Cytogenetics: Applications

Chiyan Lau

Introduction

In the previous chapter, we discussed the laboratory techniques used to detect various types of genetic abnormalities, from single nucleotide changes to changes that affect entire chromosomes. We discussed the principles behind some of the more commonly used cytogenetic and molecular genetic techniques. In this chapter, we will look at how these techniques are used clinically in various diseases/conditions including cancer and constitutional disorders, and we will discuss some of the considerations that may go into deciding what would be appropriate genetic tests to perform in these clinical scenarios.

Cancer Cytogenetics

One important area of application for cytogenetic techniques is in cancer management, to detect somatic genetic changes in the neoplastic cells. This is particularly relevant for haematological malignancies, but there are increasing numbers of solid tumours where cytogenetics has a role. In cancer, cytogenetic investigations can be used to help with diagnosis, inform prognosis, or help prioritise treatment options. The following are some examples. We shall also discuss situations where molecular genetic techniques may be more appropriate.

C. Lau (✉)
SEALS Genetics, NSW Health Pathology, Level 4, Campus Centre,
Prince of Wales Hospital, Randwick, NSW 2031, Australia
e-mail: chiyan.lau@health.nsw.gov.au

S.R. Lakhani, S.B. Fox (eds.), *Molecular Pathology in Cancer Research*,
DOI 10.1007/978-1-4939-6643-1_4

Diagnosis and Monitoring

Chronic Myelogenous Leukaemia

Chronic myelogenous leukaemia (CML) is a myeloproliferative neoplasm, a clonal proliferation of haematopoietic stem cells of one of the myeloid lineages. One of the defining features of CML is the presence of the Philadelphia chromosome (Ph), which is present in 90–95 % of CML cases [1]. The Ph chromosome is the result of a reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11.2). This structural rearrangement results in the creation of a fusion gene formed from the 5′ part of BCR on chromosome 22 and the 3′ part of ABL1 on chromosome 9. ABL1 is a tyrosine kinase, and the active promoter element of BCR in myeloid cells overactivate the transcription of the fusion BCR-ABL1 product, leading to constitutive activation of tyrosine kinase signalling, resulting in deregulated cell proliferation [2].

The Ph chromosome looks like a shortened chromosome 22, and was historically detected by standard karyotyping on bone marrow aspirate [3]. It is now more commonly detected by FISH using fusion probes, where the ABL1 breakpoint region on chromosome 9 is targeted with a FISH probe labelled with one colour (e.g. red), and the BCR breakpoint region on chromosome 22 is targeted with a probe labelled with a different colour (e.g. green). In a normal cell with no Ph chromosome, there will be two red and two green signals. If a t(9;22) translocation is present in the cell, there will be two yellow fusion signals, together with one red and one green signal. The use of FISH has an advantage over karyotyping in that ~5–10 % of CML cases do not have a typical Ph chromosome. Some of these are atypical translocations involving a third or even fourth chromosome, while others are cryptic translocations which cannot be detected by karyotyping [4]. In these atypical Ph-negative cases, BCR-ABL1 fusion is still present, and therefore can be detected by FISH. Another advantage of FISH is that it can be performed on interphase cells, thus eliminating the need for cell culture.

At the time of diagnosis, the number of CML cells in the patient's blood is high, therefore BCR-ABL1 fusions are quite easy to detect by FISH. However, once the patient has undergone treatment, e.g., with the kinase inhibitor imatinib (Glivec), the number of cells from the CML clone would decrease and eventually fall below the limit of detection of karyotyping and FISH if treatment is successful ('Complete Cytogenetic Response') [5]. In order to continue to monitor the patient for signs of relapse at this stage of the disease, a more sensitive method is required, such as real-time PCR [6]. This molecular method quantifies the number of copies of BCR-ABL1 transcripts in the patient sample, and allows early detection of relapse before the CML clone has expanded above cytogenetically detectable levels. Loss of disease control may be due to ABL1 kinase site mutations leading to acquired resistance to therapy, and can be detected by ABL1 sequencing [7]. Depending on the mutation, changes in treatment (e.g. to newer generation kinase inhibitors such as nilotinib or dasatinib) may be possible to maintain control over the CML [8, 9]. Therefore, while cytogenetic methods are useful at the time of CML diagnosis, molecular methods are the investigations of choice during minimal residual disease monitoring [10].

Prognosis and Management

Multiple Myeloma

Multiple myeloma (MM) is a malignant proliferation of plasma cells. It is a heterogeneous disorder, with several subtypes differing in prognosis and the underlying genetics. There are a number of recurrent chromosomal abnormalities in MM which are predictive of prognosis, therefore standard cytogenetics and FISH analyses can be useful in MM.

Multiple myeloma can be broadly divided by genetic changes into two main groups: hyperdiploid and non-hyperdiploid. The hyperdiploid group (h-MM) shows numerous trisomies (resulting in chromosome number >46) and generally has a better prognosis. This group has a low prevalence for translocations involving the immunoglobulin heavy chain gene locus (IGH) at 14q32. In contrast, the non-hyperdiploid group (nh-MM), which includes hypodiploid, pseudodiploid, and near-tetraploid cases, tends to have a poorer prognosis. This group is enriched for 14q32 IGH translocations, although not all IGH translocations confer a poor prognosis. Specifically, the t(11;14) IGH/CCND1 translocation appears to have a neutral or even favourable prognosis, while the t(4;14) IGH/FGFR3-MMSET translocation and t(14;16) IGH/MAF translocation are associated with poorer survival. Other cytogenetic changes which are markers of poor prognosis in MM include deletion of the TP53 gene at 17p13 which codes for the p53 tumour suppressor protein, 1q21 gains, 1p21 deletions, and cytogenetically detected monosomy 13 and 13q deletions [11, 12].

Interphase FISH can be used to detect these chromosomal changes. One strategy is to use a 14q32 break-apart probe to determine if the IGH locus is involved in a translocation, and if so, specific fusion probes for t(11;14), t(4;14), and t(14;16) can be used to determine the fusion partner. Karyotyping can also provide useful information on other chromosomal abnormalities which may be present, but can be problematic because plasma cells from the MM clone often show poor growth in culture. This results in an apparently normal karyotype result since the metaphases are dominated by normal cells. Sole use of karyotyping for MM cytogenetics is therefore not recommended.

For FISH testing in MM, to maximise sensitivity, it is recommended that either purified plasma cells are used, or that FISH analysis/scoring is confined to plasma cells by performing cytoplasmic immunoglobulin-enhanced FISH (cIg-FISH) [13] or CD138 immunostaining [14]. The reason for this is that the bone marrow aspirate specimen for cytogenetics often has a low concentration of plasma cells from the MM clone, and this is further lowered by the effects of haemodilution. Standard interphase FISH without cell enrichment would have a low sensitivity of abnormality detection on such a sample. Enrichment of the sample for plasma cells can be achieved by using anti-CD138-conjugated beads. Alternatively, the use of cIg-FISH allows identification of plasma cells during FISH analysis by immunofluorescently staining cytoplasmic kappa or lambda light chains. This allows FISH scoring to be restricted to plasma cells. These strategies maximise the chance that FISH analysis is obtained on cells relevant to the disease process.

Microarrays also play a role in current research in MM, for example, to identify new cytogenetic changes which may have prognostic significance [15]. However, standard arrays are unable to characterise translocations which play an important prognostic role in MM, and at the present time, arrays are not widely used for clinical testing in MM.

Chronic Lymphocytic Leukaemia

Chronic lymphocytic leukaemia (CLL) is a neoplastic disorder of mature B lymphocytes, where the neoplastic cells are commonly present in peripheral blood, bone marrow, spleen, and lymph nodes [16]. A number of recurrent cytogenetic abnormalities are commonly found in CLL cases, including deletions at 13q14.3, trisomy 12, and deletions at 11q22-23 (including the ATM gene) and 17p13 (including the TP53 gene) [17]. Among these, the deletions at 11q and 17p are associated with adverse prognosis, while isolated deletion at 13q or the absence of cytogenetic changes is associated with a more favourable disease outcome. These cytogenetic changes, when used in conjunction with other clinical and laboratory information, may also help to guide choice of therapy [18]. Commonly, a FISH panel consisting of probes which map to 13q14.3, ATM, TP53, and CEP12 (centromere of chromosome 12) are used for detection of the common cytogenetic changes in interphase peripheral blood cells. Conventional karyotyping in CLL is more difficult and less sensitive than FISH owing to the poor growth of CLL cells in culture, and karyotyping has a lower resolution for small deletions. Microarray-based testing (especially SNP arrays) may become a viable alternative to FISH [19], since it also does not require dividing cells, has a high enough resolution for small deletions and is a survey of the entire genome (and can therefore detect additional chromosomal abnormalities other than those targeted by specific FISH probes), although currently it has not yet been widely adopted in clinical CLL testing outside of research settings.

In addition to cytogenetic changes, somatic hypermutation status at the IGHV locus (immunoglobulin heavy chain V region) also has prognostic significance, with CLLs which show hypermutation having a better prognosis than unmutated cases [20, 21]. Also, there is emerging evidence that pathogenic mutations in the TP53, BIRC3, SF3B1, and NOTCH1 genes also influence prognosis [22, 23]. These changes cannot be detected by cytogenetic techniques, but require molecular sequencing methods for detection.

Acute Leukaemias

In patients affected by acute leukaemias, including acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL), disease classification and prognosis depend substantially on the genetic abnormalities in the malignant clone [24]. There are a number of recurrent cytogenetic rearrangements which define particular subtypes of AML. These include translocations and inversions such as t(8;21)(q22;q22)

[RUNX1-RUNX1T1], inv(16)(p13.1q22)/t(16;16)(p13.1;q22) [CBFB-MYH11], and t(15;17)(q22;q12) [PML-RARA], which are associated with better chances of long-term survival [25, 26]. The PML-RARA rearrangement is specifically associated with a subtype of AML known as acute promyelocytic leukaemia (APL), which responds to treatment with all-trans retinoic acid (ATRA) [27]. Other rearrangements such as inv(3)(q21q26.2)/t(3;3)(q21;q26.2) RPN1-EVI1 and t(6;9)(p23;q34) DEK-NUP214, as well as monosomies of chromosome 5 and 7, or complex karyotype (defined as 3 or more clonal cytogenetic abnormalities) are associated with poorer outcomes [25, 26]. Likewise, in ALL of precursor B cell origin (B-ALL), some cytogenetic changes have prognostic significance and are also used to define disease subgroups and determine treatment options. For example, rearrangements involving the MLL gene at 11q23 (especially t(4;11) MLL-AF4 translocations) are associated with poor prognosis [28]. The presence of the Philadelphia chromosome t(9;22) also confers a poor prognosis in adult ALL, although the availability of imatinib treatment for this subgroup has improved patient survival [29].

Conventional karyotyping of bone marrow aspirate specimens is the standard method for detecting these cytogenetic abnormalities in acute leukaemias. However, in malignancy karyotyping, often the chromosome quality is poor, therefore FISH testing (e.g. using fusion probes for specific rearrangements) is sometimes used to confirm karyotype findings.

In addition to cytogenetic changes, mutations in genes such as FLT3, NPM1, and CEBPA also have prognostic significance in acute leukaemias, but these require molecular methods for detection [26, 30].

The prognostic information from cytogenetic (and molecular) studies allow the clinician to adjust the aggressiveness of treatment and balance the risks and benefits of offering allogeneic stem cell transplant, which is ultimately the treatment option that offers a chance of cure in poor risk patients but is associated with significant morbidity and mortality.

Non Small Cell Lung Cancer

Non-small cell lung cancer (NSCLC) is an aggregate category of lung cancers which includes adenocarcinoma, squamous cell carcinoma, large cell carcinoma as well as a number of rarer histologic subtypes. Most cases are discovered at an advanced stage of disease and prognosis is poor. Traditionally, in metastatic NSCLC the main pharmacological treatment option was combination cytotoxic chemotherapy including platinum-based agents. Recently a number of genetic abnormalities have been found in NSCLC (especially adenocarcinoma) which allow targeted treatment of those subgroups of patients whose NSCLC carry specific mutations [31].

One of the specific mutations in NSCLC is a chromosomal structural rearrangement involving the anaplastic lymphoma kinase (ALK) gene, which codes for a receptor tyrosine kinase. This rearrangement is found in approximately 2–7 % of NSCLCs, although it appears to be enriched in never- or light-smokers

[32, 33]. The most common form is a paracentric inversion on the short arm of chromosome 2 which creates a fusion product between the 5′ end of the EML4 gene at 2p21 and the 3′ end of the ALK gene at 2p23.2 [34]. This leads to activation of kinase signalling in the affected cell. The clinical importance of this ALK rearrangement is the availability of a small molecule ALK inhibitor crizotinib which has been shown to improve outcomes in patients who carry the ALK rearrangement [33].

The most common way to detect ALK rearrangements is by the use of a dual colour break-apart FISH probe located at the 3′ end of ALK [32]. Karyotyping is inappropriate because commonly the only specimen type available in NSCLC are formalin fixed paraffin embedded (FFPE) tumour biopsies, which do not contain viable cells capable of dividing. The use of break-apart FISH also has the advantage of being able to detect atypical ALK rearrangements where the fusion partner is not EML4. Theoretically, a molecular strategy (e.g. real-time PCR) could also be designed to detect the specific EML4-ALK fusion event, but due to potential variability in breakpoints particularly in EML4 [35], this would be impractical especially in a clinical laboratory setting.

Another type of tumour-specific mutation in NSCLC is activating mutations of the epidermal growth factor receptor (EGFR) gene. The majority of reported mutations are small in-frame deletions in exon 19, and point mutations in exon 21 of the gene, including a missense mutation which replaces a leucine at amino acid position 858 by arginine (p.Leu858Arg) [36]. These mutations lead to constitutive activation of kinase activity in EGFR. Patients with activating mutations in EGFR show improved response to anti-EGFR therapy, such as the tyrosine kinase inhibitors (TKIs), gefitinib, and erlotinib [37]. Detection of these activating mutations require molecular methods, since the DNA changes are too small to detect by cytogenetic methods including FISH. Direct PCR/Sanger sequencing of the EGFR gene is one way to identify the mutations. This method has the advantage of identifying the exact mutation in the gene, and is also able to detect rare mutations. The disadvantage is that if the mutation load in the specimen is low (e.g. less than ~20 %), then sequencing may not be able to detect the change, although there is some evidence that the analytical sensitivity of Sanger sequencing may be higher for at least some mutations [38]. Also, tumour DNA extracted from FFPE specimens tend to be lower quality and more fragmented, and may be difficult to sequence. Therefore a more commonly used method in the clinical laboratory is a targeted mutation panel using strategies such as real-time PCR, with specific PCR primers and probes which target a panel of common activating EGFR mutations (and/or mutations which confer resistance to TKI inhibitors) [39].

In addition to sequence variations, some NSCLCs have amplifications in the copy number of EGFR genes. For these tumours, FISH probes against EGFR may be used to detect the gene amplification, which will show up as multiple signals per cell under fluorescence microscopy [40]. However, molecular methods are required to determine if the amplified EGFR contains activating sequence variations.

Breast Cancer

Breast cells express receptors on the cell surface which respond to extracellular growth signals. These include oestrogen receptors (ER), progesterone receptors (PR), and HER2 receptors. Human epidermal growth factor receptor 2 (HER2) is a receptor tyrosine kinase which belongs to the same protein family as EGFR, and is encoded by the ERBB2 gene on chromosome 17. In normal cells, there are two copies of the ERBB2 gene, one on each chromosome 17. In some breast cancers (~20%), there is amplification in the copy number of the ERBB2 gene, leading to overexpression of HER2 receptors on the cell surface. These HER2-positive breast cancers have an aggressive disease course, but respond favourably to monoclonal antibodies directed against HER2, such as trastuzumab (Herceptin) and pertuzumab (Perjeta®) [41]. Testing for ERBB2 copy number has clinical utility since patients with HER2-negative breast cancer do not benefit from anti-HER2 treatment, and trastuzumab is also known to have cardiac toxicity [42]. Therefore currently trastuzumab therapy is only recommended in patients with HER2-positive cancers.

ERBB2 amplification can be detected by FISH on an FFPE tumour specimen. HER2-positive cells show multiple signals for the ERBB2 FISH probe, while normal cells only show two signals. Alternatively, some centres use a related non-fluorescence in situ hybridisation (ISH) method for detection of ERBB2 copy number, such as CISH (chromogenic ISH). These methods allow detection of signal in bright field microscopy rather than requiring fluorescence microscopy. Yet another alternative is the detection of HER2 protein overexpression rather than gene amplification. This method utilises immunohistochemistry (IHC) staining for the HER2 protein. CISH and IHC allow co-examination of tissue morphology and HER2 status, and are less expensive compared to FISH analyses, although some tumours show discordance between protein expression and gene amplification results [43, 44].

In some breast cancer patients, there is a strong family history (e.g. with multiple closely related relatives affected, who may also have developed breast cancer at a younger age than average), which suggests a familial rather than sporadic form of breast cancer. Germline mutations in some genes have been associated with familial breast cancer. The most recognised of these are BRCA1 and BRCA2 which are associated with autosomal dominant forms of breast (and ovarian) cancer predisposition. In contrast to detection of ERBB2 amplification, which is performed on tumour material, mutation screening of BRCA1/BRCA2 genes for familial cancer predisposition requires germline DNA (e.g. from peripheral blood specimens), because the aim here is to determine if there is a *heritable* mutation in these genes. The method used is most commonly a combination of direct Sanger sequencing and MLPA, since the types of mutations reported in BRCA1 and BRCA2 include sequence variations as well as whole exon deletions/duplications. FISH and karyotyping are generally not applicable, since the deletions/duplications are usually below the resolution of these techniques. Microarrays, on the other hand, can technically detect the exonic deletions/duplications, but is seldom used in the clinical laboratory for this indication because of factors such as cost.

Some centres are moving to a massively parallel sequencing (MPS) approach for BRCA1 and BRCA2 mutation screening [45]. Theoretically, MPS can detect both sequence variations and whole exon deletions/duplications with the one technique. However, at the time of writing, there are still technical issues with clinical implementation of MPS, especially with detection of copy number changes and bioinformatic analyses, so this is still at a research and development stage in most clinical centres.

Germline Disorders

Another major area of application for cytogenetic techniques is the diagnosis of constitutional genetic disorders, either in the postnatal or prenatal period of life. The following are some examples of how cytogenetics are used in these clinical settings.

Postnatal Testing

Intellectual Disability/Developmental Delay

Intellectual disability (ID) and developmental delay (DD) are common presentations in the paediatric population with a wide range of severity. These encompass disorders in one or more neurodevelopmental domains, including motor skills (gross and fine), psychosocial, language, and cognitive development. Both environmental factors and genetic factors may contribute to ID/DD.

One of the most common genetic causes of ID/DD is Down syndrome (DS), or trisomy 21 [46]. DS patients commonly present with very typical and recognisable facial features (dysmorphism), intellectual disability, and a range of other complications which may include heart and other organ defects, immune deficiency, etc. The majority of Down syndrome patients have three separate copies of chromosome 21 in every cell of the body. However, in some DS patients, the extra copy of chromosome 21 is fused to another acrocentric chromosome (chromosome 13, 14, 15, 21 or 22) at the centromere (as a Robertsonian translocation), rather than being free in the cell. The translocation may have arisen de novo, but may also be inherited from one of the parents, in which case the recurrence risk of aneuploidy in a subsequent pregnancy would be increased. Therefore the detection of translocation is important for genetic counselling. Also, a small proportion of DS patients have mosaic trisomy 21, where some of the patient's cells have two chromosome 21 s but other cells have three copies. The presentation of mosaic DS is variable and may be milder than typical DS patients.

The most informative and appropriate test for Down syndrome is conventional karyotyping, because of the known possibility of mosaicism and translocation. Microarrays and FISH would also be able to detect the extra chromosome 21.

However, microarrays have a lower sensitivity for mosaic Down syndrome, and neither array nor FISH can detect a Robertsonian translocation. On the other hand, a karyotype can, in the one test, detect and confirm the presence of the extra chromosome 21, and determine if the extra copy is free or translocated to another chromosome. If mosaicism is present, karyotype has a higher sensitivity for detecting the abnormality than microarray.

Apart from Down syndrome, many other chromosomal deletions and duplications also cause ID/DD. The clinical presentations in these cases may often be non-specific. In the past, karyotyping was used as the standard screening test for non-specific ID/DD, with an abnormality detection rate of ~3–5 %. More recently, microarray testing has become the preferred test, due to its ability to detect submicroscopic copy number changes (CNCs), i.e., microdeletions/microduplications. Many of these CNCs also show significant association with autism spectrum disorder (ASD) and/or multiple congenital anomalies (MCA). Therefore, in the setting of ID/DD, ASD, or MCA, microarrays are now recommended as a first line investigation, with an abnormality detection rate of up to ~15 % [47].

However, with the increased resolution of microarrays come new issues. Some of the many CNCs detected by microarrays turn out to be relatively common in the general population and are now believed to be benign 'normal' variation. However, some of the detected CNCs appear to be rare, and have not been reported either in the normal population or in affected patients. These CNCs are called variants of uncertain clinical significance (VUCS). Other CNCs have been reported at a low frequency in normal individuals, but appear to be 'enriched' in individuals with certain phenotypes such as ASD and schizophrenia. Some of these are now thought to be 'susceptibility variants' with variable penetrance or expressivity for the associated phenotypes. In addition, microarray testing sometimes uncovers 'incidental findings' such as deletion of genes associated with familial cancer syndromes (e.g. BRCA1, BRCA2, APC, etc.). These findings do not explain the patient's presenting complaint of ID/DD, but may have important clinical implications for other members in the extended family or for the patient later in life. These findings raise issues of consent and disclosure, and present challenges for clinical management of the patient and family. They highlight the importance of adequate counselling and informed consent prior to embarking on genetic testing [48].

Syndromic Presentations

Sometimes ID/DD patients present with additional phenotypic features and/or facial dysmorphism which suggest a specific syndromic diagnosis. In some cases where the syndrome is associated with a specific chromosomal deletion/duplication, it is possible to target the genomic region with a locus-specific FISH probe. For example, in a child with conotruncal heart defects and cleft lip/palate, it is possible to perform FISH using a probe which localises to the 22q11.2 DiGeorge/velocardiofacial syndrome (DGS/VCFS) critical region [49]. Observation of only one signal for this probe would indicate heterozygous deletion of the locus and confirm the

clinical diagnosis of VCFS. However, depending on the clinical circumstances, microarray testing may in fact be a more efficient investigation, especially if there is genetic heterogeneity (i.e. several genomic loci associated with the syndrome).

Single Gene Disorders

Apart from deletions/duplications, other genetic defects such as sequence variations or triplet repeat expansion may also lead to ID/DD. These are not detectable by cytogenetic methods, and will require the application of molecular techniques. One important example is Fragile X syndrome.

The molecular mechanism underlying Fragile X is an expansion in the CGG trinucleotide repeat at the 5′ upstream region of the FMR1 gene. The number of CGG repeats is variable in the population, but is normally <45. In affected individuals, the number of CGG repeats expand to >200 ('full mutation' range), which leads to methylation of the promoter region of the FMR1 gene and silencing of expression of the FMR1 protein product (FMRP). Intermediate repeat sizes (56–200 repeats) are known as pre-mutations, and can be found in asymptomatic males or carrier females. Pre-mutation expansions do not cause ID/DD, but may have other late-onset health implications such as premature ovarian failure or tremor-ataxia syndrome [50].

The expansion in the trinucleotide repeat creates a folate-sensitive fragile site (FRAXA) on the affected X chromosome, and historically a special cytogenetic method (karyotype after culture of cells in a modified folate-deficient media) was used to detect the fragile site [51, 52]. However, this method is rarely performed today due to costs, low sensitivity, and slow turn-around time. It should be noted that routine karyotyping using standard culture techniques cannot detect the fragile site. FISH or microarray testing also cannot detect the triplet repeat expansions. Instead, molecular methods are now the method of choice for diagnosis of Fragile X. A common approach is to use two complementary molecular methods to detect the entire range of possible triplet repeat sizes. For smaller repeats, PCR followed by fragment analysis is used. PCR primers are designed to amplify the genomic region containing the CGG repeats. The size of the PCR product is determined by fragment analysis, which is used to calculate the number of repeats. This method provides a highly precise estimate of the repeat size (usually to ±1 to 3 repeats) at the low end of the repeat size range (up to a maximum of ~100 repeats), but for individuals with full mutations (>200 repeats), the repeat tract is too long to amplify by PCR. Therefore, if no amplification product is detected by PCR/fragment analysis in an affected male, Southern blot would be performed, using a probe which binds to the restriction fragment containing the CGG repeat. The size of the restriction fragment provides only a rough estimate of the repeat size, but is able to detect expansions in excess of a thousand repeats [53, 54].

It is worth pointing out that in extremely rare instances, FMR1 gene deletions and sequence variations have also been reported to cause Fragile X syndrome [55, 56]. In these very rare cases, FISH, microarray, and MLPA could be used to detect deletions, and PCR/Sanger sequencing could be used to detect sequence variations. But for the vast majority of Fragile X patients (>99 %), fragment analysis and Southern blot are the mainstay of diagnosis.

Infertility/Recurrent Pregnancy Loss

Another application of cytogenetic testing is for couples who suffer from infertility or recurrent pregnancy loss. There are many possible underlying causes for this clinical presentation, including anatomical, endocrine, and genetic factors either in the male or female partner. One important genetic factor which may contribute to this presentation is balanced chromosomal rearrangements, such as balanced reciprocal translocations, which may be present in the male or female partner.

In a balanced translocation, there is no net gain or loss of chromosomal material in the carrier. Therefore, in most cases there are no phenotypic consequences in the carrier unless the breakpoints interrupt an important gene. However, when the gonadal cells in a carrier undergo meiosis, there is a high likelihood that some of the gametes produced will be unbalanced, depending on the way the chromosomes segregate. If the resulting unbalanced gametes were used in fertilisation, the zygote formed would contain a chromosomal imbalance (typically a partial trisomy and a concomitant partial monosomy of the chromosomes involved in the translocation). The level of imbalance in these zygotes is such that many are not compatible with full-term gestation, resulting in recurrent miscarriage.

Microarray analysis cannot detect balanced rearrangements in carriers since there is no net copy number gain or loss. FISH testing is also impractical as a screening test since there is no way to predict which chromosomes are potentially involved. Karyotype analysis is therefore the most appropriate method in this clinical setting.

In addition to providing a precise diagnosis for the couple, cytogenetic investigation is also of value for planning subsequent use of artificial reproductive techniques including pre-implantation genetic diagnosis (PGD). With PGD, knowing the specific rearrangement in the carrier parent allows specific FISH probes to be designed to screen in vitro fertilised embryos. It then allows selective implantation of only those embryos which contain a balanced chromosomal complement.

Prenatal Testing

In the prenatal setting, cytogenetic testing is often requested as a result of concerns over the risk of aneuploidy, due to advanced maternal age or ‘high risk’ results of biochemical maternal serum screening. Other common indications include the finding of abnormalities on foetal ultrasound.

The most common prenatal diagnostic test is a conventional karyotype conducted on an amniotic fluid (AF) specimen or chorionic villus sample (CVS). These are invasive tests and carry a finite risk of miscarriage (estimated to be 1/100 to 1/200, depending on the centre) [57, 58]. These types of samples contain cells of fetal origin that will divide in culture, thus allowing the use of standard cytogenetic techniques to directly visualise the chromosomes in metaphase and confirm a trisomy or any other chromosomal abnormality if present. The turn-around time for a result is typically in the order of 7–10 days.

Many expectant mothers who have a 'high risk' for aneuploidy are understandably anxious and often wish to have a faster answer. Another method which may be used in this setting is aneuploidy FISH testing. This test is performed as interphase FISH using probes which target the centromeres or specific loci on chromosomes 13, 18, and 21 (and optionally chromosome X and Y) on uncultured fetal cells [59]. This test provides a faster turn-around time, typically within 24–48 h. The cells are scored for the number of signals for each probe. Two signals for each of the chromosome 13, 18, and 21 probes is the 'normal' pattern. The presence of three signals for the chromosome 21 probe would strongly suggest Trisomy 21. However, this test cannot be considered a definitive test. The absence of a third signal does not exclude the potential for duplication of a part of the chromosome which is not targeted by the FISH probe. Abnormalities of chromosomes other than 13, 18, 21, X, and Y, or unusual rearrangements, cannot be detected. Also, if trisomy is detected, it cannot distinguish between translocation trisomy and a free extra chromosome.

For these reasons, aneuploidy FISH testing should only be considered an extra screening step to fast-track an abnormal result. Whether the FISH result is positive or negative, it should always be followed up with karyotype for a definitive diagnosis.

More recently, some centres have started to offer microarrays for prenatal testing [60]. The technical principles of prenatal array are similar to the use of microarrays postnatally. As discussed already, microarray may have less sensitivity for mosaic results compared to karyotyping or FISH, especially if the level of abnormal cells is low (e.g. below 10–20 %), but can detect submicroscopic copy number changes. This increased resolution may be perceived as an advantage, but it can also lead to challenges to interpretation of results. For example, if a copy number change corresponds to a well-known microdeletion or microduplication syndrome, and is consistent with malformations seen on fetal ultrasound, then the interpretation may be straightforward, in which case the microarray testing has provided a diagnosis where karyotyping could not. However, if the copy number change is a VUCS or susceptibility variant or incidental finding, then interpretation of the finding and counselling of the parents will be challenging.

References

1. Vardiman JW, Melo JV, Baccarani M, Thiele J (2008) Chronic myelogenous leukaemia, BCR-ABL1 positive. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues. WHO classification of tumours, 4th edn, vol 2. IARC, Lyon, pp 32–39
2. Deininger MW, Goldman JM, Melo JV (2000) The molecular biology of chronic myeloid leukemia. Blood 96(10):3343–3356
3. Rowley JD (1973) Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243(5405):290–293
4. Melo JV (1996) The diversity of BCR-ABL fusion proteins and their relationship to leukemia phenotype. Blood 88(7):2375–2384

5. Fausel C (2007) Targeted chronic myeloid leukemia therapy: seeking a cure. J Manag Care Pharm 13(8 Suppl A):8–12
6. Branford S, Hughes TP, Rudzki Z (1999) Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Br J Haematol 107(3):587–599
7. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, Baccarani M, Cortes J, Cross NC, Druker BJ, Gabert J, Grimwade D, Hehlmann R, Kamel-Reid S, Lipton JH, Longtine J, Martinelli G, Saglio G, Soverini S, Stock W, Goldman JM (2006) Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood 108(1):28–37. doi:10.1182/blood-2006-01-0092
8. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, Cortes J, O'Brien S, Nicaise C, Bleickardt E, Blackwood-Chirchir MA, Iyer V, Chen TT, Huang F, Decillis AP, Sawyers CL (2006) Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med 354(24):2531–2541. doi:10.1056/NEJMoa055229
9. Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B, Tanaka C, Manley P, Rae P, Mietlowski W, Bochinski K, Hochhaus A, Griffin JD, Hoelzer D, Albitar M, Dugan M, Cortes J, Alland L, Ottmann OG (2006) Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. N Engl J Med 354(24):2542–2551. doi:10.1056/NEJMoa055104
10. Hughes T (2006) ABL kinase inhibitor therapy for CML: baseline assessments and response monitoring. Hematology Am Soc Hematol Educ Program: 211–218.doi:10.1182/asheducation-2006.1.211
11. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, Morgan G, Van Ness B, Chesi M, Minvielle S, Neri A, Barlogie B, Kuehl WM, Liebisch P, Davies F, Chen-Kiang S, Durie BG, Carrasco R, Sezer O, Reiman T, Pilarski L, Avet-Loiseau H; International Myeloma Working Group (2009) International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia 23(12):2210–2221. doi:10.1038/leu.2009.174
12. Munshi NC, Anderson KC, Bergsagel PL, Shaughnessy J, Palumbo A, Durie B, Fonseca R, Stewart AK, Harousseau JL, Dimopoulos M, Jagannath S, Hajek R, Sezer O, Kyle R, Sonneveld P, Cavo M, Rajkumar SV, San Miguel J, Crowley J, Avet-Loiseau H; International Myeloma Workshop Consensus Panel 2 (2011) Consensus recommendations for risk stratification in multiple myeloma: report of the International Myeloma Workshop Consensus Panel 2. Blood 117(18):4696–4700. doi:10.1182/blood-2010-10-300970
13. VanWier S, Fonseca R (2005) Detection of chromosome 13 deletions by fluorescent in situ hybridization. Methods Mol Med 113:59–69. doi:10.1385/1-59259-916-8:59
14. Cook JR, Hartke M, Pettay J, Tubbs RR (2006) Fluorescence in situ hybridization analysis of immunoglobulin heavy chain translocations in plasma cell myeloma using intact paraffin sections and simultaneous CD138 immunofluorescence. J Mol Diagn 8(4):459–465. doi:10.2353/jmoldx.2006.050149
15. Avet-Loiseau H, Li C, Magrangeas F, Gouraud W, Charbonnel C, Harousseau JL, Attal M, Marit G, Mathiot C, Facon T, Moreau P, Anderson KC, Campion L, Munshi NC, Minvielle S (2009) Prognostic significance of copy-number alterations in multiple myeloma. J Clin Oncol 27(27):4585–4590. doi:10.1200/JCO.2008.20.6136
16. Muller-Hermelink HK, Montserrat E, Catovsky D, Campo E, Harris NL, Stein H (2008) Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues. WHO classification of tumours, 4th edn, vol 2. IARC, Lyon, pp 180–182
17. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343(26):1910–1916. doi:10.1056/NEJM200012283432602
18. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ; International Workshop on Chronic Lymphocytic Leukemia (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia:

a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 111(12):5446–5456. doi:10.1182/blood-2007-06-093906
19. Pfeifer D, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, Fisch P, Timmer J, Veelken H (2007) Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood 109(3):1202–1210. doi:10.1182/blood-2006-07-034256
20. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94(6):1840–1847
21. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94(6):1848–1854
22. Rossi D, Rasi S, Spina V, Bruscaggin A, Monti S, Ciardullo C, Deambrogi C, Khiabanian H, Serra R, Bertoni F, Forconi F, Laurenti L, Marasca R, Dal-Bo M, Rossi FM, Bulian P, Nomdedeu J, Del Poeta G, Gattei V, Pasqualucci L, Rabadan R, Foa R, Dalla-Favera R, Gaidano G (2013) Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia. Blood 121(8):1403–1412. doi:10.1182/blood-2012-09-458265
23. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS, Zhang L, Zhang W, Vartanov AR, Fernandes SM, Goldstein NR, Folco EG, Cibulskis K, Tesar B, Sievers QL, Shefler E, Gabriel S, Hacohen N, Reed R, Meyerson M, Golub TR, Lander ES, Neuberg D, Brown JR, Getz G, Wu CJ (2011) SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 365(26):2497–2506. doi:10.1056/NEJMoa1109016
24. Mrozek K, Heerema NA, Bloomfield CD (2004) Cytogenetics in acute leukemia. Blood Rev 18(2):115–136. doi:10.1016/S0268-960X(03)00040-7
25. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 92(7):2322–2333
26. National Comprehensive Cancer Network (2013) Acute myeloid leukemia. NCCN clinical practice guidelines in oncology
27. Tallman MS, Andersen JW, Schiffer CA, Appelbaum FR, Feusner JH, Ogden A, Shepherd L, Willman C, Bloomfield CD, Rowe JM, Wiernik PH (1997) All-trans-retinoic acid in acute promyelocyticleukemia.NEnglJMed337(15):1021–1028.doi:10.1056/NEJM199710093371501
28. Mrozek K, Harper DP, Aplan PD (2009) Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Hematol Oncol Clin North Am 23(5):991–1010. doi:10.1016/j.hoc.2009.07.001, v
29. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, Wang C, Davies SM, Gaynon PS, Trigg M, Rutledge R, Burden L, Jorstad D, Carroll A, Heerema NA, Winick N, Borowitz MJ, Hunger SP, Carroll WL, Camitta B (2009) Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 27(31):5175–5181. doi:10.1200/JCO.2008.21.2514
30. How J, Sykes J, Minden MD, Gupta V, Yee KW, Schimmer AD, Schuh AC, Kamel-Reid S, Brandwein JM (2013) The prognostic impact of FLT3-ITD and NPM1 mutations in patients with relapsed acute myeloid leukemia and intermediate-risk cytogenetics. Blood Cancer J 3, e116. doi:10.1038/bcj.2013.14
31. National Comprehensive Cancer Network (2013) Non-small cell lung cancer. NCCN clinical practice guidelines in oncology
32. Perner S, Wagner PL, Demichelis F, Mehra R, Lafargue CJ, Moss BJ, Arbogast S, Soltermann A, Weder W, Giordano TJ, Beer DG, Rickman DS, Chinnaiyan AM, Moch H, Rubin MA (2008) EML4-ALK fusion lung cancer: a rare acquired event. Neoplasia 10(3):298–302

33. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, Ou SH, Dezube BJ, Janne PA, Costa DB, Varella-Garcia M, Kim WH, Lynch TJ, Fidias P, Stubbs H, Engelman JA, Sequist LV, Tan W, Gandhi L, Mino-Kenudson M, Wei GC, Shreeve SM, Ratain MJ, Settleman J, Christensen JG, Haber DA, Wilner K, Salgia R, Shapiro GI, Clark JW, Iafrate AJ (2010) Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 363(18):1693–1703. doi:10.1056/NEJMoa1006448
34. Shaw AT, Solomon B (2011) Targeting anaplastic lymphoma kinase in lung cancer. Clin Cancer Res 17(8):2081–2086. doi:10.1158/1078-0432.CCR-10-1591
35. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar J-S, Squire J, Thunnissen E, Ladanyi M (2013) Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Mol Diagn 15(4):415–453
36. Sharma SV, Bell DW, Settleman J, Haber DA (2007) Epidermal growth factor receptor mutations in lung cancer. Nat Rev Cancer 7(3):169–181. doi:10.1038/nrc2088
37. Paz-Ares L, Soulieres D, Melezinek I, Moecks J, Keil L, Mok T, Rosell R, Klughammer B (2010) Clinical outcomes in non-small-cell lung cancer patients with EGFR mutations: pooled analysis. J Cell Mol Med 14(1–2):51–69. doi:10.1111/j.1582-4934.2009.00991.x
38. Young EC, Owens MM, Adebiyi I, Bedenham T, Butler R, Callaway J, Cranston T, Crosby C, Cree IA, Dutton L, Faulkes C, Faulkner C, Howard E, Knight J, Huang Y, Lavender L, Lazarou LP, Liu H, Mair D, Milano A, Sandell S, Skinner A, Wallace A, Williams M, Spivey V, Goodall J, Frampton J, Ellard S, Clinical Molecular Genetics Society (CMGS) Scientific Subcommittee (2013) A comparison of methods for EGFR mutation testing in non-small cell lung cancer. Diagn Mol Pathol 22(4):190–195. doi:10.1097/PDM.0b013e318294936c
39. Wong AT, To RM, Wong CL, Chan WK, Ma ES (2013) Evaluation of 2 real-time PCR assays for in vitro diagnostic use in the rapid and multiplex detection of EGFR gene mutations in NSCLC. Diagn Mol Pathol 22(3):138–143. doi:10.1097/PDM.0b013e31827fedcc
40. Wang F, Fu S, Shao Q, Zhou YB, Zhang X, Zhang X, Xue C, Lin JG, Huang LX, Zhang L, Zhang WM, Shao JY (2013) High EGFR copy number predicts benefits from tyrosine kinase inhibitor treatment for non-small cell lung cancer patients with wild-type EGFR. J Transl Med 11(1):90. doi:10.1186/1479-5876-11-90
41. Hudis CA (2007) Trastuzumab — mechanism of action and use in clinical practice. N Engl J Med 357(1):39–51. doi:10.1056/NEJMra043186
42. Telli ML, Hunt SA, Carlson RW, Guardino AE (2007) Trastuzumab-related cardiotoxicity: calling into question the concept of reversibility. J Clin Oncol 25(23):3525–3533. doi:10.1200/JCO.2007.11.0106
43. Di Palma S, Collins N, Faulkes C, Ping B, Ferns G, Haagsma B, Layer G, Kissin MW, Cook MG (2007) Chromogenic in situ hybridisation (CISH) should be an accepted method in the routine diagnostic evaluation of HER2 status in breast cancer. J Clin Pathol 60(9):1067–1068. doi:10.1136/jcp.2006.043356
44. Penault-Llorca F, Bilous M, Dowsett M, Hanna W, Osamura RY, Ruschoff J, van de Vijver M (2009) Emerging technologies for assessing HER2 amplification. Am J Clin Pathol 132(4):539–548. doi:10.1309/AJCPV2I0HGPMGBSQ
45. Feliubadalo L, Lopez-Doriga A, Castellsague E, del Valle J, Menendez M, Tornero E, Montes E, Cuesta R, Gomez C, Campos O, Pineda M, Gonzalez S, Moreno V, Brunet J, Blanco I, Serra E, Capella G, Lazaro C (2013) Next-generation sequencing meets genetic diagnostics: development of a comprehensive workflow for the analysis of BRCA1 and BRCA2 genes. Eur J Hum Genet 21(8):864–870. doi:10.1038/ejhg.2012.270
46. Gardner RJM, Sutherland GR, Shaffer LG (2011) Chromosome abnormalities and genetic counselling. Oxford monographs on medical genetics, 4th edn, vol 61. Oxford University Press, New York
47. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB,

Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH (2010) Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 86(5):749–764. doi:10.1016/j.ajhg.2010.04.006
48. Schaaf CP, Wiszniewska J, Beaudet AL (2011) Copy number and SNP arrays in clinical diagnostics. Annu Rev Genomics Hum Genet 12:25–51. doi:10.1146/annurev-genom-092010-110715
49. Yu S, Graf WD, Shprintzen RJ (2012) Genomic disorders on chromosome 22. Curr Opin Pediatr 24(6):665–671. doi:10.1097/MOP.0b013e328358acd0
50. Kronquist KE, Sherman SL, Spector EB (2008) Clinical significance of tri-nucleotide repeats in Fragile X testing: a clarification of American College of Medical Genetics guidelines. Genet Med 10(11):845–847. doi:10.1097/GIM.0b013e31818b0c8a
51. Sutherland GR (1977) Fragile sites on human chromosomes: demonstration of their dependence on the type of tissue culture medium. Science 197(4300):265–266
52. Harvey J, Judge C, Wiener S (1977) Familial X-linked mental retardation with an X chromosome abnormality. J Med Genet 14(1):46–50
53. Human Genetics Society of Australasia (2012) Best practice Fragile X testing and analysis guidelines for Australasian laboratories
54. Spector EB, Kronquist KE (2006) Technical standards and guidelines for Fragile X testing. ACMG Standards and Guidelines for Clinical Genetics Laboratories
55. Hammond LS, Macias MM, Tarleton JC, Shashidhar Pai G (1997) Fragile X syndrome and deletions in FMR1: new case and review of the literature. Am J Med Genet 72(4):430–434
56. Wang YC, Lin ML, Lin SJ, Li YC, Li SY (1997) Novel point mutation within intron 10 of FMR-1 gene causing fragile X syndrome. Hum Mutat 10(5):393–399. doi:10.1002/(SICI)1098-1004(1997)10:5<393::AID-HUMU10>3.0.CO;2-V
57. Tabor A, Alfirevic Z (2010) Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther 27(1):1–7. doi:10.1159/000271995
58. Scott F, Peters H, Boogert T, Robertson R, Anderson J, McLennan A, Kesby G, Edelman D (2002) The loss rates for invasive prenatal testing in a specialised obstetric ultrasound practice. Aust N Z J Obstet Gynaecol 42(1):55–58
59. Klinger K, Landes G, Shook D, Harvey R, Lopez L, Locke P, Lerner T, Osathanondh R, Leverone B, Houseal T et al (1992) Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). Am J Hum Genet 51(1):55–65
60. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L (2012) Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 367(23):2175–2184. doi:10.1056/NEJMoa1203382