

### 3. RISK ASSESSMENT

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Throughout the history of medicine, patients and their relatives at risk of a disorder have been sought information and advise on the consequences of a disorder, the probability of developing or transmitting it and of the ways in which this may be prevented or avoided. Genetic risk assessment is the basic essence of clinical genetics. Clinical geneticists focus on probability and risk estimate, and on communicating both of these to patients via genetic counselling in ways that optimize decision making about their reproductive options or prophylactic measures to decrease their disease risk. In the molecular genetics era this demand on clinical genetics has been growing and arises also as claim of symptomless healthy persons.

#### 3.1 General recommendation for genetic risk assessment

##### 3.1.1 Risk assessment and genetic counselling

Risk assessment is inseparably interwoven with genetic counselling. When the persons ask for counselling they want to know their individual risks, while the risk estimates are transmitted to the individual in genetic counselling settings. Risk assessment has to be provided or supervised by a health-care professional trained for genetic counselling.

The three main elements of genetic counselling are:

- *Finding diagnosis* - Without diagnosis all advice has an insecure foundation. A clear diagnosis should be made as firm as possible before risk estimates are given to those seeking advice. Collecting genetic information is the first and most important step and is best achieved by drawing up a pedigree. Recent advances in molecular genetics have begun to elucidate the genetic mutations underlying many single-gene diseases. However, it is important to emphasise that an abnormal test result in itself is not equal with a clinical diagnosis. The result of genetic tests should always be related to the associated clinical condition. It is irrelevant binding a test result to an individual, by saying simply that he/she has more/less/variant DNA without relating the aberration to a definitive clinical condition.
- *Risk assessment* - Information on genetic risks is rarely an absolute 'yes' or 'no'. Risk figures in genetic counselling may be given either as odds or as percentages. Some people prefer to use odds and to quote risks as 1 in 10, 1 in 100, etc. Others prefer to use such figures as 10 per cent, 1 per cent. In the genomic era, complex mathematical models, algorithms and softwares are known for the multivariable logistic regression analyses of research data. Whatever method is used, there are pitfalls in interpretation which must be avoided, and this may require much practice.

Risk estimates may be based on different sorts of information and may be of greater or lesser reliability. The main categories are as follows (P.S.Harper).

*Empiric risks* Here the estimate is based on observed data rather than theoretical predictions; this is the form of risk estimate available for most of the more common non-mendelian or chromosomal disorders.

*Mendelian risks* Mendelian risk estimates can only be given when a clear basis of single gene inheritance can be recognized for disorder. They are perhaps the most satisfactory form of risk estimate because they commonly allow a clear differentiation into categories of negligible risk and high risk.

*Modified genetic risks* The essential feature is that a 'prior' genetic risk, based on mendelian inheritance, may be modified by 'conditional' information, usually genetic, but sometimes from other sources. Such modifying information may drastically alter the risk estimate and should always be used when available.

*Composite risks* Most empiric risks really fall into this mixed situation. A clinical entity may have different genetic background with different inheritance, resulting in an intermediate risk depending on the relative frequency of the various forms. Obviously this intermediate risk does not really exist at all – the family must represent one or other of the extreme positions. With improved resolution of genetic heterogeneity it may be possible to distinguish the individual components, while even within a single family additional information may resolve the situation.

- *Communication of the genetic information* - Communicative role ensuring that those seeking information actually benefit from it. Clinical geneticists and genetic counsellors should make genetic information available according to universal ethical principle which respect the individual's dignity, autonomy, religious and cultural beliefs. Free informed consent, privacy, confidentiality have to be regarded as strict conditions when persons are helped in their decision making.

Genetic counselling cannot be compulsory, no more than any other medical act, however, it should be offered and strongly recommended before and after genetic testing.

*Pre-test genetic counselling* has to inform the individuals what the test is for, include up-to-date, reliable description about symptoms and natural history of the disease, prospects of prevention or treatment, inheritance pattern, the risk of disease in the counsellee's situation, available reproductive choices, reliability and limitations of the test concerned, and possible psychological impact and other consequences of the test result to the counsellee and his/her family/relatives.

*Post-test genetic counselling* - in addition to the main points of pre-test counselling, a plan to inform relatives in relation to their risk has to be agreed with the counsellee (or, if necessary, a decision to discuss this further, after a time to reflect). Implications to the individual (including a follow-up plan, when relevant) and his/her near relatives should be discussed.

### 3.1.2 Some complexities in risk assessment

#### *Penetrance*

In many (but not all) single-gene, mainly autosomal dominant (AD) diseases a person with a specific genotype is virtually certain to develop the associated disease. The likelihood that a person carrying a disease-associated genotype will develop the disease is known as the penetrance of the genotype. Eg., both Huntington disease and cystic fibrosis are virtually 100% penetrant. The mutations in the BRCA1 gene are highly penetrant, but not completely so; estimates of the lifetime penetrance of these mutations vary from about 60-85%. Variations in penetrance are caused by the modifying effects of other genes and/or by environmental factors.

### *Inherited or new mutations*

The mutations underlying some, mainly autosomal recessive (AR) disorders (eg. cystic fibrosis) appear to have arisen many generations ago, with very few new mutations arising. There are other diseases, however, mostly AD disorders, for which new mutations appear to be more frequent, so an affected individual may carry a mutation that is not present in either parent. If this is the case, the parents usually have a low risk of having another child with the same disease.

### *Genetic heterogeneity*

Some single-gene diseases, clinically fairly well circumscribed entities, may be caused by different mutations. Genetic heterogeneity resulting from disease-causing mutations at different genetic loci is called non-allelic or locus heterogeneity. When different mutations at the same locus cause the same disease, this is known as allelic heterogeneity. Allelic heterogeneity is very common.

### *Variable expressivity*

Even if a disease genotype is fully penetrant, the severity and symptoms of the disease can vary in different affected individuals, presumably because they are influenced by other genetic and environmental factors. Variable expressivity can be considered the rule rather than the exception for virtually all genetic disease.

## **3.2 Risk assessment based on clinical data**

### **3.2.1 Chromosomal abnormalities**

The great majority of chromosomal disorders have an extremely low risk of recurrence in a family, except those of translocation type.

There is a well-known relationship between the incidence of trisomy 21, the most important chromosome disorder and maternal age. The population incidence is around 1 in 650 live births, while the risk of Down syndrome reaches 1% at the age of 40 years. Paternal age is of little significance. Other trisomies are rare as live births, while extremely common in spontaneous abortions.

Familial accumulation of chromosome rearrangements is possible when the abnormality is translocation type. The recurrence risk in such a situation depends on whether there is an abnormality in the parental chromosomes. If the chromosomes are normal, as in the great majority, the risk to further offspring is minimal. If one parent has an abnormal karyotype (balanced translocation), risks are 1-100% depending on the chromosomes involved and the parental origin.

During the past few years a new type of chromosomal anomaly was delineated by molecular cytogenetics techniques recognizing small (submicroscopic) deletions in a number of different malformation syndromes. The recurrence risk in these *microdeletion syndromes* is low, since the great majority of such cases are de novo rearrangements.

### **3.2.2 Single gene (or mendelian) disorders**

If the clinical and genetic information for a family with a particular disease suggest single gene disorder, then it is likely that precise risk can be given regarding its occurrence in other family members. Mendelian inheritance may be established on the basis of the pedigree, by a

combination of clinical diagnosis and the pedigree, or entirely on the clinical diagnosis (sporadic cases).

Identifying carriers of genetic disorders in families or populations at risk plays an important part in preventing genetic disease. In families in which there is a genetic disorders some members *must* be carriers because of the way in which the condition is inherited. These *obligate carriers* can be identified by drawing a family pedigree and do not require testing as their genetic status is not in doubt.

It should be emphasised that mendelian inheritance can not be regarded as a rigid and unvarying mechanism following a fixed set of rules. One of the most fascinating developments of recent years has been the discovery of fundamental biological mechanisms underlying non-mendelian monogenic inheritance (see below).

- *Autosomal dominant (AD) inheritance*

Although in theory AD inheritance is the simplest mode for risk assessment, in practice it provides some of the most difficult problems with special traps. The 50% risk of developing a condition for the offspring of an affected person may be modified by the age-of-onset of the disorder, the homo- or heterozygous state of the affected person (which is usually unknown), the lack of penetrance, variation in expressivity, and various factors underlying variability in mendelian disorders such as genomic imprinting, anticipation due to unstable DNA, gametic mosaicism, modifying alleles, somatic mutation (see: non-mendelian monogenic inheritance).

In AD condition, obligate carrier is a person with affected parent and child. Testing for carrier state applies only to disorders that either are variable in their manifestation or have a late onset. Because of possibility of germline mosaicism (the real frequency is not known), the parental carrier state can not be excluded with certainty in families with single affected child suggesting new mutation.

- *Autosomal recessive (AR) disorders*

The principal difficulty with AR inheritance is to be sure that this is indeed the mode of inheritance in a particular family, since the great majority of cases of an AR disorder are born to healthy but heterozygous parents, whose high risk can not be detected from isolated case. Where the diagnosis makes this mode of inheritance certain, or in the minority of families where the genetic pattern is clear, risk prediction is relatively simple (see mendelian rules).

It is important to know how to estimate the chance of being a carrier for an AR disorder, both for family members and for the general population. The parents and children of a patient are obligate carriers, while second-degree relatives will have a 50% chance of being a carrier. Testing may be appropriate for the healthy siglings of an affected person (and their partner), and for consanguineous couples with a positive family history on both parental lines (and not just one!). The possibility of new mutation can be ignored, lack of penetrance is rarely encountered, and variation in expression is much less than in AD disorders.

The actual risk depends on the frequency of heterozygotes in the population, what can be estimated indirectly from the disease frequency by the Hardy-Weinberg equilibrium (direct observation are exceptionally available). The main opportunity for preventing AR disorders would be in population screening programmes to identify individuals at risk.

One of the other problems with AR disease includes the risk assessment when both parents are affected by an AR condition (deafness, albinism). The risk to the offspring in such situation will depend on whether the parents share the same disease-causing genes (high risk), or they carry different alleles (risk is not increased).

- *X-linked disorders*

The term 'dominant' and 'recessive' must be used with caution, because a much greater degree of variability in the heterozygous female is seen than is the case with autosomal disorders. This is largely the result of X-chromosome inactivation (Lyon hypothesis).

Recognition of an X linked pedigree pattern is often overlooked and hence, risk assessment may be inappropriate and may be mistaken for AD inheritance. Aside from the new mutations (a mother of an affected boy is not always a carrier!), problems could be resulted from some particular conditions, eg. the disease is X-linked dominant, males with the disease do not reproduce, or the X-linked dominant disease is lethal in the male, etc.

The carrier state of the mother of an affected son may be particularly difficult to assess because of the possibility of new mutation in the child. Obligate carrier is a woman with two affected sons, or one affected son and another affected male maternal relative; the daughters of an affected man are also obligate carriers. Anyway, obligate carriers should be identified, even in the molecular era, since information from carrier testing is not always easy because of the variability of X-inactivation and gene expression in heterozygotes females.

- *Isolated cases*

Pedigrees showing only one affected person are the type most commonly encountered in clinical practice. Various causes must be considered, and risk assessment depends entirely on reaching an accurate diagnosis in the affected person. AR and X-linked recessive disorders are brought up first since, as a rule, such inheritances manifest themselves by a single case emerging in the family. Isolated cases with AD disorders may be resulted from new mutation, non-paternity, or germ-line mosaicism. In AD conditions, new mutations can usually be distinguished from transmitted cases. In X-linked recessive disorders, however, it may be extremely difficult to tell whether an isolated case represent a new mutation or whether the mother is a carrier.

- *Mitochondrial inheritance*

It is well known that any disorder following mitochondrial inheritance should be exclusively maternal in its transmission. All daughters of an affected or carrier females are themselves at risk of transmitting the disorder, as well as of becoming affected, while all sons are at risk of becoming affected (but not as transmitter).

### 3.2.3 Common complex disorders

Most common disorders (birth defects, chronic later-onset diseases) do not follow any of the clear patterns of mendelian inheritance. Yet, to some degree these conditions show a familial tendency, therefore, families where such disorders occur increasingly seek genetic counselling for risk assessment.

The fact that many of these disorders (alternative terms are '*multifactorial*' or '*polygenic*') form the basis of activity for most medical specialists and primary care doctors, creates special challenges. Available risk information for non-geneticists is often inadequate and changing



rapidly as advances in research alter specific genetic factors. This is mainly the reason why the genetic education of non-geneticists health professionals is a European primary.

The well known general rules of risk estimation in common complex disorders are based on epidemiological data available in handbooks and web sites, and such information provides the most satisfactory basis for risk assessment until the genetic basis can be resolved further. However, these empiric risk figures are not universal in their application. Eg., data on one population may not be applicable to others. Improved identification of specific causative factors may radically change risk estimates. Risks may depend also on individual factors, not only on the diagnosis.

Problems include also the involvement of more than one gene (gene-gene interaction), each of which may have only a small effect on disease susceptibility; uncertainties in disease diagnosis; different genetic polymorphisms underlying disease in different populations, and the large effects of environment and lifestyle on the development of disease (gene-environmental interactions). Although each of the underlying genes is inherited according to Mendel's rules, the disease itself is not inherited in any simple mendelian way.

*Cancer* is often described as a 'genetic disease' following rules of multifactorial inheritance, in the sense that it is caused by genetic alterations and influenced by environmental factors. However, the genetic alterations that lead to cancerous behaviour occur in the somatic cells of the body and are not passed on to the next generation. Therefore, risk assessment, instead of estimate the risk of disease transmission to the offspring, is focused on the outcome of cancer, and the ways in which this may be prevented or avoided by prophylactic measurements (decision-making before, eg. mastectomy in breast cancer). There are only some germ-line mutations (that is, mutations that are present in all the cells of the body including the sex cells) that predispose people who carry them developing cancer, and these are heritable.

### 3.3 Risk assessment based on molecular genetic testing

In growing number of diseases, genetic risk assessment is possible on the basis of genetic test results indicating whether a person is a carrier of a disease-causing mutation, or results determining whether an individual has a specific genetic susceptibility to a disease. *Clinical validity* and *clinical utility* are going to be increasingly important criteria in the professionals' offer whether or not to apply a genetic test.

#### 3.3.1 Need for genetic counselling in various form of testing

The offer and application of molecular genetic tests should be a part of comprehensive clinical genetic service, and associated with genetic counselling which may vary according to the different form of testing.

*Diagnostic testing* (genetic test performed in a symptomatic individual to diagnose or rule out a genetic condition): such tests may have a status that is similar to other diagnostic tests, and pre-test counselling might be unnecessary. However, if the test result is positive, the patient needs post-test counselling and the relatives will need risk assessment in genetic counselling setting.

*Presymptomatic testing* (genetic test in a healthy high-risk family member for a later-onset monogenic disorder): even if the family has already been counselled, further pre- and post-test genetic counselling has to be offered.

*Susceptibility testing* (simultaneous testing of several genetic markers): referred also as risk profiling for common complex disorders, it is only emerging. The clinical validity and utility of these tests needs to be proven. At present, it seems very likely, that they will be prescribed mainly by specialists other than clinical geneticists; and the need for proper risk assessment and genetic counselling by a genetic specialist will depend on the possible implications of the results of the test for the person and his/her near relatives. The same applies to pharmacogenetic testing, which tests for a genetic susceptibility for adverse drug reactions or for the efficacy of a drug treatment with a given genotype.

*Carrier testing*: genetic test that detects a gene mutation that will not have any consequence to the health of that individual; however, if inherited, alone (in case of X-linked inheritance, AD premutation or chromosomal translocation) or in combination with another mutation in the same gene from the other parent (in case of AR inheritance), it may confer a high risk of disease in the offspring. Pre- and post-test genetic counselling needs to be offered.

*Prenatal testing* (genetic test performed during a pregnancy): risk for a certain condition in the foetus should be assessed and pre- and post-test genetic counselling for the prospective parents needs to be offered.

*Preimplantation genetic diagnosis* means testing the presence of a mutation or chromosomal change in one or two cells of an embryo in a family with a previously known risk for a mendelian or chromosomal disorder in order to select the unaffected embryos to be implanted. Risk assessment, pre- and post-test genetic counselling for the prospective parents has to be offered.

### **3.3.2 The clinical application of molecular testing for risk assessment**

#### **3.3.2.1 Single gene (mendelian) disorders**

Molecular analysis is potentially possible for any single-gene disorder, especially those where the gene has been isolated or mapped. However, it is necessary to point at the growing gap between what has been discovered and what is available in service.

When interpreting the finding of an apparently specific mutation, firm proof for the association between cause and effect are needed. In the early stages of research after a gene is isolated, it may be far from clear whether a particular change is a causative mutation or is a harmless normal variation (polymorphism) unrelated to the disease state (population data, testing of the healthy parents, or protein truncation test are needed to resolve this question).

Another important point about risk assessment is that genetic testing will only reveal the presence or absence of the factor(s) being tested for. Because of genetic heterogeneity, it is important wherever possible to identify the specific mutation associated with disease in an affected member of a family. If a mutation can be found, it is then possible with great accuracy to determine whether other family members carry the same mutation.

A negative result from genetic testing, although it lowers the probability that the individual carries a disease-causing mutation in that gene, cannot eliminate it altogether. The residual risk

has several components: the possibilities of a mistake in the test itself (no test can be 100% accurate!), allelic heterogeneity, and new mutations.

In mitochondrial diseases, it is not known whether there is any correlation between the proportion of abnormal mitochondria found in blood and the risk of developing or transmitting the disorder. Genetic tests are equally unhelpful in prenatal diagnosis. Thus the conclusion at present has to be that while the recognition of mitochondrial inheritance by pedigree pattern and molecular analysis is important in identifying genetic risks and in removing risk from descendents in the male line, genetic tests are of limited use in resolving the situation for those known to be at risk.

### **3.3.2.2 Presymptomatic genetic testing for risk assessment**

Presymptomatic genetic testing is the use of genetic testing to tell whether a symptomless individual will develop a genetic disease later in life. It can only be used where the disease-associated mutation is known and is highly penetrant. Eg., in Huntington disease DNA testing at any age, even prenatally, will reveal whether the mutation is present, changing that person's individual risk from 50% to either 100% or zero. According to good clinical practice, presymptomatic tests for future severe illnesses with no options for treatment or prevention should never be performed without pre- and post-test genetic counselling, as well as psychosocial evaluation and follow-up.

### **3.3.2.3 Carrier testing**

Carrier testing for autosomal-recessive disease is widely practiced in developed countries with ethnicities characterized by particularly high disease-causing allele frequencies, such as beta-thalassaemia in Mediterranean countries, or Tay-Sachs disease in Ashkenazi Jews. Such testing is usually carried out in the format of formal, community run programmes, with clinical validity and utility well established.

### **3.3.2.4 Predictive testing of genetic susceptibility for common complex diseases**

The growing ability to map and isolate specific genes involved with common disorders has allowed two main categories to be defined:

- *disorders containing a significant mendelian subset*, resulting from the action of a single major gene with relatively high penetrance in the family. – The recognition of such a subset of cases gives extremely high risks for family members or very low risk if the particular gene mutation can be excluded. It is no longer meaningful to derive overall theoretical risks eg. in breast or colon cancer, since the separation of the mendelian forms will affect the recurrence risks for the remaining.
- *disorders where the genes/variants are of low penetrance*, each (and usually also the environmental factors) being of moderate or small influence. – The ability to detect different susceptibility genes or allelic variants associating with an increased risk of diseases like asthma, diabetes, psychoses, etc. will influence risk assessment and genetic counselling. However, because of incomplete penetrance of these genes or variants, they cannot be used with certainty to predict the development of disease. Furthermore, the genes (usually many) interact in complex ways with each other and with environmental and lifestyle factors to determine whether disease will develop.

Research for most common disorders is still at an early stage in resolving the number and nature of genes involved, and risk prediction is weak. The use of genetic susceptibility tests in



risk prediction is at present of minimal help and potentially of considerable harm. However, this situation may change in the future, and genetic susceptibility testing may find a place in mainstream clinical medicine.

### 3.3.2.5 Genetic testing/screening to predict the future health status of a healthy individual

Predictive genetic testing of seemingly healthy people can principally be applied individually or collectively. The aim is to detect individuals who are at risk of developing a particular disease or of responding badly to a particular treatment.

An *individual approach* is chosen when the person is at recognizably elevated prior risk to develop a specific disease because of a relevant family history, such as late-onset disorders, particularly familial cancer (breast, bowel, thyroid, and others) and neurodegenerative disorders, even to children, if there is a proven clinical benefit ensuing from testing before adulthood.

A *population screening* could either be applied generally (e.g. newborn screening for metabolic disorders), or to subgroups preselected on the basis of risk factors. The use of molecular genetic screening currently has some legitimacy in *certain monogenic condition*, but no established value with respect to *common complex diseases*. The risk estimates applied to individuals is generally very uncertain, with wide margin of error. The failure to replicate the majority of initial results of association studies between genetic variants and disease risk is likely to be at least partly due to inadequate sample sizes, poor or inappropriate statistical analysis, poor study design, indirect assessment of causal pathways, complexity of the phenotypes studied, and the complexity of allelic or genotypic contributions to phenotype. Further pragmatic randomised controlled trials need to be done to determine whether genetic testing has clinical benefit in management of common complex disorders

#### Recommended literature:

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