Stem Cell and next-generation sequencing (ngs) Research Literatures

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Abstract: Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as medical diagnosis, biotechnology, forensic biology, virology and biological systematics. The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species. An example of the results of automated chain-termination DNA sequencing. The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on two-dimensional chromatography. Following the development of fluorescence-based sequencing methods with a DNA sequencer, DNA sequencing has become easier and orders of magnitude faster. This article introduces recent research reports as references in the related studies.
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Key words: stem cell; next-generation sequencing (ngs); life; research; literature

Introduction

Stem cells are derived from embryonic and non-embryonic tissues. Most stem cell studies are for animal stem cells and plants have also stem cell. Stem cells were discovered in 1981 from early mouse embryos. Stem cells have the potential to develop into all different cell types in the living body. Stem cell is a body repair system. When a stem cell divides it can be still a stem cell or become adult cell, such as a brain cell. Stem cells are unspecialized cells and can renew themselves by cell division, and stem cells can also differentiate to adult cells with special functions. Stem cells replace the old cells and repair the damaged tissues. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin. DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as medical diagnosis, biotechnology, forensic biology, virology and biological systematics. The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species. An example of the results of automated chain-termination DNA sequencing. The first DNA sequences were obtained in the early 1970s by academic researchers using laborious methods based on two-dimensional chromatography. Following the development of fluorescence-based sequencing methods with a DNA sequencer, DNA sequencing has become easier and orders of magnitude faster. This article introduces recent research reports as references in the related studies.

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references in the related studies.

Abou Tayoun, A. N., et al. (2016). "Improving hearing loss gene testing: a systematic review of gene evidence toward more efficient next-generation sequencing-based diagnostic testing and interpretation." Genet Med **18**(6): 545-553.

PURPOSE: With next generation sequencing technology improvement and cost reductions, it has become technically feasible to sequence a large number of genes in one diagnostic test. This is especially relevant for diseases with large genetic and/or phenotypic heterogeneity, such as hearing loss. However, variant interpretation remains the major bottleneck. This is further exacerbated by the lack in the clinical genetics community of consensus criteria for defining the evidence necessary to include genes on targeted disease panels or in genomic reports, and the consequent risk of reporting variants in genes with no relevance to disease. METHODS: We describe a systematic evidence-based approach for assessing gene-disease associations and for curating relevant genes for different disease aspects, including mode of inheritance, phenotypic severity, and mutation spectrum. RESULTS: By applying this approach to clinically available hearing loss gene panels with a total of 163 genes, we show that a significant number (45%) of genes lack sufficient evidence of association with disease and thus are expected to increase uncertainty and patient anxiety, in addition to intensifying the interpretation burden. Information about all curated genes is summarized. Our retrospective analysis of 539 hearing loss cases tested by our previous OtoGenomeV2 panel demonstrates the impact of including genes with weak disease association in laboratory wet-bench and interpretation processes. CONCLUSION: Our study is, to our knowledge, the first to highlight the urgent need for defining the clinical validity of gene-disease relationships for more efficient and accurate clinical testing and reporting. Genet Med 18 6, 545-553.

Adamopoulos, P. G., et al. (2017). "Identification and molecular cloning of novel transcripts of the human kallikrein-related peptidase 10 (KLK10) gene using next-generation sequencing." Biochem Biophys Res Commun **487**(4): 776-781.

Tissue kallikrein and kallikrein-related peptidases (KLKs) form the largest group of serine proteases in the human genome, sharing many structural and functional characteristics. Multiple alternative transcripts have been reported for the most human KLK genes, while many of them are aberrantly expressed in various malignancies, thus possessing significant prognostic and/or diagnostic value. Alternative splicing of cancer-related genes is a common cellular mechanism accounting for cancer cell transcriptome complexity, as it affects cell cycle control, proliferation, apoptosis, invasion, and metastasis. In this study, we describe the identification and molecular cloning of eight novel transcripts of the human KLK10 gene using 3' rapid amplification of cDNA ends (3' RACE) and next-generation sequencing (NGS), as well as their expression analysis in a wide panel of cell lines, originating from several distinct cancerous and normal tissues. Bioinformatic analysis revealed that the novel KLK10 transcripts contain new alternative splicing events between already annotated exons as well as novel exons. In addition, investigation of their expression profile in a wide panel of cell lines was performed with nested RT-PCR using variant-specific pairs of primers. Since many KLK mRNA transcripts possess clinical value, these newly discovered alternatively spliced KLK10 transcripts appear as new potential biomarkers for diagnostic and/or prognostic purposes or as targets for therapeutic strategies.

Adamopoulos, P. G., et al. (2016). "Identification of novel alternative splice variants of the BCL2L12 gene in human cancer cells using next-generation sequencing methodology." Cancer Lett **373**(1): 119-129.

The next-generation sequencing (NGS) technology has enabled genome-wide studies, providing massively parallel DNA sequencing. NGS applications constitute a revolution in molecular biology and genetics and have already paved new ways in cancer research. BCL2L12 is an apoptosis-related gene, previously cloned from members of our research group. Like most members of the BCL2 gene family, it is highly implicated in various types of cancer and hematological malignancies. In the present study, we used NGS to discover novel alternatively spliced variants of the apoptosis-related BCL2L12 gene in many human cancer cell lines, after 3'-RACE nested PCR. Extensive computational analysis uncovered new alternative splicing events and patterns, resulting in novel alternative transcripts of the BCL2L12 gene. PCR was then performed to validate NGS data and identify the derived novel transcripts of the BCL2L12 gene. Therefore, 50 novel BCL2L12 splice variants were discovered. Since BCL2L12 is involved in the apoptotic machinery, the quantification of distinct BCL2L12 transcripts in human samples may have clinical applications in different types of cancer.

Afrin, S., et al. (2018). "Targeted Next-Generation Sequencing for Detecting MLL Gene Fusions in Leukemia." Mol Cancer Res **16**(2): 279-285.

Mixed lineage leukemia (MLL) gene rearrangements characterize approximately 70% of infant and 10% of adult and therapy-related leukemia. Conventional clinical diagnostics, including cytogenetics and fluorescence in situ hybridization (FISH) fail to detect MLL translocation partner genes (TPG) in many patients. Long-distance inverse (LDI)-PCR, the "gold standard" technique that is used to characterize MLL breakpoints, is laborious and requires a large input of genomic DNA (gDNA). To overcome the limitations of current techniques, a targeted next-generation sequencing (NGS) approach that requires low RNA input was tested. Anchored multiplex PCR-based enrichment (AMP-E) was used to rapidly identify a broad range of MLL fusions in patient specimens. Libraries generated using Archer FusionPlex Heme and Myeloid panels were sequenced using the Illumina platform. Diagnostic specimens (n = 39) from pediatric leukemia patients were tested with AMP-E and validated by LDI-PCR. In concordance with LDI-PCR, the AMP-E method successfully identified TPGs without prior knowledge. AMP-E identified 10 different MLL fusions in the 39 samples. Only two specimens were discordant; AMP-E successfully identified a MLL-MLLT1 fusion where LDI-PCR had failed to determine the breakpoint, whereas a MLL-MLLT3 fusion was not detected by AMP-E due to low expression of the fusion transcript. Sensitivity assays demonstrated that AMP-E can detect MLL-AFF1 in MV4-11 cell dilutions of 10(-7) and transcripts down to 0.005 copies/ng.Implications: This study demonstrates a NGS methodology with improved sensitivity compared with current diagnostic methods for MLL-rearranged leukemia. Furthermore, this assay rapidly and reliably identifies MLL partner genes and patient-specific fusion sequences that could be used for monitoring minimal residual disease. Mol Cancer Res; 16(2); 279-85. (c)2017 AACR.

Aitken, S. and R. Bharathavikru (2016). "Bioinformatic Analysis of Next-Generation Sequencing Data to Identify WT1-Associated Differential Gene and Isoform Expression." Methods Mol Biol **1467**: 211-219.

Differential gene expression analysis has been conventionally performed by microarray techniques; however with the recent advent of next-generation sequencing (NGS) approaches, it has become easier to analyze the coding as well as the noncoding components. Additionally, NGS data analysis also provides information regarding the expression changes of specific isoforms. There are several bioinformatics tools available to analyze NGS data but with different parameters. This chapter provides a comparative insight into these tools by utilizing NGS datasets available from Wt1 knockout and embryonic stem cell line model.

Al Balwi, M. A., et al. (2017). "Analysis of CCR5 gene polymorphisms in 321 healthy Saudis using Next Generation Sequencing." Hum Immunol **78**(4): 384-386.

AIMS: To investigate the extent of CCR5 polymorphism in the healthy Saudi population. METHOD: A total of 321 healthy Saudi individuals were sequenced using the ion Ampliseq Exome kit (Life Technologies, USA) on genomic DNA following manufacturer's protocol. Whole Exome Sequencing (WES) reads were aligned to the human reference genome (hg19 build) with Torrent Suite Software (v5.0.2) and the variants were called using the Torrent Variant Caller plugin (v5.0) and imported into Ion Reporter Server (v5.0) for the annotation. CCR5 coding exons variants were filtered and checked against the NHLBI GO Exome Sequencing Project (NHLBI), NCBI Reference dbSNPs database, 1000 genomes and Exome Aggregation Consortium datasets (ExAC). RESULTS: A total of 475 variants were identified. Table 1 shows polymorphisms/mutations detected within exons that introduced an amino acid change, deletion or copy number variants (CNV). Three mutations are predicted to influence CCR5 function, including the 32bp deletion (Rs333). Four polymorphisms were detected, plus two CNV. CONCLUSIONS: This is the first report on sequencing the full CCR5 gene using NGS in the Saudi population. Here we demonstrate seven polymorphisms/mutations that were reported before. All were detected within very low frequency including the delta 32 mutation. However, we report for the first time copy number variants at two CCR5 gene locations; 45072265 and 38591712.

Amato, T., et al. (2016). "Clonality Analysis of Immunoglobulin Gene Rearrangement by Next-Generation Sequencing in Endemic Burkitt Lymphoma Suggests Antigen Drive Activation of BCR as Opposed to Sporadic Burkitt Lymphoma." Am J Clin Pathol **145**(1): 116-127.

OBJECTIVES: Recent studies using next-generation sequencing (NGS) analysis disclosed the importance of the intrinsic activation of the B-cell receptor (BCR) pathway in the pathogenesis of sporadic Burkitt lymphoma (sBL) due to mutations of TCF3/ID3 genes. Since no definitive data are available on the genetic landscape of endemic Burkitt (eBL), we first assessed the mutation frequency of TCF3/ID3 in eBL compared with sBL and subsequently the somatic hypermutation status of the BCR to answer whether an extrinsic activation of BCR signaling could also be demonstrated in Burkitt lymphoma. METHODS: We

assessed the mutations of TCF3/ID3 by RNAseq and the BCR status by NGS analysis of the immunoglobulin genes (IGs). RESULTS: We detected mutations of TCF3/ID3 in about 30% of the eBL cases. This rate is significantly lower than that detected in sBL (64%). The NGS analysis of IGs revealed intraclonal diversity, suggesting an active targeted somatic hypermutation process in eBL compared with sBL. CONCLUSIONS: These findings support the view that the antigenic pressure plays a key role in the pathogenetic pathways of eBL, which may be partially distinct from those driving sBL development.

Angelini, C. I. (2015). "LGMD phenotype due to a new gene and dysferlinopathy investigated by next-generation sequencing." Neurol Genet **1**(4): e39.

In this issue of Neurology (R) Genetics, Endo et al. (1) report 3 cases of limb-girdle muscular dystrophy (LGMD) phenotype with mental retardation or hyperCKemia found by next-generation sequencing (NGS) to have a variant in the POMGNT2 gene, which has so far been recognized only as causing congenital muscular dystrophy (CMD).

Aouinti, S., et al. (2015). "IMGT/HighV-QUEST Statistical Significance of IMGT Clonotype (AA) Diversity per Gene for Standardized Comparisons of Next Generation Sequencing Immunoprofiles of Immunoglobulins and T Cell Receptors." PLoS One **10**(11): e0142353.

The adaptive immune responses of humans and of other jawed vertebrate species (gnasthostomata) are characterized by the B and T cells and their specific antigen receptors, the immunoglobulins (IG) or antibodies and the T cell receptors (TR) (up to 2.1012 different IG and TR per individual). IMGT, the international ImMunoGeneTics information system (http://www.imgt.org), was created in 1989 by Marie-Paule Lefranc (Montpellier University and CNRS) to manage the huge and complex diversity of these antigen receptors. IMGT built on IMGT-ONTOLOGY concepts of identification (keywords), description (labels), classification (gene and allele nomenclature) and numerotation (IMGT unique numbering), is at the origin of immunoinformatics, a science at the interface between immunogenetics and bioinformatics. IMGT/HighV-QUEST, the first web portal, and so far the only one, for the next generation sequencing (NGS) analysis of IG and TR, is the paradigm for immune repertoire standardized outputs and immunoprofiles of the adaptive immune responses. It provides the identification of the variable (V), diversity (D) and joining (J) genes and alleles, analysis of the V-(D)-J junction and complementarity determining region 3 (CDR3) and the characterization of the 'IMGT clonotype (AA)' (AA for amino acid) diversity and expression. IMGT/HighV-QUEST compares outputs of different batches, up to one million nucleotide sequencesfor the statistical module. These high throughput IG and TR repertoire immunoprofiles are of prime importance in vaccination, cancer, infectious diseases, autoimmunity and lymphoproliferative disorders, however their comparative statistical analysis still remains a challenge. We present a standardized statistical procedure to analyze IMGT/HighV-QUEST outputs for the evaluation of the significance of the IMGT clonotype (AA) diversity differences in proportions, per gene of a given group, between NGS IG and TR repertoire immunoprofiles. The procedure is generic and suitable for evaluating significance of the IMGT clonotype (AA) diversity and expression per gene, and for any IG and TR immunoprofiles of any species.

Armengol, G., et al. (2016). "Driver Gene Mutations in Stools of Colorectal Carcinoma Patients Detected by Targeted Next-Generation Sequencing." J Mol Diagn **18**(4): 471-479.

Detection of driver gene mutations in stool DNA represents a promising noninvasive approach for screening colorectal cancer (CRC). Amplicon-based next-generation sequencing (NGS) is a good option to study mutations in many cancer genes simultaneously and from a low amount of DNA. Our aim was to assess the feasibility of identifying mutations in 22 cancer driver genes with Ion Torrent technology in stool DNA from a series of 65 CRC patients. The assay was successful in 80% of stool DNA samples. NGS results showed 83 mutations in cancer driver genes, 29 hotspot and 54 novel mutations. One to five genes were mutated in 75% of cases. TP53, KRAS, FBXW7, and SMAD4 were the top mutated genes, consistent with previous studies. Of samples with mutations, 54% presented concomitant mutations in different genes. Phosphatidylinositol 3-kinase/mitogen-activated protein kinase pathway genes were mutated in 70% of samples, with 58% having alterations in KRAS, NRAS, or BRAF. Because mutations in these genes can compromise the efficacy of epidermal growth factor receptor blockade in CRC patients, identifying mutations that confer resistance to some targeted treatments may be useful to guide therapeutic decisions. In conclusion, the data presented herein show that NGS procedures on stool DNA represent a promising tool to detect genetic mutations that could be used in the future for diagnosis, monitoring, or treating CRC.

Au, C. H., et al. (2016). "Clinical evaluation of panel testing by next-generation sequencing (NGS) for gene mutations in myeloid neoplasms." Diagn Pathol **11**: 11.

BACKGROUND: Genomic techniques in recent years have allowed the identification of many mutated genes important in the pathogenesis of acute myeloid leukemia (AML). Together with cytogenetic aberrations, these gene mutations are powerful prognostic markers in AML and can be used to guide patient management, for example selection of optimal post-remission therapy. The mutated genes also hold promise as therapeutic targets themselves. We evaluated the applicability of a gene panel for the detection of AML mutations in a diagnostic molecular pathology laboratory. METHODS: Fifty patient samples comprising 46 AML and 4 other myeloid neoplasms were accrued for the study. They consisted of 19 males and 31 females at a median age of 60 years (range: 18-88 years). A total of 54 genes (full coding exons of 15 genes and exonic hotspots of 39 genes) were targeted by 568 amplicons that ranged from 225 to 275 bp. The combined coverage was 141 kb in sequence length. Amplicon libraries were prepared by TruSight myeloid sequencing panel (Illumina, CA) and paired-end sequencing runs were performed on a MiSeq (Illumina) genome sequencer. Sequences obtained were analyzed by in-house bioinformatics pipeline, namely BWA-MEM, Samtools, GATK, Pindel, Ensembl Variant Effect Predictor and a novel algorithm ITDseek. RESULTS: The mean count of sequencing reads obtained per sample was 3.81 million and the mean sequencing depth was over 3000X. Seventy-seven mutations in 24 genes were detected in 37 of 50 samples (74 %). On average, 2 mutations (range 1-5) were detected per positive sample. TP53 gene mutations were found in 3 out of 4 patients with complex and unfavorable cytogenetics. Comparing NGS results with that of conventional molecular testing showed a concordance rate of 95.5 %. After further resolution and application of a novel bioinformatics algorithm ITDseek to aid the detection of FLT3 internal tandem duplication (ITD), the concordance rate was revised to 98.2 %. CONCLUSIONS: Gene panel testing by NGS approach was applicable for sensitive and accurate detection of actionable AML gene mutations in the clinical laboratory to individualize patient management. A novel algorithm ITDseek was presented that improved the detection of FLT3-ITD of varying length, position and at low allelic burden.

Bach, J. E., et al. (2016). "Mutational spectrum and deep intronic variants in the factor VIII gene of haemophilia A patients. Identification by next generation sequencing." Hamostaseologie **36**(Suppl. 2): S25-s28.

Haemophilia A (HA) is caused by a broad spectrum of different mutation types in the factor VIII gene (F8). In our patient cohort of more than 2600 HA patients as well as in other published studies, the most frequent cause are missense mutations in different F8 exons or the recurrent intron 22 inversion. Some exons and several specific nucleotide positions represent hot spots for point mutations in the examined cohort. About 4 % of cases remain without mutation after routine HA diagnostic methods including inversion PCRs, Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA). Deep intronic mutations cannot be detected by current standard HA diagnostics but have been reported for several genetic disorders. However, next generation sequencing (NGS) of the whole genomic sequence of the F8 gene allows to identify deep intronic variants. CONCLUSION: In general, NGS provides an effective approach to screen for different HA causing mutation types in the F8 gene.

Bach, J. E., et al. (2015). "Identification of deep intronic variants in 15 haemophilia A patients by next generation sequencing of the whole factor VIII gene." Thromb Haemost **114**(4): 757-767.

Current screening methods for factor VIII gene (F8) mutations can reveal the causative alteration in the vast majority of haemophilia A patients. Yet, standard diagnostic methods fail in about 2% of cases. This study aimed at analysing the entire intronic sequences of the F8 gene in 15 haemophilia A patients by next generation sequencing. All patients had a mild to moderate phenotype and no mutation in the coding sequence and splice sites of the F8 gene could be diagnosed so far. Next generation sequencing data revealed 23 deep intronic candidate variants in several F8 introns, including six recurrent variants and three variants that have been described before. One patient additionally showed a deletion of 9.2 kb in intron 1, mediated by Alu-type repeats. Several bioinformatic tools were used to score the variants in comparison to known pathogenic F8 mutations in order to predict their deleteriousness. Pedigree analyses showed a correct segregation pattern for three of the presumptive mutations. In each of the 15 patients analysed, at least one deep intronic variant in the F8 gene was identified and predicted to alter F8 mRNA splicing. Reduced F8 mRNA levels and/or stability would be well compatible with the patients' mild to moderate haemophilia A phenotypes. The next generation sequencing approach used proved an efficient method to screen the complete F8 gene and could be applied as a one-stop sequencing method for molecular diagnostics of haemophilia A.

Banerjee, S., et al. (2017). "Next generation sequencing identified novel heterozygous nonsense mutation in CNGB1 gene associated with retinitis pigmentosa in a Chinese patient." Oncotarget **8**(51): 88345-88350.

Retinitis pigmentosa (RP) is a severe hereditary eye disease characterized by progressive degeneration of photoreceptors and subsequent loss of vision. Retinitis pigmentosa (RP) is a clinically and genetically heterogeneous group of retinal diseases. Germline mutations of CNGB1 is associated with retinitis pigmentosa. We have identified and investigated a 34-year-old Chinese man with markedly have night vision blindness and loss of midperipheral visual field. The proband also lose his far peripheral visual field and also central vision. Proband's retinal pigment deposits visible on fundus examination and primary loss of rod photoreceptor cells followed by secondary loss of cone photoreceptors. Target exome capture based next generation sequencing and Sanger sequencing identified novel nonsense mutation, c.1917G>A and a reported mutation, c.2361C>A, in the CNGB1 gene. Both the nonsense mutations are predicted to lead to the formation of a premature stop codon which finally results into formation of truncated CNGB1 protein product which finally predicted to be disease causing. According to the variant classification guidelines of ACMG, these two variants are categorized as "likely pathogenic" variants. Our findings expand the mutational spectra of CNGB1 and are valuable in the mutation-based pre- and post-natal screening and genetic diagnosis for retinitis pigmentosa.

Bardak, H., et al. (2017). "Next-generation sequencing analysis of the ARMS2 gene in Turkish exudative age-related macular degeneration patients." Genet Mol Res **16**(1).

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries. It is a complex disease with both genetic and environmental risk factors. To improve clinical management of this condition, it is important to develop risk assessment and prevention strategies for environmental influences, and establish a more effective treatment approach. The aim of the present study was to investigate age-related maculopathy susceptibility protein 2 (ARMS2) gene sequences among Turkish patients with exudative AMD. In addition to 39 advanced exudative AMD patients, 250 healthy individuals for whom exome sequencing data were available were included as a control group. Patients with a history of known environmental and systemic AMD risk factors were excluded. Genomic DNA was isolated from peripheral blood and analyzed using next-generation sequencing. All coding exons of the ARMS2 gene were assessed. Three different ARMS2 sequence variations (rs10490923, rs2736911, and rs10490924) were identified in both the patient and control group. Within the control group, two further ARMS2 gene variants (rs7088128 and rs36213074) were also detected. Logistic regression analysis revealed a relationship between the rs10490924 polymorphism and AMD in the Turkish population.

Bartels, S., et al. (2016). "Routine clinical mutation profiling using next generation sequencing and a customized gene panel improves diagnostic precision in myeloid neoplasms." Oncotarget **7**(21): 30084-30093.

Microscopic examination of myelodysplastic syndromes (MDS) and myelodysplastic-myeloproliferative neoplasms (MDS/MPN) may be challenging because morphological features can overlap with those of reactive states. Demonstration of clonal hematopoiesis provides a diagnostic clue and has become possible by comprehensive mutation profiling of a number of frequently mutated genes, some of them with large coding regions. To emphasize the potential benefit of NGS in hematopathology we present sequencing results from routinely processed formalin-fixed and paraffin-embedded (FFPE) bone marrow trephines (n = 192). A customized amplicon-based gene panel including 23 genes frequently mutated in myeloid neoplasms was established and implemented. Thereby, 629,691 reads per sample (range 179,847-1,460,412) and a mean coverage of 2,702 (range 707-6,327) could be obtained, which are sufficient for comprehensive mutational profiling. Seven samples failed in sequencing (3.6%). In 185 samples we found in total 269 pathogenic variants (mean 1.4 variants per patient, range 0-5), 125 Patients exhibit at least one pathogenic mutation (67.6%). Variants show allele frequencies ranging from 6.7% up to 95.7%. Most frequently mutated genes were TET2 (28.7%), SRSF2 (19.5%), ASXL1 (8.6%) and U2AF1 (8.1%). The mutation profiling increases the diagnostic precision and adds prognostic information.

Bastida, J. M., et al. (2016). "Design and application of a 23-gene panel by next-generation sequencing for inherited coagulation bleeding disorders." Haemophilia **22**(4): 590-597.

INTRODUCTION: Molecular testing of Inherited bleeding coagulation disorders (IBCDs) not only offers confirmation of diagnosis but also aids in genetic counselling, prenatal diagnosis and in certain cases genotype-phenotype correlations are important for predicting the clinical course of the disease and to allow tailor-made follow-up of individuals. Until recently, genotyping has been mainly performed by Sanger sequencing, a technique known to be time consuming and expensive. Currently, next-generation sequencing (NGS) offers a new potential approach that enables the simultaneous investigation of multiple genes at manageable cost. AIM: The aim of this study

was to design and to analyse the applicability of a 23-gene NGS panel in the molecular diagnosis of patients with IBCDs. METHODS: A custom target enrichment library was designed to capture 31 genes known to be associated with IBCDs. Probes were generated for 296 targets to cover 86.3 kb regions (all exons and flanking regions) of these genes. Twenty patients with an IBCDs phenotype were studied using NGS technology. RESULTS: In all patients, our NGS approach detected causative mutations. Twenty-one pathogenic variants were found; while most of them were missense (18), three deletions were also identified. Six novel mutations affecting F8, FGA, F11, F10 and VWF genes, and 15 previously reported variants were detected. NGS and Sanger sequencing were 100% concordant. CONCLUSION: Our results demonstrate that this approach could be an accurate, reproducible and reliable tool in the rapid genetic diagnosis of IBCDs.

Baurand, A., et al. (2017). "Incomplete Timothy syndrome secondary to a mosaic mutation of the CACNA1C gene diagnosed using next-generation sequencing." Am J Med Genet A **173**(2): 531-536.

Autosomal dominant genetic diseases can occur de novo and in the form of somatic mosaicism, which can give rise to a less severe phenotype, and make diagnosis more difficult given the sensitivity limits of the methods used. We report the case of female child with a history of surgery for syndactyly of the hands and feet, who was admitted at 6 years of age to a pediatric intensive care unit following cardiac arrest. The electrocardiogram (ECG) showed a long QT interval that on occasions reached 500 ms. Despite the absence of facial dysmorphism and the presence of normal psychomotor development, a diagnosis of Timothy syndrome was made given the association of syndactyly and the ECG features. Sanger sequencing of the CACNA1C gene, followed by sequencing of the genes KCNQ1, KCNH2, KCNE1, KCNE2, were negative. The subsequent analysis of a panel of genes responsible for hereditary cardiac rhythm disorders using Haloplex technology revealed a recurrent mosaic p.Gly406Arg missense mutation of the CACNA1C gene in 18% of the cells. This mosaicism can explain the negative Sanger analysis and the less complete phenotype in this patient. Given the other cases in the literature, mosaic mutations in Timothy syndrome appear more common than previously thought. This case demonstrates the importance of using next-generation sequencing to identify mosaic mutations when the clinical picture supports a specific mutation that is not identified using conventional testing. (c) 2016 Wiley Periodicals, Inc.

Beadling, C., et al. (2016). "A Multiplexed Amplicon Approach for Detecting Gene Fusions by Next-Generation Sequencing." J Mol Diagn **18**(2): 165-175.

Chromosomal rearrangements that result in oncogenic gene fusions are clinically important drivers of many cancer types. Rapid and sensitive methods are therefore needed to detect a broad range of gene fusions in clinical specimens that are often of limited quantity and quality. We describe a next-generation sequencing approach that uses a multiplex PCR-based amplicon panel to interrogate fusion transcripts that involve 19 driver genes and 94 partners implicated in solid tumors. The panel also includes control assays that evaluate the 3'/5' expression ratios of 12 oncogenic kinases, which might be used to infer gene fusion events when the partner is unknown or not included on the panel. There was good concordance between the solid tumor fusion gene panel and other methods, including fluorescence in situ hybridization, real-time PCR, Sanger sequencing, and other next-generation sequencing panels, because 40 specimens known to harbor gene fusions were correctly identified. No specific fusion reads were observed in 59 fusion-negative specimens. The 3'/5' expression ratio was informative for fusions that involved ALK, RET, and NTRK1 but not for BRAF or ROS1 fusions. However, among 37 ALK or RET fusion-negative specimens, four exhibited elevated 3'/5' expression ratios, indicating that fusions predicted solely by 3'/5' read ratios require confirmatory testing.

Beltrame, L., et al. (2015). "Profiling cancer gene mutations in longitudinal epithelial ovarian cancer biopsies by targeted next-generation sequencing: a retrospective study." Ann Oncol **26**(7): 1363-1371.

BACKGROUND: The majority of patients with stage III-IV epithelial ovarian cancer (EOC) relapse after initially responding to platinum-based chemotherapy, and develop resistance. The genomic features involved in drug resistance are unknown. To unravel some of these features, we investigated the mutational profile of genes involved in pathways related to drug sensitivity in a cohort of matched tumors obtained at first surgery (Ft-S) and second surgery (Sd-S). PATIENTS AND METHODS: Matched biopsies (33) taken at Ft-S and Sd-S were selected from the 'Pandora' tumor tissue collection. DNA libraries for 65 genes were generated using the TruSeq Custom Amplicon kit and sequenced on MiSeq (Illumina). Data were analyzed using a high-performance cluster computing platform (Cloud4CARE project) and independently validated. RESULTS: A total of 2270 somatic mutations were identified (89.85% base substitutions 8.19% indels, and 1.92% unknown). Homologous recombination

(HR) genes and TP53 were mutated in the majority of Ft-S, while ATM, ATR, TOP2A and TOP2B were mutated in the entire dataset. Only 2% of mutations were conserved between matched Ft-S and Sd-S. Mutations detected at second surgery clustered patients in two groups characterized by different mutational profiles in genes associated with HR, PI3K, miRNA biogenesis and signal transduction. CONCLUSIONS: There was a low level of concordance between Ft-S and Sd-S in terms of mutations in genes involved in key processes of tumor growth and drug resistance. This result suggests the importance of future longitudinal analyses to improve the clinical management of relapsed EOC.

Ben-Ari Fuchs, S., et al. (2016). "GeneAnalytics: An Integrative Gene Set Analysis Tool for Next Generation Sequencing, RNAseq and Microarray Data." OMICS **20**(3): 139-151.

Postgenomics data are produced in large volumes by life sciences and clinical applications of novel omics diagnostics and therapeutics for precision medicine. To move from "data-to-knowledge-to-innovation," a crucial missing step in the current era is, however, our limited understanding of biological and clinical contexts associated with data. Prominent among the emerging remedies to this challenge are the gene set enrichment tools. This study reports on GeneAnalytics (geneanalytics.genecards.org), a comprehensive and easy-to-apply gene set analysis tool for rapid contextualization of expression patterns and functional signatures embedded in the postgenomics Big Data domains, such as Next Generation Sequencing (NGS), RNAseq, and microarray experiments. GeneAnalytics' differentiating features include in-depth evidence-based scoring algorithms, an intuitive user interface and proprietary unified data. GeneAnalytics employs the LifeMap Science's GeneCards suite, including the GeneCards (R)--the human gene database; the MalaCards-the human diseases database; and the PathCards--the biological pathways database. Expression-based analysis in GeneAnalytics relies on the LifeMap Discovery (R)--the embryonic development and stem cells database, which includes manually curated expression data for normal and diseased tissues, enabling advanced matching algorithm for gene-tissue association. This assists in evaluating differentiation protocols and discovering biomarkers for tissues and cells. Results are directly linked to gene, disease, or cell "cards" in the GeneCards suite. Future developments aim to enhance the GeneAnalytics algorithm as well as visualizations, employing varied graphical display items. Such attributes make GeneAnalytics a broadly applicable postgenomics data analyses and interpretation tool for translation of data to knowledge-based innovation in various Big Data fields such as precision medicine, ecogenomics, nutrigenomics, pharmacogenomics, vaccinomics, and others yet to emerge on the postgenomics horizon.

Bergougnoux, A., et al. (2018). "Multicenter validation study for the certification of a CFTR gene scanning method using next generation sequencing technology." Clin Chem Lab Med.

BACKGROUND: Many European laboratories offer molecular genetic analysis of the CFTR gene using a wide range of methods to identify mutations causative of cystic fibrosis (CF) and CFTR-related disorders (CFTR-RDs). Next-generation sequencing (NGS) strategies are widely used in diagnostic practice, and CE marking is now required for most in vitro diagnostic (IVD) tests in Europe. The aim of this multicenter study, which involved three European laboratories specialized in CF molecular analysis, was to evaluate the performance of Multiplicom's CFTR MASTR Dx kit to obtain CE-IVD certification. METHODS: A total of 164 samples, previously analyzed with well-established "reference" methods for the molecular diagnosis of the CFTR gene, were selected and re-sequenced using the Illumina MiSeq benchtop NGS platform. Sequencing data were analyzed using two different bioinformatic pipelines. Annotated variants were then compared to the previously obtained reference data. RESULTS AND CONCLUSIONS: The analytical sensitivity, specificity and accuracy rates of the Multiplicom CFTR MASTR assay exceeded 99%. Because different types of CFTR mutations can be detected in a single workflow, the CFTR MASTR assay simplifies the overall process and is consequently well suited for routine diagnostics.

Bevilacqua, J., et al. (2017). "Clinical utility of a 377 gene custom next-generation sequencing epilepsy panel." J Genet **96**(4): 681-685.

Epilepsy is one of the most common neurological disorders with about 500 genes thought to be involved across the phenotypic spectrum (Busch et al. 2014; Ran et al. 2014), which includes monogenic, multigenic, epistatic and pleiotropic phenotype manifestations (Busch et al. 2014; Thomas et al. 2014), driving the need for a comprehensive diagnostic test. Next-generation sequencing (NGS) allows for the simultaneous investigation of a large number of genes, making it a very attractive option for a condition as diverse as epilepsy at a low cost compared to traditional Sanger sequencing (Lemke et al. 2012; Nemeth et al. 2013). Our 377 gene epilepsy NGS test was developed to include genes known to cause or have published association with epilepsy and seizure-

related disorders. Given the scale of information that is generated, the efficacy of an NGS panel depends on a number of factors, including the genes present on the panel, prebioinformatic and postbioinformatic analysis protocols, as well as reporting criteria, prompting the current study, a retrospective analysis of 305 cases tested for the epilepsy panel.

Boutte, J., et al. (2015). "Haplotype Detection from Next-Generation Sequencing in High-Ploidy-Level Species: 45S rDNA Gene Copies in the Hexaploid Spartina maritima." G3 (Bethesda) **6**(1): 29-40.

Gene and whole-genome duplications are widespread in plant nuclear genomes, resulting in sequence heterogeneity. Identification of duplicated genes may be particularly challenging in highly redundant genomes, especially when there are no diploid parents as a reference. Here, we developed a pipeline to detect the different copies in the ribosomal RNA gene family in the hexaploid grass Spartina maritima from next-generation sequencing (Roche-454) reads. The heterogeneity of the different domains of the highly repeated 45S unit was explored by identifying single nucleotide polymorphisms (SNPs) and assembling reads based on shared polymorphisms. SNPs were validated using comparisons with Illumina sequence data sets and by cloning and Sanger (re)sequencing. Using this approach, 29 validated polymorphisms and 11 validated haplotypes were reported (out of 34 and 20, respectively, that were initially predicted by our program). The rDNA domains of S. maritima have similar lengths as those found in other Poaceae, apart from the 5'-ETS, which is approximately two-times longer in S. maritima. Sequence homogeneity was encountered in coding regions and both internal transcribed spacers (ITS), whereas high intragenomic variability was detected in the intergenic spacer (IGS) and the external transcribed spacer (ETS). Molecular cytogenetic analysis by fluorescent in situ hybridization (FISH) revealed the presence of one pair of 45S rDNA signals on the chromosomes of S. maritima instead of three expected pairs for a hexaploid genome, indicating loss of duplicated homeologous loci through the diploidization process. The procedure developed here may be used at any ploidy level and using different sequencing technologies.

Burns, C., et al. (2017). "Multiple Gene Variants in Hypertrophic Cardiomyopathy in the Era of Next-Generation Sequencing." Circ Cardiovasc Genet **10**(4).

BACKGROUND: Multiple likely pathogenic/pathogenic (LP/P; >/=2) variants in patients with hypertrophic cardiomyopathy were described 10 years ago with a prevalence of 5%. We sought to re-examine the significance of multiple rare variants in patients with hypertrophic cardiomyopathy in the setting of comprehensive and targeted panels. METHODS AND RESULTS: Of 758 hypertrophic cardiomyopathy probands, we included 382 with >/=45 cardiomyopathy genes screened. There were 224 (59%) with >/=1 rare variant (allele frequency </=0.02%). Variants were analyzed using varying sized gene panels to represent comprehensive or targeted testing. Based on a 45-gene panel, 127 (33%) had a LP/P variant, 139 (36%) had variants of uncertain significance, and 66 (17%) had multiple rare variants. A targeted 8-gene panel yielded 125 (32%) LP/P variants, 52 (14%) variants of uncertain significance, and 14 (4%) had multiple rare variants. No proband had 2 LP/P variants. Including affected family members (total n=412), cluster-adjusted analyses identified a phenotype effect, with younger age (odds ratio, 0.95; 95% confidence interval, 0.92-0.98; P=0.004) and family history of sudden cardiac death (odds ratio, 3.5; 95% confidence interval, 1.3-9.9; P=0.02) significantly more likely in multiple versus single variant patients when considering an 8-gene panel but not larger panels. Those with multiple variants had worse event-free survival from all-cause death, cardiac transplantation, and cardiac arrest (log-rank P=0.008). CONCLUSIONS: No proband had multiple LP/P variants in contrast to previous reports. However, multiple rare variants regardless of classification were seen in 4% and contributed to earlier disease onset and cardiac events. Our findings support a cumulative variant hypothesis in hypertrophic cardiomyopathy.

Cacho, R. A., et al. (2014). "Next-generation sequencing approach for connecting secondary metabolites to biosynthetic gene clusters in fungi." Front Microbiol **5**: 774.

Genomics has revolutionized the research on fungal secondary metabolite (SM) biosynthesis. To elucidate the molecular and enzymatic mechanisms underlying the biosynthesis of a specific SM compound, the important first step is often to find the genes that responsible for its synthesis. The accessibility to fungal genome sequences allows the bypass of the cumbersome traditional library construction and screening approach. The advance in next-generation sequencing (NGS) technologies have further improved the speed and reduced the cost of microbial genome sequencing in the past few years, which has accelerated the research in this field. Here, we will present an example work flow for identifying the gene cluster encoding the biosynthesis of SMs of interest using an NGS approach. We will also review the different strategies that can be employed to

pinpoint the targeted gene clusters rapidly by giving several examples stemming from our work.

Choudhary, A., et al. (2014). "Evaluation of an integrated clinical workflow for targeted next-generation sequencing of low-quality tumor DNA using a 51-gene enrichment panel." BMC Med Genomics **7**: 62.

BACKGROUND: Improvements in both performance and cost for next-generation sequencing (NGS) have spurred its rapid adoption for clinical applications. We designed and optimized a pan-cancer target-enrichment panel for 51 well-established oncogenes and tumor suppressors, in conjunction with a bioinformatic pipeline informed by in-process controls and pre- and post-analytical quality control measures. METHODS: The evaluation of this workflow consisted of sequencing mixtures of intact DNA to establish analytical sensitivity and precision, utilization of heuristics to identify systematic artifacts, titration studies of intact and FFPE samples for input optimization, and incorporation of orthogonal sequencing strategies to increase both positive predictive value and variant detection. We also used 128 FFPE samples to assess clinical accuracy and incorporated the previously described quantitative functional index (QFI) for sample qualification as part of detailing complete system performance. RESULTS: We observed a concordance correlation coefficient of 0.99 between the observed versus expected percent variant at 250 ng input across 4 independent sequencing runs. A subset of the systematic variants were confirmed to be barely detectable on an independent sequencing platform (Wilcox signed-rank test p-value <10(-16)), and the incorporation of orthogonal sequencing strategies increased the harmonic mean of sensitivity and positive predictive value of mutation detection by 41%. In one cohort of FFPE tumor samples, coverage and inter-platform concordance were positively correlated with the QFI, emphasizing the need for pre-analytical sample quality control to reduce the risk of false positives and negatives. In a separate cohort of FFPE samples, the 51-gene panel achieved 78% sensitivity (95% CI = 56.3, 92.5) with 100% PPV (95% CI = 81.5, 100.0) based on known mutations at 7.9% median abundance. By sequencing specimens using an orthogonal NGS technology, sensitivity was improved to 87.0% (95% CI = 66.4,97.2) while maintaining PPV. CONCLUSIONS: The results highlight the value of process integration in a comprehensive targeted NGS system, enabling both discovery and diagnostic applications, particularly when sequencing low-quality cancer specimens.

Claesen, J. and T. Burzykowski (2015). "A hidden Markov-model for gene mapping based on whole-genome next generation sequencing data." Stat Appl Genet Mol Biol **14**(1): 21-34.

The analysis of polygenic, phenotypic characteristics such as quantitative traits or inheritable diseases requires reliable scoring of many genetic markers covering the entire genome. The advent of high-throughput sequencing technologies provides a new way to evaluate large numbers of single nucleotide polymorphisms as genetic markers. Combining the technologies with pooling of segregants, as performed in bulk segregant analysis, should, in principle, allow the simultaneous mapping of multiple genetic loci present throughout the genome. We propose a hidden Markov-model to analyze the marker data obtained by the bulk segregant next generation sequencing. The model includes several states, each associated with a different probability of observing the same/different nucleotide in an offspring as compared to the parent. The transitions between the molecular markers imply transitions between the states of the model. After estimating the transition probabilities and state-related probabilities of nucleotide (dis)similarity, the most probable state for each SNP is selected. The most probable states can then be used to indicate which genomic regions may be likely to contain trait-related genes. The application of the model is illustrated on the data from a study of ethanol tolerance in yeast. Software is written in R. R-functions, R-scripts and documentation are available on www.ibiostat.be/software/bioinformatics.

Clark, K. F. and S. J. Greenwood (2016). "Next-Generation Sequencing and the Crustacean Immune System: The Need for Alternatives in Immune Gene Annotation." Integr Comp Biol **56**(6): 1113-1130.

Next-generation sequencing has been a huge benefit to investigators studying non-model species. High-throughput gene expression studies, which were once restricted to animals with extensive genomic resources, can now be applied to any species. Transcriptomic studies using RNA-Seq can discover hundreds of thousands of transcripts from any species of interest. The power and limitation of these techniques is the sheer size of the dataset that is acquired. Parsing these large datasets is becoming easier as more bioinformatic tools are available for biologists without extensive computer programming expertise. Gene annotation and physiological pathway tools such as Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology enable the application of the vast amount of information acquired from model organisms to non-model species. While noble in nature, utilization of these tools can inadvertently misrepresent transcriptomic data from

non-model species via annotation omission. Annotation followed by molecular pathway analysis highlights pathways that are disproportionately affected by disease, stress, or the physiological condition being examined. Problems occur when gene annotation procedures only recognizes a subset, often 50% or less, of the genes differently expressed from a non-model organisms. Annotated transcripts normally belong to highly conserved metabolic or regulatory genes that likely have a secondary or tertiary role, if any at all, in immunity. They appear to be disproportionately affected simply because conserved genes are most easily annotated. Evolutionarily induced specialization of physiological pathways is a driving force of adaptive evolution, but it results in genes that have diverged sufficiently to prevent their identification and annotation through conventional gene or protein databases. The purpose of this manuscript is to highlight some of the challenges faced when annotating crustacean immune genes by using an American lobster (Homarus americanus) transcriptome as an example. Immune genes have evolved rapidly over time, facilitating speciation and adaption to highly divergent ecological niches. Complete and proper annotation of immune genes from invertebrates has been challenging. Modulation of the crustacean immune system occurs in a variety of physiological responses including biotic and abiotic stressors, molting and reproduction. A simple method for the identification of a greater number of potential immune genes is proposed, along with a short introductory primer on crustacean immune response. The intended audience is not the advanced bioinformatic user, but those investigating physiological responses who require rudimentary understanding of crustacean immunological principles, but where immune gene regulation is not their primary interest.

Cunha, K. S., et al. (2016). "Hybridization Capture-Based Next-Generation Sequencing to Evaluate Coding Sequence and Deep Intronic Mutations in the NF1 Gene." Genes (Basel) **7**(12).

Neurofibromatosis 1 (NF1) is one of the most common genetic disorders and is caused by mutations in the NF1 gene. NF1 gene mutational analysis presents a considerable challenge because of its large size, existence of highly homologous pseudogenes located throughout the human genome, absence of mutational hotspots, and diversity of mutations types, including deep intronic splicing mutations. We aimed to evaluate the use of hybridization capture-based next-generation sequencing to screen coding and noncoding NF1 regions. Hybridization capture-based next-generation sequencing, with genomic DNA as starting material, was used to sequence the whole NF1 gene (exons and introns) from 11 unrelated individuals and 1 relative, who all had NF1. All of them met the NF1 clinical diagnostic criteria. We showed a mutation detection rate of 91% (10 out of 11). We identified eight recurrent and two novel mutations, which were all confirmed by Sanger methodology. In the Sanger sequencing confirmation, we also included another three relatives with NF1. Splicing alterations accounted for 50% of the mutations. One of them was caused by a deep intronic mutation (c.1260 + 1604A > G). Frameshift truncation and missense mutations corresponded to 30% and 20% of the pathogenic variants, respectively. In conclusion, we show the use of a simple and fast approach to screen, at once, the entire NF1 gene (exons and introns) for different types of pathogenic variations, including the deep intronic splicing mutations.

Day, S. E., et al. (2016). "Next-generation sequencing methylation profiling of subjects with obesity identifies novel gene changes." Clin Epigenetics **8**: 77.

BACKGROUND: Obesity is a metabolic disease caused by environmental and genetic factors. However, the epigenetic mechanisms of obesity are incompletely understood. The aim of our study was to investigate the role of skeletal muscle DNA methylation in combination with transcriptomic changes in obesity. RESULTS: Muscle biopsies were obtained basally from lean (n = 12; BMI = 23.4 +/- 0.7 kg/m (2)) and obese (n = 10; BMI = 32.9 +/- 0.7 kg/m (2)) participants in combination with euglycemic-hyperinsulinemic clamps to assess insulin sensitivity. We performed reduced representation bisulfite sequencing (RRBS) next-generation methylation and microarray analyses on DNA and RNA isolated from vastus lateralis muscle biopsies. There were 13,130 differentially methylated cytosines (DMC; uncorrected $P < 0.05$) that were altered in the promoter and untranslated (5' and 3'UTR) regions in the obese versus lean analysis. Microarray analysis revealed 99 probes that were significantly (corrected $P < 0.05$) altered. Of these, 12 genes (encompassing 22 methylation sites) demonstrated a negative relationship between gene expression and DNA methylation. Specifically, sorbin and SH3 domain containing 3 (SORBS3) which codes for the adapter protein vinexin was significantly decreased in gene expression (fold change -1.9) and had nine DMCs that were significantly increased in methylation in obesity (methylation differences ranged from 5.0 to 24.4 %). Moreover, differentially methylated region (DMR) analysis identified a region in the 5'UTR (Chr.8:22,423,530-22,423,569) of SORBS3 that was increased in methylation by 11.2 % in the obese group. The negative relationship observed between DNA methylation and gene expression for SORBS3 was

validated by a site-specific sequencing approach, pyrosequencing, and qRT-PCR. Additionally, we performed transcription factor binding analysis and identified a number of transcription factors whose binding to the differentially methylated sites or region may contribute to obesity. CONCLUSIONS: These results demonstrate that obesity alters the epigenome through DNA methylation and highlights novel transcriptomic changes in SORBS3 in skeletal muscle.

De Castro, M., et al. (2015). "Determining the prevalence of McArdle disease from gene frequency by analysis of next-generation sequencing data." Genet Med **17**(12): 1002-1006.

PURPOSE: McArdle disease is one of the most common glycogen storage disorders. Although the exact prevalence is not known, it has been estimated to be 1 in 100,000 patients in the United States. More than 100 mutations in PYGM have been associated with this disorder. McArdle disease has significant clinical variability: Some patients present with severe muscle pain and weakness; others have only mild, exercise-related symptoms. METHODS: Next-generation sequencing data allow estimation of disease prevalence with minimal ascertainment bias. We analyzed gene frequencies in two cohorts of patients based on exome sequencing results. We categorized variants into three groups: a curated set of published mutations, variants of uncertain significance, and likely benign variants. RESULTS: An initial estimate based on the frequency of six common mutations predicts a disease prevalence of 1/7,650 (95% confidence interval (CI) 1/5,362-1/11,108), which greatly deviates from published estimates. A second method using the two most common mutations predicts a prevalence of 1/42,355 (95% CI 1/24,536-1/76,310) in Caucasians. CONCLUSIONS: These results suggest that the currently accepted prevalence of McArdle disease is an underestimate and that some of the currently considered pathogenic variants are likely benign.

De Summa, S., et al. (2017). "GATK hard filtering: tunable parameters to improve variant calling for next generation sequencing targeted gene panel data." BMC Bioinformatics **18**(Suppl 5): 119.

BACKGROUND: NGS technology represents a powerful alternative to the standard Sanger sequencing in the context of clinical setting. The proprietary software that are generally used for variant calling often depend on preset parameters that may not fit in a satisfactory manner for different genes. GATK, which is widely used in the academic world, is rich in parameters for variant calling. However the self-adjusting parameter calibration of GATK requires data from a large number of exomes. When these are not available, which is the standard condition of a diagnostic laboratory, the parameters must be set by the operator (hard filtering). The aim of the present paper was to set up a procedure to assess the best parameters to be used in the hard filtering of GATK. This was pursued by using classification trees on true and false variants from simulated sequences of a real dataset data. RESULTS: We simulated two datasets, with different coverages, including all the sequence alterations identified in a real dataset according to their observed frequencies. Simulated sequences were aligned with standard protocols and then regression trees were built up to identify the most reliable parameters and cutoff values to discriminate true and false variant calls. Moreover, we analyzed flanking sequences of region presenting a high rate of false positive calls observing that such sequences present a low complexity make up. CONCLUSIONS: Our results showed that GATK hard filtering parameter values can be tailored through a simulation study based-on the DNA region of interest to ameliorate the accuracy of the variant calling.

Devarajan, B., et al. (2015). "Targeted next generation sequencing of RB1 gene for the molecular diagnosis of Retinoblastoma." BMC Cancer **15**: 320.

BACKGROUND: The spectrum of RB1gene mutations in Retinoblastoma (RB) patients and the necessity of multiple traditional methods for complete variant analysis make the molecular diagnosis a cumbersome, labor-intensive and time-consuming process. Here, we have used targeted next generation sequencing (NGS) approach with in-house analysis pipeline to explore its potential for the molecular diagnosis of RB. METHODS: Thirty-three patients with RB and their family members were selected randomly. DNA from patient blood and/or tumor was used for RB1 gene targeted sequencing. The raw reads were obtained from Illumina Miseq. An in-house bioinformatics pipeline was developed to detect both single nucleotide variants (SNVs) and small insertions/deletions (InDels) and to distinguish between somatic and germline mutations. In addition, ExomeCNV and Cn. MOPS were used to detect copy number variations (CNVs). The pathogenic variants were identified with stringent criteria, and were further confirmed by conventional methods and cosegregation in families. RESULTS: Using our approach, an array of pathogenic variants including SNVs, InDels and CNVs were detected in 85% of patients. Among the variants detected, 63% were germline and 37% were somatic. Interestingly, nine novel pathogenic variants (33%) were also detected in our study. CONCLUSIONS: We demonstrated for the first time that targeted NGS is an efficient approach for the identification of wide spectrum of pathogenic variants

in RB patients. This study is helpful for the molecular diagnosis of RB in a comprehensive and time-efficient manner.

Devran, Z., et al. (2015). "Development of molecular markers tightly linked to Pvr4 gene in pepper using next-generation sequencing." Mol Breed **35**(4): 101.

It is imperative to identify highly polymorphic and tightly linked markers of a known trait for molecular marker-assisted selection. Potyvirus resistance 4 (Pvr4) locus in pepper confers resistance to three pathotypes of potato virus Y and to pepper mottle virus. We describe the use of next-generation sequencing technology to generate molecular markers tightly linked to Pvr4. Initially, comparative genomics was carried out, and a syntenic region of tomato on chromosome ten was used to generate PCR-based markers and map Pvr4. Subsequently, the genomic sequence of pepper was used, and more than 5000 single-nucleotide variants (SNVs) were identified within the interval. In addition, we identified nucleotide binding site-leucine-rich repeat-type disease resistance genes within the interval. Several of these SNVs were converted to molecular markers desirable for large-scale molecular breeding programmes.

Elbers, J. P., et al. (2018). "Identifying genome-wide immune gene variation underlying infectious disease in wildlife populations - a next generation sequencing approach in the gopher tortoise." BMC Genomics **19**(1): 64.

BACKGROUND: Infectious disease is the single greatest threat to taxa such as amphibians (chytrid fungus), bats (white nose syndrome), Tasmanian devils (devil facial tumor disease), and black-footed ferrets (canine distemper virus, plague). Although understanding the genetic basis to disease susceptibility is important for the long-term persistence of these groups, most research has been limited to major-histocompatibility and Toll-like receptor genes. To better understand the genetic basis of infectious disease susceptibility in a species of conservation concern, we sequenced all known/predicted immune response genes (i.e., the immunomes) in 16 Florida gopher tortoises, Gopherus polyphemus. All tortoises produced antibodies against Mycoplasma agassizii (an etiologic agent of infectious upper respiratory tract disease; URTD) and, at the time of sampling, either had (n = 10) or lacked (n = 6) clinical signs. RESULTS: We found several variants associated with URTD clinical status in complement and lectin genes, which may play a role in Mycoplasma immunity. Thirty-five genes deviated from neutrality according to Tajima's D. These genes were enriched in functions relating to macromolecule and protein modifications, which are vital to immune system functioning. CONCLUSIONS: These results are suggestive of genetic differences that might contribute to disease severity, a finding that is consistent with other mycoplasmal diseases. This has implications for management because tortoises across their range may possess genetic variation associated with a more severe response to URTD. More generally: 1) this approach demonstrates that a broader consideration of immune genes is better able to identify important variants, and; 2) this data pipeline can be adopted to identify alleles associated with disease susceptibility or resistance in other taxa, and therefore provide information on a population's risk of succumbing to disease, inform translocations to increase genetic variation for disease resistance, and help to identify potential treatments.

Ellingford, J. M., et al. (2018). "Assessment of the incorporation of CNV surveillance into gene panel next-generation sequencing testing for inherited retinal diseases." J Med Genet **55**(2): 114-121.

BACKGROUND: Diagnostic use of gene panel next-generation sequencing (NGS) techniques is commonplace for individuals with inherited retinal dystrophies (IRDs), a highly genetically heterogeneous group of disorders. However, these techniques have often failed to capture the complete spectrum of genomic variation causing IRD, including CNVs. This study assessed the applicability of introducing CNV surveillance into first-tier diagnostic gene panel NGS services for IRD. METHODS: Three read-depth algorithms were applied to gene panel NGS data sets for 550 referred individuals, and informatics strategies used for quality assurance and CNV filtering. CNV events were confirmed and reported to referring clinicians through an accredited diagnostic laboratory. RESULTS: We confirmed the presence of 33 deletions and 11 duplications, determining these findings to contribute to the confirmed or provisional molecular diagnosis of IRD for 25 individuals. We show that at least 7% of individuals referred for diagnostic testing for IRD have a CNV within genes relevant to their clinical diagnosis, and determined a positive predictive value of 79% for the employed CNV filtering techniques. CONCLUSION: Incorporation of CNV analysis increases diagnostic yield of gene panel NGS diagnostic tests for IRD, increases clarity in diagnostic reporting and expands the spectrum of known disease-causing mutations.

Forleo, C., et al. (2017). "Targeted next-generation sequencing detects novel gene-phenotype associations and expands the mutational spectrum in cardiomyopathies." PLoS One **12**(7): e0181842.

Cardiomyopathies are a heterogeneous group of primary diseases of the myocardium, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC), with higher morbidity and mortality. These diseases are genetically diverse and associated with rare mutations in a large number of genes, many of which overlap among the phenotypes. To better investigate the genetic overlap between these three phenotypes and to identify new genotype-phenotype correlations, we designed a custom gene panel consisting of 115 genes known to be associated with cardiomyopathic phenotypes and channelopathies. A cohort of 38 unrelated patients, 16 affected by DCM, 14 by HCM and 8 by ARVC, was recruited for the study on the basis of more severe phenotypes and family history of cardiomyopathy and/or sudden death. We detected a total of 142 rare variants in 40 genes, and all patients were found to be carriers of at least one rare variant. Twenty-eight of the 142 rare variants were also predicted as potentially pathogenic variants and found in 26 patients. In 23 out of 38 patients, we found at least one novel potential gene-phenotype association. In particular, we detected three variants in OBSCN gene in ARVC patients, four variants in ANK2 gene and two variants in DLG1, TRPM4, and AKAP9 genes in DCM patients, two variants in PSEN2 gene and four variants in AKAP9 gene in HCM patients. Overall, our results confirmed that cardiomyopathic patients could carry multiple rare gene variants; in addition, our investigation of the genetic overlap among cardiomyopathies revealed new gene-phenotype associations. Furthermore, as our study confirms, data obtained using targeted next-generation sequencing could provide a remarkable contribution to the molecular diagnosis of cardiomyopathies, early identification of patients at risk for arrhythmia development, and better clinical management of cardiomyopathic patients.

Fujii, H., et al. (2018). "Application of next-generation sequencing to detect acyclovir-resistant herpes simplex virus type 1 variants at low frequency in thymidine kinase gene of the isolates recovered from patients with hematopoietic stem cell transplantation." J Virol Methods **251**: 123-128.

Ion Torrent next-generation sequencing (NGS) technology was applied to study the mode of emergence of acyclovir (ACV)-resistant (ACVr) herpes simplex virus type 1 (HSV-1) in patients with hematopoietic stem cell transplantation (HSCT) by quantitatively detecting mutations in the viral thymidine kinase (vTK) gene in the HSV-1 isolates recovered from HSCT patients. All of the mutations detected with the Sanger sequencing method in the vTK genes of HSV-1 isolates were also detected with the NGS assay. Furthermore, different mutations, which conferred ACV resistance and were not detected with the Sanger sequencing method, were also detected in a quantitative manner by using the NGS assay. The approach described here is applicable to studying the emergence process of vTK gene mutation-associated ACVr HSV-1 more in detail than the Sanger method. The NGS assay makes it possible to make a diagnosis of vTK gene mutation-associated ACVr HSV-1 infections at the early stage, which the ratio of ACVr HSV-1 is much lower than that of ACV-sensitive (ACVs) HSV-1.

Fuller, M. Y., et al. (2018). "Next-Generation Sequencing Identifies Gene Mutations That Are Predictive of Malignancy in Residual Needle Rinses Collected From Fine-Needle Aspirations of Thyroid Nodules." Arch Pathol Lab Med **142**(2): 178-183.

CONTEXT: - Thyroid nodules have a prevalence of approximately 70% in adults. Fine-needle aspiration (FNA) is a minimally invasive, cost-effective, standard method to collect tissue from thyroid nodules for cytologic examination. However, approximately 15% of thyroid FNA specimens cannot be unambiguously diagnosed as benign or malignant. OBJECTIVE: - To investigate whether clinically actionable data can be obtained using next-generation sequencing of residual needle rinse material. DESIGN: - A total of 24 residual needle rinse specimens with malignant (n = 6), indeterminate (n = 9), or benign (n = 9) thyroid FNA diagnoses were analyzed in our clinical molecular diagnostics laboratory using next-generation sequencing assays designed to detect gene mutations and translocations that commonly occur in thyroid cancer. Results were correlated with surgical diagnoses and clinical outcomes. RESULTS: - Interpretable data were generated from 23 of 24 residual needle rinse specimens. Consistent with its well-known role in thyroid malignancy, BRAF V600E mutations were detected in 4 malignant cases. An NRAS mutation was detected in 1 benign case. No mutations were detected from specimens with indeterminate diagnoses. CONCLUSIONS: - Our data demonstrate that residual thyroid FNA needle rinses are an adequate source of material for molecular diagnostic testing. Importantly, detection of a mutation implicated in thyroid malignancy was predictive of the final surgical diagnosis and clinical outcome. Our strategy to triage thyroid nodules with indeterminate cytology with molecular testing eliminates the need to perform additional FNA passes into dedicated media or to schedule additional invasive procedures. Further investigation with a larger sample size to confirm the clinical utility of our proposed strategy is underway.

Gao, M. L., et al. (2016). "[Target gene sequence capture and next generation sequencing technology to diagnose four children with Alagille syndrome]." Zhonghua Er Ke Za Zhi **54**(6): 441-445.

OBJECTIVE: To make genetic diagnosis of Alagille syndrome (ALGS) patients using target gene sequence capture and next generation sequencing technology. METHOD: Target gene sequence capture and next generation sequencing were used to detect ALGS gene of 4 patients. They were hospitalized at the Affiliated Hospital, Capital Institute of Pediatrics between January 2014 and December 2015, referred to clinical diagnosis of ALGS typical and atypical respectively in 2 cases. Blood samples were collected from patients and their parents and genomic DNA was extracted from lymphocytes. Target gene sequence capture and next generation sequencing was detected. Sanger sequencing was used to confirm the results of the patients and their parents. RESULT: Cholestasis, heart defects, inverted triangular face and butterfly vertebrae were presented as main clinical features in 4 male patients. The first hospital visiting ages ranged from 3 months and 14 days to 3 years and 1 month. The age of onset ranged from 3 days to 42 days (median 23 days). According to the clinical diagnostic criteria of ALGS, patient 1 and patient 2 were considered as typical ALGS. The other 2 patients were considered as atypical ALGS. Four Jagged 1(JAG1) pathogenic mutations were detected. Three different missense mutations were detected in patient 1 to patient 3 with ALGS (c.839C>T (p.W280X), c. 703G>A (p.R235X), c. 1720C>T (p.V574M)). The JAG1 mutation of patient 3 was first reported. Patient 4 had one novel insertion mutation (c.1779_1780insA (p.Ile594AsnfsTer23)). Parental analysis verified that the JAG1 missense mutation of 3 patients were de novo. The results of sanger sequencing was consistent with the results of the next generation sequencing. CONCLUSION: Target gene sequence capture combined with next generation sequencing can detect two pathogenic genes in ALGS and test genes of other related diseases in infantile cholestatic diseases simultaneously and presents a high throughput, high efficiency and low cost. It may provide molecular diagnosis and treatment for clinicians with good clinical application prospects.

Ghavidel, F. Z., et al. (2015). "A nonhomogeneous hidden markov model for gene mapping based on next-generation sequencing data." J Comput Biol **22**(2): 178-188.

The analysis of polygenetic characteristics for mapping quantitative trait loci (QTL) remains an important challenge. QTL analysis requires two or more strains of organisms that differ substantially in the (poly-)genetic trait of interest, resulting in a heterozygous offspring. The offspring with the trait of interest is selected and subsequently screened for molecular markers such as single-nucleotide polymorphisms (SNPs) with next-generation sequencing. Gene mapping relies on the co-segregation between genes and/or markers. Genes and/or markers that are linked to a QTL influencing the trait will segregate more frequently with this locus. For each identified marker, observed mismatch frequencies between the reads of the offspring and the parental reference strains can be modeled by a multinomial distribution with the probabilities depending on the state of an underlying, unobserved Markov process. The states indicate whether the SNP is located in a (vicinity of a) QTL or not. Consequently, genomic loci associated with the QTL can be discovered by analyzing hidden states along the genome. The aforementioned hidden Markov model assumes that the identified SNPs are equally distributed along the chromosome and does not take the distance between neighboring SNPs into account. The distance between the neighboring SNPs could influence the chance of co-segregation between genes and markers. To address this issue, we propose a nonhomogeneous hidden Markov model with a transition matrix that depends on a set of distance-varying observed covariates. The application of the model is illustrated on the data from a study of ethanol tolerance in yeast.

Giugliano, T., et al. (2016). "Identification of an intragenic deletion in the SGCB gene through a re-evaluation of negative next generation sequencing results." Neuromuscul Disord **26**(6): 367-369.

A large mutation screening of 504 patients with muscular dystrophy or myopathy has been performed by next generation sequencing (NGS). Among this cohort of patients, we report a case with a severe form of muscular dystrophy with a proximal weakness in the limb-girdle muscles. Her biopsy revealed typical dystrophic features and immunohistochemistry for alpha- and gamma-sarcoglycans showed an absent reaction, addressing the clinical diagnosis toward a sarcoglycanopathy. Considering that no causative point mutation was detected in any of the four sarcoglycan genes, we re-evaluated the NGS data by careful quantitative analysis of the specific reads mapping on the four sarcoglycan genes. A complete absence of reads from the sixth exon of the beta-sarcoglycan gene was found. Subsequent array comparative genomic hybridization (CGH) analysis confirmed the result with the identification of a novel 3.3 kb intragenic deletion in the SGCB gene. This case illustrates the importance of a multidisciplinary approach involving clinicians and molecular

geneticists and the need for a careful re-evaluation of NGS data.

Gleeson, F. C., et al. (2016). "Molecular cytology genotyping of primary and metastatic GI stromal tumors by using a custom two-gene targeted next-generation sequencing panel with therapeutic intent." Gastrointest Endosc **84**(6): 950-958.e953.

BACKGROUND AND AIMS: In an era of precision medicine, customized genotyping of GI stromal tumors by screening for driver mutations will become the standard of care. The fidelity of genotype concordance between paired cytology smears and surgical pathology specimens is unknown. In patients with either primary or metastatic sporadic disease, we sought to determine the frequency of KIT and PDGFRA pathogenic alterations within such specimens, imatinib sensitivity, and the concordance of pathogenic alterations between paired specimens. METHODS: DNA obtained from cytology smears from 36 patients, 24 of whom had paired surgical pathology specimens, underwent targeted next-generation sequencing by using a custom panel to evaluate somatic mutations within KIT (exon 2, 9, 10, 11, 13, 14, 15, 17, 18) and PDGFRA (exon 12, 14, 15, 18) genes. Patients with KIT and PDGRFA wild-type genes completed the Qiagen Human Comprehensive Cancer GeneRead DNAseq Targeted Array V2. RESULTS: Genotyping revealed KIT and PDGFRA mutations in 68% and 15% of patients. The wild-type population did not harbor mutations in BRAF, RAS family, SDHB, SETD2, or NF1. Imatinib sensitivity based on the oncogenic kinase mutation prevalence was estimated to be 68%. Mutational concordance between paired cytology and surgical pathology specimens was 96%. CONCLUSIONS: Our data have demonstrated the ability to stratify either primary or metastatic gastrointestinal stromal tumors by mutational subtype using a targeted next-generation sequencing 2 gene mutation panel. We highlight the ability to use cytology specimens obtained via minimally invasive techniques as a surrogate to surgical specimens given the high mutational landscape concordance between paired specimens.

Gold, W. A. and J. Christodoulou (2015). "The Utility of Next-Generation Sequencing in Gene Discovery for Mutation-Negative Patients with Rett Syndrome." Front Cell Neurosci **9**: 266.

Rett syndrome (RTT) is a rare, severe disorder of neuronal plasticity that predominantly affects girls. Girls with RTT usually appear asymptomatic in the first 6-18 months of life, but gradually develop severe motor, cognitive, and behavioral abnormalities that persist for life. A predominance of neuronal and synaptic dysfunction, with altered excitatory-inhibitory neuronal synaptic transmission and synaptic plasticity, are overarching features of RTT in children and in mouse models. Over 90% of patients with classical RTT have mutations in the X-linked methyl-CpG-binding (MECP2) gene, while other genes, including cyclin-dependent kinase-like 5 (CDKL5), Forkhead box protein G1 (FOXG1), myocyte-specific enhancer factor 2C (MEF2C), and transcription factor 4 (TCF4), have been associated with phenotypes overlapping with RTT. However, there remain a proportion of patients who carry a clinical diagnosis of RTT, but who are mutation negative. In recent years, next-generation sequencing technologies have revolutionized approaches to genetic studies, making whole-exome and even whole-genome sequencing possible strategies for the detection of rare and de novo mutations, aiding the discovery of novel disease genes. Here, we review the recent progress that is emerging in identifying pathogenic variations, specifically from exome sequencing in RTT patients, and emphasize the need for the use of this technology to identify known and new disease genes in RTT patients.

Gomez, J., et al. (2015). "Next generation sequencing search for uromodulin gene variants related with impaired renal function." Mol Biol Rep **42**(9): 1353-1358.

Uromodulin gene (UMOD) mutations have been linked to rare forms of mendelian dominant medullary cystic kidney disease and familial hyperuricemia. In addition, common single nucleotide polymorphisms in the UMOD promoter have been associated with the risk for impaired renal function and chronic kidney disease. Our main purpose was to identify UMOD variants related with impaired renal function in an elderly population. The UMOD gene was next generation sequenced in a total of 100 healthy individuals with normal or reduced renal function [measured as the rate of estimated glomerular filtration (eGFR)]. The identified missense changes and the common promoter rs12917707 polymorphism were determined in individuals with reduced (n = 88) and normal (n = 442) eGFR values. Allele and genotype frequencies were compared between the groups. We only identified a rare UMOD misense change, p.V458L, and the rare leu allele was significantly more frequent in a cohort of individuals with reduced (eGFR < 60) compared to normal eGFR (P = 0.02). The common rs12917707 polymorphism previously linked to renal function and kidney disease was not associated with impaired filtration rate in our cohort. We found a significant effect of the rare p.V458L variant on the value of estimated glomerular filtration. This finding deserves further validation in larger cohorts.

Gupta, S., et al. (2017). "Inherited DNA repair gene mutations detected by tumor next generation sequencing in urinary tract cancers." Fam Cancer **16**(4): 545-550.

Interpretation of next-generation sequencing (NGS) of tumor tissue in patients with advanced Urinary Tract Cancer (UTC) is performed to guide treatment selection but may reveal pathogenic variants with germline implications. We identified three patients with UTC with unexpected germline DNA repair gene mutations. Specific testing for these was prompted by the detection of these mutations by tumor NGS. All three patients were nonsmokers with a strong family history of cancer. Two patients had upper tract UTC with age at diagnosis in the 40 s. One had a family history suggestive of hereditary breast/ovarian predisposition and a FANCA mutation detected on NGS was confirmed to be germline. The second patient had a family history suggestive of Lynch syndrome but was found to have a germline BRCA2 mutation that was suggested by NGS. The third patient had bladder cancer at an advanced age, a family history of late-onset gastrointestinal malignancies that did not meet criteria for clinical testing for a hereditary cancer predisposition syndrome. NGS identified an MUTYH mutation, and targeted testing confirmed a monoallelic germline MUTYH mutation. Detection of variants with germline implications by tumor NGS may be clinically relevant for patients and their families and warrant genetic counseling and germline genetic testing. The prevalence of germline DNA repair defects in the context of inherited predisposition to UTC merits further study.

Guseva, N. V., et al. (2016). "The NAB2-STAT6 gene fusion in solitary fibrous tumor can be reliably detected by anchored multiplexed PCR for targeted next-generation sequencing." Cancer Genet **209**(7-8): 303-312.

Solitary fibrous tumor (SFT) is a mesenchymal tumor of fibroblastic origin, which can affect any region of the body. 10-15% of SFTs metastasize and metastatic tumors are uniformly lethal with no effective therapies. The behavior of SFT is difficult to predict based on morphology. Recently, an intrachromosomal gene fusion between NAB2 and STAT6 was identified as the defining driving genetic event of SFT and different fusion types correlated with tumor histology and behavior. Due to the proximity of NAB2 and STAT6 on chromosome 12, this fusion may be missed by fluorescence in-situ hybridization. We evaluated 12 SFTs from 10 patients. All tumors showed strong nuclear staining for STAT6 by immunohistochemistry (IHC). The same formalin-fixed, paraffin-embedded blocks for IHC were used for gene fusion detection by a next-generation sequencing (NGS)-based assay. Targeted RNA fusion sequencing for gene fusions was performed using the Universal RNA Fusion Detection Kit, the Archer () FusionPlex () Sarcoma Panel and the Ion Torrent PGM, and data were analyzed using the Archer Analysis Pipeline 3.3. All tumors were positive for NAB2-STAT6 fusion. Six types of fusions were detected: NAB2ex4-STAT6ex2, NAB2ex2-STAT6ex5, NAB2ex6-STAT6ex16, NAB2ex6-STAT6ex17, NAB2ex3-STAT6ex18 and NAB2intron6-STAT6Ex17. The NGS findings were confirmed by RT-PCR followed by Sanger sequencing. No STAT6 fusion was detected in selected morphologic mimics of SFT. The assay also allows for detection of novel fusions and can detect NAB2-STAT6 fusions at a single-base resolution.

Han, X., et al. (2018). "Gene mutation patterns of Chinese acute myeloid leukemia patients by targeted next-generation sequencing and bioinformatic analysis." Clin Chim Acta **479**: 25-37.

PURPOSES: The conventional risk stratification of acute myeloid leukemia (AML), based on cytogenetics, cannot meet the demand for accurate prognostic evaluations. In recent years, gene mutations are found to be potential markers for more accurate risk stratification, but reports on mutation screening of Chinese AML are limited. We aim to display the mutation patterns of Chinese AML patients, reveal the genotype-phenotype correlations and make a comparison with Caucasians patients. METHODS: Genome DNA from 78 patients' bone marrow were extracted for targeted gene mutation panel by next-generation sequencing (NGS) technology. Statistics and bioinformatics were used to analyze the correlations between gene mutations and clinical features, as well as the comparison of our results with the Cancer Genome Atlas Research Network (TCGA) public AML dataset. RESULTS: We found patients with mutations of FLT3 and TET2 had higher bone marrow blasts, peripheral blasts and white blood cell (WBC) count, mutations of SRSF2 were related with age, and mutations of FLT3-ITD, DNMT3A, IDH1, TET2 and SRSF2 were risk factors for overall survival. What's more, we discovered 15 novel mutations and difference of mutational incidence in 6 genes between Chinese and Caucasians AML. Bioinformatic analysis revealed some relationship between gene mutations and expressions as well as drug sensitivities. CONCLUSIONS: We made an investigation on the mutation patterns of Chinese AML patients by NGS technique and revealed correlations between gene mutations and clinical features. Thus we recommend

routine testing of suspected genes for better prognostic prediction and individualized treatment.

Hawi, Z., et al. (2017). "Rare DNA variants in the brain-derived neurotrophic factor gene increase risk for attention-deficit hyperactivity disorder: a next-generation sequencing study." Mol Psychiatry **22**(4): 580-584.

Attention-deficit hyperactivity disorder (ADHD) is a prevalent and highly heritable disorder of childhood with negative lifetime outcomes. Although candidate gene and genome-wide association studies have identified promising common variant signals, these explain only a fraction of the heritability of ADHD. The observation that rare structural variants confer substantial risk to psychiatric disorders suggests that rare variants might explain a portion of the missing heritability for ADHD. Here we believe we performed the first large-scale next-generation targeted sequencing study of ADHD in 152 child and adolescent cases and 188 controls across an a priori set of 117 genes. A multi-marker gene-level analysis of rare (<1% frequency) single-nucleotide variants (SNVs) revealed that the gene encoding brain-derived neurotrophic factor (BDNF) was associated with ADHD at Bonferroni corrected levels. Sanger sequencing confirmed the existence of all novel rare BDNF variants. Our results implicate BDNF as a genetic risk factor for ADHD, potentially by virtue of its critical role in neurodevelopment and synaptic plasticity.

Held, E., et al. (2016). "Comparing machine learning and logistic regression methods for predicting hypertension using a combination of gene expression and next-generation sequencing data." BMC Proc **10**(Suppl 7): 141-145.

Machine learning methods continue to show promise in the analysis of data from genetic association studies because of the high number of variables relative to the number of observations. However, few best practices exist for the application of these methods. We extend a recently proposed supervised machine learning approach for predicting disease risk by genotypes to be able to incorporate gene expression data and rare variants. We then apply 2 different versions of the approach (radial and linear support vector machines) to simulated data from Genetic Analysis Workshop 19 and compare performance to logistic regression. Method performance was not radically different across the 3 methods, although the linear support vector machine tended to show small gains in predictive ability relative to a radial support vector machine and logistic regression. Importantly, as the number of genes in the models was increased, even when those genes contained causal rare variants, model predictive ability showed a statistically significant decrease in performance for both the radial support vector machine and logistic regression. The linear support vector machine showed more robust performance to the inclusion of additional genes. Further work is needed to evaluate machine learning approaches on larger samples and to evaluate the relative improvement in model prediction from the incorporation of gene expression data.

Helsten, T., et al. (2016). "Cell-Cycle Gene Alterations in 4,864 Tumors Analyzed by Next-Generation Sequencing: Implications for Targeted Therapeutics." Mol Cancer Ther **15**(7): 1682-1690.

Alterations in the cyclin-dependent kinase (CDK)-retinoblastoma (RB) machinery disrupt cell-cycle regulation and are being targeted in drug development. To understand the cancer types impacted by this pathway, we analyzed frequency of abnormalities in key cell-cycle genes across 4,864 tumors using next-generation sequencing (182 or 236 genes; Clinical Laboratory Improvement Amendments laboratory). Aberrations in the cell-cycle pathway were identified in 39% of cancers, making this pathway one of the most commonly altered in cancer. The frequency of aberrations was as follows: CDKN2A/B (20.1% of all patients), RB1 (7.6%), CCND1 (6.1%), CCNE1 (3.6%), CDK4 (3.2%), CCND3 (1.8%), CCND2 (1.7%), and CDK6 (1.7%). Rates and types of aberrant cell-cycle pathway genes differed between cancer types and within histologies. Analysis of coexisting and mutually exclusive genetic aberrations showed that CCND1, CCND2, and CCND3 aberrations were all positively associated with CDK6 aberrations [OR and P values, multivariate analysis: CCND1 and CDK6 (OR = 3.5; P < 0.0001), CCND2 and CDK6 (OR = 4.3; P = 0.003), CCND3 and CDK6 (OR = 3.6; P = 0.007)]. In contrast, RB1 alterations were negatively associated with multiple gene anomalies in the cell-cycle pathway, including CCND1 (OR = 0.25; P = 0.003), CKD4 (OR = 0.10; P = 0.001), and CDKN2A/B (OR = 0.21; P < 0.0001). In conclusion, aberrations in the cell-cycle pathway were very common in diverse cancers (39% of 4,864 neoplasms). The frequencies and types of alterations differed between and within tumor types and will be informative for drug development strategies. Mol Cancer Ther; 15(7); 1682-90. (c)2016 AACR.

Hernan, I., et al. (2015). "Two novel frameshift mutations in BRCA2 gene detected by next generation sequencing in a survey of Spanish patients of breast cancer." Clin Transl Oncol **17**(7): 576-580.

PURPOSE: To analyze BRCA1 and BRCA2 genes using a cost-effective and rapid approach based

on next generation sequencing (NGS) technology. METHODS: A population of Spanish cancer patients with a personal or familial history of breast and/or ovarian cancer was analyzed for germline mutations in BRCA1 and BRCA2 genes. The methodology relies on a 5 multiplex PCR assay coupled to NGS. RESULTS: Ten pathogenic mutations (four in BRCA1 and six in BRCA2 gene) were identified in a Spanish population. The deletion c.1792delA, in exon 10, and the duplication c.5869dupA, in exon 11 of BRCA2 gene were not previously reported and should be considered as pathogenic due to its frameshift nature. CONCLUSION: Two novel frameshift mutations in BRCA2 gene were detected using the multiplex PCR-based assay following by NGS.

Hirsch, B., et al. (2018). "Multicenter validation of cancer gene panel-based next-generation sequencing for translational research and molecular diagnostics." Virchows Arch.

The simultaneous detection of multiple somatic mutations in the context of molecular diagnostics of cancer is frequently performed by means of amplicon-based targeted next-generation sequencing (NGS). However, only few studies are available comparing multicenter testing of different NGS platforms and gene panels. Therefore, seven partner sites of the German Cancer Consortium (DKTK) performed a multicenter interlaboratory trial for targeted NGS using the same formalin-fixed, paraffin-embedded (FFPE) specimen of molecularly pre-characterized tumors (n = 15; each n = 5 cases of Breast, Lung, and Colon carcinoma) and a colorectal cancer cell line DNA dilution series. Detailed information regarding pre-characterized mutations was not disclosed to the partners. Commercially available and custom-designed cancer gene panels were used for library preparation and subsequent sequencing on several devices of two NGS different platforms. For every case, centrally extracted DNA and FFPE tissue sections for local processing were delivered to each partner site to be sequenced with the commercial gene panel and local bioinformatics. For cancer-specific panel-based sequencing, only centrally extracted DNA was analyzed at seven sequencing sites. Subsequently, local data were compiled and bioinformatics was performed centrally. We were able to demonstrate that all pre-characterized mutations were re-identified correctly, irrespective of NGS platform or gene panel used. However, locally processed FFPE tissue sections disclosed that the DNA extraction method can affect the detection of mutations with a trend in favor of magnetic bead-based DNA extraction methods. In conclusion, targeted NGS is a very robust method for simultaneous detection of various mutations in FFPE tissue specimens if certain pre-analytical conditions are carefully considered.

Hsu, Y. L., et al. (2017). "Identification of novel gene expression signature in lung adenocarcinoma by using next-generation sequencing data and bioinformatics analysis." Oncotarget **8**(62): 104831-104854.

Lung adenocarcinoma is one of the leading causes of cancer-related death worldwide. We showed transcriptomic profiles in three pairs of tumors and adjacent non-tumor lung tissues using next-generation sequencing (NGS) to screen protein-coding RNAs and microRNAs. Combined with meta-analysis from the Oncomine and Gene Expression Omnibus (GEO) databases, we identified a representative genetic expression signature in lung adenocarcinoma. There were 9 upregulated genes, and 8 downregulated genes in lung adenocarcinoma. The analysis of the effects from each gene expression on survival outcome indicated that 6 genes (AGR2, SPDEF, CDKN2A, CLDN3, SFN, and PHLDA2) play oncogenic roles, and 7 genes (PDK4, FMO2, CPED1, GNG11, IL33, BTNL9, and FABP4) act as tumor suppressors in lung adenocarcinoma. In addition, we also identified putative genetic interactions, in which there were 5 upregulated microRNAs with specific targets - hsa-miR-183-5p-BTNL9, hsa-miR-33b-5p-CPED1, hsa-miR-429-CPED1, hsa-miR-182-5p-FMO2, and hsa-miR-130b-5p-IL33. These 5 microRNAs have been shown to be associated with tumorigenesis in lung cancer. Our findings suggest that these genetic interactions play important roles in the progression of lung adenocarcinoma. We propose that this molecular change of genetic expression may represent a novel signature in lung adenocarcinoma, which may be developed for diagnostic and therapeutic strategies in the future.

Huang, X., et al. (2017). "Next-generation sequencing reveals a novel NDP gene mutation in a Chinese family with Norrie disease." Indian J Ophthalmol **65**(11): 1161-1165.

PURPOSE: Norrie disease (ND) is a rare X-linked genetic disorder, the main symptoms of which are congenital blindness and white pupils. It has been reported that ND is caused by mutations in the NDP gene. Although many mutations in NDP have been reported, the genetic cause for many patients remains unknown. In this study, the aim is to investigate the genetic defect in a five-generation family with typical symptoms of ND. METHODS: To identify the causative gene, next-generation sequencing based target capture sequencing was performed. Segregation analysis of the candidate variant was performed in additional family members using Sanger sequencing.

RESULTS: We identified a novel missense variant (c.314C>A) located within the NDP gene. The mutation cosegregated within all affected individuals in the family and was not found in unaffected members. By happenstance, in this family, we also detected a known pathogenic variant of retinitis pigmentosa in a healthy individual. CONCLUSION: c.314C>A mutation of NDP gene is a novel mutation and broadens the genetic spectrum of ND.

Huijun, Y. and L. Yu (2014). "[Application of next generation sequencing in gene identification and genetic diagnosis of hereditary hearing loss]." Yi Chuan **36**(11): 1112-1120.

More than 50% cases with hearing loss are caused by genetic defects. With the development of genomics technology, molecular genetics of hearing loss has become a cutting-edge field under investigation in otology. The next generation sequencing has provided a new model originated from sequencing data to genetic disease study, and has made a revolutionary change in the strategy of gene identification for genetic diseases. Due to the application of next generation sequencing technology, gene identification of hearing loss has been accelerated in recent years, and moreover, the new technology and strategy were explored to clinical application. In this review, we briefly introduce the current situation of hereditary hearing loss research, and the application and perspective of next generation sequencing in this field.

Iacocca, M. A., et al. (2017). "Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia." J Lipid Res **58**(11): 2202-2209.

Familial hypercholesterolemia (FH) is a heritable condition of severely elevated LDL cholesterol, caused predominantly by autosomal codominant mutations in the LDL receptor gene (LDLR). In providing a molecular diagnosis for FH, the current procedure often includes targeted next-generation sequencing (NGS) panels for the detection of small-scale DNA variants, followed by multiplex ligation-dependent probe amplification (MLPA) in LDLR for the detection of whole-exon copy number variants (CNVs). The latter is essential because approximately 10% of FH cases are attributed to CNVs in LDLR; accounting for them decreases false negative findings. Here, we determined the potential of replacing MLPA with bioinformatic analysis applied to NGS data, which uses depth-of-coverage analysis as its principal method to identify whole-exon CNV events. In analysis of 388 FH patient samples, there was 100% concordance in LDLR CNV detection between these two methods: 38 reported CNVs identified by MLPA were also successfully detected by our NGS method, while 350 samples negative for CNVs by MLPA were also negative by NGS. This result suggests that MLPA can be removed from the routine diagnostic screening for FH, significantly reducing associated costs, resources, and analysis time, while promoting more widespread assessment of this important class of mutations across diagnostic laboratories.

Jang, J. S., et al. (2016). "Custom Gene Capture and Next-Generation Sequencing to Resolve Discordant ALK Status by FISH and IHC in Lung Adenocarcinoma." J Thorac Oncol **11**(11): 1891-1900.

INTRODUCTION: We performed a genomic study in lung adenocarcinoma cases with discordant anaplastic lymphoma receptor tyrosine kinase gene (ALK) status by fluorescent in situ hybridization (FISH) and immunohistochemical (IHC) analysis. METHODS: DNA from formalin-fixed paraffin-embedded tissues of 16 discordant (four FISH-positive/IHC-negative and 12 FISH-negative/IHC-positive) cases by Vysis ALK Break Apart FISH and ALK IHC testing (ALK1 clone) were subjected to whole gene capture and next-generation sequencing (NGS) of nine genes, including ALK, echinoderm microtubule associated protein like 4 gene (EML4), kinesin family member 5B gene (KIF5B), staphylococcal nuclease and tudor domain containing 1 gene (SND1), BRAF, ret proto-oncogene (RET), ezrin gene (EZR), ROS1, and telomerase reverse transcriptase (TERT). All discordant cases (except one FISH-negative/IHC-positive case without sufficient tissue) were analyzed by IHC with D5F3 antibody. In one case with fresh frozen tissue, whole transcriptome sequencing was also performed. Twenty-six concordant (16 FISH-positive/IHC-positive and 10 FISH-negative/IHC-negative) cases were included as controls. RESULTS: In four ALK FISH-positive/IHC-negative cases, no EML4-ALK fusion gene was observed by NGS, but in one case using fresh frozen tissue, we identified EML4-baculoviral AIP repeat containing 6 gene (BIRC6) and AP2 associated kinase 1 gene (AAK1)-ALK fusion genes. Whole transcriptome sequencing revealed a highly expressed EML4-BIRC6 fusion transcript and a minimally expressed AAK1 transcript. Among the 12 FISH-negative/IHC-positive cases, no evidence of ALK gene rearrangement was detected by NGS. Eleven of 12 FISH-negative/IHC-positive cases detected by ALK1 clone were concordant by repeat ALK IHC with D5F3 antibody (i.e., FISH-negative/IHC-negative by D5F3 clone). Among the 16 ALK FISH-positive/IHC-positive positive controls, whole gene capture identified ALK gene fusion in 15 cases, including in one case with Huntington interacting protein 1 gene (HIP1)-ALK. No ALK fusion gene was observed in

any of the 10 FISH-negative/IHC-negative cases. Other fusion genes involving ROS1, EZR, BRAF, and SND1 were also found. CONCLUSIONS: ALK FISH results appeared to be false-positive in three of four FISH-positive/IHC-negative cases, whereas no false-negative ALK FISH case was identified among 12 ALK FISH-negative/IHC-positive cases by ALK1 clone, which was in keeping with the concordant FISH-negative/IHC-negative status by D5F3 clone. Our targeted whole gene capture approach using formalin-fixed paraffin embedded samples was effective for detecting rearrangements involving ALK and other actionable oncogenes.

Jesmok, E. M., et al. (2016). "Next-Generation Sequencing of the Bacterial 16S rRNA Gene for Forensic Soil Comparison: A Feasibility Study." J Forensic Sci **61**(3): 607-617.

Soil has the potential to be valuable forensic evidence linking a person or item to a crime scene; however, there is no established soil individualization technique. In this study, the utility of soil bacterial profiling via next-generation sequencing of the 16S rRNA gene was examined for associating soils with their place of origin. Soil samples were collected from ten diverse and nine similar habitats over time, and within three habitats at various horizontal and vertical distances. Bacterial profiles were analyzed using four methods: abundance charts and nonmetric multidimensional scaling provided simplification and visualization of the massive datasets, potentially aiding in expert testimony, while analysis of similarities and k-nearest neighbor offered objective statistical comparisons. The vast majority of soil bacterial profiles (95.4%) were classified to their location of origin, highlighting the potential of bacterial profiling via next-generation sequencing for the forensic analysis of soil samples.

Ji, H., et al. (2015). "Next generation sequencing of the hepatitis C virus NS5B gene reveals potential novel S282 drug resistance mutations." Virology **477**: 1-9.

Identifying HCV drug resistance mutations (DRMs) is increasingly important as new direct acting antiviral therapies (DAA) become available. Tagged pooled pyrosequencing (TPP) was originally developed as cost-effective approach for detecting low abundance HIV DRMs. Using 127 HCV-positive samples from a Canadian injection drug user cohort, we demonstrated the suitability and efficiency of TPP for evaluating DRMs in HCV NS5B gene. At a mutation identification threshold of 1%, no nucleoside inhibitor DRMs were detected among these DAA naive subjects. Clinical NS5B resistance to non-nucleoside inhibitors and interferon/ribavirin was predicted to be low within this cohort. S282T mutation, the primary mutation selected by sofosbuvir in vitro, was not identified while S282G/C/R variants were detected in 9 subjects. Further characterization on these new S282 variants using in silico molecular modeling implied their potential association with resistance. Combining TPP with in silico analysis detects NS5B polymorphisms that may explain differences in treatment outcomes.

Jia, B., et al. (2017). "Learning gene regulatory networks from next generation sequencing data." Biometrics **73**(4): 1221-1230.

In recent years, next generation sequencing (NGS) has gradually replaced microarray as the major platform in measuring gene expressions. Compared to microarray, NGS has many advantages, such as less noise and higher throughput. However, the discreteness of NGS data also challenges the existing statistical methodology. In particular, there still lacks an appropriate statistical method for reconstructing gene regulatory networks using NGS data in the literature. The existing local Poisson graphical model method is not consistent and can only infer certain local structures of the network. In this article, we propose a random effect model-based transformation to continuize NGS data and then we transform the continuized data to Gaussian via a semiparametric transformation and apply an equivalent partial correlation selection method to reconstruct gene regulatory networks. The proposed method is consistent. The numerical results indicate that the proposed method can lead to much more accurate inference of gene regulatory networks than the local Poisson graphical model and other existing methods. The proposed data-continuized transformation fills the theoretical gap for how to transform discrete data to continuous data and facilitates NGS data analysis. The proposed data-continuized transformation also makes it feasible to integrate different types of data, such as microarray and RNA-seq data, in reconstruction of gene regulatory networks.

Jiang, J. L., et al. (2017). "Digital gene expression analysis of Takifugu rubripes brain after acute hypoxia exposure using next-generation sequencing." Comp Biochem Physiol Part D Genomics Proteomics **24**: 12-18.

The adverse effects of hypoxia are confined to biochemical, physiological, developmental and behavioral processes, especially injury of the brain. In this study, a subset of genes in the brain of Takifugu rubripes were analyzed using digital gene expression (DGE) profiles and next-generation sequencing after acute hypoxia. Among 32 differentially expressed genes, 29 were up-regulated and 3 were down-

regulated following hypoxia exposure. Using Gene Ontology analysis, it was found that transcription and translation, metabolism, and the stress response were affected by exposure to hypoxia. KEGG analysis revealed that the neuroactive ligand-receptor interaction pathway was significantly enriched in hypoxia-exposed T. rubripes. To further confirm the differential expression of genes, quantitative real-time PCR was performed to test six candidate genes, with the following five genes exhibiting the same expression patterns as the sequencing results: Proto-oncogene c-fos, Kruppel-like factor 2, immediate early response 2, proopiomelanocortin A and rhodopsin. This work is the first to identify and annotate genes in T. rubripes affected by hypoxia stress. This investigation provides data for understanding the molecular mechanism of fish adaptation to hypoxia and provides a reference for rationally setting dissolved oxygen levels in aquaculture.

Johar, A. S., et al. (2015). "Candidate gene discovery in autoimmunity by using extreme phenotypes, next generation sequencing and whole exome capture." Autoimmun Rev **14**(3): 204-209.

Whole exome sequencing (WES) is a widely used strategy for detection of protein coding and splicing variants associated with inherited diseases. Many studies have shown that the strategy has been broad and proficient due to its ability in detecting a high proportion of disease causing variants, using only a small portion of the genome. In this review we outline the main steps involved in WES, the comprehensive analysis of the massive data obtained including the genomic capture, amplification, sequencing, alignment, curating, filtering and genetic analysis to determine the presence of candidate variants with potential pathogenic/functional effect. Further, we propose that the multiple autoimmune syndrome, an extreme phenotype of autoimmune disorders, is a very well suited trait to tackle genomic variants of major effect underpinning the lost of self-tolerance.

Jouali, F., et al. (2016). "First application of next-generation sequencing in Moroccan breast/ovarian cancer families and report of a novel frameshift mutation of the BRCA1 gene." Oncol Lett **12**(2): 1192-1196.

At present, breast cancer is the most common type of cancer in females. The majority of cases are sporadic, but 5-10% are due to an inherited predisposition to develop breast and ovarian cancers, which are transmitted as an autosomal dominant form with incomplete penetrance. The beneficial effects of clinical genetic testing, including next generation sequencing (NGS) for BRCA1/2 mutations, is major; in particular, it benefits the care of patients and the counseling of relatives that are at risk of breast cancer, in order to reduce breast cancer mortality. BRCA genetic testing was performed in 15 patients with breast cancer and a family with positivity for the heterozygous c.6428C>A mutation of the BRCA2 gene. Informed consent was obtained from all the subjects. Genomic DNAs were extracted and the NGS for genes was performed using the Ion Torrent Personal Genome Machine (PGM) with a 316 chip. The reads were aligned with the human reference HG19 genome to elucidate variants in the BRCA1 and BRCA2 genes. Mutations detected by the PGM platform were confirmed by target direct Sanger sequencing on a second patient DNA sample. In total, 4 BRCA variants were identified in 6 families by NGS. Of these, 3 mutations had been previously reported: c.2126insA of BRCA1, and c.1310_1313delAAGA and c.7235insG of BRCA2. The fourth variant, c.3453delT in BRCA1, has, to the best of our knowledge, never been previously reported. The present study is the first to apply NGS of the BRCA1 and BRCA2 genes to a Moroccan population, prompting additional investigation into local founder mutations and variant characteristics in the region. The variants with no clear clinical significance may present a diagnostic challenge when performing targeted resequencing. These results confirm that an NGS approach based on Ampliseq libraries and PGM sequencing is a highly efficient, speedy and high-throughput mutation detection method, which may be preferable in lower income countries.

Judkins, T., et al. (2015). "Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk." BMC Cancer **15**: 215.

BACKGROUND: Germline DNA mutations that increase the susceptibility of a patient to certain cancers have been identified in various genes, and patients can be screened for mutations in these genes to assess their level of risk for developing cancer. Traditional methods using Sanger sequencing focus on small groups of genes and therefore are unable to screen for numerous genes from several patients simultaneously. The goal of the present study was to validate a 25-gene panel to assess genetic risk for cancer in 8 different tissues using next generation sequencing (NGS) techniques. METHODS: Twenty-five genes associated with hereditary cancer syndromes were selected for development of a panel to screen for risk of these cancers using NGS. In an initial technical assessment, NGS results for BRCA1 and BRCA2 were compared with Sanger sequencing in 1864 anonymized DNA samples from patients who

had undergone previous clinical testing. Next, the entire gene panel was validated using parallel NGS and Sanger sequencing in 100 anonymized DNA samples. Large rearrangement analysis was validated using NGS, microarray comparative genomic hybridization (CGH), and multiplex ligation-dependent probe amplification analyses (MLPA). RESULTS: NGS identified 15,877 sequence variants, while Sanger sequencing identified 15,878 in the BRCA1 and BRCA2 comparison study of the same regions. Based on these results, the NGS process was refined prior to the validation of the full gene panel. In the validation study, NGS and Sanger sequencing were 100% concordant for the 3,923 collective variants across all genes for an analytical sensitivity of the NGS assay of >99.92% (lower limit of 95% confidence interval). NGS, microarray CGH and MLPA correctly identified all expected positive and negative large rearrangement results for the 25-gene panel. CONCLUSION: This study provides a thorough validation of the 25-gene NGS panel and indicates that this analysis tool can be used to collect clinically significant information related to risk of developing hereditary cancers.

Kaderbhai, C. G., et al. (2016). "Use of dedicated gene panel sequencing using next generation sequencing to improve the personalized care of lung cancer." Oncotarget **7**(17): 24860-24870.

Advances in Next Generation Sequencing (NGS) technologies have improved the ability to detect potentially targetable mutations. However, the integration of NGS into clinical management in an individualized manner remains challenging. In this single-center observational study, we performed a dedicated NGS panel studying 41 cancer-related genes in 50 consecutive patients with metastatic non-small-cell lung cancer between May 2012 and October 2014. Molecular analysis could be performed in 48 patients with a good quality check. One hundred and thirty-three mutations, whose twenty-four unique mutations, were detected. At least one mutation was found in 46 patients. In 58% of cases, the Molecular Tumor Board (MTB) was able to recommend treatment with a targeted agent based on the evaluation of the tumor genetic profile and treatment history. Nine patients (18%) were subsequently treated with a MTB-recommended targeted therapy; four patients experienced a clinical benefit with a partial response or stabilization lasting more than 4 months. In this case series involving patients with metastatic non-small cell lung cancer, we show that including integrative clinical sequencing data into routine clinical management was feasible and could impact on patient therapeutic proposal.

Kappel, K., et al. (2017). "Species identification in mixed tuna samples with next-generation sequencing targeting two short cytochrome b gene fragments." Food Chem **234**: 212-219.

Conventional Sanger sequencing of PCR products is the gold standard for species authentication of seafood products. However, this method is inappropriate for the analysis of products that might contain mixtures of species, such as tinned tuna. The purpose of this study was to test whether next-generation sequencing (NGS) can be a solution for the authentication of mixed products. Nine tuna samples containing mixtures of up to four species were prepared and subjected to an NGS approach targeting two short cytochrome b gene (cytb) fragments on the Illumina MiSeq platform. Sequence recovery was precise and admixtures of as low as 1% could be identified, depending on the species composition of the mixtures. Duplicate samples as well as two individual NGS runs produced very similar results. A first test of three commercial tinned tuna samples indicated the presence of different species in the same tin, although this is forbidden by EU law.

Katz-Summercorn, A., et al. (2017). "Application of a multi-gene next-generation sequencing panel to a non-invasive oesophageal cell-sampling device to diagnose dysplastic Barrett's oesophagus." J Pathol Clin Res **3**(4): 258-267.

The early detection and endoscopic treatment of patients with the dysplastic stage of Barrett's oesophagus is a key to preventing progression to oesophageal adenocarcinoma. However, endoscopic surveillance protocols are hampered by the invasiveness of repeat endoscopy, sampling bias, and a subjective histopathological diagnosis of dysplasia. In this case-control study, we investigated the use of a non-invasive, pan-oesophageal cell-sampling device, the Cytosponge, coupled with a cancer hot-spot panel to identify patients with dysplastic Barrett's oesophagus. Formalin-fixed, paraffin-embedded (FFPE) Cytosponge samples from 31 patients with non-dysplastic and 28 with dysplastic Barrett's oesophagus with good available clinical annotation were selected for inclusion. Samples were microdissected and amplicon sequencing performed using a panel covering >2800 COSMIC hot-spot mutations in 50 oncogenes and tumour suppressor genes. Strict mutation criteria were determined and duplicates were run to confirm any mutations with an allele frequency <12%. When compared with endoscopy and biopsy as the gold standard the panel achieved a 71.4% sensitivity (95% CI 51.3-86.8) and 90.3% (95% CI 74.3-98.0) specificity for diagnosing dysplasia. TP53 had the highest rate of mutation in 14/28 dysplastic samples (50%). CDKN2A was

mutated in 6/28 (21.4%), ERBB2 in 3/28 (10.7%), and 5 other genes at lower frequency. The only gene from this panel found to be mutated in the non-dysplastic cases was CDKN2A in 3/31 cases (9.7%) in keeping with its known loss early in the natural history of the disease. Hence, it is possible to apply a multi-gene cancer hot-spot panel and next-generation sequencing to microdissected, FFPE samples collected by the Cytosponge, in order to distinguish non-dysplastic from dysplastic Barrett's oesophagus. Further work is required to maximize the panel sensitivity.

Kelly, P. A. (2017). "Next Generation Sequencing and Multi-Gene Panel Testing: Implications for the Oncology Nurse." Semin Oncol Nurs **33**(2): 208-218.

OBJECTIVES: To review past, current, and future events in genetics and discuss how genetic testing information personalizes cancer screening, detection, and treatment. A case study is presented to illustrate key points. DATA SOURCES: National guidelines, evidence-based summaries, peer-reviewed studies, editorials, and web sites. CONCLUSION: Multi-gene testing using next-generation sequencing has changed the landscape for hereditary cancer syndromes. IMPLICATIONS FOR NURSING PRACTICE: Nurses have key roles in personalizing health care including recognizing the complexities of genetic testing, assessing family history, understanding gene/environment factors, referring for genetics consultations, and promoting registry studies. In order to be effective, nurses must stay current with the rapidly-changing technology and guidelines for genetic evaluations and testing.

Kim, S. T., et al. (2015). "Prospective blinded study of somatic mutation detection in cell-free DNA utilizing a targeted 54-gene next generation sequencing panel in metastatic solid tumor patients." Oncotarget **6**(37): 40360-40369.

Sequencing of the mutant allele fraction of circulating cell-free DNA (cfDNA) derived from tumors is increasingly utilized to detect actionable genomic alterations in cancer. We conducted a prospective blinded study of a comprehensive cfDNA sequencing panel with 54 cancer genes. To evaluate the concordance between cfDNA and tumor DNA (tDNA), sequencing results were compared between cfDNA from plasma and genomic tumor DNA (tDNA). Utilizing next generation digital sequencing technology (DST), we profiled approximately 78,000 bases encoding 512 complete exons in the targeted genes in cfDNA from plasma. Seventy-five patients were prospectively enrolled between February 2013 and March 2014, including 61 metastatic cancer patients and 14 clinical stage II CRC patients with matched plasma and tissue samples. Using the 54-gene panel, we detected at least one somatic mutation in 44 of 61 tDNA (72.1%) and 29 of 44 (65.9%) cfDNA. The overall concordance rate of cfDNA to tDNA was 85.9%, when all detected mutations were considered. We collected serial cfDNAs during cetuximab-based treatment in 2 metastatic KRAS wild-type CRC patients, one with acquired resistance and one with primary resistance. We demonstrate newly emerged KRAS mutation in cfDNA 1.5 months before radiologic progression. Another patient had a newly emerged PIK3CA H1047R mutation on cfDNA analysis at progression during cetuximab/irinotecan chemotherapy with gradual increase in allele frequency from 0.8 to 2.1%. This blinded, prospective study of a cfDNA sequencing showed high concordance to tDNA suggesting that the DST approach may be used as a non-invasive biopsy-free alternative to conventional sequencing using tumor biopsy.

Kim, Y. Y., et al. (2017). "Genetic alterations in mesiodens as revealed by targeted next-generation sequencing and gene co-occurrence network analysis." Oral Dis **23**(7): 966-972.

OBJECTIVE: Mesiodens is the most common type of supernumerary tooth which includes a population prevalence of 0.15%-1.9%. Alongside evidence that the condition is heritable, mutations in single genes have been reported in few human supernumerary tooth cases. Gene sequencing methods in tradition way are time-consuming and labor-intensive, whereas next-generation sequencing and bioinformatics are cost-effective for large samples and target sizes. MATERIALS AND METHODS: We describe the application of a targeted next-generation sequencing (NGS) and bioinformatics approach to samples from 17 mesiodens patients. Subjects were diagnosed on the basis of panoramic radiograph. A total of 101 candidate genes which were captured custom genes were sequenced on the Illumina HiSeq 2500. Multistep bioinformatics processing was performed including variant identification, base calling, and in silico analysis of putative disease-causing variants. RESULTS: Targeted capture identified 88 non-synonymous, rare, exonic variants involving 42 of the 101 candidate genes. Moreover, we investigated gene co-occurrence relationships between the genomic alterations and identified 88 significant relationships among 18 most recurrent driver alterations. CONCLUSION: Our search for co-occurring genetic alterations revealed that such alterations interact cooperatively to drive mesiodens. We discovered a gene co-occurrence network in mesiodens patients with functionally enriched gene groups in the sonic

hedgehog (SHH), bone morphogenetic proteins (BMP), and wingless integrated (WNT) signaling pathways.

Knopp, C., et al. (2015). "Syndromic ciliopathies: From single gene to multi gene analysis by SNP arrays and next generation sequencing." Mol Cell Probes **29**(5): 299-307.

Joubert syndrome (JS) and related disorders (JSRD), Meckel syndrome (MKS) and Bardet-Biedl syndrome (BBS) are autosomal recessive ciliopathies with a broad clinical and genetic overlap. In our multiethnic cohort of 88 MKS, 61 JS/JSRD and 66 BBS families we performed genetic analyses and were able to determine mutation frequencies and detection rates for the most frequently mutated MKS genes. On the basis of determined mutation frequencies, a next generation gene panel for JS/JSRD and MKS was established. Furthermore 35 patients from 26 unrelated consanguineous families were investigated by SNP array-based homozygosity mapping and subsequent DNA sequencing of known candidate genes according to runs of homozygosity size in descending order. This led to the identification of the causative homozygous mutation in 62% of unrelated index cases. Based on our data we discuss various strategies for diagnostic mutation detection in the syndromic ciliopathies JS/JSRD, MKS and BBS.

Kono, N., et al. (2017). "Deciphering antigen-responding antibody repertoires by using next-generation sequencing and confirming them through antibody-gene synthesis." Biochem Biophys Res Commun **487**(2): 300-306.

Vast diversity and high specificity of antigen recognition by antibodies are hallmarks of the acquired immune system. Although the molecular mechanisms that yield the extremely large antibody repertoires are precisely understood, comprehensive description of the global antibody repertoire generated in individual bodies has been hindered by the lack of powerful measures. To obtain holistic understanding of the antibody-repertoire space, we used next-generation sequencing (NGS) to analyze the deep profiles of naive and antigen-responding repertoires of the IgM, IgG1, and IgG2c classes formed in individual mice. The overall landscapes of naive IgM repertoires were almost the same for each mouse, whereas those of IgG1 and IgG2c differed considerably among naive individuals. Next, we immunized mice with a model antigen, nitrophenol (NP)-hapten linked to chicken gamma-globulin (CGG) carrier, and compared the antigen-responding repertoires in individual mice. To extract the complete antigen response, we developed an intelligible method for detecting common components of antigen-responding repertoires. The major responding antibodies were IGHV1-72/IGHD1-1/IGHJ2 for NP-hapten and IGHV9-3/IGHD3-1/IGHJ2 for CGG-carrier protein. The antigen-binding specificities of the identified antibodies were confirmed through ELISA after antibody-gene synthesis and expression of the corresponding NGS reads. Thus, we deciphered antigen-responding antibody repertoires by inclusively analyzing the antibody-repertoire space generated in individual bodies by using NGS, which avoided inadvertent omission of key antibody repertoires.

Kou, T., et al. (2017). "Clinical sequencing using a next-generation sequencing-based multiplex gene assay in patients with advanced solid tumors." Cancer Sci **108**(7): 1440-1446.

Advances in next-generation sequencing (NGS) technologies have enabled physicians to test for genomic alterations in multiple cancer-related genes at once in daily clinical practice. In April 2015, we introduced clinical sequencing using an NGS-based multiplex gene assay (OncoPrime) certified by the Clinical Laboratory Improvement Amendment. This assay covers the entire coding regions of 215 genes and the rearrangement of 17 frequently rearranged genes with clinical relevance in human cancers. The principal indications for the assay were cancers of unknown primary site, rare tumors, and any solid tumors that were refractory to standard chemotherapy. A total of 85 patients underwent testing with multiplex gene assay between April 2015 and July 2016. The most common solid tumor types tested were pancreatic (n = 19; 22.4%), followed by biliary tract (n = 14; 16.5%), and tumors of unknown primary site (n = 13; 15.3%). Samples from 80 patients (94.1%) were successfully sequenced. The median turnaround time was 40 days (range, 18-70 days). Potentially actionable mutations were identified in 69 of 80 patients (86.3%) and were most commonly found in TP53 (46.3%), KRAS (23.8%), APC (18.8%), STK11 (7.5%), and ATR (7.5%). Nine patients (13.0%) received a subsequent therapy based on the NGS assay results. Implementation of clinical sequencing using an NGS-based multiplex gene assay was feasible in the clinical setting and identified potentially actionable mutations in more than 80% of patients. Current challenges are to incorporate this genomic information into better therapeutic decision making.

Kringel, D., et al. (2017). "Next-generation sequencing of the human TRPV1 gene and the regulating co-players LTB4R and LTB4R2 based on a custom AmpliSeq panel." PLoS One **12**(6): e0180116.

BACKGROUND: Transient receptor potential cation channel subfamily V member 1 (TRPV1) are sensitive to heat, capsaicin, pungent chemicals and other noxious stimuli. They play important roles in the

pain pathway where in concert with proinflammatory factors such as leukotrienes they mediate sensitization and hyperalgesia. TRPV1 is the target of several novel analgesics drugs under development and therefore, TRPV1 genetic variants might represent promising candidates for pharmacogenetic modulators of drug effects. METHODS: A next-generation sequencing (NGS) panel was created for the human TRPV1 gene and in addition, for the leukotriene receptors BLT1 and BLT2 recently described to modulate TRPV1 mediated sensitisation processes rendering the coding genes LTB4R and LTB4R2 important co-players in pharmacogenetic approaches involving TRPV1. The NGS workflow was based on a custom AmpliSeq panel and designed for sequencing of human genes on an Ion PGM Sequencer. A cohort of 80 healthy subjects of Western European descent was screened to evaluate and validate the detection of exomic sequences of the coding genes with 25 base pair exon padding. RESULTS: The amplicons covered approximately 97% of the target sequence. A median of 2.81 x 106 reads per run was obtained. This identified approximately 140 chromosome loci where nucleotides deviated from the reference sequence GRCh37 hg19 comprising the three genes TRPV1, LTB4R and LTB4R2. Correspondence between NGS and Sanger derived nucleotide sequences was 100%. CONCLUSIONS: Results suggested that the NGS approach based on AmpliSeq libraries and Ion Personal Genome Machine (PGM) sequencing is a highly efficient mutation detection method. It is suitable for large-scale sequencing of TRPV1 and functionally related genes. The method adds a large amount of genetic information as a basis for complete analysis of TRPV1 ion channel genetics and its functional consequences.

Kuhn, M., et al. (2016). "Utility of a next-generation sequencing-based gene panel investigation in German patients with genetically unclassified limb-girdle muscular dystrophy." J Neurol **263**(4): 743-750.

Limb-girdle muscular dystrophies (LGMDs) are genetically heterogeneous and the diagnostic work-up including conventional genetic testing using Sanger sequencing remains complex and often unsatisfactory. We performed targeted sequencing of 23 LGMD-related genes and 15 genes in which alterations result in a similar phenotype in 58 patients with genetically unclassified LGMDs. A genetic diagnosis was possible in 19 of 58 patients (33 %). LGMD2A was the most common form, followed by LGMD2L and LGMD2I. In two patients, pathogenic mutations were identified in genes that are not classified as LGMD genes (glycogen branching enzyme and valosin-containing protein). Thus, a focused next-generation sequencing-based gene panel is a rather satisfactory tool for the diagnosis in unclassified LGMDs.

Kwong, A., et al. (2016). "Detection of Germline Mutation in Hereditary Breast and/or Ovarian Cancers by Next-Generation Sequencing on a Four-Gene Panel." J Mol Diagn **18**(4): 580-594.

Mutation in BRCA1/BRCA2 genes accounts for 20% of familial breast cancers, 5% to 10% of which may be due to other less penetrant genes which are still incompletely studied. Herein, a four-gene panel was used to examine the prevalence of BRCA1, BRCA2, TP53, and PTEN in hereditary breast and ovarian cancers in Southern Chinese population. In this cohort, 948 high-risk breast and/or ovarian patients were recruited for genetic screening by next-generation sequencing (NGS). The performance of our NGS pipeline was evaluated with 80 Sanger-validated known mutations and eight negative cases. With appropriate bioinformatics analysis pipeline, the detection sensitivity of NGS is comparable with Sanger sequencing. The prevalence of BRCA1/BRCA2 germline mutations was 9.4% in our Chinese cohort, of which 48.8% of the mutations arose from hotspot mutations. With the use of a tailor-made algorithm, HomopolymerQZ, more mutations were detected compared with single mutation detection algorithm. The frequencies of PTEN and TP53 were 0.21% and 0.53%, respectively, in the Southern Chinese patients with breast and/or ovarian cancers. High-throughput NGS approach allows the incorporation of control cohort that provides an ethnicity-specific data for polymorphic variants. Our data suggest that hotspot mutations screening such as SNaPshot could be an effective preliminary screening alternative adopted in a standard clinical laboratory without NGS setup.

Lanktree, M. B., et al. (2017). "Clinical evaluation of a hemochromatosis next-generation sequencing gene panel." Eur J Haematol **98**(3): 228-234.

BACKGROUND: Next-generation sequencing of an iron metabolism gene panel could identify pathogenic mutations, improving on standard hemochromatosis genetic testing and providing a molecular diagnosis in patients with suspected iron overload. METHODS: A next-generation sequencing panel of 15 genes with known roles in iron metabolism was constructed. A total of 190 patients were sequenced: 94 from a tertiary hemochromatosis clinic and 96 submitted for HFE testing with biochemical evidence of iron overload [elevated ferritin (>450 mug/L) or transferrin saturation (>55%)] obtained from a chart review. RESULTS: From the hemochromatosis clinic cohort, six patients were

diagnosed with non-HFE hemochromatosis due to homozygous hemojuvelin (HFE2) mutations. Ten additional heterozygous pathogenic mutations were observed. From the chart review cohort, a C-terminus ferritin light chain (FTL) frameshift mutation was observed consistent with neuroferritinopathy. Heterozygous deletion of HFE2 and four additional rare pathogenic or likely pathogenic heterozygous mutations were identified in seven patients. CONCLUSIONS: An iron metabolism gene panel provided a molecular diagnosis in six patients with non-HFE iron overload and is suitable for diagnostic purposes in exceptional cases in specialized clinics. Further research will be required to assess the modifier effect of rare heterozygous mutations in iron metabolism genes.

Lim, E. C., et al. (2015). "Next-generation sequencing using a pre-designed gene panel for the molecular diagnosis of congenital disorders in pediatric patients." Hum Genomics **9**: 33.

BACKGROUND: Next-generation sequencing (NGS) has revolutionized genetic research and offers enormous potential for clinical application. Sequencing the exome has the advantage of casting the net wide for all known coding regions while targeted gene panel sequencing provides enhanced sequencing depths and can be designed to avoid incidental findings in adult-onset conditions. A HaloPlex panel consisting of 180 genes within commonly altered chromosomal regions is available for use on both the Ion Personal Genome Machine (PGM) and MiSeq platforms to screen for causative mutations in these genes. METHODS: We used this Haloplex ICCG panel for targeted sequencing of 15 patients with clinical presentations indicative of an abnormality in one of the 180 genes. Sequencing runs were done using the Ion 318 Chips on the Ion Torrent PGM. Variants were filtered for known polymorphisms and analysis was done to identify possible disease-causing variants before validation by Sanger sequencing. When possible, segregation of variants with phenotype in family members was performed to ascertain the pathogenicity of the variant. RESULTS: More than 97% of the target bases were covered at >20x. There was an average of 9.6 novel variants per patient. Pathogenic mutations were identified in five genes for six patients, with two novel variants. There were another five likely pathogenic variants, some of which were unreported novel variants. CONCLUSIONS: In a cohort of 15 patients, we were able to identify a likely genetic etiology in six patients (40%). Another five patients had candidate variants for which further evaluation and segregation analysis are ongoing. Our results indicate that the HaloPlex ICCG panel is useful as a rapid, high-throughput and cost-effective screening tool for 170 of the 180 genes. There is low coverage for some regions in several genes which might have to be supplemented by Sanger sequencing. However, comparing the cost, ease of analysis, and shorter turnaround time, it is a good alternative to exome sequencing for patients whose features are suggestive of a genetic etiology involving one of the genes in the panel.

Lin, Y., et al. (2015). "Gene mutations in gastric cancer: a review of recent next-generation sequencing studies." Tumour Biol **36**(10): 7385-7394.

Gastric cancer (GC) is one of the most common malignancies worldwide. Although some driver genes have been identified in GC, the molecular compositions of GC have not been fully understood. The development of next-generation sequencing (NGS) provides a high-throughput and systematic method to identify all genetic alterations in the cancer genome, especially in the field of mutation detection. NGS studies in GC have discovered some novel driver mutations. In this review, we focused on novel gene mutations discovered by NGS studies, along with some well-known driver genes in GC. We organized mutated genes from the perspective of related biological pathways. Mutations in genes relating to genome integrity (TP53, BRCA2), chromatin remodeling (ARID1A), cell adhesion (CDH1, FAT4, CTNNA1), cytoskeleton and cell motility (RHOA), Wnt pathway (CTNNB1, APC, RNF43), and RTK pathway (RTKs, RAS family, MAPK pathway, PIK pathway) are discussed. Efforts to establish a molecular classification based on NGS data which is valuable for future targeted therapy for GC are introduced. Comprehensive dissection of the molecular profile of GC cannot only unveil the molecular basis for GC but also identify genes of clinical utility, especially potential and specific therapeutic targets for GC.

Lindquist, K. E., et al. (2017). "Clinical framework for next generation sequencing based analysis of treatment predictive mutations and multiplexed gene fusion detection in non-small cell lung cancer." Oncotarget **8**(21): 34796-34810.

Precision medicine requires accurate multi-gene clinical diagnostics. We describe the implementation of an Illumina TruSight Tumor (TST) clinical NGS diagnostic framework and parallel validation of a NanoString RNA-based ALK, RET, and ROS1 gene fusion assay for combined analysis of treatment predictive alterations in non-small cell lung cancer (NSCLC) in a regional healthcare region of Sweden (Scandinavia). The TST panel was clinically validated in 81 tumors (99% hotspot mutation concordance), after which 533 consecutive NSCLCs were collected

during one-year of routine clinical analysis in the healthcare region (~90% advanced stage patients). The NanoString assay was evaluated in 169 of 533 cases. In the 533-sample cohort 79% had 1-2 variants, 12% >2 variants and 9% no detected variants. Ten gene fusions (five ALK, three RET, two ROS1) were detected in 135 successfully analyzed cases (80% analysis success rate). No ALK or ROS1 FISH fusion positive case was missed by the NanoString assay. Stratification of the 533-sample cohort based on actionable alterations in 11 oncogenes revealed that 66% of adenocarcinomas, 13% of squamous carcinoma (SqCC) and 56% of NSCLC not otherwise specified harbored >/=1 alteration. In adenocarcinoma, 10.6% of patients (50.3% if including KRAS) could potentially be eligible for emerging therapeutics, in addition to the 15.3% of patients eligible for standard EGFR or ALK inhibitors. For squamous carcinoma corresponding proportions were 4.4% (11.1% with KRAS) vs 2.2%. In conclusion, multiplexed NGS and gene fusion analyses are feasible in NSCLC for clinical diagnostics, identifying notable proportions of patients potentially eligible for emerging molecular therapeutics.

Liu, X., et al. (2016). "Next-Generation Sequencing of Pulmonary Sarcomatoid Carcinoma Reveals High Frequency of Actionable MET Gene Mutations." J Clin Oncol **34**(8): 794-802.

PURPOSE: To further understand the molecular pathogenesis of pulmonary sarcomatoid carcinoma (PSC) and develop new therapeutic strategies in this treatment-refractory disease. MATERIALS AND METHODS: Whole-exome sequencing in a discovery set (n = 10) as well as targeted MET mutation screening in an independent validation set (n = 26) of PSC were performed. Reverse transcriptase polymerase chain reaction and Western blotting were performed to validate MET exon 14 skipping. Functional studies for validation of the oncogenic roles of MET exon 14 skipping were conducted in lung adenosquamous cell line H596 (MET exon 14 skipped and PIK3CA mutated) and gastric adenocarcinoma cell line Hs746T (MET exon 14 skipped). Response to MET inhibitor therapy with crizotinib in a patient with advanced PSC and MET exon 14 skipping was evaluated to assess clinical translatability. RESULTS: In addition to confirming mutations in known cancer-associated genes (TP53, KRAS, PIK3CA, MET, NOTCH, STK11, and RB1), several novel mutations in additional genes, including RASA1, CDH4, CDH7, LAMB4, SCAF1, and LMTK2, were identified and validated. MET mutations leading to exon 14 skipping were identified in eight (22%) of 36 patient cases; one of these tumors also harbored a concurrent PIK3CA mutation. Short interfering RNA silencing of MET and MET inhibition with crizotinib showed marked effects on cell viability and decrease in downstream AKT and mitogen-activated protein kinase activation in Hs746T and H596 cells. Concurrent PIK3CA mutation required addition of a second agent for successful pathway suppression and cell viability effect. Dramatic response to crizotinib was noted in a patient with advanced chemotherapy-refractory PSC carrying a MET exon 14 skipping mutation. CONCLUSION: Mutational events of MET leading to exon 14 skipping are frequent and potentially targetable events in PSC.

Liu, Y., et al. (2017). "Gene mutation spectrum and genotype-phenotype correlation in a cohort of Chinese osteogenesis imperfecta patients revealed by targeted next generation sequencing." Osteoporos Int **28**(10): 2985-2995.

The achievement of more accurate diagnosis would greatly benefit the management of patients with osteogenesis imperfecta (OI). In this study, we present the largest OI sample in China as screened by next generation sequencing. In particular, we successfully identified 81 variants, which included 45 novel variants. We further did a genotype-phenotype analysis, which helps make a better understanding of OI. INTRODUCTION: This study aims to reveal the gene mutation spectrum and the genotype-phenotype relationship among Chinese OI patients by next generation sequencing (NGS). METHODS: We developed a NGS-based panel for targeted sequencing of all exons of 14 genes related to OI, and performed diagnostic gene sequencing for a cohort of 103 Chinese OI patients from 101 unrelated families. Mutations identified by NGS were further confirmed by Sanger sequencing and co-segregation analysis. RESULTS: Of the 103 patients from 101 unrelated OI families, we identified 79 mutations, including 43 novel mutations (11 frameshift, 17 missense, 5 nonsense, 9 splice site, and 1 chromosome translocation) in 90 patients (87.4%). Mutations in genes encoding type I collagen, COL1A1 (n = 37), and COL1A2 (n = 29) accounts for 73.3% of all molecularly diagnosed patients, followed by IFITM5 (n = 9, 10%), SERPINF1 (n = 4, 4.4%), WNT1 (n = 4, 4.4%), FKBP10 (n = 3, 3.3%), TMEM38B (n = 3, 3.3%), and PLOD2 (n = 1, 1.1%). This corresponds to 75 autosomal dominant inherited (AD) OI patients and 15 autosomal recessive (AR) inherited patients. Compared with AD inherited OI patients, AR inherited patients had lower bone mineral density (BMD) at spine (P = 0.05) and less frequent blue sclera (P = 0.001). Patients with type I collagen qualitative defects had lower femoral neck BMD Z-score (P = 0.034) and were shorter compared with patients with type I collagen quantitative defects (P = 0.022). CONCLUSION: We revealed the gene mutation spectrum in Chinese OI patients, and novel mutations

identified here expanded the mutation catalog and genotype and phenotype relationships among OI patients.

Livhits, M. J., et al. (2018). "Gene Expression Classifier versus Targeted Next-Generation Sequencing in the Management of Indeterminate Thyroid Nodules." J Clin Endocrinol Metab.

Context: Molecular testing has reduced the need for diagnostic hemithyroidectomy for indeterminate thyroid nodules. No studies have directly compared molecular testing techniques. Objective: Compare diagnostic performance of Afirma Gene Expression Classifier (GEC) and ThyroSeq v2 next-generation sequencing assay. Design: Parallel randomized trial, monthly block randomization of patients with Bethesda III / IV cytology to GEC or ThyroSeq v2. Setting: University of California, Los Angeles. Participants: Patients who underwent thyroid biopsy (April 2016 - June 2017). Interventions: Testing with GEC or ThyroSeq v2. Main Outcome Measures: Molecular test performance. Results: Of 1,372 thyroid nodules, 176 (13%) had indeterminate cytology and 149 of 157 eligible indeterminate nodules (95%) were included in the study. Of nodules tested with GEC, 49% were suspicious, 43% were benign, and 9% were insufficient. Of nodules tested with ThyroSeq v2, 19% were mutation positive, 77% were mutation negative, and 4% were insufficient. The specificities of GEC and ThyroSeq v2 were 66% and 91%, respectively (P=0.002); the positive predictive value of GEC and ThyroSeq v2 were 39% and 57%, respectively. Diagnostic hemithyroidectomy was avoided in 28 patients tested with GEC (39%) and 49 patients tested with ThyroSeq v2 (62%). Surveillance ultrasounds were available for 46 nodules (45 remained stable). Conclusions: ThyroSeq v2 had higher specificity compared to Afirma GEC and allowed more patients to avoid surgery. Long-term surveillance is necessary to assess the false-negative rate of these particular molecular tests. Further studies are required for comparison with other available molecular diagnostics and for newer tests as they are developed.

Lucas, S. J., et al. (2014). "Next-generation sequencing of flow-sorted wheat chromosome 5D reveals lineage-specific translocations and widespread gene duplications." BMC Genomics **15**: 1080.

BACKGROUND: The ~17 Gb hexaploid bread wheat genome is a high priority and a major technical challenge for genomic studies. In particular, the D sub-genome is relatively lacking in genetic diversity, making it both difficult to map genetically, and a target for introgression of agriculturally useful traits. Elucidating its sequence and structure will therefore facilitate wheat breeding and crop improvement. RESULTS: We generated shotgun sequences from each arm of flow-sorted Triticum aestivum chromosome 5D using 454 FLX Titanium technology, giving 1.34x and 1.61x coverage of the short (5DS) and long (5DL) arms of the chromosome respectively. By a combination of sequence similarity and assembly-based methods, ~74% of the sequence reads were classified as repetitive elements, and coding sequence models of 1314 (5DS) and 2975 (5DL) genes were generated. The order of conserved genes in syntenic regions of previously sequenced grass genomes were integrated with physical and genetic map positions of 518 wheat markers to establish a virtual gene order for chromosome 5D. CONCLUSIONS: The virtual gene order revealed a large-scale chromosomal rearrangement in the peri-centromeric region of 5DL, and a concentration of non-syntenic genes in the telomeric region of 5DS. Although our data support the large-scale conservation of Triticeae chromosome structure, they also suggest that some regions are evolving rapidly through frequent gene duplications and translocations. SEQUENCE ACCESSIONS: EBI European Nucleotide Archive, Study no. ERP002330.

Luthra, R., et al. (2017). "A Targeted High-Throughput Next-Generation Sequencing Panel for Clinical Screening of Mutations, Gene Amplifications, and Fusions in Solid Tumors." J Mol Diagn **19**(2): 255-264.

Clinical next-generation sequencing (NGS) assay choice requires careful consideration of panel size, inclusion of appropriate markers, ability to detect multiple genomic aberration types, compatibility with low quality and quantity of nucleic acids, and work flow feasibility. Herein, in a high-volume clinical molecular diagnostic laboratory, we have validated a targeted high-multiplex PCR-based NGS panel (OncoMine Comprehensive Assay) coupled with high-throughput sequencing using Ion Proton sequencer for routine screening of solid tumors. The panel screens 143 genes using low amounts of formalin-fixed, paraffin-embedded DNA (20 ng) and RNA (10 ng). A large cohort of 121 tumor samples representing 13 tumor types and 6 cancer cell lines was used to assess the capability of the panel to detect 148 single-nucleotide variants, 49 insertions or deletions, 40 copy number aberrations, and a subset of gene fusions. High levels of analytic sensitivity and reproducibility and robust detection sensitivity were observed. Furthermore, we demonstrated the critical utility of sequencing paired normal tissues to improve the accuracy of detecting somatic mutations in a background of germline variants. We also validated use of the Ion Chef automated bead templating and chip loading system, which represents a major work

flow improvement. In summary, we present data establishing the OncoMine Comprehensive Assay-Ion Proton platform to be well suited for implementation as a routine clinical NGS test for solid tumors.

Lyu, X., et al. (2016). "A novel BCR-ABL1 fusion gene identified by next-generation sequencing in chronic myeloid leukemia." Mol Cytogenet **9**: 47.

BACKGROUND: BCR-ABL1 fusion proteins contain constitutively active tyrosine kinases that are potential candidates for targeted therapy with tyrosine kinase inhibitors such as imatinib in chronic myeloid leukemia (CML). However, uncharacterized BCR-ABL1 fusion genes can be missed by quantitative RT-PCR (qRT-PCR)-based routine screening methods, causing adverse effect on drug selection and treatment outcome. CASE PRESENTATION: In this study, we demonstrated that the next-generation sequencing (NGS) can be employed to overcome this obstacle. Through NGS, we identified a novel BCR-ABL1 fusion gene with breakpoints in the BCR intron 14 and the ABL1 intron 2, respectively, in a rare case of CML. Its mRNA with an e14a3 junction was then detected using customized RT-PCR followed by Sanger sequencing. Subsequently, the patient received targeted medicine imatinib initially at 400 mg/day, and later 300 mg/day due to intolerance reactions. With this personalized treatment, the patient's condition was significantly improved. Interestingly, this novel fusion gene encodes a fusion protein containing a compromised SH3 domain, which is usually intact in the majority of CML cases, suggesting that dysfunctional SH3 domain may be associated with altered drug response and unique clinicopathological manifestations observed in this patient. CONCLUSION: We identified a novel BCR-ABL1 fusion gene using NGS in a rare case of CML while routine laboratory procedures were challenged, demonstrating the power of NGS as a diagnostic tool for detecting novel genetic mutations. Moreover, our new finding regarding the novel fusion variant will provide useful insights to improve the spectrum of the genomic abnormalities recognizable by routine molecular screening.

Ma, M., et al. (2016). "Next-generation sequencing identifies novel mutations in the FBN1 gene for two Chinese families with Marfan syndrome." Mol Med Rep **14**(1): 151-158.

Marfan syndrome (MFS) is an autosomal dominant heterogeneous disorder of connective tissue characterized by the early development of thoracic aneurysms/dissections, together with defects of the ocular and skeletal systems. Loss-of-function mutations in fibrillin-1 (FBN1) encoded by the gene, FBN1 (MFS1), and in the transforming growth factor beta receptor 2 (TGFBR2) gene, TGFBR2 (MFS2), are major causes of this disorder. In the present study, a rapid and costeffective method for genetically diagnosing MFS was described and used to identify diseasecausing mutations in two unrelated pedigrees with MFS in mainland China. Using targeted semiconductor sequencing, two pathogenic mutations in four MFS patients of the two pedigrees were identified, including a novel frameshift insertion, p.G2120fsX2160, and a reported nonsense mutation, p.Arg529X (rs137854476), in the FBN1 gene. In addition, a rare, probably benign Chinesespecific polymorphism in the FBN1 gene was also revealed.

Maksemous, N., et al. (2016). "Next-generation sequencing identifies novel CACNA1A gene mutations in episodic ataxia type 2." Mol Genet Genomic Med **4**(2): 211-222.

Episodic Ataxia type 2 (EA2) is a rare autosomal dominantly inherited neurological disorder characterized by recurrent disabling imbalance, vertigo, and episodes of ataxia lasting minutes to hours. EA2 is caused most often by loss of function mutations of the calcium channel gene CACNA1A. In addition to EA2, mutations in CACNA1A are responsible for two other allelic disorders: familial hemiplegic migraine type 1 (FHM1) and spinocerebellar ataxia type 6 (SCA6). Herein, we have utilized next-generation sequencing (NGS) to screen the coding sequence, exon-intron boundaries, and Untranslated Regions (UTRs) of five genes where mutation is known to produce symptoms related to EA2, including CACNA1A. We performed this screening in a group of 31 unrelated patients with EA2 symptoms. Both novel and known mutations were detected through NGS technology, and confirmed through Sanger sequencing. Genetic testing showed in total 15 mutation bearing patients (48%), of which nine were novel mutations (6 missense and 3 small frameshift deletion mutations) and six known mutations (4 missense and 2 nonsense).These results demonstrate the efficiency of our NGS-panel for detecting known and novel mutations for EA2 in the CACNA1A gene, also identifying a novel missense mutation in ATP1A2 which is not a normal target for EA2 screening.

Maksemous, N., et al. (2016). "Targeted next generation sequencing identifies novel NOTCH3 gene mutations in CADASIL diagnostics patients." Hum Genomics **10**(1): 38.

BACKGROUND: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a monogenic, hereditary, small vessel disease of the brain causing stroke and vascular dementia in adults. CADASIL has previously been shown to be caused by varying

mutations in the NOTCH3 gene. The disorder is often misdiagnosed due to its significant clinical heterogeneic manifestation with familial hemiplegic migraine and several ataxia disorders as well as the location of the currently identified causative mutations. The aim of this study was to develop a new, comprehensive and efficient single assay strategy for complete molecular diagnosis of NOTCH3 mutations through the use of a custom next-generation sequencing (NGS) panel for improved routine clinical molecular diagnostic testing. RESULTS: Our custom NGS panel identified nine genetic variants in NOTCH3 (p.D139V, p.C183R, p.R332C, p.Y465C, p.C597W, p.R607H, p.E813E, p.C977G and p.Y1106C). Six mutations were stereotypical CADASIL mutations leading to an odd number of cysteine residues in one of the 34 NOTCH3 gene epidermal growth factor (EGF)-like repeats, including three new typical cysteine mutations identified in exon 11 (p.C597W; c.1791C>G); exon 18 (p.C977G; c.2929T>G) and exon 20 (p.Y1106C; c.3317A>G). Interestingly, a novel missense mutation in the CACNA1A gene was also identified in one CADASIL patient. All variants identified (novel and known) were further investigated using in silico bioinformatic analyses and confirmed through Sanger sequencing. CONCLUSIONS: NGS provides an improved and effective methodology for the diagnosis of CADASIL. The NGS approach reduced time and cost for comprehensive genetic diagnosis, placing genetic diagnostic testing within reach of more patients.

Malapelle, U., et al. (2017). "Development of a gene panel for next-generation sequencing of clinically relevant mutations in cell-free DNA from cancer patients." Br J Cancer **116**(6): 802-810.

BACKGROUND: When tumour tissue is unavailable, cell-free DNA (cfDNA)can serve as a surrogate for genetic analyses. Because mutated alleles in cfDNA are usually below 1%, next-generation sequencing (NGS)must be narrowed to target only clinically relevant genes. In this proof-of-concept study, we developed a panel to use in ultra-deep sequencing to identify such mutations in cfDNA. METHODS: Our panel ('SiRe') covers 568 mutations in six genes (EGFR, KRAS, NRAS, BRAF, cKIT and PDGFRalpha)involved in non-small-cell lung cancer (NSCLC), gastrointestinal stromal tumour, colorectal carcinoma and melanoma. We evaluated the panel performance in three steps. First, we analysed its analytical sensitivity on cell line DNA and by using an artificial reference standard with multiple mutations in different genes. Second, we analysed cfDNA from cancer patients at presentation (n=42), treatment response (n=12) and tumour progression (n=11); all patients had paired tumour tissue and cfDNA previously genotyped with a Taqman-derived assay (TDA). Third, we tested blood samples prospectively collected from NSCLC patients (n=79) to assess the performance of SiRe in clinical practice. RESULTS: SiRe had a high analytical performance and a 0.01% lower limit of detection. In the retrospective series, SiRe detected 40 EGFR, 11 KRAS, 1 NRAS and 5 BRAF mutations (96.8% concordance with TDA). In the baseline samples, SiRe had 100% specificity and 79% sensitivity relative to tumour tissue. Finally, in the prospective series, SiRe detected 8.7% (4/46) of EGFR mutations at baseline and 42.9% (9/21) of EGFR p.T790M in patients at tumour progression. CONCLUSIONS: SiRe is a feasible NGS panel for cfDNA analysis in clinical practice.

Maltese, P. E., et al. (2018). "A Next Generation Sequencing custom gene panel as first line diagnostic tool for atypical cases of syndromic obesity: Application in a case of Alstrom syndrome." Eur J Med Genet **61**(2): 79-83.

Obesity phenotype can be manifested as an isolated trait or accompanied by multisystem disorders as part of a syndromic picture. In both situations, same molecular pathways may be involved to different degrees. This evidence is stronger in syndromic obesity, in which phenotypes of different syndromes may overlap. In these cases, genetic testing can unequivocally provide a final diagnosis. Here we describe a patient who met the diagnostic criteria for Alstrom syndrome only during adolescence. Genetic testing was requested at 25 years of age for a final confirmation of the diagnosis. The genetic diagnosis of Alstrom syndrome was obtained through a Next Generation Sequencing genetic test approach using a custom-designed gene panel of 47 genes associated with syndromic and non-syndromic obesity. Genetic analysis revealed a novel homozygous frameshift variant p. (Arg1550Lysfs*10) on exon 8 of the ALMS1 gene. This case shows the need for a revision of the diagnostic criteria guidelines, as a consequence of the recent advent of massive parallel sequencing technology. Indications for genetic testing reported in these currently accepted diagnostic criteria for Alstrom syndrome, were drafted when sequencing was expensive and time consuming. Nowadays, Next Generation Sequencing testing could be considered as first line diagnostic tool not only for Alstrom syndrome but, more generally, for all those atypical or not clearly distinguishable cases of syndromic obesity, thus avoiding delayed diagnosis and treatments. Early diagnosis permits a better follow-up and pre-symptomatic interventions.

Marino, P., et al. (2018). "Cost of cancer diagnosis using next-generation sequencing targeted

gene panels in routine practice: a nationwide French study." Eur J Hum Genet **26**(3): 314-323.

It is currently unclear if next-generation sequencing (NGS) technologies can be implemented in the diagnosis setting at an affordable cost. The aim of this study was to measure the total cost of performing NGS in clinical practice in France, in both germline and somatic cancer genetics. The study was performed on 15 French representative cancer molecular genetics laboratories performing NGS panels' tests. The production cost was estimated using a micro-costing method with resources consumed collected in situ in each laboratory from a healthcare provider perspective. In addition, we used a top-down methodology for specific post-sequencing steps including bioinformatics, technical validation, and biological validation. Additional non-specific costs were also included. Costs were detailed per step of the process (from the pre-analytical phase to delivery of results), and per cost driver (consumables, staff, equipment, maintenance, overheads). Sensitivity analyses were performed. The mean total cost of NGS for targeted gene panels was estimated to 607euro (+/-207) in somatic genetics and 550euro (+/-140) in germline oncogenetic analysis. Consumables were the highest cost driver of the sequencing process. The sensitivity analysis showed that a 25% reduction of consumables resulted in a 15% decrease in total NGS cost in somatic genetics, and 13% in germline analysis. Additional costs accounted for 30-32% of the total NGS costs. Beyond cost assessment considerations, the diffusion of NGS technologies will raise questions about their efficiency when compared to more targeted approaches, and their added value in a context of routine diagnosis.

Messerer, M., et al. (2017). "Investigation of horizontal gene transfer of pathogenicity islands in Escherichia coli using next-generation sequencing." PLoS One **12**(7): e0179880.

Horizontal gene transfer (HGT) contributes to the evolution of bacteria. All extraintestinal pathogenic Escherichia coli (ExPEC) harbour pathogenicity islands (PAIs), however relatively little is known about the acquisition of these PAIs. Due to these islands, ExPEC have properties to colonize and invade its hosts efficiently. Even though these PAIs are known to be acquired by HGT, only very few PAIs do carry mobilization and transfer genes required for the transmission by HGT. In this study, we apply for the first time next-generation sequencing (NGS) and in silico analyses in combination with in vitro experiments to decipher the mechanisms of PAI acquisition in ExPEC. For this, we investigated three neighbouring E. coli PAIs, namely the high-pathogenicity island (HPI), the pks and the serU island. As these PAIs contain no mobilization and transfer genes, they are immobile and dependent on transfer vehicles. By whole genome sequencing of the entire E. coli reference (ECOR) collection and by applying a phylogenetic approach we could unambiguously demonstrate that these PAIs are transmitted not only vertically, but also horizontally. Furthermore, we could prove in silico that distinct groups of PAIs were transferred "en bloc" in conjunction with the neighbouring chromosomal backbone. We traced this PAI transfer in vitro using an F' plasmid. Different lengths of transferred DNA were exactly detectable in the sequenced transconjugants indicating NGS as a powerful tool for determination of PAI transfer.

Mukherjee, S., et al. (2016). "Chromosomal microarray provides enhanced targetable gene aberration detection when paired with next generation sequencing panel in profiling lung and colorectal tumors." Cancer Genet **209**(4): 119-129.

The development of targeted therapies based on specific genomic alterations has altered the treatment and management of lung and colorectal cancers. Chromosomal microarray (CMA) has allowed identification of copy number variations (CNVs) in lung and colorectal cancers in great detail, and next-generation sequencing (NGS) is used extensively to analyze the genome of cancers for molecular subtyping and use of molecularly guided therapies. The main objective of this study was to evaluate the utility of combining CMA and NGS for a comprehensive genomic assessment of lung and colorectal adenocarcinomas, especially for detecting drug targets. We compared the results from NGS and CMA data from 60 lung and 51 colorectal tumors. From CMA analysis, 33% were amplified, 89% showed gains, 75% showed losses and 41% demonstrated loss of heterozygosity; pathogenic variants were identified in 81% of colon and 67% lung specimens through NGS. KRAS mutations commonly occurred with loss in TP53 and there was significant loss of BRCA1 and NF1 among male patients with lung cancer. For clinically actionable targets, 23% had targetable CNVs when no pathogenic variants were detected by NGS. The data thus indicate that combining the two approaches provides significant benefit in a routine clinical setting not available by NGS alone.

Murphy, D. A., et al. (2017). "Detecting Gene Rearrangements in Patient Populations Through a 2-Step Diagnostic Test Comprised of Rapid IHC Enrichment Followed by Sensitive Next-Generation Sequencing." Appl Immunohistochem Mol Morphol **25**(7): 513-523.

Targeted therapy combined with companion diagnostics has led to the advancement of next-generation sequencing (NGS) for detection of molecular alterations. However, using a diagnostic test to identify patient populations with low prevalence molecular alterations, such as gene rearrangements, poses efficiency, and cost challenges. To address this, we have developed a 2-step diagnostic test to identify NTRK1, NTRK2, NTRK3, ROS1, and ALK rearrangements in formalin-fixed paraffin-embedded clinical specimens. This test is comprised of immunohistochemistry screening using a pan-receptor tyrosine kinase cocktail of antibodies to identify samples expressing TrkA (encoded by NTRK1), TrkB (encoded by NTRK2), TrkC (encoded by NTRK3), ROS1, and ALK followed by an RNA-based anchored multiplex polymerase chain reaction NGS assay. We demonstrate that the NGS assay is accurate and reproducible in identification of gene rearrangements. Furthermore, implementation of an RNA quality control metric to assess the presence of amplifiable nucleic acid input material enables a measure of confidence when an NGS result is negative for gene rearrangements. Finally, we demonstrate that performing a pan-receptor tyrosine kinase immunohistochemistry staining enriches detection of the patient population for gene rearrangements from 4% to 9% and has a 100% negative predictive value. Together, this 2-step assay is an efficient method for detection of gene rearrangements in both clinical testing and studies of archival formalin-fixed paraffin-embedded specimens.

Mutze, U., et al. (2017). "Multigene panel next generation sequencing in a patient with cherry red macular spot: Identification of two novel mutations in NEU1 gene causing sialidosis type I associated with mild to unspecific biochemical and enzymatic findings." Mol Genet Metab Rep **10**: 1-4.

BACKGROUND: Lysosomal storage diseases (LSD) often manifest with cherry red macular spots. Diagnosis is based on clinical features and specific biochemical and enzymatic patterns. In uncertain cases, genetic testing with next generation sequencing can establish a diagnosis, especially in milder or atypical phenotypes. We report on the diagnostic work-up in a boy with sialidosis type I, presenting initially with marked cherry red macular spots but non-specific urinary oligosaccharide patterns and unusually mild excretion of bound sialic acid. METHODS: Biochemical, enzymatic and genetic tests were performed in the patient. The clinical and electrophysiological data was reviewed and a genotype-phenotype analysis was performed. In addition a systematic literature review was carried out. CASE REPORT AND RESULTS: Cherry red macular spots were first noted at 6 years of age after routine screening myopia. Physical examination, psychometric testing, laboratory investigations as well as cerebral MRI were unremarkable at 9 years of age. So far no clinical myoclonic seizures occurred, but EEG displays generalized epileptic discharges and visual evoked potentials are prolonged bilaterally. Urine thin layer chromatography showed an oligosaccharide pattern compatible with different LSD including sialidosis, galactosialidosis, GM1 gangliosidosis or mucopolysaccharidosis type IV B. Urinary bound sialic acid excretion was mildly elevated in spontaneous and 24 h urine samples. In cultured fibroblasts, alpha-sialidase activity was markedly decreased to < 1%; however, bound and free sialic acid were within normal range. Diagnosis was eventually established by multigene panel next generation sequencing of genes associated to LSD, identifying two novel, compound heterozygous variants in NEU1 gene (c.699C > A, p.S233R in exon 4 and c.803A > G; p.Y268C in Exon 5 in NEU1 transcript NM_000434.3), leading to amino acid changes predicted to impair protein function. DISCUSSION: Sialidosis should be suspected in patients with cherry red macular spots, even with non-significant urinary sialic acid excretion. Multigene panel next generation sequencing can establish a definite diagnosis, allowing for counseling of the patient and family.

Nakano, E., et al. (2015). "Targeted next-generation sequencing effectively analyzed the cystic fibrosis transmembrane conductance regulator gene in pancreatitis." Dig Dis Sci **60**(5): 1297-1307.

BACKGROUND: The cystic fibrosis transmembrane conductance regulator (CFTR) gene, responsible for the development of cystic fibrosis, is known as a pancreatitis susceptibility gene. Direct DNA sequencing of PCR-amplified CFTR gene segments is a first-line method to detect unknown mutations, but it is a tedious and labor-intensive endeavor given the large size of the gene (27 exons, 1,480 amino acids). Next-generation sequencing (NGS) is becoming standardized, reducing the cost of DNA sequencing, and enabling the generation of millions of reads per run. We here report a comprehensive analysis of CFTR variants in Japanese patients with chronic pancreatitis using NGS coupling with target capture. METHODS: Exon sequences of the CFTR gene from 193 patients with chronic pancreatitis (121 idiopathic, 46 alcoholic, 17 hereditary, and nine familial) were captured by HaloPlex target enrichment technology, followed by NGS. RESULTS: The sequencing data covered 91.6 % of the coding regions of the CFTR gene by >/= 20 reads with a mean read depth of 449. We could identify 12 non-synonymous

variants including three novel ones [c.A1231G (p.K411E), c.1753G>T (p.E585X) and c.2869delC (p.L957fs)] and seven synonymous variants including three novel ones in the exonic regions. The frequencies of the c.4056G>C (p.Q1352H) and the c.3468G>T (p.L1156F) variants were higher in patients with chronic pancreatitis than those in controls. CONCLUSIONS: Target sequence capture combined with NGS is an effective method for the analysis of pancreatitis susceptibility genes.

Nikiforov, Y. E., et al. (2015). "Impact of the Multi-Gene ThyroSeq Next-Generation Sequencing Assay on Cancer Diagnosis in Thyroid Nodules with Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance Cytology." Thyroid **25**(11): 1217-1223.

BACKGROUND: Fine-needle aspiration (FNA) cytology is a common approach to evaluate thyroid nodules. It offers definitive diagnosis of a benign or malignant nodule in the majority of cases. However, 10-25% of nodules yield one of three indeterminate cytologic diagnoses, leading to suboptimal management of these patients. Atypia of undetermined significance/follicular lesion of undermined significance (AUS/FLUS) is a common indeterminate diagnosis, with the cancer risk ranging from 6% to 48%. This study assessed whether a multi-gene next-generation sequencing (NGS) assay can offer significant improvement in diagnosis in AUS/FLUS nodules. METHODS: From May 2014 to March 2015, 465 consecutive FNA samples with the cytologic diagnosis of AUS/FLUS underwent prospective molecular testing using the ThyroSeq v2.1 panel. The panel included 14 genes analyzed for point mutations and 42 types of gene fusions occurring in thyroid cancer. In addition, eight genes were assessed for expression in order to evaluate the cell composition of FNA samples. Ninety-eight (21%) of these nodules had definitive surgical (n = 96) or nonsurgical (n = 2) follow-up and were used to determine the assay performance. RESULTS: Among 465 AUS/FLUS nodules, three were found to be composed of parathyroid cells and 462 of thyroid follicular cells. Of the latter, 31 (6.7%) were positive for mutations. The most frequently mutated genes were NRAS and HRAS, and overall point mutations in seven different genes and five types of gene fusions were identified in these nodules. Among 98 nodules with known outcome, histologic analysis revealed 22 (22.5%) cancers. ThyroSeq v2.1 was able to classify 20/22 cancers correctly, showing a sensitivity of 90.9% [confidence interval (CI) 78.8-100], specificity of 92.1% [CI 86.0-98.2], positive predictive value of 76.9% [CI 60.7-93.1], and negative predictive value of 97.2% [CI 78.8-100], with an overall accuracy of 91.8% [CI 86.4-97.3]. CONCLUSIONS: The results of the study demonstrate that the ThyroSeq v2.1 multi-gene NGS panel of molecular markers provides both high sensitivity and high specificity for cancer detection in thyroid nodules with AUS/FLUS cytology, which should allow improved management for these patients.

Nishio, S. Y., et al. (2017). "Laser-capture micro dissection combined with next-generation sequencing analysis of cell type-specific deafness gene expression in the mouse cochlea." Hear Res **348**: 87-97.

Cochlear implantation (CI), which directly stimulates the cochlear nerves, is the most effective and widely used medical intervention for patients with severe to profound sensorineural hearing loss. The etiology of the hearing loss is speculated to have a major influence of CI outcomes, particularly in cases resulting from mutations in genes preferentially expressed in the spiral ganglion region. To elucidate precise gene expression levels in each part of the cochlea, we performed laser-capture micro dissection in combination with next-generation sequencing analysis and determined the expression levels of all known deafness-associated genes in the organ of Corti, spiral ganglion, lateral wall, and spiral limbs. The results were generally consistent with previous reports based on immunocytochemistry or in situ hybridization. As a notable result, the genes associated with many kinds of syndromic hearing loss (such as Clpp, Hars2, Hsd17b4, Lars2 for Perrault syndrome, Polr1c and Polr1d for Treacher Collins syndrome, Ndp for Norrie Disease, Kal for Kallmann syndrome, Edn3 and Snai2 for Waardenburg Syndrome, Col4a3 for Alport syndrome, Sema3e for CHARGE syndrome, Col9a1 for Sticker syndrome, Cdh23, Cib2, Clrn1, Pcdh15, Ush1c, Ush2a, Whrn for Usher syndrome and Wfs1 for Wolfram syndrome) showed higher levels of expression in the spiral ganglion than in other parts of the cochlea. This dataset will provide a base for more detailed analysis in order to clarify gene functions in the cochlea as well as predict CI outcomes based on gene expression data.

Okazaki, T., et al. (2016). "Clinical Diagnosis of Mendelian Disorders Using a Comprehensive Gene-Targeted Panel Test for Next-Generation Sequencing." Yonago Acta Med **59**(2): 118-125.

BACKGROUND: Genetic diagnoses provide beneficial information to patients and families. However, traditional genetic diagnoses are often difficult even for experienced clinicians and require recognition of characteristic patterns of signs or symptoms to guide targeted genetic testing for the confirmation of diagnoses. Next-generation sequencing (NGS) is a powerful genetic diagnostic tool. However, whole-genome and whole-exome

sequencing (WES) are expensive, and the interpretation of results is difficult. Hence, target gene capture sequencing of gene panels has recently been applied to genetic diagnoses. Herein, we demonstrate that targeted sequencing approaches using gene panel testing are highly efficient for the diagnosis of Mendelian disorders. METHODS: NGS using TruSight one gene panel was performed in 17 families and 20 patients, and we developed a bioinformatic pipeline at our institution for detecting mutations. RESULTS: We detected causative mutations in 6 of 17 (35%) families. In particular, 11 (65%) families had syndromic diagnosis and 6 (35%) had no syndromic diagnosis before NGS testing. The number of positive diagnoses was 5 of 11 (45%) in the syndromic group and were 1 of 6 (17%) among patients of the no syndromic diagnosis group. CONCLUSION: Diagnostic yields in the present study were higher than in previous reports of genetic and chromosomal tests and WES. The present comprehensive gene-targeted panel test is a powerful diagnostic tool for Mendelian disorders.

Omoyinmi, E., et al. (2017). "Clinical impact of a targeted next-generation sequencing gene panel for autoinflammation and vasculitis." PLoS One **12**(7): e0181874.

BACKGROUND: Monogenic autoinflammatory diseases (AID) are a rapidly expanding group of genetically diverse but phenotypically overlapping systemic inflammatory disorders associated with dysregulated innate immunity. They cause significant morbidity, mortality and economic burden. Here, we aimed to develop and evaluate the clinical impact of a NGS targeted gene panel, the "Vasculitis and Inflammation Panel" (VIP) for AID and vasculitis. METHODS: The Agilent SureDesign tool was used to design 2 versions of VIP; VIP1 targeting 113 genes, and a later version, VIP2, targeting 166 genes. Captured and indexed libraries (QXT Target Enrichment System) prepared for 72 patients were sequenced as a multiplex of 16 samples on an Illumina MiSeq sequencer in 150bp paired-end mode. The cohort comprised 22 positive control DNA samples from patients with previously validated mutations in a variety of the genes; and 50 prospective samples from patients with suspected AID in whom previous Sanger based genetic screening had been non-diagnostic. RESULTS: VIP was sensitive and specific at detecting all the different types of known mutations in 22 positive controls, including gene deletion, small INDELS, and somatic mosaicism with allele fraction as low as 3%. Six/50 patients (12%) with unclassified AID had at least one class 5 (clearly pathogenic) variant; and 11/50 (22%) had at least one likely pathogenic variant (class 4). Overall, testing with VIP resulted in a firm or strongly suspected molecular diagnosis in 16/50 patients (32%). CONCLUSIONS: The high diagnostic yield and accuracy of this comprehensive targeted gene panel validate the use of broad NGS-based testing for patients with suspected AID.

Page, K., et al. (2017). "Next Generation Sequencing of Circulating Cell-Free DNA for Evaluating Mutations and Gene Amplification in Metastatic Breast Cancer." Clin Chem **63**(2): 532-541.

BACKGROUND: Breast cancer tissues are heterogeneous and show diverse somatic mutations and somatic copy number alterations (CNAs). We used a novel targeted next generation sequencing (NGS) panel to examine cell-free DNA (cfDNA) to detect somatic mutations and gene amplification in women with metastatic breast cancer (MBC). METHODS: cfDNA from pretreated patients (n = 42) and 9 healthy controls were compared with matched lymphocyte DNA by NGS, using a custom 158 amplicon panel covering hot-spot mutations and CNAs in 16 genes, with further validation of results by droplet digital PCR. RESULTS: No mutations were identified in cfDNA of healthy controls, whereas exactly half the patients with metastatic breast cancer had at least one mutation or amplification in cfDNA (mean 2, range 1-6) across a total of 13 genes. Longitudinal follow up showed dynamic changes to mutations and gene amplification in cfDNA indicating clonal and subclonal response to treatment that was more dynamic than cancer antigen 15-3 (CA15-3). Interestingly, at the time of blood sampling disease progression was occurring in 7 patients with erb-b2 receptor tyrosine kinase 2 (ERBB2) gene amplification in their cfDNA and 3 of these patients were human epidermal growth factor receptor 2 (HER2) negative at diagnosis, suggesting clonal evolution to a more aggressive phenotype. Lastly, 6 patients harbored estrogen receptor 1 (ESR1) mutations in cfDNA, suggesting resistance to endocrine therapy. Overall 9 of 42 patients (21%) had alterations in cfDNA that could herald a change in treatment. CONCLUSIONS: Targeted NGS of cfDNA has potential for monitoring response to targeted therapies through both mutations and gene amplification, for analysis of dynamic tumor heterogeneity and stratification to targeted therapy.

Pang, S. Y., et al. (2017). "The role of gene variants in the pathogenesis of neurodegenerative disorders as revealed by next generation sequencing studies: a review." Transl Neurodegener **6**: 27.

The clinical diagnosis of neurodegenerative disorders based on phenotype is difficult in heterogeneous conditions with overlapping symptoms. It does not take into account the disease etiology or the

highly variable clinical course even amongst patients diagnosed with the same disorder. The advent of next generation sequencing (NGS) has allowed for a system-wide, unbiased approach to identify all gene variants in the genome simultaneously. With the plethora of new genes being identified, genetic rather than phenotype-based classification of Mendelian diseases such as spinocerebellar ataxia (SCA), hereditary spastic paraplegia (HSP) and Charcot-Marie-Tooth disease (CMT) has become widely accepted. It has also become clear that gene variants play a role in common and predominantly sporadic neurodegenerative diseases such as Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). The observation of pleiotropy has emerged, with mutations in the same gene giving rise to diverse phenotypes, which further increases the complexity of phenotype-genotype correlation. Possible mechanisms of pleiotropy include different downstream effects of different mutations in the same gene, presence of modifier genes, and oligogenic inheritance. Future directions include development of bioinformatics tools and establishment of more extensive public genotype/phenotype databases to better distinguish deleterious gene variants from benign polymorphisms, translation of genetic findings into pathogenic mechanisms through in-vitro and in-vivo studies, and ultimately finding disease-modifying therapies for neurodegenerative disorders.

Petersson, F., et al. (2018). "Renal Cell Carcinoma With Leiomyomatous Stroma: A Group of Tumors With Indistinguishable Histopathologic Features, But 2 Distinct Genetic Profiles: Next-Generation Sequencing Analysis of 6 Cases Negative for Aberrations Related to the VHL gene." Appl Immunohistochem Mol Morphol **26**(3): 192-197.

We have studied a cohort of renal cell carcinomas (RCCs) with smooth-muscle stroma (N=6), which lacked any of following genetic aberrations: mutations in the VHL-gene-coding sequence, loss of heterozygosity of chromosome 3p, or hypermethylation of VHL. Using targeted next-generation sequencing, no intronic VHL mutations or mutations in selected genes involved in angiogenesis and genes frequently mutated in clear cell RCC were identified. Tumors were also tested for the presence of hotspot mutations in the TCEB1 gene with negative results in all cases. We conclude that there exists a group of RCCs with abundant leiomyomatous stroma, where the epithelial component is indistinguishable from conventional clear cell RCC and distinct from clear cell (tubulo-) papillary RCC and that these tumors lack aberrations related to the function of the VHL gene, mutations in genes involved in angiogenesis, and hotspot mutations in the TCEB1 gene.

Piga, D., et al. (2016). "New Mutations in NEB Gene Discovered by Targeted Next-Generation Sequencing in Nemaline Myopathy Italian Patients." J Mol Neurosci **59**(3): 351-359.

Nemaline myopathy represents a group of clinically and genetically heterogeneous neuromuscular disorders. Different clinical-genetic entities have been characterized in the last few years, with implications for diagnostics and genetic counseling. Fifty percent of nemaline myopathy forms are due to NEB mutations, but genetic analysis of this large and complex gene by Sanger sequencing is time consuming and expensive. We selected 10 Italian patients with clinical and biopsy features suggestive for nemaline myopathy and negative for ACTA1, TPM2 and TPM3 mutations. We applied a targeted next-generation sequencing strategy designed to analyse NEB coding regions, the relative full introns and the promoter. We also evaluated copy number variations (by CGH array) and transcriptional changes by RNA Sanger sequencing, whenever possible. This combined strategy revealed 11 likely pathogenic variants in 8 of 10 patients. The molecular diagnosis was fully achieved in 3 of 8 patients, while only one heterozygous mutation was observed in 5 subjects. This approach revealed to be a fast and cost-effective way to analyse the large NEB gene in a small group of patients and might be promising for the detection of pathological variants of other genes featuring large coding regions and lacking mutational hotspots.

Pongor, L., et al. (2015). "A genome-wide approach to link genotype to clinical outcome by utilizing next generation sequencing and gene chip data of 6,697 breast cancer patients." Genome Med **7**: 104.

BACKGROUND: The use of somatic mutations for predicting clinical outcome is difficult because a mutation can indirectly influence the function of many genes, and also because clinical follow-up is sparse in the relatively young next generation sequencing (NGS) databanks. Here we approach this problem by linking sequence databanks to well annotated gene-chip datasets, using a multigene transcriptomic fingerprint as a link between gene mutations and gene expression in breast cancer patients. METHODS: The database consists of 763 NGS samples containing mutational status for 22,938 genes and RNA-seq data for 10,987 genes. The gene chip database contains 5,934 patients with 10,987 genes plus clinical characteristics. For the prediction, mutations present in a sample are first translated into a 'transcriptomic fingerprint' by running ROC analysis on mutation and RNA-seq data. Then

correlation to survival is assessed by computing Cox regression for both up- and downregulated signatures. RESULTS: According to this approach, the top driver oncogenes having a mutation prevalence over 5 % included AKT1, TRANK1, TRAPPC10, RPGR, COL6A2, RAPGEF4, ATG2B, CNTRL, NAA38, OSBPL10, POTEF, SCLT1, SUN1, VWDE, MTUS2, and PIK3CA, and the top tumor suppressor genes included PHEX, TP53, GGA3, RGS22, PXDNL, ARFGEF1, BRCA2, CHD8, GCC2, and ARMC4. The system was validated by computing correlation between RNA-seq and microarray data (r (2) = 0.73, P < 1E-16). Cross-validation using 20 genes with a prevalence of approximately 5 % confirmed analysis reproducibility. CONCLUSIONS: We established a pipeline enabling rapid clinical validation of a discovered mutation in a large breast cancer cohort. An online interface is available for evaluating any human gene mutation or combinations of maximum three such genes (http://www.g-2-o.com).

Poon, K. S., et al. (2016). "Targeted next-generation sequencing of the ATP7B gene for molecular diagnosis of Wilson disease." Clin Biochem **49**(1-2): 166-171.

OBJECTIVES: In recent years, next-generation sequencing (NGS) technologies, which enable high throughput sample processing at relatively lower costs, are adopted in both research and clinical settings. A multiplex PCR-based NGS assay to identify mutations in the ATP7B gene for routine molecular diagnosis of Wilson disease was evaluated in comparison with the gold standard direct Sanger sequencing. DESIGN AND METHODS: Five multiplex PCRs to amplify the partial promoter, 5' untranslated and the entire coding regions of the ATP7B gene were designed. Indexed paired-end libraries were generated from the pooled amplicons using Nextera XT DNA Sample Preparation Kit and subjected to NGS on the MiSeq platform. DNA from the peripheral blood of 12 patients with Wilson disease, 2 B-lymphocyte cell lines and 3 external quality assurance samples were sequenced by the MiSeq and Sanger sequencing. RESULTS: Complete coverage was achieved across the targeted bases without any drop-out sequences. The observed read depth in a single run with 20 samples was >100X. Comparison of the NGS results against Sanger sequencing data on a panel of clinical specimens, cell lines and European Molecular Genetics Quality Networks (EMQN) quality assurance samples showed 100% concordance in identifying pathogenic mutations. CONCLUSION: With the capability of generating relatively higher throughput in a short time period, the NGS assay is a viable alternative to Sanger sequencing for detecting ATP7B mutations causally linked to Wilson disease in the clinical diagnostic laboratory.

Preiksaitiene, E., et al. (2015). "A novel missense mutation in the NSDHL gene identified in a Lithuanian family by targeted next-generation sequencing causes CK syndrome." Am J Med Genet A **167**(6): 1342-1348.

The NSDHL gene encodes 3beta-hydroxysteroid dehydrogenase involved in one of the later steps of the cholesterol biosynthetic pathway. Mutations in this gene can cause CHILD syndrome (OMIM 308050) and CK syndrome (OMIM 300831). CHILD syndrome is an X-linked dominant, male lethal disorder caused by mutations in the NSDHL gene that result in the loss of the function of the NSDHL protein. CK syndrome is an allelic X-linked recessive disorder. So far, 13 patients with CK syndrome from two families have been reported on. We present a new five-generation family with affected males manifesting clinical features of CK syndrome. Next generation sequencing was targeted to a custom panel of 542 genes with known or putative implication on intellectual disability. Missense mutation p.Gly152Asp was identified in the NSDHL gene in the DNA sample of the affected male. Mutation carrier status was confirmed for all the obligate carriers in the family. The clinical features of the affected males in the family manifested as weak fetal movements, severe intellectual disability, seizures, spasticity, atrophy of optic discs, microcephaly, plagiocephaly, skeletal abnormalities, and minor facial anomalies, including a high nasal bridge, strabismus, and micrognathia. A highly significant preferential transmission of the mutation was observed in this and previous families segregating CK syndrome. Our report expands the clinical spectrum of this syndrome to include weak fetal movements, spasticity, and plagiocephaly, and transmission ratio distortion. The various findings in these patients increase our understanding of the diversity of the clinical presentation of cholesterol biosynthesis disorders.

Quijada-Alamo, M., et al. (2017). "Next-generation sequencing and FISH studies reveal the appearance of gene mutations and chromosomal abnormalities in hematopoietic progenitors in chronic lymphocytic leukemia." J Hematol Oncol **10**(1): 83.

BACKGROUND: Chronic lymphocytic leukemia (CLL) is a highly genetically heterogeneous disease. Although CLL has been traditionally considered as a mature B cell leukemia, few independent studies have shown that the genetic alterations may appear in CD34+ hematopoietic progenitors. However, the presence of both chromosomal aberrations and gene mutations in CD34+ cells from the same patients has not been

explored. METHODS: Amplicon-based deep next-generation sequencing (NGS) studies were carried out in magnetically activated-cell-sorting separated CD19+ mature B lymphocytes and CD34+ hematopoietic progenitors (n = 56) to study the mutational status of TP53, NOTCH1, SF3B1, FBXW7, MYD88, and XPO1 genes. In addition, ultra-deep NGS was performed in a subset of seven patients to determine the presence of mutations in flow-sorted CD34+CD19- early hematopoietic progenitors. Fluorescence in situ hybridization (FISH) studies were performed in the CD34+ cells from nine patients of the cohort to examine the presence of cytogenetic abnormalities. RESULTS: NGS studies revealed a total of 28 mutations in 24 CLL patients. Interestingly, 15 of them also showed the same mutations in their corresponding whole population of CD34+ progenitors. The majority of NOTCH1 (7/9) and XPO1 (4/4) mutations presented a similar mutational burden in both cell fractions; by contrast, mutations of TP53 (2/2), FBXW7 (2/2), and SF3B1 (3/4) showed lower mutational allele frequencies, or even none, in the CD34+ cells compared with the CD19+ population. Ultra-deep NGS confirmed the presence of FBXW7, MYD88, NOTCH1, and XPO1 mutations in the subpopulation of CD34+CD19- early hematopoietic progenitors (6/7). Furthermore, FISH studies showed the presence of 11q and 13q deletions (2/2 and 3/5, respectively) in CD34+ progenitors but the absence of IGH cytogenetic alterations (0/2) in the CD34+ cells. Combining all the results from NGS and FISH, a model of the appearance and expansion of genetic alterations in CLL was derived, suggesting that most of the genetic events appear on the hematopoietic progenitors, although these mutations could induce the beginning of tumoral cell expansion at different stage of B cell differentiation. CONCLUSIONS: Our study showed the presence of both gene mutations and chromosomal abnormalities in early hematopoietic progenitor cells from CLL patients.

Rae, W., et al. (2018). "Clinical efficacy of a next-generation sequencing gene panel for primary immunodeficiency diagnostics." Clin Genet **93**(3): 647-655.

Primary immunodeficiencies (PIDs) are rare monogenic inborn errors of immunity that result in impairment of functions of the human immune system. PIDs have a broad phenotype with increased morbidity and mortality, and treatment choices are often complex. With increased accessibility of next-generation sequencing (NGS), the rate of discovery of genetic causes for PID has increased exponentially. Identification of an underlying monogenic diagnosis provides important clinical benefits for patients with the potential to alter treatments, facilitate genetic counselling, and pre-implantation diagnostics. We investigated a NGS PID panel of 242 genes within clinical care across a range of PID phenotypes. We also evaluated Phenomizer to predict causal genes from human phenotype ontology (HPO) terms. Twenty-seven participants were recruited, and a total of 15 reportable variants were identified in 48% (13/27) of the participants. The panel results had implications for treatment in 37% (10/27) of participants. Phenomizer identified the genes harbouring variants from HPO terms in 33% (9/27) of participants. This study shows the clinical efficacy that genetic testing has in the care of PID. However, it also highlights some of the disadvantages of gene panels in the rapidly moving field of PID genomics and current challenges in HPO term assignment for PID.

Ranjzad, F., et al. (2018). "Identification of Three Novel Frameshift Mutations in the PKD1 Gene in Iranian Families with Autosomal Dominant Polycystic Kidney Disease Using Efficient Targeted Next-Generation Sequencing." Kidney Blood Press Res **43**(2): 471-478.

BACKGROUND/AIMS: Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited cystic kidney diseases caused by mutations in two large multi-exon genes, PKD1 and PKD2. High allelic heterogeneity and duplication of PKD1 exons 1-32 as six pseudo genes on chromosome 16 complicate molecular analysis of this disease. METHODS: We applied targeted next-generation sequencing (NGS) in 9 non-consanguineous unrelated Iranian families with ADPKD to identify the genes hosting disease-causing mutations. This approach was confirmed by Sanger sequencing. RESULTS: Here, we determined three different novel frameshift mutations and four previously reported nonsense mutations in the PKD1 gene encoding polycystin1 in heterozygotes. CONCLUSION: This study demonstrates the effectiveness of NGS in significantly reducing the cost and time for simultaneous sequence analysis of PKD1 and PKD2, simplifying the genetic diagnostics of ADPKD. Although a probable correlation between the mutation types and phenotypic outcome is possible, however for more extensive studies in future, the consideration of renal hypouricemia (RHUC) and PKD1 coexistence may be helpful. The novel frameshift mutations reported by this study are p. Q1997X, P. D73X and p. V336X.

Rathi, V., et al. (2017). "Clinical validation of the 50 gene AmpliSeq Cancer Panel V2 for use on a next generation sequencing platform using formalin fixed, paraffin embedded and fine needle aspiration tumour specimens." Pathology **49**(1): 75-82.

The advent of massively parallel sequencing has caused a paradigm shift in the ways cancer is treated, as personalised therapy becomes a reality. More and more laboratories are looking to introduce next generation sequencing (NGS) as a tool for mutational analysis, as this technology has many advantages compared to conventional platforms like Sanger sequencing. In Australia all massively parallel sequencing platforms are still considered in-house in vitro diagnostic tools by the National Association of Testing Authorities (NATA) and a comprehensive analytical validation of all assays, and not just mere verification, is a strict requirement before accreditation can be granted for clinical testing on these platforms. Analytical validation of assays on NGS platforms can prove to be extremely challenging for pathology laboratories. Although there are many affordable and easily accessible NGS instruments available, there are no standardised guidelines as yet for clinical validation of NGS assays. We present an accreditation development procedure that was both comprehensive and applicable in a setting of hospital laboratory for NGS services. This approach may also be applied to other NGS applications in service laboratories.

Revollo, J., et al. (2017). "Spectrum of benzo [a]pyrene-induced mutations in the Pig-a gene of L5178YTk (+/-) cells identified with next generation sequencing." Mutat Res **824**: 1-8.

We used Sanger sequencing and next generation sequencing (NGS) for analysis of mutations in the endogenous X-linked Pig-a gene of clonally expanded L5178YTk (+/-) cells. The clones developed from single cells that were sorted on a flow cytometer based upon the expression pattern of the GPI-anchored marker, CD90, on their surface. CD90-deficient and CD90-proficient cells were sorted from untreated cultures and CD90-deficient cells were sorted from cultures treated with benzo [a]pyrene (B [a]P). Pig-a mutations were identified in all clones developed from CD90-deficient cells; no Pig-a mutations were found in clones of CD90-proficient cells. The spectrum of B [a]P-induced Pig-a mutations was dominated by basepair substitutions, small insertions and deletions at G:C, or at sequences rich in G:C content. We observed high concordance between Pig-a mutations determined by Sanger sequencing and by NGS, but NGS was able to identify mutations in samples that were difficult to analyze by Sanger sequencing (e.g., mixtures of two mutant clones). Overall, the NGS method is a cost and labor efficient high throughput approach for analysis of a large number of mutant clones.

Ricordel, C., et al. (2018). "Mutational Landscape of DDR2 Gene in Lung Squamous Cell Carcinoma Using Next-generation Sequencing." Clin Lung Cancer **19**(2): 163-169.e164.

BACKGROUND: Lung cancer represents the leading cause of cancer-related death worldwide. Despite great advances in lung cancer management with the recent emergence of molecular targeted therapies for non-squamous non-small-cell lung cancer, no dramatic improvements have been achieved in lung squamous cell carcinoma (SCC). Mutations in discoidin domain receptor 2 (DDR2) gene were recently identified as promising molecular targets in this histology. The aim of this study is to describe the DDR2 mutational landscape of lung SCC and investigate the associated clinical factors. METHODS: Next-generation sequencing of the DDR2 gene was performed on 271 samples of lung SCC. Patients followed in our institution from January 2011 to August 2014 were retrospectively selected for data collection. Other driver gene alterations (EGFR, KRAS, BRAF, HER2, and PI3KCA) were analyzed using pyrosequencing. RESULTS: A total of 11 patients harboring a DDR2 mutation was detected among the 271 sequenced lung SCC samples (4%). We describe 10 unreported mutations, comprising a novel DDR2 exon 7 splice mutant. DDR2 mutations were not mutually exclusive with other driver gene alterations. One hundred thirty-six patients were included for clinical comparison and logistic regression analysis. No difference was detected between DDR2-mutant and DDR2 wild-type lung SCC regarding clinical characteristics or survival. CONCLUSION: DDR2 mutations were observed in 4% of cases of lung SCC of European descent. DDR2-mutated tumors can exhibit other driver gene alterations. No clinical characteristics were significantly associated with DDR2 mutation.

Roucher Boulez, F., et al. (2015). "A splicing mutation in the DMD gene detected by next-generation sequencing and confirmed by mRNA and protein analysis." Clin Chim Acta **448**: 146-149.

BACKGROUND: Dystrophinopathies, either the severe Duchenne Muscular Dystrophy (DMD) or the milder Becker Muscular Dystrophy (BMD), are X-linked recessive disorders caused by mutations in the DMD gene. DMD is one of the longest human genes. Large deletions or duplications account for 60-80% of the mutations. Remaining anomalies consist in point mutations or small rearrangements. Routinely, the molecular diagnosis is done by a Multiplex Ligation-dependent Probe Amplification (MLPA) or array Comparative Genome Hybridization (aCGH), followed, if negative, by Sanger sequencing of all exons. METHODS: In this study, massive parallel sequencing (MPS) or next generation sequencing (NGS) was used to make a rapid and costless

molecular diagnosis in a young boy suspected of DMD. RESULTS: A small deletion: NM_004006.2:c.2803+5_2803+8del was identified. The diagnosis was performed in one single manipulation and within a week. The consequence of this intronic mutation is a skipping of exon 21 confirmed by mRNA and protein analysis. CONCLUSIONS: NGS appears to be an efficient new strategy in DMD molecular diagnosis. It highlights the major evolution of the diagnostic strategy towards high throughput technologies, where bioinformatics analysis becomes the real challenge for variations detection. This is the first study reporting in vivo impact of this intronic mutation.

Rubattu, S., et al. (2016). "A Next-Generation Sequencing Approach to Identify Gene Mutations in Early- and Late-Onset Hypertrophic Cardiomyopathy Patients of an Italian Cohort." Int J Mol Sci **17**(8).

Sequencing of sarcomere protein genes in patients fulfilling the clinical diagnostic criteria for hypertrophic cardiomyopathy (HCM) identifies a disease-causing mutation in 35% to 60% of cases. Age at diagnosis and family history may increase the yield of mutations screening. In order to assess whether Next-Generation Sequencing (NGS) may fulfil the molecular diagnostic needs in HCM, we included 17 HCM-related genes in a sequencing panel run on PGM IonTorrent. We selected 70 HCM patients, 35 with early (</=25 years) and 35 with late (>/=65 years) diagnosis of disease onset. All samples had a 98.6% average of target regions, with coverage higher than 20x (mean coverage 620x). We identified 41 different mutations (seven of them novel) in nine genes: MYBPC3 (17/41 = 41%); MYH7 (10/41 = 24%); TNNT2, CAV3 and MYH6 (3/41 = 7.5% each); TNNI3 (2/41 = 5%); GLA, MYL2, and MYL3 (1/41=2.5% each). Mutation detection rate was 30/35 (85.7%) in early-onset and 8/35 (22.9%) in late-onset HCM patients, respectively ($p < 0.0001$). The overall detection rate for patients with positive family history was 84%, and 90.5% in patients with early disease onset. In our study NGS revealed higher mutations yield in patients with early onset and with a family history of HCM. Appropriate patient selection can increase the yield of genetic testing and make diagnostic testing cost-effective.

Schenkel, L. C., et al. (2016). "Clinical Next-Generation Sequencing Pipeline Outperforms a Combined Approach Using Sanger Sequencing and Multiplex Ligation-Dependent Probe Amplification in Targeted Gene Panel Analysis." J Mol Diagn **18**(5): 657-667.

Advances in next-generation sequencing (NGS) have facilitated parallel analysis of multiple genes enabling the implementation of cost-effective, rapid, and high-throughput methods for the molecular diagnosis of multiple genetic conditions, including the identification of BRCA1 and BRCA2 mutations in high-risk patients for hereditary breast and ovarian cancer. We clinically validated a NGS pipeline designed to replace Sanger sequencing and multiplex ligation-dependent probe amplification analysis and to facilitate detection of sequence and copy number alterations in a single test focusing on a BRCA1/BRCA2 gene analysis panel. Our custom capture library covers 46 exons, including BRCA1 exons 2, 3, and 5 to 24 and BRCA2 exons 2 to 27, with 20 nucleotides of intronic regions both 5' and 3' of each exon. We analyzed 402 retrospective patients, with previous Sanger sequencing and multiplex ligation-dependent probe amplification results, and 240 clinical prospective patients. One-hundred eighty-three unique variants, including sequence and copy number variants, were detected in the retrospective (n = 95) and prospective (n = 88) cohorts. This standardized NGS pipeline demonstrated 100% sensitivity and 100% specificity, uniformity, and high-depth nucleotide coverage per sample (approximately 7000 reads per nucleotide). Subsequently, the NGS pipeline was applied to the analysis of larger gene panels, which have shown similar uniformity, sample-to-sample reproducibility in coverage distribution, and sensitivity and specificity for detection of sequence and copy number variants.

Schwaederle, M., et al. (2015). "Next generation sequencing demonstrates association between tumor suppressor gene aberrations and poor outcome in patients with cancer." Cell Cycle **14**(11): 1730-1737.

Next generation sequencing is transforming patient care by allowing physicians to customize and match treatment to their patients' tumor alterations. Our goal was to study the association between key molecular alterations and outcome parameters. We evaluated the characteristics and outcomes (overall survival (OS), time to metastasis/recurrence, and best progression-free survival (PFS)) of 392 patients for whom next generation sequencing (182 or 236 genes) had been performed. The Kaplan-Meier method and Cox regression models were used for our analysis, and results were subjected to internal validation using a resampling method (bootstrap analysis). In a multivariable analysis (Cox regression model), the parameters that were statistically associated with a poorer overall survival were the presence of metastases at diagnosis ($P = 0.014$), gastrointestinal histology ($P < 0.0001$), PTEN ($P < 0.0001$), and CDKN2A alterations ($P = 0.0001$). The variables associated with a shorter time to metastases/recurrence were gastrointestinal histology ($P = 0.004$), APC (P =

0.008), PTEN (P = 0.026) and TP53 (P = 0.044) alterations. TP53 (P = 0.003) and PTEN (P = 0.034) alterations were independent predictors of a shorter best PFS. A personalized treatment approach (matching the molecular aberration with a cognate targeted drug) also correlated with a longer best PFS (P = 0.046). Our study demonstrated that, across diverse cancers, anomalies in specific tumor suppressor genes (PTEN, CDKN2A, APC, and/or TP53) were independently associated with a worse outcome, as reflected by time to metastases/recurrence, best PFS on treatment, and/or overall survival. These observations suggest that molecular diagnostic tests may provide important prognostic information in patients with cancer.

Seed, G., et al. (2017). "Gene Copy Number Estimation from Targeted Next-Generation Sequencing of Prostate Cancer Biopsies: Analytic Validation and Clinical Qualification." Clin Cancer Res **23**(20): 6070-6077.

Purpose: Precise detection of copy number aberrations (CNA) from tumor biopsies is critically important to the treatment of metastatic prostate cancer. The use of targeted panel next-generation sequencing (NGS) is inexpensive, high throughput, and easily feasible, allowing single-nucleotide variant calls, but CNA estimation from this remains challenging. Experimental Design: We evaluated CNVkit for CNA identification from amplicon-based targeted NGS in a cohort of 110 fresh castration-resistant prostate cancer biopsies and used capture-based whole-exome sequencing (WES), array comparative genomic hybridization (aCGH), and FISH to explore the viability of this approach. Results: We showed that this method produced highly reproducible CNA results (r = 0.92), with the use of pooled germline DNA as a coverage reference supporting precise CNA estimation. CNA estimates from targeted NGS were comparable with WES (r = 0.86) and aCGH (r = 0.7); for key selected genes (BRCA2, MYC, PIK3CA, PTEN, and RB1), CNA estimation correlated well with WES (r = 0.91) and aCGH (r = 0.84) results. The frequency of CNAs in our population was comparable with that previously described (i.e., deep deletions: BRCA2 4.5%; RB1 8.2%; PTEN 15.5%; amplification: AR 45.5%; gain: MYC 31.8%). We also showed, utilizing FISH, that CNA estimation can be impacted by intratumor heterogeneity and demonstrated that tumor microdissection allows NGS to provide more precise CNA estimates. Conclusions: Targeted NGS and CNVkit-based analyses provide a robust, precise, high-throughput, and cost-effective method for CNA estimation for the delivery of more precise patient care. Clin Cancer Res; 23(20); 6070-7. (c)2017 AACR.

Seong, M. W., et al. (2015). "Clinical applications of next-generation sequencing-based gene panel in patients with muscular dystrophy: Korean experience." Clin Genet.

Muscular dystrophy (MD) is a genetically and clinically heterogeneous group of disorders. Here, we performed targeted sequencing of 18 limb-girdle MD (LGMD)-related genes in 35 patients who were highly suspected of having MD. We identified one or more pathogenic variants in 23 of 35 patients (65.7%), and a genetic diagnosis was performed in 20 patients (57.1%). LGMD2B was the most common LGMD type, followed by LGMD1B, LGMD2A, and LGMD2G. Among the three major LGMD types in this group, LGMD1B was correlated with the lowest creatine kinase (CK) levels and the earliest onset, whereas LGMD2B was correlated with the highest CK levels and the latest onset. Thus, next-generation sequencing-based gene panels can be a helpful tool for the diagnosis of MDs, particularly in young children and those displaying atypical symptoms.

Shang, H., et al. (2018). "Targeted Next-Generation Sequencing of a Deafness Gene Panel (MiamiOtoGenes) Analysis in Families Unsuitable for Linkage Analysis." Biomed Res Int **2018**: 3103986.

Hearing loss (HL) is a common sensory disorder in humans with high genetic heterogeneity. To date, over 145 loci have been identified to cause nonsyndromic deafness. Furthermore, there are countless families unsuitable for the conventional linkage analysis. In the present study, we used a custom capture panel (MiamiOtoGenes) to target sequence 180 deafness-associated genes in 5 GJB2 negative deaf probands with autosomal recessive nonsyndromic HL from Iran. In these 5 families, we detected one reported and six novel mutations in 5 different deafness autosomal recessive (DFNB) genes (TRIOBP, LHFPL5, CDH23, PCDH15, and MYO7A). The custom capture panel in our study provided an efficient and comprehensive diagnosis for known deafness genes in small families.

Sharma Bhai, P., et al. (2017). "Next-Generation Sequencing Reveals a Nonsense Mutation (p.Arg364Ter) in MRE11A Gene in an Indian Patient with Familial Breast Cancer." Breast Care (Basel) **12**(2): 114-116.

BACKGROUND: The MRN complex consisting of MRE11A-RAD50-NBS1 proteins is involved in the repair of double-strand breaks, and mutations in genes coding for the MRN complex have been identified in families with breast and ovarian cancer. CASE REPORT: In a BRCA-negative family with positive history of breast and endometrial cancer, next-generation sequencing-based panel testing identified a

mutation in the MRE11A gene (NM_005590 c.1090C>T: p.Arg364Ter). This mutation results in a shorter mutated protein lacking 2 DNA binding domains (the GAR domain and the RAD50 binding site), abolishing the function of protein. CONCLUSION: This case provides insight into the role of the MRE11A gene in causing breast cancer susceptibility in families, and supports the use of multigene panel testing in cases with hereditary predisposition to breast cancer.

Shin, S., et al. (2017). "Detection of Immunoglobulin Heavy Chain Gene Clonality by Next-Generation Sequencing for Minimal Residual Disease Monitoring in B-Lymphoblastic Leukemia." Ann Lab Med **37**(4): 331-335.

Minimal residual disease (MRD) following B-lymphoblastic leukemia (B-ALL) treatment has gained prognostic importance. Clonal immunoglobulin heavy chain (IGH) gene rearrangement is a useful follow-up marker in B-ALL owing to its high positivity rate. We evaluated the performance and clinical applicability of a next-generation sequencing (NGS) assay for IGH rearrangement in B-ALL MRD monitoring. IGH rearrangement was tested by using fluorescence PCR-fragment analysis and the NGS assay in eight B-ALL patients. The NGS assay was run on two platforms: the Ion Torrent PGM (Thermo Fisher Scientific, USA) (18 samples from 1st to 7th patients) and the MiSeq system (Illumina, USA) (four samples from 8th patient). All initial diagnostic samples and four follow-up samples were positive for clonal IGH rearrangement with fluorescence PCR-fragment analysis and the NGS assay, and six follow-up samples were positive only with NGS. In one case with BCR-ABL1 translocation, BCR-ABL1 quantitative PCR was negative but the NGS IGH assay was positive just prior to full-blown relapse, suggesting the high sensitivity and clinical utility of the NGS assay. The NGS assay is proposed for MRD monitoring in B-ALL Additional studies are needed to confirm the clinical implications of cases showing positive results only in NGS.

Sillanpaa, S., et al. (2017). "Next-Generation Sequencing Combined with Specific PCR Assays To Determine the Bacterial 16S rRNA Gene Profiles of Middle Ear Fluid Collected from Children with Acute Otitis Media." mSphere **2**(2).

The aim of the study was to analyze the bacteriome of acute otitis media with a novel modification of next-generation sequencing techniques. Outpatient children with acute otitis media were enrolled in the study, and middle ear fluids were collected during 90 episodes from 79 subjects aged 5 to 42 months (median age, 19 months). The bacteriome profiles of middle ear fluid samples were determined by a nested-PCR amplification of the 16S rRNA gene (V4 region), followed by mass sequencing. The profiling results were compared to the results of specific PCR assays targeting selected prevalent pathogens. Bacteriome profiling using nested amplification of low-volume samples was aided by a bioinformatic subtraction of signal contaminants from the recombinant polymerase, achieving a sensitivity slightly lower than that of specific PCR detection. Streptococcus pneumoniae was detected in 28 (31%) samples, Haemophilus influenzae in 24 (27%), Moraxella catarrhalis in 18 (20%), Staphylococcus spp. in 21 (23%), Turicella otitidis in 5 (5.6%), Alloiococcus otitidis in 3 (3.3%), and other bacteria in 14 (16%) using bacteriome profiling. S. pneumoniae was the dominant pathogen in 14 (16%) samples, H. influenzae in 15 (17%), M. catarrhalis in 5 (5.6%), T. otitidis in 2, and Staphylococcus auricularis in 2. Weaker signals of Prevotella melaninogenica, Veillonella dispar, and Veillonella montpellierensis were noted in several samples. Fourteen samples (16%) were not explainable by bacterial pathogens; novel causative agents were not detected. In conclusion, unbiased bacteriome profiling helped in depicting the true mutual quantitative ratios of ear bacteria, but at present, its complicated protocol impedes its routine clinical use. IMPORTANCE Although S. pneumoniae, H. influenzae, and M. catarrhalis have been long established as the most important pathogens in acute otitis media using culture and specific PCR assays, the knowledge of their mutual quantitative relations and possible roles of other bacteria is incomplete. The advent of unbiased bacteriome 16S rRNA gene profiling has allowed the detection of nearly all bacteria present in the sample, and it helps in depicting their mutual quantitative ratios. Due to the difficulties in performing mass sequencing in low-volume samples, only a few bacteriome-profiling studies of otitis media have been published, all limited to cases of chronic otitis media. Here, we present a study on samples obtained from young children with acute otitis media, successfully using a strategy of nested PCR coupled with mass sequencing, and demonstrate that the method can confer quantitative information hardly obtainable by other methods.

Solloch, U. V., et al. (2017). "Frequencies of gene variant CCR5-Delta32 in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers." Hum Immunol **78**(11-12): 710-717.

Homozygous carriers of CCR5-Delta32, a gene variant of CC-type chemokine receptor 5 (CCR5), are highly resistant to infections with human immunodeficiency virus type 1 (HIV-1) and therefore

preferred stem cell donors for HIV-infected patients. We analyzed CCR5 typing data of 1,333,035 potential hematopoietic stem cell donors enlisted with three national DKMS donor centers. Allele and genotype frequencies were determined for 87 countries of origin as self-assessed by the donors. CCR5-Delta32 allele frequencies ranged from 16.4% in the Norwegian sample to 0 in donors from Ethiopia. The highest CCR5-Delta32/Delta32 genotype frequency was found in the sample from the Faroe Islands (2.3%), whereas in 27 samples, predominantly of donors from Africa, Asia and South America, none of the individuals carried this genotype. The characteristic CCR5-Delta32 allele frequency decline from Northern to Southeastern Eurasia supports findings of earlier studies. With available HLA haplotype frequency information for the patient's ethnicity, our data allows upfront estimation of the probability that an HLA-matched donor with CCR5-Delta32/Delta32 genotype can be found for a patient in need of hematopoietic stem cell transplantation.

Stenson, P. D., et al. (2017). "The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies." Hum Genet **136**(6): 665-677.

The Human Gene Mutation Database (HGMD ((R))) constitutes a comprehensive collection of published germline mutations in nuclear genes that underlie, or are closely associated with human inherited disease. At the time of writing (March 2017), the database contained in excess of 203,000 different gene lesions identified in over 8000 genes manually curated from over 2600 journals. With new mutation entries currently accumulating at a rate exceeding 17,000 per annum, HGMD represents de facto the central unified gene/disease-oriented repository of heritable mutations causing human genetic disease used worldwide by researchers, clinicians, diagnostic laboratories and genetic counsellors, and is an essential tool for the annotation of next-generation sequencing data. The public version of HGMD (http://www.hgmd.org) is freely available to registered users from academic institutions and non-profit organisations whilst the subscription version (HGMD Professional) is available to academic, clinical and commercial users under license via QIAGEN Inc.

Stevanato, P., et al. (2017). "Targeted Next-Generation Sequencing Identification of Mutations in Disease Resistance Gene Analogs (RGAs) in Wild and Cultivated Beets." Genes (Basel) **8**(10).

Resistance gene analogs (RGAs) were searched bioinformatically in the sugar beet (Beta vulgaris L.) genome as potential candidates for improving resistance against different diseases. In the present study, Ion Torrent sequencing technology was used to identify mutations in 21 RGAs. The DNA samples of ninety-six individuals from six sea beets (Beta vulgaris L. subsp. maritima) and six sugar beet pollinators (eight individuals each) were used for the discovery of single-nucleotide polymorphisms (SNPs). Target amplicons of about 200 bp in length were designed with the Ion AmpliSeq Designer system in order to cover the DNA sequences of the RGAs. The number of SNPs ranged from 0 in four individuals to 278 in the pollinator R740 (which is resistant to rhizomania infection). Among different groups of beets, cytoplasmic male sterile lines had the highest number of SNPs (132) whereas the lowest number of SNPs belonged to O-types (95). The principal coordinates analysis (PCoA) showed that the polymorphisms inside the gene Bv8_184910_pkon (including the CCCTCC sequence) can effectively differentiate wild from cultivated beets, pointing at a possible mutation associated to rhizomania resistance that originated directly from cultivated beets. This is unlike other resistance sources that are introgressed from wild beets. This gene belongs to the receptor-like kinase (RLK) class of RGAs, and is associated to a hypothetical protein. In conclusion, this first report of using Ion Torrent sequencing technology in beet germplasm suggests that the identified sequence CCCTCC can be used in marker-assisted programs to differentiate wild from domestic beets and to identify other unknown disease resistance genes in beet.

Suryavanshi, M., et al. (2017). "Detection of false positive mutations in BRCA gene by next generation sequencing." Fam Cancer **16**(3): 311-317.

BRCA1 and BRCA2 genes are implicated in 20-25% of hereditary breast and ovarian cancers. New age sequencing platforms have revolutionized massively parallel sequencing in clinical practice by providing cost effective, rapid, and sensitive sequencing. This study critically evaluates the false positives in multiplex panels and suggests the need for careful analysis. We employed multiplex PCR based BRCA1 and BRCA2 community Panel with ion torrent PGM machine for evaluation of these mutations. Out of all 41samples analyzed for BRCA1 and BRCA2 five were found with 950_951 insA (Asn319fs) at Chr13:32906565 position and one sample with 1032_1033 insA (Asn346fs) at Chr13:32906647, both being frame-shift mutations in BRCA2 gene. 950_951 insA (Asn319fs) mutation is reported as pathogenic allele in NCBI dbSNP. On examination of IGV for all these samples, it was seen that both mutations had 'A' nucleotide insertion at 950, and 1032 position in exon 10 of BRCA2 gene. Sanger Sequencing did not

confirm these insertions. Next-generation sequencing shows great promise by allowing rapid mutational analysis of multiple genes in human cancer but our results indicate the need for careful sequence analysis to avoid false positive results.

Szopa, M., et al. (2016). "A family with the Arg103Pro mutation in the NEUROD1 gene detected by next-generation sequencing - Clinical characteristics of mutation carriers." Eur J Med Genet **59**(2): 75-79.

UNLABELLED: Until now only a few families with early onset autosomal diabetes due to the NEUROD1 gene mutations have been identified. Moreover, only some of them meet strict MODY (maturity-onset diabetes of the young) criteria. Next-generation sequencing (NGS) provides an opportunity to detect more pathogenic mutations in this gene. Here, we evaluated the segregation of the Arg103Pro mutation in the NEUROD1 gene in a pedigree in which it was detected, and described the clinical characteristics of the mutation carriers. METHODS: We included 156 diabetic probands of MODY families, among them 52 patients earlier tested for GCK-MODY and/or HNF1A-MODY by Sanger sequencing with negative results. Genetic testing was performed by targeted NGS sequencing using a panel of 28 monogenic diabetes genes. RESULTS: As detected by NGS, one patient had the missense Arg103Pro (CGC/CCC) mutation in the gene NEUROD1 changing the amino-acid structure of the DNA binding domain of this transcription factor. We confirmed this sequence difference by Sanger sequencing. This family had previously been tested with negative results for HNF1A gene mutations. 17 additional members of this family were invited for further testing. We confirmed the presence of the mutation in 11 subjects. Seven adult mutation carriers (all but one) from three generations had been already diagnosed with diabetes. There were 3 individuals with the Arg103Pro mutation diagnosed before the age of 30 years in the family. The range of age of the four unaffected mutation carriers (3 minors and 1 adult) was 3-48 years. Interestingly, one mutation carrier had a history of transient neonatal hypoglycemia, of which the clinical course resembled episodes typical for HNF4A-MODY. CONCLUSIONS: We report a family with autosomal dominant diabetes related to a new NEUROD1 mutation, one of very few meeting MODY criteria. The use of the NGS method will facilitate identification of more families with rare forms of MODY.

Talebi, F., et al. (2017). "Next-Generation Sequencing Reveals One Novel Missense Mutation in COL1A2 Gene in an Iranian Family with Osteogenesis imperfecta." Iran Biomed J **21**(5): 338-341.

BAckground: Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous disorder characterized by bone loss and bone fragility. The aim of this study was to investigate the variants of three genes involved in the pathogenesis of OI. Methods: Molecular genetic analyses were performed for COL1A1, COL1A2, and CRTAP genes in an Iranian family with OI. The DNA samples were analyzed by next-generation sequencing (NGS) gene panel and Sanger sequencing. Results: Five different variants were identified in COL1A1 and COL1A2, including two variants in COL1A1 and three variants in COL1A2. Among the five causative COL1A1 and COL1A2 variants, one novel variants, c.1081 G>A, was found in COL1A2, which was identified in two siblings. Conclusion: Our finding extends the variant spectrum of the COL1A2 gene and has important implications for genetic counseling of families. The NGS is a powerful molecular diagnostic strategy for OI, a heterogeneous disorder.

Talebi, F., et al. (2017). "Identification of a novel missence mutation in FGFR3 gene in an Iranian family with LADD syndrome by Next-Generation Sequencing." Int J Pediatr Otorhinolaryngol **97**: 192-196.

Lacrimo-auriculo-dento-digital syndrome (LADD) is a multiple congenital anomaly and a genetically heterogeneous disorder. The aim of this study was to identify the pathogenic gene in an Iranian family with LADD syndrome and review the literature on reported mutations that involved in pathogenesis of LADD syndrome. One novel variant, c.1882 G > A, in fibroblast growth factor receptor 3 (FGFR3) was identified by next generation sequencing and Sanger sequencing. The heterozygous FGFR3 c.1882 G > A variant results in substitution of aspartic acid with asparagine at amino acid 628 (p.D628N) and co-segregated with the phenotype in the LADD family. Our findings suggest that the heterozygous FGFR3 c.1882 G > A variant might be the pathogenic mutation, because this amino acid is conserved in several species. Our data extend the mutation spectrum of the FGFR3 gene and have important implications for genetic counseling for the families. This is the second report of FGFR3 involvement in syndromic deafness in humans, and confirms the gene's positive role in inner ear development. In addition, this is the first FGFR3 mutation recognized in the Iranian LADD family.

Taniguchi-Ikeda, M., et al. (2016). "Next-generation sequencing discloses a nonsense mutation in the dystrophin gene from long preserved dried

umbilical cord and low-level somatic mosaicism in the proband mother." J Hum Genet **61**(4): 351-355.

Duchene muscular dystrophy (DMD) is a progressive muscle wasting disease, caused by mutations in the dystrophin (DMD) on the X chromosome. One-third of patients are estimated to have de novo mutations. To provide in-depth genetic counseling, the comprehensive identification of mutations is mandatory. However, many DMD patients did not undergo genetic diagnosis because detailed genetic diagnosis was not available or their mutational types were difficult to identify. Here we report the genetic testing of a sporadic DMD boy, who died >20 years previously. Dried umbilical cord preserved for 38 years was the only available source of genomic DNA. Although the genomic DNA was severely degraded, multiplex ligation-dependent probe amplification analysis was performed but no gross mutations found. Sanger sequencing was attempted but not conclusive. Next-generation sequencing (NGS) was performed by controlling the tagmentation during library preparation. A nonsense mutation in DMD (p.Arg2095*) was clearly identified in the proband. Consequently, the identical mutation was detected as an 11% mosaic mutation from his healthy mother. Finally, the proband's sister was diagnosed as a non-carrier of the mutation. Thus using NGS we have identified a pathogenic DMD mutation from degraded DNA and low-level somatic mosaicism, which would have been overlooked using Sanger sequencing.

Tekin, D., et al. (2016). "A next-generation sequencing gene panel (MiamiOtoGenes) for comprehensive analysis of deafness genes." Hear Res **333**: 179-184.

Extreme genetic heterogeneity along with remarkable variation in the distribution of causative variants across in different ethnicities makes single gene testing inefficient for hearing loss. We developed a custom capture/next-generation sequencing gene panel of 146 known deafness genes with a total target size of approximately 1 MB. The genes were identified by searching databases including Hereditary Hearing Loss Homepage, the Human Genome Mutation Database (HGMD), Online Mendelian Inheritance in Man (OMIM) and most recent peer-reviewed publications related to the genetics of deafness. The design covered all coding exons, UTRs and 25 bases of intronic flanking sequences for each exon. To validate our panel, we used 6 positive controls with variants in known deafness genes and 8 unsolved samples from individuals with hearing loss. Mean coverage of the targeted exons was 697X. On average, each sample had 99.8%, 96.2% and 92.7% of the targeted region coverage of 1X, 50X and 100X reads, respectively. Analysis detected all known variants in nuclear genes. These results prove the accuracy and reliability of the custom capture experiment.

Thomas, M. G., et al. (2017). "Development and clinical utility of a novel diagnostic nystagmus gene panel using targeted next-generation sequencing." Eur J Hum Genet **25**(6): 725-734.

Infantile nystagmus (IN) is a genetically heterogeneous disorder arising from variants of genes expressed within the developing retina and brain. IN presents a diagnostic challenge and patients often undergo numerous investigations. We aimed to develop and assess the utility of a next-generation sequencing (NGS) panel to enhance the diagnosis of IN. We identified 336 genes associated with IN from the literature and OMIM. NimbleGen Human custom array was used to enrich the target genes and sequencing was performed using HiSeq2000. Using reference genome material (NA12878), we show the sensitivity (98.5%) and specificity (99.9%) of the panel. Fifteen patients with familial IN were sequenced using the panel. Two authors were masked to the clinical diagnosis. We identified variants in 12/15 patients in the following genes: FRMD7 (n=3), CACNA1F (n=2), TYR (n=5), CRYBA1 (n=1) and TYRP1 (n=1). In 9/12 patients, the clinical diagnosis was consistent with the genetic diagnosis. In 3/12 patients, the results from the genetic diagnoses (TYR, CRYBA1 and TYRP1 variants) enabled revision of clinical diagnoses. In 3/15 patients, we were unable to determine a genetic diagnosis. In one patient, copy number variation analysis revealed a FRMD7 deletion. This is the first study establishing the clinical utility of a diagnostic NGS panel for IN. We show that the panel has high sensitivity and specificity. The genetic information from the panel will lead to personalised diagnosis and management of IN and enable accurate genetic counselling. This will allow development of a new clinical care pathway for IN.

Todorovic Balint, M., et al. (2016). "Gene Mutation Profiles in Primary Diffuse Large B Cell Lymphoma of Central Nervous System: Next Generation Sequencing Analyses." Int J Mol Sci **17**(5).

The existence of a potential primary central nervous system lymphoma-specific genomic signature that differs from the systemic form of diffuse large B cell lymphoma (DLBCL) has been suggested, but is still controversial. We investigated 19 patients with primary DLBCL of central nervous system (DLBCL CNS) using the TruSeq Amplicon Cancer Panel (TSACP) for 48 cancer-related genes. Next generation sequencing (NGS) analyses have revealed that over 80% of potentially protein-changing mutations were located in eight genes (CTNNB1, PIK3CA, PTEN, ATM, KRAS, PTPN11, TP53 and JAK3), pointing to the

potential role of these genes in lymphomagenesis. TP53 was the only gene harboring mutations in all 19 patients. In addition, the presence of mutated TP53 and ATM genes correlated with a higher total number of mutations in other analyzed genes. Furthermore, the presence of mutated ATM correlated with poorer event-free survival (EFS) (p = 0.036). The presence of the mutated SMO gene correlated with earlier disease relapse (p = 0.023), inferior event-free survival (p = 0.011) and overall survival (OS) (p = 0.017), while mutations in the PTEN gene were associated with inferior OS (p = 0.048). Our findings suggest that the TP53 and ATM genes could be involved in the molecular pathophysiology of primary DLBCL CNS, whereas mutations in the PTEN and SMO genes could affect survival regardless of the initial treatment approach.

Vahidnezhad, H., et al. (2017). "Gene-Targeted Next Generation Sequencing Identifies PNPLA1 Mutations in Patients with a Phenotypic Spectrum of Autosomal Recessive Congenital Ichthyosis: The Impact of Consanguinity." J Invest Dermatol **137**(3): 678-685.

Autosomal recessive congenital ichthyosis is a heterogeneous group of disorders associated with mutations in at least nine distinct genes. To ascertain the molecular basis of ichthyosis patients in Iran, a country of approximately 80 million people with a high prevalence of customary consanguineous marriages, we have developed a gene-targeted next generation sequencing array consisting of 38 genes reported in association with ichthyosis phenotypes. In a subset of nine extended consanguineous families, we found homozygous missense mutations in the PNPLA1 gene, six of them being distinct and, to our knowledge, previously unpublished. This gene encodes an enzyme with lipid hydrolase activity, important for development and maintenance of the barrier function of the epidermis. These six mutations, as well as four previously published mutations, reside exclusively within the patatin-like subdomain of PNPLA1 containing the catalytic site. The mutations clustered around the active center of the enzyme or resided at the surface of the protein possibly involved in the protein-protein interactions. Clinical features of the patients showed considerable intra- and interfamilial heterogeneity. Knowledge of the specific mutations allows identification of heterozygous carriers, assisting in genetic counseling, prenatal testing, and preimplantation genetic diagnosis in extended families at risk of recurrence of this disorder, the incidence of which is significantly increased in consanguineous marriages.

Vavoulis, D. V., et al. (2017). "Hierarchical probabilistic models for multiple gene/variant associations based on next-generation sequencing data." Bioinformatics **33**(19): 3058-3064.

Motivation: The identification of genetic variants influencing gene expression (known as expression quantitative trait loci or eQTLs) is important in unravelling the genetic basis of complex traits. Detecting multiple eQTLs simultaneously in a population based on paired DNA-seq and RNA-seq assays employs two competing types of models: models which rely on appropriate transformations of RNA-seq data (and are powered by a mature mathematical theory), or count-based models, which represent digital gene expression explicitly, thus rendering such transformations unnecessary. The latter constitutes an immensely popular methodology, which is however plagued by mathematical intractability. Results: We develop tractable count-based models, which are amenable to efficient estimation through the introduction of latent variables and the appropriate application of recent statistical theory in a sparse Bayesian modelling framework. Furthermore, we examine several transformation methods for RNA-seq read counts and we introduce arcsin, logit and Laplace smoothing as preprocessing steps for transformation-based models. Using natural and carefully simulated data from the 1000 Genomes and gEUVADIS projects, we benchmark both approaches under a variety of scenarios, including the presence of noise and violation of basic model assumptions. We demonstrate that an arcsin transformation of Laplace-smoothed data is at least as good as state-of-the-art models, particularly at small samples. Furthermore, we show that an over-dispersed Poisson model is comparable to the celebrated Negative Binomial, but much easier to estimate. These results provide strong support for transformation-based versus count-based (particularly Negative-Binomial-based) models for eQTL mapping. Availability and implementation: All methods are implemented in the free software eQTLseq: https://github.com/dvav/eQTLseq. Contact: dimitris.vavoulis@well.ox.ac.uk. Supplementary information: Supplementary data are available at Bioinformatics online.

Villate, O., et al. (2018). "Functional Analyses of a Novel Splice Variant in the CHD7 Gene, Found by Next Generation Sequencing, Confirm Its Pathogenicity in a Spanish Patient and Diagnose Him with CHARGE Syndrome." Front Genet **9**: 7.

Mutations in CHD7 have been shown to be a major cause of CHARGE syndrome, which presents many symptoms and features common to other syndromes making its diagnosis difficult. Next generation sequencing (NGS) of a panel of intellectual

disability related genes was performed in an adult patient without molecular diagnosis. A splice donor variant in CHD7 (c.5665 + 1G > T) was identified. To study its potential pathogenicity, exons and flanking intronic sequences were amplified from patient DNA and cloned into the pSAD ((R)) splicing vector. HeLa cells were transfected with this construct and a wild-type minigene and functional analysis were performed. The construct with the c.5665 + 1G > T variant produced an aberrant transcript with an insert of 63 nucleotides of intron 28 creating a premature termination codon (TAG) 25 nucleotides downstream. This would lead to the insertion of 8 new amino acids and therefore a truncated 1896 amino acid protein. As a result of this, the patient was diagnosed with CHARGE syndrome. Functional analyses underline their usefulness for studying the pathogenicity of variants found by NGS and therefore its application to accurately diagnose patients.

Visser, M., et al. (2017). "Next-generation sequencing of a large gene panel in patients initially diagnosed with idiopathic ventricular fibrillation." Heart Rhythm **14**(7): 1035-1040.

BACKGROUND: Idiopathic ventricular fibrillation (IVF) is a rare primary cardiac arrhythmia syndrome that is diagnosed in a resuscitated cardiac arrest victim, with documented ventricular fibrillation, in whom no underlying cause is identified after comprehensive clinical evaluation. In some patients, causative genetic mutations are detected that facilitate patient treatment and follow-up. The feasibility of next-generation sequencing (NGS) has increased with its greater availability and decreasing costs. OBJECTIVE: The aim of this study was to assess the diagnostic yield of NGS in patients with IVF. METHODS: A total of 33 patients initially diagnosed with IVF were included (mean age 53 +/- 15 years; 14(42%) men). In all included patients, NGS of 33 genes and the DPP6 haplotype revealed no pathogenic mutations. Genetic screening comprised NGS of a panel of 179 additional genes. Variants with a minor allele frequency of <0.05% were assessed for pathogenicity by using existing mutation databases and in silico predictive algorithms. RESULTS: In 1 of 33 patients, a likely pathogenic mutation was detected. The added yield of genetic testing with NGS of 179 additional genes is 3% in patients with IVF. In 15% of patients, 1 or multiple variants of uncertain clinical significance were detected. CONCLUSION: The added yield of genetic screening of extended NGS panels in patients initially diagnosed with IVF is minimal. Routine analysis of large diagnostic NGS panels is therefore not recommended.

Vlenterie, M., et al. (2015). "Next generation sequencing in synovial sarcoma reveals novel gene mutations." Oncotarget **6**(33): 34680-34690.

Over 95% of all synovial sarcomas (SS) share a unique translocation, t (X;18), however, they show heterogeneous clinical behavior. We analyzed multiple SS to reveal additional genetic alterations besides the translocation. Twenty-six SS from 22 patients were sequenced for 409 cancer-related genes using the Comprehensive Cancer Panel (Life Technologies, USA) on an Ion Torrent platform. The detected variants were verified by Sanger sequencing and compared to matched normal DNAs. Copy number variation was assessed in six tumors using the Oncoscan array (Affymetrix, USA). In total, eight somatic mutations were detected in eight samples. These mutations have not been reported previously in SS. Two of these, in KRAS and CCND1, represent known oncogenic mutations in other malignancies. Additional mutations were detected in RNF213, SEPT9, KDR, CSMD3, MLH1 and ERBB4. DNA alterations occurred more often in adult tumors. A distinctive loss of 6q was found in a metastatic lesion progressing under pazopanib, but not in the responding lesion. Our results emphasize t (X;18) as a single initiating event in SS and as the main oncogenic driver. Our results also show the occurrence of additional genetic events, mutations or chromosomal aberrations, occurring more frequently in SS with an onset in adults.

Wang, H., et al. (2017). "Patterns of Gene Expression in Western Corn Rootworm (Diabrotica virgifera virgifera) Neonates, Challenged with Cry34Ab1, Cry35Ab1 and Cry34/35Ab1, Based on Next-Generation Sequencing." Toxins (Basel) **9**(4).

With Next Generation Sequencing technologies, high-throughput RNA sequencing (RNAseq) was conducted to examine gene expression in neonates of Diabrotica virgifera virgifera (LeConte) (Western Corn Rootworm, WCR) challenged with individual proteins of the binary Bacillus thuringiensis insecticidal proteins, Cry34Ab1 and Cry35Ab1, and the combination of Cry34/Cry35Ab1, which together are active against rootworm larvae. Integrated results of three different statistical comparisons identified 114 and 1300 differentially expressed transcripts (DETs) in the Cry34Ab1 and Cry34/35Ab1 treatment, respectively, as compared to the control. No DETs were identified in the Cry35Ab1 treatment. Putative Bt binding receptors previously identified in other insect species were not identified in DETs in this study. The majority of DETs (75% with Cry34Ab1 and 68.3% with Cry34/35Ab1 treatments) had no significant hits in the NCBI nr database. In addition, 92 DETs were shared between Cry34Ab1 and Cry34/35Ab1

treatments. Further analysis revealed that the most abundant DETs in both Cry34Ab1 and Cry34/35Ab1 treatments were associated with binding and catalytic activity. Results from this study confirmed the nature of these binary toxins against WCR larvae and provide a fundamental profile of expression pattern of genes in response to challenge of the Cry34/35Ab1 toxin, which may provide insight into potential resistance mechanisms.

Warren, M., et al. (2017). "Gene fusions PAFAH1B1-USP6 and RUNX2-USP6 in aneurysmal bone cysts identified by next generation sequencing." Cancer Genet **212-213**: 13-18.

Aneurysmal bone cyst (ABC) is a locally aggressive, expansile, typically multilocular cystic bone tumor. ABC was previously thought to be a non-neoplastic lesion; however, it is now considered to be neoplasm that features recurrent chromosomal translocations resulting in gene fusions between ubiquitin specific peptidase 6 (USP6) and multiple partners, including COL1A1, CDH11, TRAP150, ZNF90 and OMD. Using next generation sequencing (NGS), we uncovered two fusion partners of USP6 in two ABCs: platelet activating factor acetylhydrolase 1b regulatory subunit 1 (PAFAH1B1), which is known to contribute to tumorigenesis in lung cancer, and runt-related transcription factor 2 (RUNX2), which is known to regulate osteoblastic differentiation, osteosarcoma tumorigenesis and its metastasis. In our study, the PAFAH1B1-USP6 fusion consisted of the promoter of PAFAH1B1 fused to the 5'-untranslated region (5'-UTR) of USP6 and was discovered in a typical ABC. The RUNX2-USP6 fusion had the promoter and a short coding region of RUNX2 fused to the translation start codon of USP6 and was detected in an unusually aggressive ABC with an osteosarcoma-like soft tissue extension. Our findings not only expanded the repertoire of the partner genes of USP6 in ABC but also can serve as a reference for future studies to better understand the correlation between various gene fusions and the progression of ABC.

Yang, H. J., et al. (2015). "Vision from next generation sequencing: multi-dimensional genome-wide analysis for producing gene regulatory networks underlying retinal development, aging and disease." Prog Retin Eye Res **46**: 1-30.

Genomics and genetics have invaded all aspects of biology and medicine, opening uncharted territory for scientific exploration. The definition of "gene" itself has become ambiguous, and the central dogma is continuously being revised and expanded. Computational biology and computational medicine are no longer intellectual domains of the chosen few. Next generation sequencing (NGS) technology, together with novel methods of pattern recognition and network analyses, has revolutionized the way we think about fundamental biological mechanisms and cellular pathways. In this review, we discuss NGS-based genome-wide approaches that can provide deeper insights into retinal development, aging and disease pathogenesis. We first focus on gene regulatory networks (GRNs) that govern the differentiation of retinal photoreceptors and modulate adaptive response during aging. Then, we discuss NGS technology in the context of retinal disease and develop a vision for therapies based on network biology. We should emphasize that basic strategies for network construction and analyses can be transported to any tissue or cell type. We believe that specific and uniform guidelines are required for generation of genome, transcriptome and epigenome data to facilitate comparative analysis and integration of multi-dimensional data sets, and for constructing networks underlying complex biological processes. As cellular homeostasis and organismal survival are dependent on gene-gene and gene-environment interactions, we believe that network-based biology will provide the foundation for deciphering disease mechanisms and discovering novel drug targets for retinal neurodegenerative diseases.

Yang, M., et al. (2018). "Targeted Next-Generation Sequencing Reveals a Novel Frameshift Mutation in the MERTK Gene in a Chinese Family with Retinitis Pigmentosa." Genet Test Mol Biomarkers **22**(3): 165-169.

BACKGROUND: Retinitis pigmentosa (RP) is a group of inherited retinal diseases that result in severe progressive visual impairment. AIMS: The purpose of this article was to apply targeted next-generation sequencing (NGS) to identify the causative mutation in a Chinese RP family. METHODS: Blood samples were collected from a Chinese proband diagnosed with RP and her family members. A total of 163 genes that have been previously found to be involved in inherited retinal diseases were selected for NGS. Rigorous NGS data analysis; Sanger sequencing validation; and segregation analysis were applied to evaluate a novel frameshift mutation. RESULTS: Sequence analysis revealed that the proband and her affected sister both carried a novel homozygous frameshift mutation in MERTK (p.I103Nfs*4). Other family members carrying a heterozygous mutation were unaffected. This mutation was found to cosegregate with the disease phenotype in this family. This mutation was not found in 1,000 control individuals. CONCLUSIONS: The targeted NGS strategy employed provides an efficient tool for RP pathogenic gene detection. This study identified a new autosomal

recessive mutation in the RP-related gene MERTK, which expands the spectrum of RP disease-causing mutations.

Yang, Y., et al. (2015). "Targeted next generation sequencing reveals a novel intragenic deletion of the LAMA2 gene in a patient with congenital muscular dystrophy." Mol Med Rep **11**(5): 3687-3693.

Mutations in the LAMA2 gene cause laminin alpha2 (merosin) deficient congenital muscular dystrophies, which are autosomal recessive muscle disorders. Laminin alpha2 is widely expressed in the basement membrane of skeletal muscle, the myotendinous junctions and extrasynaptically at neuromuscular synapses. In the present study, target nextgeneration sequencing was used for mutation detection, and polymerase chain reaction (PCR) analysis and Sanger sequencing were used in the identification of small deletions. Subsequently, quantitative PCR (qPCR) was performed to characterize the identified deletion encompassing exon five of the LAMA2 gene. Two causative mutations were identified using target region sequencing which provided the additional information required to facilitate clinical diagnosis. One heterozygous mutation (p. Lys682LysfsX22) was identified and confirmed by Sanger sequencing, and another heterozygous mutation (Exon5del) was found and validated by qPCR. Cosegregation analysis indicated that the Exon5del mutation originated from the proband's mother and the previously reported frameshift mutation (p. Lys682LysfsX22) was inherited from the proband's father. To the best of our knowledge, the present study was the first to report an entire exon five deletion in the LAMA2 gene.

Youssef, O., et al. (2017). "Gene mutations in stool from gastric and colorectal neoplasia patients by next-generation sequencing." World J Gastroenterol **23**(47): 8291-8299.

AIM: To study cancer hotspot mutations by next-generation sequencing (NGS) in stool DNA from patients with different gastrointestinal tract (GIT) neoplasms. METHODS: Stool samples were collected from 87 Finnish patients diagnosed with various gastric and colorectal neoplasms, including benign tumors, and from 14 healthy controls. DNA was isolated from stools by using the PSP ((R)) Spin Stool DNA Plus Kit. For each sample, 20 ng of DNA was used to construct sequencing libraries using the Ion AmpliSeq Cancer Hotspot Panel v2 or Ion AmpliSeq Colon and Lung Cancer panel v2. Sequencing was performed on Ion PGM. Torrent Suite Software v.5.2.2 was used for variant calling and data analysis. RESULTS: NGS was successful in assaying 72 GIT samples and 13 healthy controls, with success rates of the assay being 78% for stomach neoplasia and 87% for colorectal tumors. In stool specimens from patients with gastric neoplasia, five hotspot mutations were found in APC, CDKN2A and EGFR genes, in addition to seven novel mutations. From colorectal patients, 20 mutations were detected in AKT1, APC, ERBB2, FBXW7, KIT, KRAS, NRAS, SMARCB1, SMO, STK11 and TP53. Healthy controls did not exhibit any hotspot mutations, except for two novel ones. APC and TP53 were the most frequently mutated genes in colorectal neoplasms, with five mutations, followed by KRAS with two mutations. APC was the most commonly mutated gene in stools of patients with premalignant/benign GIT lesions. CONCLUSION: Our results show that in addition to colorectal neoplasms, mutations can also be assayed from stool specimens of patients with gastric neoplasms.

Yuhui, X., et al. (2017). "Complete mitochondrial genomes from two species of Chinese freshwater crabs of the genus Sinopotamon recovered using next-generation sequencing reveal a novel gene order (Brachyura, Potamidae)." Zookeys (705): 41-60.

Recent morphological and molecular evidence has challenged classical interpretations of eubrachyuran phylogeny and evolution. Complete mitochondrial genomes of two species of potamid freshwater crabs, Sinopotamon yaanense and Sinopotamon yangtsekiense were obtained using next-generation sequencing. The results revealed a novel gene order with translocations of a five-gene block and a tRNA gene in comparison to available brachyuran mitochondrial genomes. DNA sequence comparisons position the Potamidae, a primary freshwater crab family, outside of the clade for the traditional heterotreme families, and closer to the clade that includes the thoracotreme families of grapsoid and ocypodoid crabs. Mitogenomic comparisons using rapid next-generation sequencing and a much wider taxonomic sample are required for a high-resolution examination of the phylogenetic relationships within the Eubrachyura.

Zacher, A., et al. (2017). "Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel." Brain Pathol **27**(2): 146-159.

Current classification of gliomas is based on histological criteria according to the World Health Organization (WHO) classification of tumors of the central nervous system. Over the past years, characteristic genetic profiles have been identified in various glioma types. These can refine tumor diagnostics and provide important prognostic and predictive information. We report on the establishment and validation of gene panel next generation

sequencing (NGS) for the molecular diagnostics of gliomas. We designed a glioma-tailored gene panel covering 660 amplicons derived from 20 genes frequently aberrant in different glioma types. Sensitivity and specificity of glioma gene panel NGS for detection of DNA sequence variants and copy number changes were validated by single gene analyses. NGS-based mutation detection was optimized for application on formalin-fixed paraffin-embedded tissue specimens including small stereotactic biopsy samples. NGS data obtained in a retrospective analysis of 121 gliomas allowed for their molecular classification into distinct biological groups, including (i) isocitrate dehydrogenase gene (IDH) 1 or 2 mutant astrocytic gliomas with frequent alpha-thalassemia/mental retardation syndrome X-linked (ATRX) and tumor protein p53 (TP53) gene mutations, (ii) IDH mutant oligodendroglial tumors with 1p/19q codeletion, telomerase reverse transcriptase (TERT) promoter mutation and frequent Drosophila homolog of capicua (CIC) gene mutation, as well as (iii) IDH wildtype glioblastomas with frequent TERT promoter mutation, phosphatase and tensin homolog (PTEN) mutation and/or epidermal growth factor receptor (EGFR) amplification. Oligoastrocytic gliomas were genetically assigned to either of these groups. Our findings implicate gene panel NGS as a promising diagnostic technique that may facilitate integrated histological and molecular glioma classification.

Zhang, H., et al. (2015). "A pilot study of gene testing of genetic bone dysplasia using targeted next-generation sequencing." J Hum Genet **60**(12): 769-776.

Molecular diagnosis of genetic bone dysplasia is challenging for non-expert. A targeted next-generation sequencing technology was applied to identify the underlying molecular mechanism of bone dysplasia and evaluate the contribution of these genes to patients with bone dysplasia encountered in pediatric endocrinology. A group of unrelated patients (n=82), characterized by short stature, dysmorphology and X-ray abnormalities, of which mucopolysacharidoses, GM1 gangliosidosis, mucolipidosis type II/III and achondroplasia owing to FGFR3 G380R mutation had been excluded, were recruited in this study. Probes were designed to 61 genes selected according to the nosology and classification of genetic skeletal disorders of 2010 by Illumina's online DesignStudio software. DNA was hybridized with probes and then a library was established following the standard Illumina protocols. Amplicon library was sequenced on a MiSeq sequencing system and the data were analyzed by MiSeq Reporter. Mutations of 13 different genes were found in 44 of the 82 patients (54%). Mutations of COL2A1 gene and PHEX gene were found in nine patients, respectively (9/44=20%), followed by COMP gene in 8 (18%), TRPV4 gene in 4 (9%), FBN1 gene in 4 (9%), COL1A1 gene in 3 (6%) and COL11A1, TRAPPC2, MATN3, ARSE, TRPS1, SMARCAL1, ENPP1 gene mutations in one patient each (2% each). In conclusion, mutations of COL2A1, PHEX and COMP gene are common for short stature due to bone dysplasia in outpatient clinics in pediatric endocrinology. Targeted next-generation sequencing is an efficient way to identify the underlying molecular mechanism of genetic bone dysplasia.

Zhao, J., et al. (2016). "Genome-wide gene-gene interaction analysis for next-generation sequencing." Eur J Hum Genet **24**(3): 421-428.

The critical barrier in interaction analysis for next-generation sequencing (NGS) data is that the traditional pairwise interaction analysis that is suitable for common variants is difficult to apply to rare variants because of their prohibitive computational time, large number of tests and low power. The great challenges for successful detection of interactions with NGS data are (1) the demands in the paradigm of changes in interaction analysis; (2) severe multiple testing; and (3) heavy computations. To meet these challenges, we shift the paradigm of interaction analysis between two SNPs to interaction analysis between two genomic regions. In other words, we take a gene as a unit of analysis and use functional data analysis techniques as dimensional reduction tools to develop a novel statistic to collectively test interaction between all possible pairs of SNPs within two genome regions. By intensive simulations, we demonstrate that the functional logistic regression for interaction analysis has the correct type 1 error rates and higher power to detect interaction than the currently used methods. The proposed method was applied to a coronary artery disease dataset from the Wellcome Trust Case Control Consortium (WTCCC) study and the Framingham Heart Study (FHS) dataset, and the early-onset myocardial infarction (EOMI) exome sequence datasets with European origin from the NHLBI's Exome Sequencing Project. We discovered that 6 of 27 pairs of significantly interacted genes in the FHS were replicated in the independent WTCCC study and 24 pairs of significantly interacted genes after applying Bonferroni correction in the EOMI study.

Zheng, B., et al. (2016). "Targeted next-generation sequencing identification of a novel missense mutation of the SKIV2L gene in a patient with trichohepatoenteric syndrome." Mol Med Rep **14**(3): 2107-2110.

Trichohepatoenteric syndrome (THES) is a rare autosomal, recessively inherited disorder. Mutations in the tetratricopeptide repeat domain 37 (TTC37) gene

and the superkiller viralicidic activity 2like (SKIV2L) gene have been identified to cause THES. The present study reported a case of a Chinese boy, who presented clinically with intrauterine growth retardation, intractable diarrhea, facial dysmorphism, abnormal scalp hair shafts, immune disorders and liver involvement. Targeted nextgeneration sequencing and Sanger DNA sequencing showed compound heterozygous mutations of the SKIV2L gene. The present study was the first, to the best of our knowledge, to report a case of a boy with THES resulting from compound heterozygous mutations of the SKIV2L gene in China. Target sequence capture combined with highthroughput nextgeneration sequencing technologies have shown to be effective methods for the molecular genetic assessment of rare inherited disorders.

Zheng, W., et al. (2016). "Genetic mapping and molecular marker development for Pi65(t), a novel broad-spectrum resistance gene to rice blast using next-generation sequencing." Theor Appl Genet **129**(5): 1035-1044.

KEY MESSAGE: A novel R gene was mapped to a locus on chromosome 11 from 30.42 to 30.85 Mb, which was proven to be efficient in the improvement of rice blast resistance. Rice blast is a devastating fungal disease worldwide. The use of blast resistance (R) genes is the most important approach to control the disease in rice breeding. In the present study, we finely mapped a novel resistance gene Pi65(t), conferring a broad-spectrum resistance to the fungus Magnaporthe oryzae, using bulked segregant analysis in combination with next-generation sequencing technology. Segregation in a doubled haploid (DH) population and a BC1F2 population suggested that resistance to blast in Gangyu129 was likely conferred by a single dominant gene, designated Pi65(t); it was located on chromosome 11 from 30.20 to 31.20 Mb using next-generation sequencing. After screening recombinants with newly developed molecular markers, the region was narrowed down to 0.43 Mb, flanked by SNP-2 and SNP-8 at the physical location from 30.42 to 30.85 Mb based on the Nipponbare reference database in build 5. Using the software QTL IciMapping, Pi65(t) was further mapped to a locus between InDel-1 and SNP-4 with genetic distances of 0.11 and 0.98 cM, respectively. Within this region, 4 predicted R genes were found with nucleotide binding site and leucine-rich repeat (NBS-LRR) domains. We developed molecular markers to genotype 305 DH lines and found that InDel-1 was closely linked with Pi65(t). Using InDel-1, a new rice variety Chuangxin1 containing Pi65(t) was developed, and it is highly resistant to rice blast and produces a high yield in Liaoning province of China. This indicated that Pi65(t) could play a key role in the improvement of rice blast resistance.

Zhong, C., et al. (2018). "Next-generation sequencing to identify candidate genes and develop diagnostic markers for a novel Phytophthora resistance gene, RpsHC18, in soybean." Theor Appl Genet **131**(3): 525-538.

KEY MESSAGE: A novel Phytophthora sojae resistance gene RpsHC18 was identified and finely mapped on soybean chromosome 3. Two NBS-LRR candidate genes were identified and two diagnostic markers of RpsHC18 were developed. Phytophthora root rot caused by Phytophthora sojae is a destructive disease of soybean. The most effective disease-control strategy is to deploy resistant cultivars carrying Phytophthora-resistant Rps genes. The soybean cultivar Huachun 18 has a broad and distinct resistance spectrum to 12 P. sojae isolates. Quantitative trait loci sequencing (QTL-seq), based on the whole-genome resequencing (WGRS) of two extreme resistant and susceptible phenotype bulks from an F2:3 population, was performed, and one 767-kb genomic region with DeltaSNP-index >/= 0.9 on chromosome 3 was identified as the RpsHC18 candidate region in Huachun 18. The candidate region was reduced to a 146-kb region by fine mapping. Nonsynonymous SNP and haplotype analyses were carried out in the 146-kb region among ten soybean genotypes using WGRS. Four specific nonsynonymous SNPs were identified in two nucleotide-binding sites-leucine-rich repeat (NBS-LRR) genes, RpsHC18-NBL1 and RpsHC18-NBL2, which were considered to be the candidate genes. Finally, one specific SNP marker in each candidate gene was successfully developed using a tetra-primer ARMS-PCR assay, and the two markers were verified to be specific for RpsHC18 and to effectively distinguish other known Rps genes. In this study, we applied an integrated genomic-based strategy combining WGRS with traditional genetic mapping to identify RpsHC18 candidate genes and develop diagnostic markers. These results suggest that next-generation sequencing is a precise, rapid and cost-effective way to identify candidate genes and develop diagnostic markers, and it can accelerate Rps gene cloning and marker-assisted selection for breeding of P. sojae-resistant soybean cultivars.

Zhou, Q., et al. (2015). "Identification of a novel heterozygous missense mutation in the CACNA1F gene in a chinese family with retinitis pigmentosa by next generation sequencing." Biomed Res Int **2015**: 907827.

BACKGROUND: Retinitis pigmentosa (RP) is an inherited retinal degenerative disease, which is clinically and genetically heterogeneous, and the

inheritance pattern is complex. In this study, we have intended to study the possible association of certain genes with X-linked RP (XLRP) in a Chinese family. METHODS: A Chinese family with RP was recruited, and a total of seven individuals were enrolled in this genetic study. Genomic DNA was isolated from peripheral leukocytes, and used for the next generation sequencing (NGS). RESULTS: The affected individual presented the clinical signs of XLRP. A heterozygous missense mutation (c.1555C>T, p.R519W) was identified by NGS in exon 13 of the CACNA1F gene on X chromosome, and was confirmed by Sanger sequencing. It showed perfect cosegregation with the disease in the family. The mutation at this position in the CACNA1F gene of RP was found novel by database searching. CONCLUSION: By using NGS, we have found a novel heterozygous missense mutation (c.1555C>T, p.R519W) in CACNA1F gene, which is probably associated with XLRP. The findings might provide new insights into the cause and diagnosis of RP, and have implications for genetic counseling and clinical management in this family.

Zhu, J., et al. (2017). "Rapid mapping and cloning of the virescent-1 gene in cotton by bulked segregant analysis-next generation sequencing and virus-induced gene silencing strategies." J Exp Bot **68**(15): 4125-4135.

Map-based gene cloning is a vital strategy for the identification of the quantitative trait loci or genes underlying important agronomic traits. The conventional map-based cloning method is powerful but generally time-consuming and labor-intensive. In this context, we introduce an improved bulked segregant analysis method in combination with a virus-induced gene silencing (VIGS) strategy for rapid and reliable gene mapping, identification and functional verification. This method was applied to a multiple recessive marker line of upland cotton, Texas 582 (T582), and identified unique genomic positions harboring mutant loci, showing the reliability and efficacy of this method. The v1 locus was further fine-mapped. Only one gene, GhCHLI, which encodes one of the subunits of Mg chelatase, was differentially down-regulated in T582 compared with TM-1. A point mutation occurred in the AAA+ conserved region of GhCHLI and led to an amino acid substitution. Suppression of its expression by VIGS in TM-1 resulted in a yellow blade phenotype that was similar to T582. This integrated approach provides a paradigm for the rapid mapping and identification of the candidate genes underlying the genetic traits in plants with large and complex genomes in the future.

Zrhidri, A., et al. (2017). "Next Generation Sequencing identifies mutations in GNPTG gene as a cause of familial form of scleroderma-like disease." Pediatr Rheumatol Online J **15**(1): 72.

BACKGROUND: Scleroderma is a multisystem disease, characterized by fibrosis of skin and internal organs, immune dysregulation, and vasculopathy. The etiology of the disease remains unknown, but it is likely multifactorial. However, the genetic basis for this condition is defined by multiple genes that have only modest effect on disease susceptibility. METHODS: Three Moroccan siblings, born from non-consanguineous Moroccan healthy parents were referred for genetic evaluation of familial scleroderma. Whole Exome Sequencing was performed in the proband and his parents, in addition to Sanger sequencing that was carried out to confirm the results obtained. RESULTS: Mutation analysis showed two compound heterozygous mutations c.196C>T in exon 4 and c.635_636delTT in exon 9 of GNPTG gene. Sanger sequencing confirmed these mutations in the affected patient and demonstrated that their parents are heterozygous carriers. CONCLUSION: Our findings expand the mutation spectrum of the GNPTG gene and extend the knowledge of the phenotype-genotype correlation of Mucolipidosis Type III gamma. This report also highlights the diagnostic utility of Next Generation Sequencing particularly when the clinical presentation did not point to specific genes.

Zumaraga, M. P., et al. (2017). "Targeted next generation sequencing of the entire vitamin D receptor gene reveals polymorphisms correlated with vitamin D deficiency among older Filipino women with and without fragility fracture." J Nutr Biochem **41**: 98-108.

This study aimed to discover genetic variants in the entire 101 kB vitamin D receptor (VDR) gene for vitamin D deficiency in a group of postmenopausal Filipino women using targeted next generation sequencing (TNGS) approach in a case-control study design. A total of 50 women with and without osteoporotic fracture seen at the Philippine Orthopedic Center were included. Blood samples were collected for determination of serum vitamin D, calcium, phosphorus, glucose, blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and as primary source for targeted VDR gene sequencing using the Ion Torrent Personal Genome Machine. The variant calling was based on the GATK best practice workflow and annotated using Annovar tool. A total of 1496 unique variants in the whole 101-kb VDR gene were identified. Novel sequence variations not registered in the dbSNP database were found among cases and controls at a rate of 23.1% and 16.6% of total discovered variants, respectively. One disease-associated enhancer showed statistically

significant association to low serum 25-hydroxy vitamin D levels (Pearson chi-square P-value=0.009). The transcription factor binding site prediction program PROMO predicted the disruption of three transcription factor binding sites in this enhancer region. These findings show the power of TNGS in identifying sequence variations in a very large gene and the surprising results obtained in this study greatly expand the catalog of known VDR sequence variants that may represent an important clue in the emergence of vitamin D deficiency. Such information will also provide the additional guidance necessary toward a personalized nutritional advice to reach sufficient vitamin D status.

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