Constitutional genetic testing: chromosomal microarrays or exome sequencing?

Keywords
chromosomal microarray analysis, exome sequencing, genome sequencing, chromosomal aberration, constitutional genetics, cytogenetics, prenatal genetic testing, postnatal genetic testing, next-generation sequencing

Introduction

Technological advances in genetic analysis have greatly expanded the capabilities of constitutional cytogenetics testing in recent years. These advances have led to the emergence of high-resolution, genome-wide methods for chromosomal disorder analysis, namely chromosomal microarray analysis (CMA), exome sequencing (ES), and genome sequencing (GS). With the increased utility of these test methods in clinical research studies, the key challenge for lab directors and clinical scientists is to know when to use which test method for optimal outcomes.

This whitepaper provides an overview of each method, the current recommendations from professional societies, and operational and technical factors one should consider when implementing a test method. It also discusses the suitability of each method and test strategies for different clinical research studies.

Advancements in constitutional genetic testing techniques

More advanced techniques, such as CMA, ES, and GS, can detect more genetic abnormalities than traditional cytogenetic methods such as karyotyping, FISH (fluorescence *in situ* hybridization) and various types of PCR (polymerase chain reaction) based tests. The major drawbacks of such traditional methods are that they can only detect a specific subset of genetic aberrations, usually only find the defects specifically sought, and those with low resolution can only detect larger aberrations. The more advanced techniques, on the other hand, provide much more detailed information about an individual's entire genome that can aid in the identification of genetic disorders.

Chromosomal microarrays

Chromosomal microarrays combine microscale manufacturing techniques with nucleic acid hybridization chemistry. In general, microarrays are based on a solid matrix carrying a defined set of nucleic acid fragments. Labeled DNA fragments from a test sample are applied to these microarrays. The labeled complementary fragments can hybridize with the immobilized DNA fragments on the microarray and unbound DNA fragments are washed away. The microarray is then scanned for fluorescent signals, which indicate the presence of DNA sequences complementary to the immobilized fragments. Software packages for analysis, interpretation, and reporting of cytogenetic microarray data are standardized, commercially available, user-friendly, and often included with the platform.

CMA is optimized for detecting variations in the number of copies of a particular region (copy number variations, or CNVs). Some arrays also contain probes to detect a targeted panel of sequence variations at a single base (single nucleotide variations, or SNVs). Single nucleotide polymorphism (SNP) is when an SNV is present in at least 1% of the population. CMA is well established and is considered the first-tier test in many areas of cytogenetic testing. However, not all CMAs are the same. For more information and explanations on the different CMAs, please refer to our **infographics**.

aCGH and aCGH-SNP arrays

Array comparative genomic hybridization (aCGH) compares the genome from a test sample against a normal reference genome on the same microarray. The reference sample serves as an internal standard for the test DNA, and the relative signal intensity is evaluated. It is critically important that the reference and test sample DNAs are processed identically. Otherwise, the data could be compromised, making aCGH arrays more error-prone.

Most aCGH arrays contain probes for CNV only. While aCGH arrays are useful for CNV calls, without SNP probes it is not possible to reliably detect smaller genetic features such as minor deletions and duplications, absence-of-heterozygosity (AOH), or uniparental disomy (UPD).

With SNP analysis becoming ever more important in cytogenetics testing, there are newer aCGH array versions that contain SNP probes; these arrays are called aCGH-SNP arrays. They are designed to bring the advantages of SNP analysis to aCGH platforms. A study by Halder et al. demonstrated the need for arrays with SNP probes for greater confidence in the detection and identification of copy-neutral abnormalities, such as Silver-Russell syndrome, Beckwith-Wiedemann syndrome, and Prader-Willi syndrome[1]. Although medium-density aCGH-SNP arrays with >100,000 CNV probes and 30,000 or fewer unique SNP probes were suitable for detecting UPD, a study by Mason Suares et al. found that these arrays miscall AOH regions arising from identity by descent[2].

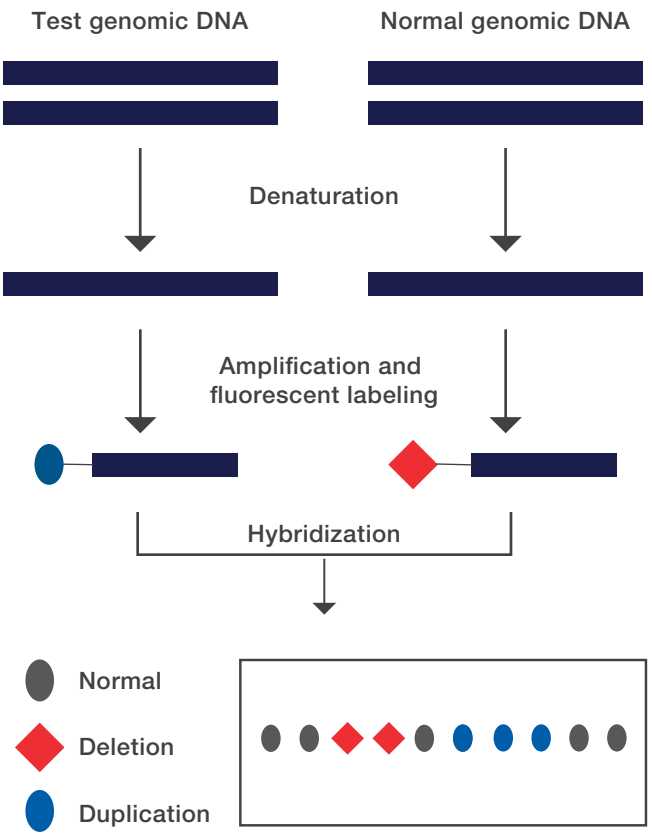

Figure 1: General workflow for aCGH and aCGH-SNP arrays
Genomic DNA from a test and a normal reference sample are processed and labeled with different fluorescent dyes (blue and red) and hybridized to the same array. Deletions and duplications are indicated by an abundance of one color over the other.

SNP arrays

SNP arrays, unlike aCGH arrays, contain immobilized DNA fragments specifically designed to detect allele frequency in the genomic DNA of a test sample. Another difference from aCGH arrays is that analysis of genomic DNA from a reference sample is not needed for comparison. The analysis is performed *in silico* against reference genome databases. Analyzing only the test sample DNA eliminates the need to process the reference and test sample DNA exactly the same way, resulting in more precise data.

Most SNP arrays typically contain less than two million probes, most of which are polymorphic for SNP analysis. CNV detection is imputed from hybridization intensity and allele frequency from these polymorphic SNP probes.

Hybrid-SNP arrays

Hybrid-SNP arrays are the latest generation of arrays that combine the best features of SNP arrays and aCGH arrays. They are high-resolution and high-density with up to seven million probes, containing both polymorphic (SNP) and non-polymorphic (copy number) probes. These higher-density arrays give greater confidence to the detection and positive identification of copy-neutral abnormalities. Like SNP arrays, only the test sample is processed and analyzed, resulting in more precise data analysis. Some hybrid-SNP arrays also contain probes to detect a targeted panel of SNVs.

Hybrid-SNP arrays can detect:

- CNV (from aneuploidies to small CNV >25 kb for losses and >50 kb for gains)
- Structural variants, unbalanced
- Suspected uniparental disomies (UPDs), whole genome UPD, copy-neutral loss of heterozygosity (cnLOH), or absence-of-heterozygosity (AOH)
- Mosaicism
- Sample heterogeneity, clonal diversity
- Triploidy
- Tetraploidy (depending on the mechanism)
- Hypo- and hyperploidy
- Zygosity
- Consanguinity
- Finding the parent of origin (requires parental genomic analysis)
- Twin-twin or maternal cell contamination
- Allele-specific changes
- Genomic contamination

While the nomenclature used between aCGH-SNP arrays, SNP arrays, and hybrid-SNP arrays sound similar, there are major technical differences that may affect discovery yield. It is important to confirm and ensure the right array is used in the study for the clinical indications researched.

In general, CMAs cannot detect balanced chromosomal translocations, trinucleotide repeat expansion disorders, or SNVs. Alternative test methods should be performed if such aberrations are suspected.

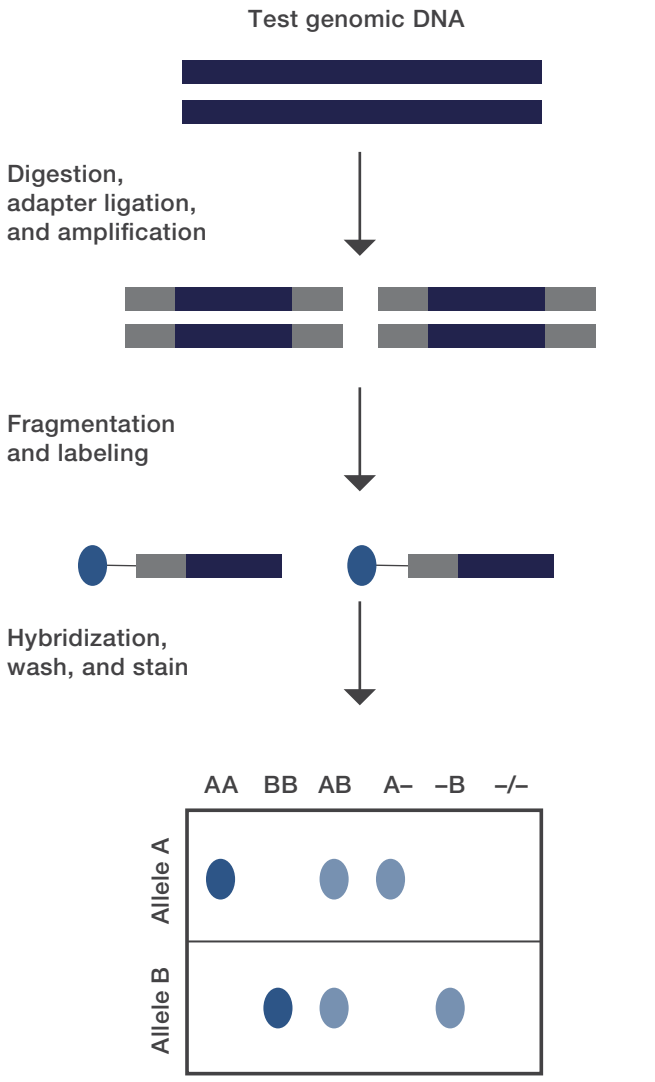

Figure 2: General workflow for SNP and hybrid-SNP arrays
Genomic DNA from the test sample alone is processed, labeled, and hybridized to the array. The fluorescence signal intensity is compared to a reference data set to determine allele frequency and copy number. Homozygous samples (AA or BB) will show signals on the Allele A or Allele B position on the array. Heterozygous samples (AB) will show signals on both positions. Samples with a deletion (A– or –B) will show signals with reduced intensity on the Allele A or Allele B position. Samples with deletions of both alleles (–/–) will not show signals above the background level. Samples with duplications (not shown) will show an increased signal intensity relative to the reference.

Next-generation sequencing

Based on a massive parallel method, next-generation sequencing (NGS) can sequence millions or even billions of DNA fragments to detect many different genetic variations from multiple samples at once. Each NGS platform has its proprietary chemistry and algorithm for DNA sequencing and data analysis; however, the general steps are the same: (1) library preparation, which involves fragmenting the genome randomly, (2) amplification of the libraries, (3) massively parallel sequencing of the libraries using platform-specific chemistry and signal detectors, and (4) conversion of raw signals to sequencing data using platform-specific algorithm and bioinformatics. The sequencing reads are mapped to a reference genome for the identifications of SNVs, insertions and deletions (indels), and, more recently, CNVs using specialized software tools. Data analysis and interpretation require a complex bioinformatics pipeline and expert-level bioinformaticians. Numerous software tools are available and continue to improve to simplify the process and increase user-friendliness.

Due to continued advancements leading to a better understanding of the technology's capabilities and limitations, reduced cost, and faster turnaround time, the use of NGS in genetic testing is expanding to increase discovery yield and improve outcomes.

Exome sequencing

Exome sequencing (ES) sequences only the coding regions (exons) of more than 20,000 genes, representing about 1.5% of the entire human genome. ES can be a much faster and less expensive research method than GS to detect disease-associated genetic variants or confirm a clinical observation. Only the exome sequences are captured and isolated for sequencing. Since most disease-associated genetic variants occur in the exome, it is efficient to focus the search specifically on this part of the genome. Nevertheless, variants in the non-coding regions are missed by ES. It has also been shown that ES is not ideal for detecting larger DNA structural variations[3], trinucleotide repeat expansions, and methylation anomalies, and may have limited capabilities for detecting copy number variants[4].

ES can detect:

- SNV
- Indel
- CNV (depending on the read depth, coverage, and algorithm used)
- Mosaicism (depending on read depth)
- AOH/LOH (depending on the coverage and algorithm used)

Unlike CMAs, accurately detecting CNVs using NGS methods is challenging and lacks standardization. There are numerous different calling methods available, yet no consensus and limited guidelines. Here are summaries from a few publications on their findings, approaches, and recommendations.

Lincoln et al. conducted a research study to evaluate different NGS workflows in the detection of technically challenging variants for clinical genetic tests. They found that they had to use multiple algorithms to call these variant types and the sensitivity of different workflows performed differently. Off-the-shelf bioinformatics solutions performed poorly. They also found pathogenic variants had low or no coverage in some ES workflows, which could affect sensitivity—especially for small CNVs. They stated that confirmatory testing should be mandatory in some cases due to NGS's tendency to achieve high sensitivity but poor specificity for some of these variants[5].

Due to the lack of comprehensive guides for use of ES-based CNV detection tools for research studies in clinical settings, Zhao et al. conducted a comparison study of four different tools: CoNIFER, cn.MOPS, CNVkit, and exomeCopy. Three aspects of each tool were evaluated: (1) the sensitivity and specificity of detection for various CNV coverages, sizes, and types, (2) the overlapping consistency, and (3) the computational cost, including time (CPU usage and running time) and computer space (storage space required by the algorithm). Findings concluded that no one tool performs well under all conditions and that different tools are recommended based on CNV size and type, turnaround time, and computing power[6].

Coutilier et al. took a different approach by combining four callers (Manta, Delly, ERDS, CNVnator) and a regenotyping tool (SV2) to obtain the best compromise between sensitivity and practicality. In comparison to the aCGH array, they found this approach brought more resolution to the call breakpoints and detected a higher but manageable number of calls. However, they do acknowledge that using multiple techniques increases cost and turn-around time[7].

Finally, a review article published in *The Journal of Clinical Investigation* by Schuler et al. provides a comprehensive discussion on the strengths and limitations of different genetic testing methods, with a heavy emphasis on NGS[3].

Genome sequencing

Genome sequencing (GS) analyzes the entire genome at the nucleotide level, including coding and non-coding regions, generating a massive amount of data. No other method can provide the same level of detail and overall coverage. Despite GS providing more genetic information, it is not recommended for routine constitutional genetic testing. It could be useful for rare genetic disorders when no other test methods have returned a result but, GS takes significantly longer to perform, requires more data storage, has a more complex analysis process, and is more costly compared to ES. There is still a lack of deep knowledge on the function of a considerable part of the human genome; therefore, much of the GS results are of unknown clinical relevance.

GS can detect:

- SNV
- Indels
- CNV (depending on the read depth, coverage, and algorithm used)
- Structural variants, balanced and unbalanced
- Mosaicism (depending on read depth)
- AOH/LOH
- Repeat expansion

Recommendations from professional societies

With continued technological advancements and increased applications in clinical research labs, lab directors and clinical scientists need to be aware of and stay up to date on the recommendations and guidelines issued by various professional societies. Here we provide a snapshot of the current recommendations.

American College of Obstetricians and Gynecologists (ACOG)

In 2016, ACOG issued a Committee Opinion that made recommendations on the use of CMA and NGS for prenatal testing.

ACOG proposes that CMA be used as the primary prenatal genetic testing method of choice. This is based on the major advantage of CMA over standard karyotyping in terms of discovery yield. Prenatal CMA is recommended for research studies of individuals whose fetus has one or more major structural abnormalities detected on ultrasonographic examination, and who undergo invasive prenatal testing[11].

CMA is also recommended in the research evaluation of intrauterine fetal death or stillbirth, if further cytogenetic testing is desired, due to its higher probability of detecting causative abnormalities. Most genetic alterations identified by CMA, which are typically not found in the standard karyotype, are not associated with increasing maternal age; therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal testing[11].

Prenatal ES may be reasonable in select circumstances in consultation with a geneticist, but ACOG does not recommend the use of GS or ES for prenatal testing outside of clinical trials because insufficient data and validation studies have been published on these methods[11].

American Academy of Pediatrics (AAP) and American Academy of Neurology (AAN)

Both academies recommend cytogenetic testing to look for chromosomal abnormalities when an individual has DD, ID, multiple CA, autism spectrum disorders, and other symptoms with unclear origins. CMA is generally recommended as a first-tier test in these research studies. In addition, AAP suggests ES as a follow-up test when CMA and fragile X testing do not identify an etiology[12,13].

American College of Medical Genetics (ACMG)

ACMG recommends, in 2010 and reaffirmed in 2020, CMA testing for CNV in individuals with apparently nonsyndromic developmental delay (DD)/intellectual disability (ID), autism, and multiple anomalies not specific to a well-delineated genetic syndrome. They also recommend CMA testing should be performed for other disorders such as growth retardation, speech delay, and other lesser-studied indications. Since there are different CMA types, they highlighted the need for awareness of each platform's limitations and the CMA type utilized by the clinical research lab[8].

Based on available evidence, ACMG supports the use of ES and GS as either first-tier or second-tier tests in individuals with congenital anomalies (CA) with onset prior to age 1 or DD, or ID with onset prior to age 18. ES is generally recommended as a first- or second-tier test (e.g., after CMA or targeted testing) because of its higher discovery yield at a lower cost than when used only after extensive standard testing (e.g., large sequencing panels and/or multiple testing approaches) or standard testing alone. However, ACMG highlights the need for awareness of ES limitations. The decision to use ES should be based on clinical scenarios researched and shared decision-making between stakeholders[4].

For prenatal testing, ACMG stated ES may be used as a second-tier test in a fetus with one or more significant ultrasound anomalies where routine methods such as CMA and karyotyping have failed to provide definitive results. It also stated that there are no data supporting the use of ES for research into other reproductive indications, such as aneuploidy or recurrent unexplained pregnancy loss[9].

European Society of Human Genetics (ESHG)

European guidelines for constitutional cytogenomic analysis state that CMA is recommended as a first-tier test for research studies of individuals with ID, autism, neurodevelopmental disorders, and/or CA due to its high discovery yield. It is also recommended that a CMA be performed in research studies when a fetal ultrasound shows abnormal structural features[10].

ESHG's opinion on NGS is that ES is a promising tool for genetic testing. It is argued that the focus on the exome is justified because 85% of disease-causing variants are found in this part of the genome. On the other hand, the European guidelines for constitutional genetic analysis state that the widespread use of ES is currently hampered by both high costs and the need for complex analysis algorithms[10].

Implementation and operational considerations

Data quality	Resources	Time	Cost
• High-quality data requires robust and standardized computational analysis tools • Bioinformatics plays a major role in data analysis, interpretation, and reporting • ACMG technical standards provide guidelines and recommendations	• Include resources for library preparation, automation, and computational analysis • Ensure access to qualified personnel, including skilled bioinformatics • Resources should be scalable for sample volume growth	• Turnaround time (TAT) should be appropriate for decision making • TAT should include time for data analysis, interpretation, and reporting as well as for confirmatory testing, if required	• Include the cost of personnel, infrastructure, and maintenance, in addition to instrumentations • Not all platforms have the same throughput dependency for cost-per-sample calculations • Weigh the cost vs. benefits when deciding which test to use

Figure 3: Key implementation and operational considerations

In addition to understanding the analytical capabilities and limitations of each technology, as well as recommendations by professional societies, lab directors and clinical scientists must also consider the operational factors, such as data quality, resources required to perform the test, turn-around time, and cost, when selecting a test platform for research studies.

Data quality

Each technology platform has its analytical performance characteristics that will affect data quality. Data quality depends not only on the primary data but also on the analysis. Bioinformatics plays a major role in data analysis, interpretation, and reporting. Therefore, to generate high-quality data, robust computational analysis tools are required. The tools should be easy to use and flexible to generate custom reports. For CNV analysis, CMA has more robust, out-of-the-box, standardized analysis tools. As demonstrated earlier, algorithms and analysis tools are still evolving and being established for NGS.

Adhering to best practices and technical standards can help clinical research labs deliver quality genetic data and services. The ACMG technical standards for CMA[14] and for NGS[15] provide guidelines and recommendations on (1) platform verification and validation on various performance criteria, sample types, variant types, (2) bioinformatics and software considerations, (3) quality control and assurance, and (4) interpretation and reporting. And for NGS, since it is a complex technology that is still evolving, the technical standards include additional guidelines such as (1) confirmatory testing policy, (2) data storage, security, and traceability of reports, and (3) staff qualifications.

Resources

A lab's access to resources is multi-faceted. Considerations should be made for (1) space for the various instruments and workbench, (2) budget for implementing an end-to-end solution, including those for confirmatory testing, (3) computational needs, including IT staff, data storage, including back-up, (4) maintenance and service of instruments, and (5) qualified personnel. These resources should be scalable to accommodate sample volume growth.

When considering space and budget for instruments, ancillary instruments such as those for library preparation, automation, servers for data storage, and high-power computers should be included as part of the end-to-end solution.

Resource requirements for NGS and CMA are quite different. CMA generally does not require large instruments or expensive computational equipment for data analysis and storage. Due to data analysis, interpretation, and reporting for CMA being more established, robust, and user-friendly, there is less requirement for expert-level bioinformatics.

Time

Turnaround time (TAT), the time between sending a sample to the lab and receiving the results, is very important, especially in research studies for prenatal testing and in some cases, postnatal testing. Test results should be made available at a time that is appropriate for decision making.

When establishing TAT, it is important to consider assay time, including hands-on time, as well as data analysis, interpretation, and reporting time. Data analysis and interpretation time could be impacted by the computational power of the computers used in the case of NGS. The time required to perform confirmatory testing should be included as well.

Cost

Finally, testing costs must also be considered. In general, the cost per sample depends on throughput. Higher throughput, in principle, lowers the cost per sample. Higher throughput labs may therefore be able to offer genetic tests at a lower price.

The cost per sample for NGS methods depends on the number of samples sequenced per sequencing run. The more samples sequenced per run, the lower the cost per sample. For CMA, the cost per sample is less dependent on the number of samples processed per run.

For NGS to obtain a cost per sample similar to that of CMA, a high throughput instrument with high volume runs is needed. Investments in instrumentation, personnel, and infrastructure must be considered when analyzing and comparing costs and deciding which technology to use.

One must weigh the costs and benefits when deciding which test to use. What a lab can charge for a test and which test to order may also be determined by reimbursement rates or national health services policies. For private service labs, there is a market price that is determined by competition and what one is willing to pay out-of-pocket. Therefore, labs must choose a technology that allows them to meet local market conditions.

Technical considerations

Different approaches for detecting different variant types

It is important to understand the analytical limitations of each testing approach to ensure an appropriate conclusion. Research studies for different clinical scenarios require different testing strategies. One single test, like CMA or ES, might provide a result for some samples; however, for other samples, an iterative approach that requires complementary methods and additional confirmatory tests might be needed to provide an accurate interpretation.

NGS has proven to be quite successful in clinical research studies. For example, singleton ES achieved a discovery rate of 52% in children with suspected monogenic conditions in a study by Tan et al.[16] According to other studies, GS (41% yield) is generally superior to other methods including ES (24% yield) due to its ability to identify structural variants and non-coding variants[17].

However, there is currently no "one-size-fits-all" solution for genetic testing. Even though NGS is the most advanced method in terms of base accuracy, there are examples where NGS might not be the most pragmatic choice or has failed to reveal the variant responsible for the disease. Aneuploidies and mosaicism can be detected with GS, but normal karyotyping or CMA can produce the same result in less time and at a lower cost. Balanced translocations may be missed by NGS, which was demonstrated in a research study in which neurofibromatosis type 1 was confirmed by FISH after being missed by NGS[18].

It should also be noted that CMA, ES, and GS cannot detect epigenetic disorders, such as abnormal methylation patterns. Therefore, methylation studies should be considered simultaneously. Similarly, NGS requires specialized bioinformatics and highly reproducible, uniform data to detect large CNVs, repeat expansions, genomic rearrangements, and mosaicism. Complementary testing should be done if such disorders are a possible explanation for a particular research study[15].

More data is not always better

The more genetic data a method generates, the more likely a disease-causing variant is present in the data set. On the downside, more data also increases the likelihood of identifying more variants of uncertain significance (VUS) and secondary findings (SFs), and in many cases, without detecting the causal variant. All of these can complicate data interpretation and reporting, thus requiring expert-level data analysis by a qualified bioinformatician. The burden of VUS and SFs is highest with GS, followed by ES, then CMA.

Each clinical research lab should have clear guidelines and policies on the reporting of VUS and SFs, especially for prenatal testing. These policies should be consistent with professional guidance, as published by ACMG[14,15,19].

Table 1: Capabilities of the different methods for chromosomal abnormality detections

	aCGH	SNP array	Hybrid-SNP array	ES	GS
Reference DNA	Single biological sample	*in silico* database	*in silico* database	*in silico* database	*in silico* database
Density/coverage	++	+++	++++	+++++	++++++
Type of variants detected					
Aneuploidies	+	+	+	-	+/-
Balanced translocation/inversion	-	-	-	-	+/-
Large deletion/duplication	+	+	+	-	+/-
Mosaicism	+/-	+	+	+	+
Ploidy	-	+	+	-	+
Single base substitution	-	-	-	+	+
Single base insertion/deletion	-	-	-	+	+
Small deletion/duplication (>10Kb)	-	+	+	+/-	+
Absence/loss of heterozygosity (AOH/LOH)	-	+	+	+/-	+
Uniparental disomy (UPD)	-	+	+	+	+
Gene fusions	-	+/- [1]	+/- [1]	+	+
Noncoding variants	-	-	-	-	+
Methylation defects	-	-	-	-	-
Repeat expansions	-	-	-	-	+/-
VUS burden	++	++	++	++++	+++++
Cost	$$	$$	$$	$$$$	$$$$$
Turnaround time (TAT)	Days	Days	Days	1 - 3 weeks	Months

For Density/coverage + is the lowest density/coverage and ++++++ is the highest
+/– means that the method may or may not detect that variant type
For VUS + is the least number detected, +++++ is the most number detected
1 can detect unbalanced gene fusions, cannot detect balanced gene fusions

Recommendations: the right tools for the right scenario

Choosing the right genetic test method can drastically improve outcomes. The right tests can shorten the discovery journey with increased discovery yield and faster time to results. It can also reduce test costs. As discussed earlier, choosing the right test method for clinical research studies is not trivial. The choice should be driven by the clinical scenario researched, which can inform the most suitable method based on technical performance; however, one must also consider a lab's operational resources and capabilities, which can affect data quality, TAT, and cost.

For prenatal testing, time to results is critical and ultrasound findings can help drive the approach for molecular genetics testing. For example, in research studies for a fetus with one or more major structural abnormalities, or with increased nuchal translucency, CMA has been the first-tier test due to faster TAT, lower cost, high causal variant coverage, and low VUS and SFs burden. If the CMA test result is negative, ES as a second-tier test can be performed for further investigation. CMA has also been the confirmatory test in research studies for a fetus with positive CNV in non-invasive prenatal testing (NIPT). In research studies for a fetus with an aneuploidy positive NIPT result that is either unconfirmed or inconsistent with phenotype, CMA has been used for further investigation.

In research studies for postnatal testing, time to results is usually less critical and the test strategy that combines SNV and CNV detections can provide the most information for the investigation of ID/DD. Using CMA as the first-tier test, and if negative, using ES as the second-tier test is the more established approach. This approach is ideal, especially for labs transitioning from karyotyping as well as those with limited resources or who need to outsource NGS testing. A more recent approach, especially for labs with NGS capability and proper resources, ES is used for CNV detection followed by CMA as a confirmatory test.

Complementary testing increases the discovery yield

Research studies have demonstrated that combining ES with CMA, either as a complementary first-tier or second-tier test, can improve discovery yield. Here are some case studies.

Chen et al. studied the discovery yield of combining CMA and ES in 61 samples from individuals with moderate or severe DD/ID with unexplained etiology. Using a targeted-disease, low-density CMA as the first-tier test, clinically significant CNVs were identified in 12 of the samples, representing a 19.7% discovery rate. The 49 samples with negative CMA results were analyzed with ES, which further detected CNVs in 25 samples. The combined CMA and ES yielded a discovery rate of 60.7%[20].

Benson et al. determined the discovery yield of combining ES and aCGH with 101 samples from adults and children with epilepsy and co-morbid ID. Their research findings revealed an overall discovery rate of 31%, with 27% in unrelated adults and 42% in unrelated children[21].

Tammimies et al. conducted a comparative research study to determine the performance of CMA and ES with 258 samples from children with autism spectrum disorder (ASD). All 258 samples underwent CMA and 95 with ES. Of the samples, 24 received a molecular determination by CMA (9.3%), and 8 of 95 from ES (8.4%). In samples tested with both CMA and ES, a combined discovery yield of 15.8% was obtained. Based on the combined discovery yield of these two methods, the authors estimate that a molecular determination might be assigned to more than 35% of samples from ASD children with additional medical and dysmorphology features[22].

Confirmatory testing for more confident results

Confirmatory testing should be performed if an unexpected finding or aberrations that cannot be reliably detected by one method have resulted. The choice of confirmatory method depends mainly on the research question at hand and the first-tier test used. The type and number of variants that require confirmation, method availability, and cost may also play a role in the choice of confirmatory methods. Confirmatory test methods can include karyotyping, FISH, PCR, and MPLA as well as Sanger sequencing and CMA. For example, suspected large structural variants should be confirmed by FISH. For confirmation of single CNV, MLPA and qPCR are cost-effective methods. CMA, especially ones with high-resolution, whole-genome coverage, is a fast and cost-effective orthogonal method for confirming multiple CNV calls from GS/ES data.

Summary

CMA, ES, and GS are powerful genome-wide chromosomal aberration analysis tools for constitutional genetic testing. As discussed in this white paper, each method has its technical and operational advantages and limitations. CMA is more established, robust, and requires fewer resources, including bioinformatics, to implement than NGS. However, not all CMAs are the same. Hybrid-SNP arrays, with the highest density of polymorphic and non-morphic probes, can be more informative than arrays with low density or that lack polymorphic probes (aCGH arrays) or non-polymorphic probes (SNP arrays).

ES and GS are increasingly utilized by clinical research labs as first-tier or second-tier tests. Since NGS technology is still evolving for testing, one must stay up to date on professional society guidelines and recommendations. In addition, it is important for lab directors to fully assess all the resources required and market conditions (sample volume, reimbursement, and out-of-pocket pricing) before deciding to implement NGS testing.

The decision on which test method to use should be informed by clinical indications researched and prior test results, if available. However, there is no “one size fits all” method. Often multiple tests will be required to complement or confirm the initial test results. The chosen test strategy should consider TAT, cost, and the best outcome for the end user.

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