Reliable and coordinated genomic investigations of rare conditions in southern Sweden offers clinical differential diagnostics for pediatricians and pathologists

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Introduction

An established genetic diagnosis in a patient with *e.g.*, an unexplained neurological condition, a congenital abnormality or a metabolic disease is crucial for clinical decisions and habilitation, facilitates allocation of support, alleviates the psychological burden, and is essential for calculation of recurrence risk as well as potentiating prenatal diagnostics in future pregnancies. A diagnosis also empowers families to seek knowledge and support through genetic counselling as well as through online information sources and support groups such as socialstyrelsen.se/ovanligadiagnoser and rarechromo.org (Unique).

Recently, it was difficult to investigate more than one gene at a time and, given that we have more than 21000 protein coding genes, it often took a long time to identify the cause of heterogenic diseases and syndromes. Advances in two crucial techniques, genomic array and exome sequencing, today makes it possible to investigate essentially all genes in parallel.

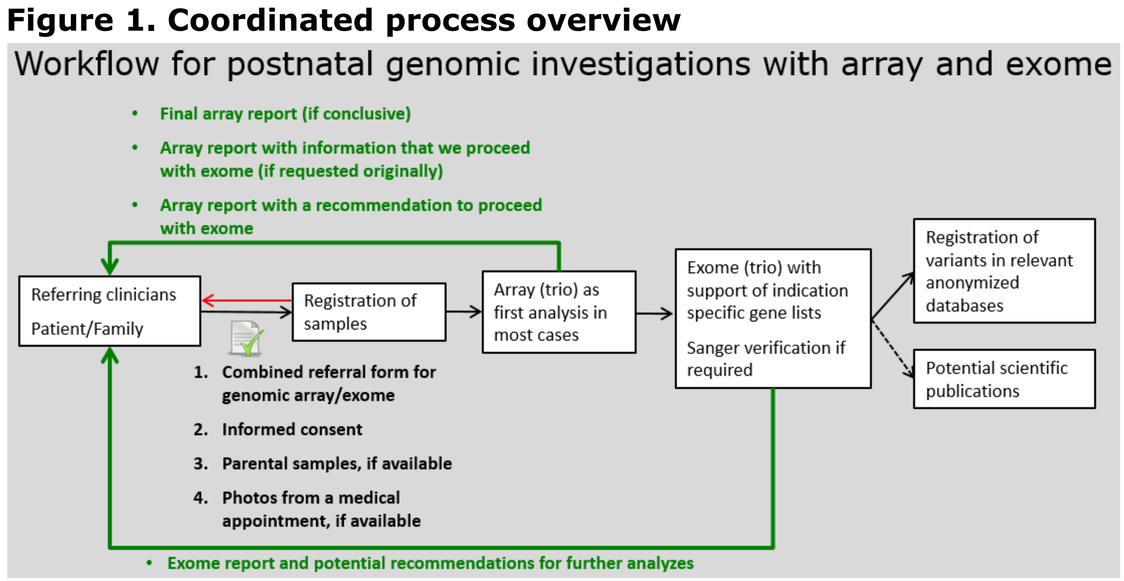
A general consensus has been established that genomic array, and sequential exome analysis, should be considered as first-line tests to aid in the evaluation of children with unexplained intellectual disability, autism spectrum disorder and/or congenital anomalies[1-5]. The expected clinical yield of these tests are for high resolution genomic array 15-20% and in combination with exome sequencing >50%[3].

Project milestones, objectives and techniques

At the Department of Clinical Genetics in Lund we have since 2010 received support from Regional decision makers, to introduce and coordinate these new technologies in close collaboration with clinicians in Southern Sweden and abroad. The efforts were acknowledged by SWEDAC in 2012 for the genomic arrays with accreditation (according to "ISO 15189:2012 Medical laboratories - Requirements for quality and competence"). In 2013 Clinical Genetics also received reference laboratory status for genomic array (Affymetrix) and in 2016 for exome sequencing (ThermoFisher).

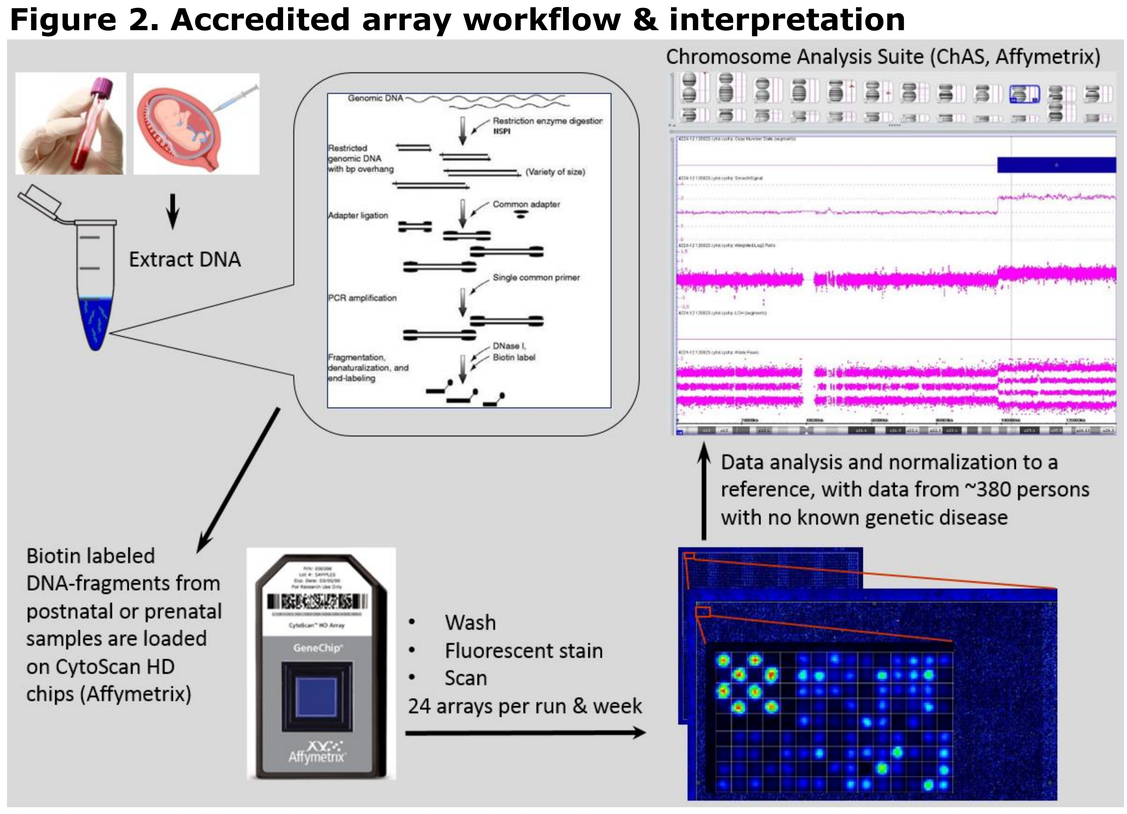
The main objective of the presented coordinated workflow (fig. 1) is to provide reliable and cost-efficient genetic investigations, using complementary[3,6] genomic array (fig. 2) and exome sequencing (fig. 3), of rare conditions with a rapid turn-around time to greatly facilitate clinical differential diagnostics in southern Sweden. Today, the turnaround time for array analysis of non-prioritized cases is less than 1 month and within 2 weeks when prioritized. For exome sequencing we are in the process of speeding up data analysis and expert clinical interpretation to achieve a total turnaround time of genomic array plus exome sequencing of less than 4 months.

Figure 1. Coordinated process overview



Published budget impact calculations suggests overall health care savings for a coordinated work-flow for array and 'low cost exome', where platform choice determines cost-efficiency[7]

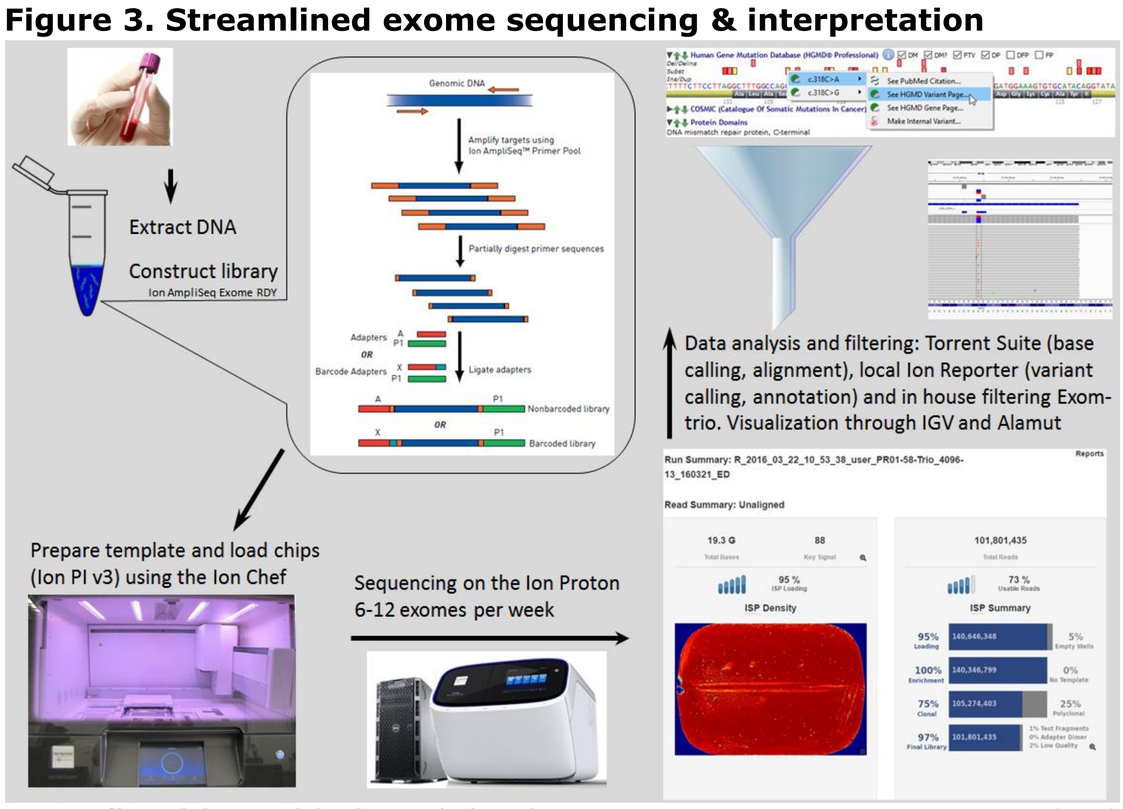
Figure 2. Accredited array workflow & interpretation



CytoScan HD (Affymetrix), our analysis-platform for genomic array is widely viewed as the golden standard of choice due to its high resolution (1 marker per ~880 bp), allowing detection of all well described microdeletion and microduplication syndromes. In total, the array includes 2.7 million markers (of which 750' are SNPs) across the entire genome.

Parental samples are, if obtainable, always analyzed together with the child (trio)
Trio-analysis aids interpretation of unclear findings and enables detection of uniparental disomy (UPD), identification of sample mix-ups, and parent of origin annotation of genetic variants. UPD occurs when a person receives two copies of a chromosome, or part of it, from one parent and no copy from the other, which can cause disease by unmasking recessive traits or by altering gene expression. The array also gives a measure of consanguinity, which greatly increases the risk of recessive disorders. At this time, we do not recommend *in silico* searches of candidate genes (followed by Sanger) – instead, after normal array results, we recommend exome trio-sequencing (fig. 3), especially for consanguineous cases.

Figure 3. Streamlined exome sequencing & interpretation



Ion AmpliSeq (ThermoFisher), our platform for exome sequencing is using a semi-automated work flow on the Ion Chef and Ion Proton machines. Sequencing gives the highest possible resolution, as it detects single base pair genetic variants. In contrast to genomic array analysis (fig. 2) exome sequencing is, at this moment, not recommended for detection of copy number changes. Therefore, genomic arrays provides a strong backbone to exome sequencing.

Development and validation of exome sequencing, from sample to interpreted results, began upon the arrival of the Proton sequencer (ThermoFisher) in 2013 and initial findings, verified by Sanger, suggests a high clinical yield. Each of our protein coding genes is usually composed of both coding sequence (exons) and noncoding sequence between the exons (introns). The term "exome" includes all exons. The current Ion AmpliSeq exome analysis (ThermoFisher) covers >97% of "Consensus Coding Sequences (CCDS) annotation" and at least 5 base pairs surrounding each exon, potentiating the detection of splicing mutations.

At the Dep. of Clinical Genetics in Lund, user friendly bioinformatic tools and databases, with graphical interfaces, have been developed, *e.g.*, Mímisbrunnr, MyUPDFinder, MyPODFinder, Exome DB (in house) and Exome-trio, to provide visualization and interpretation support of results from trio or single sample arrays and exomes. The interpretation tools include *in silico* gene panels for specific clinical indications.

Results and conclusions

Here we have described a coordinated workflow for genomic array and exome sequencing, enabling reliable genetic investigations of rare conditions, with a relatively rapid turn-around time, to facilitate clinical differential diagnostics for pediatricians and pathologists. By striving for cost effectiveness[7], using available technologies and local know-how, we can offer a competitive sequential package of array and exome trio analysis for ~5500€.

To date, the number of array samples processed surpass 3500, with an average clinical yield of ~15%. In addition, more than 100 sequencing runs, or 200 individual exome samples, have been processed. Including two preclinical exome research studies of 32 stroke patients and 39 paired or single tumor samples. Data from initial exome cases suggests an additional clinical yield of >30%.

We can conclude, in line with published studies, that together with a well described clinical examination and local expert interpretation teams, these methods can find the cause for rare diseases and syndromes in about half of the investigated patients.

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