such as asthma. In the more distant future some DNA analysis may be superseded by more functional protein assays. The extent of testing will also be governed by public opinion relating to ethical, social, and legal implications.

ELECTRONIC-DATABASE INFORMATION

Sites of interest: British Society of Human Genetics, www.bshg.org.uk; Genetic Interest Group (GIG), national alliance of patient support groups, <http://gig.org.uk>; GeneTests homepage (National Institutes of Health, USA), <http://www.geneclinics.org/>; ORPHANET database on rare diseases, <http://www.orpha.net/international/Orphanet-uk-1.htm>; and UK Genetic Testing Network, www.genetic-testingnetwork.org.uk.

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APPENDIX

GLOSSARY

Allele

One of the alternative versions of a gene that may occupy a given locus

Association

A tendency of two characters (disease, marker, etc) to occur together at non-random frequency

Bayesian calculations

A mathematical method for combining probabilities where alternative hypotheses are assigned probabilities for each item of information that are then combined together to give a relative likelihood

Conservation across species

The occurrence of the same amino acid (or nucleic acid) in the same relative position of the protein or gene in the same or related structure of different organisms

Double mutant alleles

A gene that has two mutations, both of which occur on the same maternal or paternal copy of the gene

Expressivity

The extent to which a genetic defect is expressed. If there is variable expressivity, the trait may vary in expression from mild to severe but is never completely unexpressed in individuals who have the corresponding genotype

Genetic heterogeneity

The production of the same or similar phenotypes by different genetic mechanisms. Locus heterogeneity refers to the production of identical phenotypes by mutations at two or more different chromosomal locations

Germline

The egg and sperm cells and those cells that give rise to them

Haplotype

A series of alleles found at linked loci on a single chromosome

Imprinting

Determination of the expression of a gene by its parental origin

Informative

DNA analysis able to distinguish between the maternal and paternal alleles carried by an individual

Entrance

The frequency with which a given genotype manifests itself with given symptoms of a disease

Phase (of disease)

The association between a genetic marker and the disease status used to identify the high or low risk marker

Recombination

The formation of new combinations of alleles on the same chromosome by crossing over between the loci during replication

Sensitivity

The frequency with which the test result is positive when the disorder is present

Specificity

The frequency with which a test result is negative when the disease is absent