

## **Routine Cytogenetic Analysis of Chromosome Abnormalities in Acute Lymphoblastic Leukemia**

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### **Abstract**

Acute lymphoblastic leukemia (ALL) is the most common cancer in children. Acquired chromosomal and genetic abnormalities have been used to define distinct biological and clinical subtypes of ALL. These aberrations have been shown to be associated with patient outcome and are used to direct therapy. Conventional cytogenetics analysis (CCA) is currently the gold standard to detect chromosome abnormalities in hematological malignancies. In this review we briefly describe the CCA methodology, advantages and limitations of CCA, the main chromosomal abnormalities and their prognostic significance in ALL.

**Keywords:** Acute lymphoblastic leukemia, chromosome abnormalities, prognosis.

### **1. Introduction**

Acute lymphoblastic leukemia (ALL) is a neoplastic disease characterised by clonal expansion of white cell precursors in the bone marrow, lymph nodes, thymus or spleen. ALL shows a bimodal age distribution, with a high incidence at ages 1-4 years, a decline at ages 20 to 59 years, followed by a modest rise in incidence above age 60 years [1]. About 75% of ALL occurs in children under 6 years of age. B-lineage ALL is more frequent than T-lineage ALL, accounting for 85% of childhood ALL and 75% of adult ALL [2]. The survival rate of pediatric ALL has improved to about 90% with risk stratification, but however the prognosis of infants and adults remains poor [3]. Most modern treatment protocols stratify patients according to prognostic factors such as genetics, age, white cell count, immunophenotype, and response to therapy. Acquired genetic abnormalities define distinct biological and clinical subtypes of ALL, and are one of the most powerful predictors of outcome in ALL. About 75% of childhood ALL harbor recurrent genetic abnormalities, including aneuploidy or structural chromosomal arrangements [4].

The World Health Organization (WHO) classification of B-lineage lymphoblastic

leukemia/lymphoma identifies 7 subtypes with recurrent genetic abnormalities: t(9;22)(q34;q11.2), *BCR-ABL1*; t(v;11q23), *MLL* rearranged; t(12;21)(p13;q22), *ETV6-RUNX1*; hyperdiploidy; hypodiploidy; t(5;14)(q31;q32), *IL3-IGH*; and t(1;19)(q23;p13.3), *TCF3-PBX1* [5]. Most of these specific abnormalities may be detected by routine cytogenetic analysis, except for t(12;21)(p13;q22), *ETV6-RUNX1* which is a submicroscopic rearrangement that is detectable by FISH (fluorescence in situ hybridization) or RT-PCR (reverse transcriptase polymerase chain reaction).

There are many genetic techniques that can be utilised to detect chromosomal and genomic aberrations in patients with ALL. The spectrum of genetic abnormalities in ALL is very wide and diverse, and no single technique is able to detect all types of aberration. Despite the invention of novel technologies, conventional cytogenetic analysis (CCA) is currently the foundation genetic test in ALL. In this article we briefly describe the CCA methodology, some limitations of cytogenetic analysis, the recurrent chromosomal abnormalities and their prognostic significance in ALL.

## 2. Conventional Cytogenetic Analysis

CCA is commonly used as a routine diagnostic test to detect chromosomal abnormalities in hematological malignancies. It can identify all chromosomal aberrations present simultaneously (that are visible under the microscope) in a leukemic cell.

### a) Sample requirements and cultures

The preferred sample for CCA is bone marrow aspirate collected in a heparinised container and transported as soon as possible (in ice) to the Cytogenetics Laboratory. If the bone marrow aspirate is not available, then trephine biopsies or peripheral blood (if blasts are present) may be cultured. Cell counting should be performed for optimisation of cell culture. An overnight unstimulated culture (<24 hrs) should be set up as some ALL cells may have an increased tendency for apoptosis. If there are sufficient cells, an additional 48-hr culture may be initiated. The addition of growth factor supplements such as stem cell factor, interleukins, and *Flt3*-ligand may increase chromosome quality in B-lineage ALL [6]. For T-lineage ALL, additional cultures stimulated with phytohemagglutinin may be set up.

### b) G-banded karyotyping

Karyotyping is according to the International System of Human Cytogenetic Nomenclature (ISCN2013) [7] at the time of analysis. A minimum of 20 metaphases must be analysed if there is no clonal abnormality. Chromosomal aberrations when detected must be clonal (when present in a minimum of two cells with the same extra chromosomes or structural aberrations, or at least three cells with the same chromosome loss). Near-haploidy (<30 chromosomes) and low hypodiploidy (30-39 chromosomes) may undergo clonal evolution and double-up as hyperdiploid and near-triploid metaphases respectively [8]. Near-haploidy and low hypodiploidy confer a poor prognosis and are assigned to high risk therapy. In the event of finding an atypical hyperdiploid or near-triploid karyotype with four copies of chromosomes other than 21 or the sex chromosome, one must investigate the possibility of an underlying near-haploid or low hypodiploid population. Investigations may include interphase FISH, and consideration could be given to short tandem repeat (STR) analysis and zygosity testing [9].

### c) Limitations of Cytogenetic Analysis

CCA is a biological test which depends on dividing cells for analysis. Sometimes, the processed samples yield no or only a few analyzable metaphase cells, or the karyotypes obtained were of poor quality to allow interpretation. Another limitation is the inability to detect small or subtle abnormalities such

as cryptic translocations and insertions. The t(12;21)(p13;q22) translocation is cryptic where the breakpoints involves similarly banded regions and thus cannot be identified by G-banding. Although the *BCR-ABL1* gene is usually generated by the typical t(9;22)(q22;q11.2) translocation, submicroscopic insertion of the *BCR-ABL1* fusion gene resulting in normal chromosomes 9 and 22 have also been reported [10]. These two major limitations of cytogenetics may be overcome by the incorporation of additional targeted tests such as FISH and RT-PCR.

## 3. Chromosomal Abnormalities in B-lineage ALL (B-ALL)

Chromosome abnormalities in B-ALL can be divided into 3 main groups: (i) chromosomal translocations which result in the formation of novel chimeric fusion genes leading to expression of leukemogenic proteins or over-expression of oncogenes, (ii) ploidy subgroups characterised by the gain or loss of multiple non-random chromosomes, and (iii) miscellaneous subgroups. Translocations account for about 40-50% of ALL. There is also a strong correlation between age and frequency of types of chromosomal abnormalities. Ploidy subgroups are defined according to the number of chromosomes present in the major leukemia clone, and are more prevalent in children (40%) than adults (20%) [11].

### a) Primary Chromosomal Abnormalities

#### (i) Chromosomal Translocations / gene fusions

The translocation t(12;21)(p13;q22) results in the fusion gene *ETV6-RUNX1* and is most prevalent in pediatric ALL (about 25%), but is rare in adults. The translocation is detectable using FISH or RT-PCR. Children with *ETV6-RUNX1* fusion have excellent overall survival with very low relapse rates. Patients with t(12;21) also harbour additional chromosomal abnormalities (ADA) such as deletions of 12p with the loss of *ETV6* gene (55-70% of patients), +21 (15-20%), or an extra derivative chromosome 21, der(21)t(12;21) [10-15%]. Studies have shown that these ADA do not have any impact on survival [12].

The translocation t(1;19) is present in about 3-5% of ALL. About 90-95% of the translocation t(1;19)(q23;p13) results in the fusion of *TCF3* (formerly known as *E2A*) gene with *PBX1* gene. About 50% of the translocation t(1;19) is balanced giving rise to *TCF3-PBX1* fusion, while the other 50% is unbalanced with only the derivative chromosome, der(19)t(1;19) and with no *TCF3-PBX1* fusion [13]. Patients with *TCF3-PBX1* fusion usually have a pre-B immunophenotype expressing

cytoplasmic  $\mu$ . With the use of modern intensive therapy, pediatric ALL patients with t(1;19) have an intermediate/good response [14]. In adults with t(1;19), the prognosis remains controversial, with some studies reporting poor outcomes while others found to association with outcome.

Translocations involving the *MLL* (mixed lineage leukemia) gene on 11q23 with other partner genes have been reported in both ALL and acute myeloid leukemia (AML). The most common *MLL* translocation in ALL is the t(4;11)(q21;q23)/*MLL-AFF1* (formerly known as *AF4*), with a low incidence in children (2-3%), and an increase in the incidence with age among the adults. Patients with t(4;11) have a poor prognosis, but with the use of modern aggressive therapy especially in children their outcome has improved [15]. The prognosis of patients with other *MLL* translocations is still uncertain. However, infants and young children (< 3 years) with *MLL* translocations have a very poor prognosis.

The translocation t(9;22)(q34;q11.2) results in the generation of the Philadelphia (Ph) chromosome. The *BCR-ABL1* fusion gene formed then translates a protein with tyrosine kinase activity. The t(9;22) had been reported in chronic myeloid leukemia (CML), AML and ALL. Ph-positive ALL is characterised by presence of intragenic deletion of the *IKZF1* (*Ikaros*) gene, which is not found in Ph-positive CML and AML [16]. The incidence of Ph-positive ALL increases with age from 2% in children to 29% in adults (30-39 years), and to 40% in older adults (40+ years). Although the Ph chromosome in ALL is associated with a poor prognosis, treatment with tyrosine kinase inhibitors have improved the event free survival rates of ALL patients compared to patients treated with traditional chemotherapy and stem cell transplantation [17, 18]. However, Ph-positive ALL with *IKZF1* deletions has an unfavourable outcome even with imatinib therapy compared to those with *IKZF1* wild-type [19, 20]. Gene expression profiling (GEP) has also identified 'BCR-ABL1-like' B-ALL which is found in 15% of childhood B-ALL. *BCR-ABL1*-like B-ALL shows a similar GEP to Ph+ B-ALL, and is also associated with a poor prognosis, but without *BCR-ABL1* rearrangement [21].

Immunoglobulin heavy chain (*IGH*) translocations involve deregulation of an oncogene via its juxtaposition to the *IGH* enhancer at 14q32. These translocations are recurrent but are rare in B-ALL, occurring in less than 5% of cases [22]. Five members of the *CEBP* gene family have been shown to undergo translocation with the *IGH* locus [23]. *IGH* translocations are prevalent in teenagers and

young adults with ALL and are associated with a poor outcome [24]. The t(5;14)(q31;q32)/*IGH-IL3* in B-ALL is associated with hypereosinophilia and a poor prognosis.

The three genetic mechanisms which cause deregulation of *CRLF2* (cytokine receptor-like factor 2 gene) are: a cryptic chromosomal translocation, t(X;14)(p22;q32) or t(Y;14)(p11;q32); a cryptic interstitial deletion, del(X)(p22.33)(p22.33) or del(Y)(p11.32)(p11.32); and an activating mutation. Studies on the prognosis of *CRLF2* deregulation in childhood ALL show conflicting results whereas in adult ALL the outcome is inferior.

#### (ii) Ploidy Subtypes

Hypodiploidy (less than 46 chromosomes) is seen in about 5-8% of B-ALL, with a majority of them having 45 chromosomes. The other types of hypodiploidy are much rarer, and they include near-haploidy (24-29 chromosomes), low-hypodiploidy (30-39 chromosomes) and high-hypodiploidy (40-44 chromosomes) [25]. About 1% of children (< 15 years) with ALL have a near haploid karyotype. Nullsomy, loss of both copies of a particular chromosome and structural abnormalities are rare. The chromosome loss is non random and the chromosomes frequently retained are chromosomes 10, 14, 18, 21 and the sex chromosomes (X/Y). Clonal evolution is common where the subclone is a double-up version of the primary clone. It is important to identify correctly near-haploidy especially when the double-up subclone might be mis-classified as hyperdiploidy. Near-haploidy has a very poor outcome and requires high risk therapy [26].

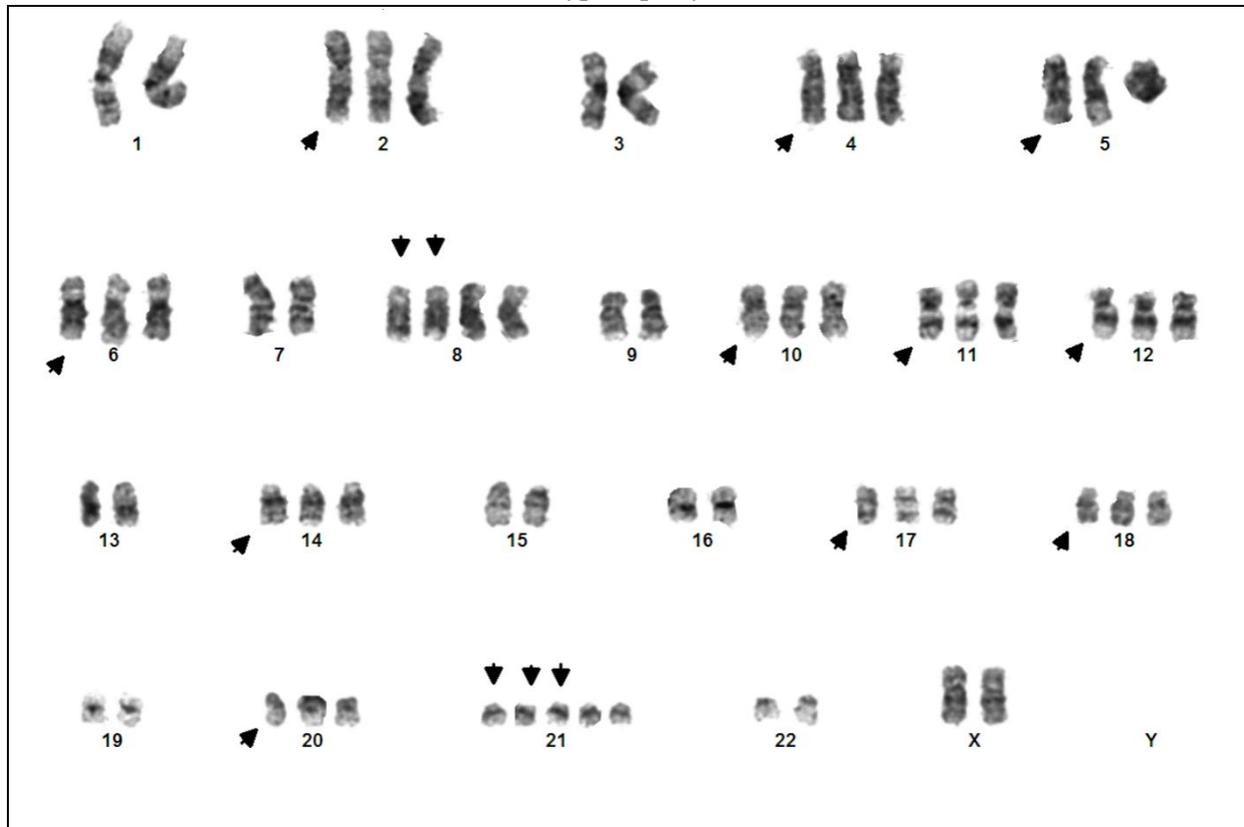
Low hypodiploidy karyotypes (30-39 chromosomes) will usually be monosomic for chromosomes 3, 7, 15, 16, 17 and disomic for chromosomes 1, 6, 11 and 18. Subclones with near-triploid karyotypes (60-78 chromosomes) are common due to the phenomenon of doubling-up in low hypodiploid clones. In karyotypes with 60-65 chromosomes, correct identification of low hypodiploidy/ near-triploidy (LHNT) and high hyperdiploidy (HH) is necessary as LHNT has a poor prognosis. The pattern of chromosome gain can be used to identify HH and LHNT. HH are trisomic for chromosomes X, 4, 6, 10, 17 & 18, and tetrasomic for chromosomes 14 and 21. Double-up low hypodiploid karyotypes are usually disomic for chromosomes 3, 7, 15, 16, 17 and tetrasomic for chromosomes 1, 6, 11 and 18 [27]. LHNT is rare among children but is present in 3-5% of adolescence adults.

HH is characterised by simultaneous gain of between 5 to 19 chromosomes, thus resulting in the leukemic cells having a total of 51 to 65

chromosomes. The chromosome gain is non-random, mainly involving chromosomes X, 4, 6, 10, 14, 17, 18, and 21 [28]. HH is the most common genetic abnormality observed in ALL, and is associated with a favourable outcome. It is present in about 35% of

children compared to 10% in adults. About 2.8% of childhood ALL with HH harbour either t(1;19), t(9;22), der(11q23) or t(12;21) [29]. Fig. 1 shows the karyotype of a child with B-ALL having HH detected by our Cytogenetics Laboratory.

**Fig. 1 Karyotype of a female child with B-lineage acute lymphoblastic leukemia showing high hyperdiploidy**



62,XX,+2,+4,+5,+6,+8,+8,+10,+11,+12,+14,+17,+18,+20,+21,+21,+21. Gain of chromosomes 2, 4, 5, 6, 8, 10, 11, 12, 14, 17, 18, 20 and 21. There is also gain of two chromosomes 8 and three chromosomes 21. Arrows indicate the chromosomes gained.

### (iii) Miscellaneous Subgroup

Intrachromosomal amplification of chromosome 21 (iAMP21) is found in about 3-5% of pediatric patients with ALL, usually between 7 and 13 years. This abnormal chromosome 21 has multiple regions of inversion, deletion, duplication and amplification along its entire length. The regions amplified always include the *RUNX1* locus and can be detected by using a FISH probe that targets *RUNX1* locus (e.g. *ETV6-RUNX1* dual fusion probe). However, there is no evidence that *RUNX1* gene is involved [30]. Patients with iAMP21 have a high risk of relapse and require more intensive therapy [31]. There is a 2700-fold increased risk of developing B-ALL with iAMP21 in people born with constitutional Robertsonian translocation [32].

The complex karyotype in ALL is defined as the presence of 5 or more unrelated chromosomal abnormalities in the absence of a known translocation. About 5-10% of Ph-negative B-cell

ALL have a complex karyotype, which is associated with a poor outcome.

### b) Secondary chromosomal abnormalities

The chromosomal abnormalities described above are considered as primary chromosomal abnormalities which define distinct biological and clinical subgroups of the disease. The t(9;22), t(12;21), iAMP21, and *MLL* rearrangements are reported to be mutually exclusive [2]. A majority of ALL patients at diagnosis harbour more than one genetic abnormality in the form of non random numerical and structural chromosome aberrations that can be detected microscopically. However, the application of genomic-wide molecular technologies (such as array comparative genomic hybridization and single nucleotide polymorphism array) also reveal many genetic abnormalities that are not detectable at the cytogenetics level [33]. These secondary additional chromosomal or genomic abnormalities (ACGA) have been found in virtually all chromosomes and all regions of the genome. It

has been shown that the majority of ACGA are deletions rather than amplifications or duplications [34]. The common ACGA are deletions of *CDKN2A/B* (30-40%), deletions/mutations of *IKZF1* gene (20%), deletions/mutations/amplifications of *PAX5* gene (20%), and deletions of *ETV6* (10-15%) [11, 35].

Chromosome abnormalities of 9p are associated with aberrations of *CDKN2A/B* and *PAX5*. Chromosome abnormalities such as del(13q), monosomy 13, 17p abnormalities are associated with a poor outcome in pediatric ALL, but when exist as secondary abnormalities with a favourable outcome

abnormality such as high hyperdiploidy or *ETV6-RUNX1*, the good outcome of the abnormality is not abrogated [14]. *IGH* translocation have been reported to coexist together with primary rearrangements (e.g. High hyperdiploidy, t(12;21), t(9;22); *MLL* rearrangements, iAMP21 ) either in the same or separate clones, and when present, *IGH* translocation usually arise as secondary events. The *IGH* partner genes include *CRLF2*, *CEBPA*, *CEBPB*, *TRA/D@*, *IGF2BP1* and *IGK@* [36]. Table 1 shows the non-random chromosome/genetic abnormalities, genes involved, and prognosis in B-ALL.

**Table 1: Non-random chromosomal/ genetic abnormalities, genes involved and prognosis in B-lineage acute lymphoblastic leukemia [11]**

Chromosome / Genetic Abnormalities	Genes	Prognosis
t(12;21)(p13;q22)	<i>ETV6-RUNX1</i>	Excellent
t(1;19)(q23;p13)	<i>TCF3-PBX1</i>	Children: intermediate/good Adults: intermediate/poor Infants: very poor
t(17;19)(q22;p13)	<i>TCF3-HLF</i>	Extremely poor
<i>MLL</i> (11q23) translocations	<i>MLL</i> gene	Poor
t(9;22)(q34;q11.2)	<i>BCR-ABL1</i>	Very poor (unless treated with tyrosine kinase inhibitors)
<i>IGH</i> (14q32) translocations	<i>IGH</i> gene	Poor
<i>CRLF2</i> alterations	<i>CRLF2</i> gene	Children: Intermediate/ poor Adolescents & young adults: poor
iAMP21		Very poor (unless treated on high risk protocol)
Complex karyotype (> 4 chr abnormalities)		Very poor
Near haploidy (<30 chrs)		Very poor
Low hypodiploidy/ near triploidy (30-39/ 60-78 chrs)		Very poor
High hyperdiploidy (51-65/67 chrs)		Excellent

Note: The prognostic impact of any given chromosome/ genetic abnormality depends not only on the treatment protocol, but may be modified by other risk factors such as age and presence of other genetic abnormalities.

Abbreviations: chrs: chromosomes; iAMP21: intrachromosomal amplification of chromosome 21

#### 4. Chromosomal Abnormalities in T-lineage ALL (T-ALL)

T-ALL has clinical, biochemical, immunologic, chromosomal aberrations that are distinct from B-ALL, an unfavourable prognosis, thus requiring more intensive therapy [37]. CCA revealed that chromosomal aberrations are found in about 60-70% T-ALL compared to 80-90% in B-ALL. Most of the recurrent chromosomal abnormalities in T-ALL are different from those in B-ALL. HH which is common in B-ALL are uncommon in T-ALL. Tetraploidy is present in about 3% of T-ALL but is of no prognostic significance. The nonrandom breakpoints within the T-cell receptor (*TCR*) gene cluster in T-ALL are: *TCRA* and *TCRD* at 14q11.2, *TCRB* at 7q34, and *TRCG* at 7p15. Other chromosome abnormalities associated with T-ALL include breakpoints with 8q24 (*MYC*), 1p32 (*TALI*), 9q32 (*TAL2*), 19p13 (*LYL1*), 21q22 (*OLIG2*), 11p15 (*LMO1*), 11p13 (*LMO2*), 10q24 (*TLX1*), 1p34 (*LCK*), and 9q34.3 (*NOTCH1*) [38].

A high number of T-ALL has cryptic cytogenetic abnormalities that can be detected by FISH or other molecular methods. CCA revealed that about 5-8% of T-ALL with abnormal karyotype have 7q34 (*TCRB*) abnormalities. However, molecular cytogenetics studies showed a higher incidence of *TCRB* rearrangements (20%). Cryptic deletions of the *TALI* (at 1p32) and the tumor suppressor gene *CDKN2A* (at 9p21) are found in 60% and 80% of patients respectively. At present there is no genetic abnormality in T-ALL that has been used for risk assessment. Overall survival outcomes are unaffected by the type of chromosome aberrations in T-ALL [38, 39]. The prognostic significance of T-ALL probably depends on the type and intensity of therapy given.

#### 5. Discussion & Future Direction

Currently, CCA is the gold standard method for the identification of recurrent chromosomal abnormalities [40]. However, CCA has a low resolution (about 5-7 Mb), and is unable to detect

submicroscopic genetic alterations. In a diagnostic cytogenetics laboratory for hematological malignancies, supplementary tests such as FISH and RT-PCR studies are usually performed as well. Although the five-year survival rate of children with ALL has exceeded 85%, survival after relapse remains poor [41]. Cytogenetic and molecular cytogenetic studies revealed chromosomal abnormalities in only about 80% of ALL [42]. However, genome-wide studies utilizing microarray-based techniques and next-generation sequencing (NGS) have shown that most ALL harbour sequence and structural DNA alterations involving coding genes and noncoding elements such as noncoding RNAs and enhancer elements [43]. These genetic alterations affect key cellular pathways in lymphoid development, tumor suppression, cell cycle regulation, cytokine receptor, *ras* signaling and chromatin modifications. These DNA changes also evolve over time from diagnosis to relapse, with the acquisition of new deletions and mutations, and loss of specific genetic lesions at diagnosis [44]. The incorporation of genomic findings would improve diagnosis and risk stratification in ALL, up to the delivery of targeted therapy. Recently, Moorman *et al.*, (2014) [45] developed a novel integrated cytogenetic and genomic classification to refine risk stratification in pediatric ALL. NGS would probably be used to comprehensively identify all clinically relevant genomic alterations including sequence alterations, DNA copy-number alterations, and structural rearrangements in ALL. In future, genome sequencing when becomes cost-effective, may replace many of the diagnostic cytogenetics and molecular tests.

**Conflict of Interest:** None

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