Genetic Disease Review

An Overview of Publications Featuring Illumina® Technology

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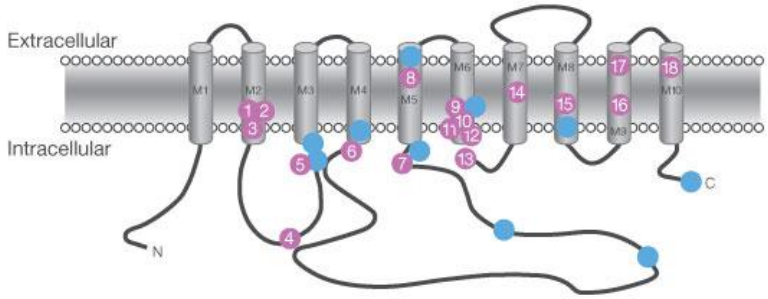
Introduction

The new generation of sequencing technology[1] has been remarkably successful in finding the causes of Mendelian and rare diseases. Mendelian studies require more than one unrelated affected individuals with disease or linkage evidence in at least one family. In recent studies next-generation sequencing (NGS) has also been used to identify causes of rare genetic conditions even when they are seen in only a single patient.[2,3] Complex diseases are caused by a combination of genetic, environmental and lifestyle factors. Some examples include Alzheimer's disease, scleroderma, asthma, Parkinson's disease, multiple sclerosis, osteoporosis, connective tissue diseases, kidney diseases, autoimmune diseases, and many more.[4,5] There is an increasing awareness that de-novo mutations may be the underlying cause of undiagnosed genetic conditions seen in the clinic. These patients typically present with a wide range of clinical features and remain undiagnosed by tools that are built on the assumption of a Mendelian disease.

NGS is creating significant interest as a tool that can objectively examine each patient's genome individually to find potentially causative mutations. This is ideal for the discovery of new mutations or investigation of high penetrance rare diseases, but it may also provide long-awaited breakthroughs to understanding complex diseases. In addition, it provides the benefit of a common, standardizable approach that can be used to address confusing clinical presentations.[6]

The ethical issues around genetic testing have been discussed extensively[7] and are out of the scope of this review. In short, a family history of disease reveals much about a patient's risk of disease, but the detailed nature of sequencing tests and the uncertainty of the interpretation raise concerns. As our understanding of genetic diseases improves and genetic testing becomes routine, it may well be possible to address those concerns so that patients can benefit from this remarkable technology.

To learn more about Illumina sequencing and microarray technologies, visit www.illumina.com.



One gene — two pathologies. *De novo mutations in ATP1A3 and encoded protein modifications are associated with alternating hemiplegia of childhood (magenta) and rapid-onset dystonia-parkinsonism (cyan). NGS is changing the way we see genetic diseases. Heinzen E. L. et al. (2012) Nat Genet. 44:1030-4.*

[1] Next Generation Sequencing (NGS) and Massively Parallel Sequencing (MPS) are often used interchangeably to refer to high throughput sequencing technologies. Sequencing by Synthesis (SBS) refers specifically to Illumina sequencing technology.
[2] Worthey, E. A., Mayer, A. N., Syverson, G. D., Helbling, D., Bonacci, B. B., et al. (2011) Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 13: 255-262
[3] Choi, M., Scholl, U. I., Ji, W., Liu, T., Tikhonova, I. R., et al. (2009) Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci U S A 106: 19096-19101
[4] Hunter, D. J. (2005) Gene-environment interactions in human diseases. Nat Rev Genet 6: 287-298
[5] Dempfle, A., Scherag, A., Hein, R., Beckmann, L., Chang-Claude, J., et al. (2008) Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. Eur J Hum Genet 16: 1164-1172
[6] Need, A. C., Shashi, V., Hitomi, Y., Schoch, K., Shianna, K. V., et al. (2012) Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49: 353-361
[7] Chadwick, R. (2011) Personal genomes: no bad news? Bioethics 25: 62-65

General Reviews

The following references are general reviews that provide an introduction to NGS and its potential use in diagnostics.

Bras, J., Guerreiro, R., and Hardy, J. (2012) Use of next-generation sequencing and other whole-genome strategies to dissect neurological disease. Nat Rev Neurosci 13: 453-464

This is an extensive overview of the successes and limitations of using NGS to study neurological diseases.

Green, E. D., Guyer, M. S., and National Human Genome Research, I. (2011) Charting a course for genomic medicine from base pairs to bedside. Nature 470: 204-213

This recent paper from National Institute of Health (NIH) represents a vision for the future of genomics. It describes the path and lists the imperatives towards an era of genomic medicine. The imperatives are making genomics-based diagnostics routine, defining the genetic components of disease, comprehensive characterization of cancer genomes, developing practical systems for clinical genomic informatics, and understanding the role of the human microbiome in health and disease.

Maxmen, A. (2011) Exome sequencing deciphers rare diseases. Cell 144: 635-637

This is a progress report from NIH's Undiagnosed Diseases Program. The program began delivering genomics to the clinic and has led to the diagnosis of 39 rare diseases. This is an indication of future clinical application of the technology. In many respects it can be seen as a model of how next-generation sequencing can be used to understand diseases that defy current clinical approaches.

Majewski, J., Schwartzentruber, J., Lalonde, E., Montpetit, A., and Jabado, N. (2011) What can exome sequencing do for you? J Med Genet 48: 580-589

This paper provides an overview of the current and future use of next generation sequencing as it relates to whole exome sequencing in human disease. The authors focus on technical capabilities, limitations and ethical issues.

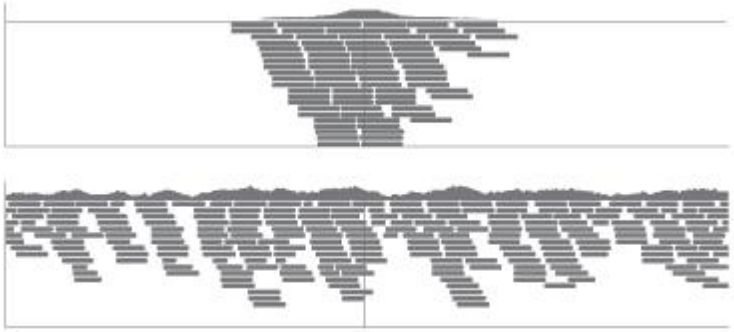
Categories of Disease

Rare and Mendelian Disease

Rare diseases are lifelong illnesses that may have a genetic component and impact fewer than 200,000 individuals in the United States.[8] In current clinical practice genetic testing is confirmatory and takes place after a clinical syndrome has been identified through discussion with the patient or parents.[9] In a typical case the suspected gene will be amplified and sequenced through Sanger sequencing. The result is a confident statement about the mutations present in the region sequenced. However, when suspected and common causes have to be eliminated first, it can lead to a lengthy diagnostic odyssey for patients with rare genetic diseases. The increasing awareness that rare genetic diseases may be caused by de novo mutations is profoundly changing our perception of these diseases.[10]

Mendelian diseases are usually defined as a mutation in a single gene that can cause a disease, which is inherited according to Mendel's laws. The Online Mendelian Inheritance in Man (OMIM) database contains a complete catalog of these genes and genetic disorders.[11]

Exome sequencing is proving to be an effective approach for identification of genetic defects in both rare and Mendelian diseases. It is estimated that 85% of the mutations that cause Mendelian diseases are located in the approximately 1% to 1.5% of the genome that comprise the exons.[12] In the context of rare or Mendelian diseases, exome sequencing has a high probability of identifying the underlying genetic cause. This approach should substantially increase the number of patients who receive a molecular diagnosis, even when the clinical presentation is ambiguous.[13]



The cost-effectiveness and deep coverage of the targeted regions in exome sequencing provide a simplified analysis, with a very high level of confidence in the results. Where the mutated regions do not lie within an exome, whole-genome sequencing provides an agnostic view of the whole genome. A comparison of whole-exome and whole-genome sequencing results is shown above. The upper panel shows whole-exome sequencing and the lower panel shows whole-genome sequencing of one exon of the amyloid precursor protein (APP) gene. Bras, J., et al. (2012). Nat Rev Neurosci 13:453-64.

[8] http://rarediseases.info.nih.gov
[9] Raffan, E. and Semple, R. K. (2011) Next generation sequencing--implications for clinical practice. Br Med Bull 99: 53-71
[10] Ku, C. S., Polychronakos, C., Tan, E. K., Naidoo, N., Pawitan, Y., et al. (2012) A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. Mol Psychiatry
[11] http://omim.org/
[12] Majewski, J., Schwartzentruber, J., Lalonde, E., Montpetit, A. and Jabado, N. (2011) What can exome sequencing do for you? J Med Genet 48: 580-589
[13] Maxmen, A. (2011) Exome sequencing deciphers rare diseases. Cell 144: 635-637

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Lines, M. A., Huang, L., Schwartzentruber, J., Douglas, S. L., Lynch, D. C., et al. (2012) Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly. Am J Hum Genet 90: 369-377

The authors used whole-exome sequencing of four unrelated affected individuals to identify heterozygous mutations of EFTUD2 in all four. The protein encoded by EFTUD2, is a highly conserved spliceosomal GTPase with a central regulatory role in the spliceosome.

Illumina Technology: HiSeq 2000 exome sequencing with >12 Gbp of 100 bp paired-end reads per sample.

Zankl, A., Duncan, E. L., Leo, P. J., Clark, G. R., Glazov, E. A., et al. (2012) Multicentric carpotarsal osteolysis is caused by mutations clustering in the amino-terminal transcriptional activation domain of MAFB. Am J Hum Genet 90: 494-501

The authors identified missense mutations clustering within a 51 bp region of the single exon of MAFB in five unrelated cases of multicentric carpotarsal osteolysis (MCTO). A further six unrelated simplex cases with MCTO were also heterozygous for previously unreported mutations within this same region, as were affected members of two families with autosomal-dominant MCTO. MAFB encodes a transcription factor that negatively regulates RANKL-induced osteoclastogenesis and is essential for normal renal development.

Illumina Technology: Genome Analyzer$_{II}$ exome sequencing with 56 bp paired-end reads.

Polvi, A., Linnankivi, T., Kivela, T., Herva, R., Keating, J. P., et al. (2012) Mutations in CTC1, encoding the CTS telomere maintenance complex component 1, cause cerebroretinal microangiopathy with calcifications and cysts. Am J Hum Genet 90: 540-549

The authors found recessively inherited compound heterozygous mutations in CTC1 in four unrelated individuals with cerebroretinal microangiopathy with calcifications and cysts (CRMCC). CTC1 encodes the CTS telomere maintenance complex component 1. Sanger sequencing revealed seven more compound heterozygous mutations in eight more unrelated affected individuals.

Illumina Technology: Genome Analyzer$_{IIx}$ exome sequencing with 100 bp paired ends.

Hood, R. L., Lines, M. A., Nikkel, S. M., Schwartzentruber, J., Beaulieu, C., et al. (2012) Mutations in SRCAP, encoding SNF2-related CREBBP activator protein, cause Floating-Harbor syndrome. Am J Hum Genet 90: 308-313

The authors identified heterozygous truncating mutations in SRCAP in five unrelated individuals with sporadic Floating-Harbor syndrome (FHS). Sanger sequencing identified mutations in SRCAP in eight more affected individuals. Mutations were de novo in all six instances in which parental DNA was available. SRCAP is an SNF2-related chromatin-remodeling factor that serves as a coactivator for CREB-binding protein (CREBBP, better known as CBP, the major cause of Rubinstein-Taybi syndrome (RTS)).

Illumina Technology: HiSeq 2000 exome sequencing with 35-40 Gb of 100 bp paired ends

Ostergaard, P., Simpson, M. A., Mendola, A., Vasudevan, P., Connell, F. C., et al. (2012) Mutations in KIF11 cause autosomal-dominant microcephaly variably associated with congenital lymphedema and chorioretinopathy. Am J Hum Genet 90: 356-362

Whole-exome sequencing revealed heterozygous KIF11 mutations in three individuals with a combination of microcephaly and lymphedema. Subsequent sequencing of KIF11 in a further 15 unrelated microcephalic probands with lymphedema and/or chorioretinopathy identified additional heterozygous mutations in 12 of them.

Illumina Technology: Genome Analyzer$_{IIx}$ exome sequencing with 76 bp paired ends.

Audo, I., Bujakowska, K., Orhan, E., Poloschek, C. M., Defoort-Dhellemmes, S., et al. (2012) Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 90: 321-330

Gibson, W. T., Hood, R. L., Zhan, S. H., Bulman, D. E., Fejes, A. P., et al. (2012) Mutations in EZH2 cause Weaver syndrome. Am J Hum Genet 90: 110-118

Huppke, P., Brendel, C., Kalscheuer, V., Korenke, G. C., Marquardt, I., et al. (2012) Mutations in SLC33A1 cause a lethal autosomal-recessive disorder with congenital cataracts, hearing loss, and low serum copper and ceruloplasmin. Am J Hum Genet 90: 61-68

Johnston, J. J., Gropman, A. L., Sapp, J. C., Teer, J. K., Martin, J. M., et al. (2012) The phenotype of a germline mutation in PIGA: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. Am J Hum Genet 90: 295-300

Jones, M. A., Ng, B. G., Bhide, S., Chin, E., Rhodenizer, D., et al. (2012) DDOST mutations identified by whole-exome sequencing are implicated in congenital disorders of glycosylation. Am J Hum Genet 90: 363-368

Lee, H., Graham, J. M., Jr., Rimoin, D. L., Lachman, R. S., Krejci, P., et al. (2012) Exome sequencing identifies PDE4D mutations in acrodysostosis. Am J Hum Genet 90: 746-751

Lim, Y. M., Koh, I., Park, Y. M., Kim, J. J., Kim, D. S., et al. (2012) Exome sequencing identifies KIAA1377 and C5orf42 as susceptibility genes for monomelic amyotrophy. Neuromuscul Disord 22: 394-400

Michot, C., Le Goff, C., Goldenberg, A., Abhyankar, A., Klein, C., et al. (2012) Exome sequencing identifies PDE4D mutations as another cause of acrodysostosis. Am J Hum Genet 90: 740-745

Sorte, H., Morkrid, L., Rodningen, O., Kulseth, M. A., Stray-Pedersen, A., et al. (2012) Severe ALG8-CDG (CDG-Ih) associated with homozygosity for two novel missense mutations detected by exome sequencing of candidate genes. Eur J Med Genet 55: 196-202

Velinov, M., Dolzhanskaya, N., Gonzalez, M., Powell, E., Konidari, I., et al. (2012) Mutations in the gene DNAJC5 cause autosomal dominant Kufs disease in a proportion of cases: study of the Parry family and 8 other families. PLoS ONE 7: e29729

Complex Disease

The vast majority of genetic diseases fall into this category. Some examples include Alzheimer's disease, scleroderma, asthma, Parkinson's disease, multiple sclerosis, osteoporosis, connective tissue diseases, kidney diseases, autoimmune diseases, and many more.[14,15,16] There are complete databases that catalog genome-wide association studies (GWAS) on these diseases.[17,18] For the most part these complex diseases are caused by a combination of genetic, environmental, and lifestyle factors. The study and treatment of these diseases should take all these contributing factors into account.

NGS offers a comprehensive set of tools to study these complex diseases. Whole-genome and exome-sequencing can be combined with transcriptome sequencing (RNA-Seq) to assess expression levels and the expression of mutated transcripts and splice variants. The combination of these tools provides a holistic approach to studying these complex diseases.

Reviews:

Bras, J., Guerreiro, R. and Hardy, J. (2012) Use of next-generation sequencing and other whole-genome strategies to dissect neurological disease. Nat Rev Neurosci 13: 453-464

Casals, F., Idaghdour, Y., Hussin, J. and Awadalla, P. (2012) Next-generation sequencing approaches for genetic mapping of complex diseases. J Neuroimmunol 248: 10-22

Ku, C. S., Cooper, D. N., Wu, M., Roukos, D. H., Pawitan, Y., et al. (2012) Gene discovery in familial cancer syndromes by exome sequencing: prospects for the elucidation of familial colorectal cancer type X. Mod Pathol 25: 1055-1068

References:

Tennessen, J. A., Bigham, A. W., O'Connor, T. D., Fu, W., Kenny, E. E., et al. (2012) Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 337: 64-69

In this study the authors sequenced 15,585 genes in 2,440 individuals of European and African ancestry. The majority of the over 500,000 SNV are rare (86% with a minor allele frequency <0.5%), novel (82%), and population-specific (82%). On average, ~95.7% of SNVs predicted to be functionally important were rare.

Illumina Technology: Genome Analyzer$_{IIx}$ or HiSeq 2000 using either paired-end 76 base or 50 base runs.

[14] Hunter, D. J. (2005) Gene-environment interactions in human diseases. Nat Rev Genet 6: 287-298
[15] Dempfle, A., Scherag, A., Hein, R., Beckmann, L., Chang-Claude, J., et al. (2008) Gene-environment interactions for complex traits: definitions, methodological requirements and challenges. Eur J Hum Genet 16: 1164-1172
[16] Johnson, A. D. and O'Donnell, C. J. (2009) An open access database of genome-wide association results. BMC Med Genet 10: 6
[17] http://www.genome.gov/gwastudies/
[18] https://www.gwascentral.org/

Kiezun, A., Garimella, K., Do, R., Stitziel, N. O., Neale, B. M., et al. (2012) Exome sequencing and the genetic basis of complex traits. Nat Genet 44: 623-630

The authors analyze exome sequencing data from 438 individuals and use this as a basis to review processing and quality control of raw sequence data, as well as evaluate the statistical properties of exome sequencing studies. They conclude that enthusiasm for exome sequencing studies to identify the genetic basis of complex traits should be combined with caution stemming from the observation that on the order of over 10,000 samples may be required to reach sufficient statistical power.

Illumina Technology: Genome Analyzer exome sequencing.

Genome-Wide Association Studies (GWAS)

GWAS are designed to detect associations between common single-nucleotide polymorphisms (SNPs) in common complex diseases such as heart disease, diabetes, autoimmune diseases, and psychiatric disorders.[19] GWAS have led to many scientific and biological discoveries but have failed to explain the bulk of the heritability.[20,21] The assumption that common risk variants can explain the vast majority of genetic heritability for any human disease, either individually or collectively, may not adequately describe the complexity of these diseases. The large cohorts and the rigorous statistical analysis that was developed for GWAS will facilitate future studies with new technologies such as NGS. The sequencing of entire genomes in large cohorts at affordable prices is likely to generate additional genes, pathways, and biological insights, as well as the potential to identify causal mutations.[22]

Disease or trait	% Variance explained by all GWAS SNPs combined
Type 1 diabetes	60 (Includes pre-GWAS loci with large effects.)
Type 2 diabetes	5-10
Obesity (BMI)	1-2
Crohn's disease	10
Ulcerative colitis	5
Multiple sclerosis	10
Ankylosing spondylitis	20
Schizophrenia	1
Bipolar disorder	2
Breast cancer	8
Von Willebrand factor	13
Height	10
Bone mineral density	5
QT interval	7
HDL cholesterol	10
Platelet count	5-10

Visscher, P. M., Brown, M. A., McCarthy, M. I. and Yang, J. (2012) Five years of GWAS discovery. Am J Hum Genet 90: 7-24

[19] A Catalog of Published Genome-Wide Association Studies. Available at: www.genome.gov/gwastudies
[20] McClellan, J. and King, M. C. (2010) Genetic heterogeneity in human disease. Cell 141: 210-217
[21] Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., et al. (2009) Finding the missing heritability of complex diseases. Nature 461: 747-753
[22] Visscher, P. M., Brown, M. A., McCarthy, M. I. and Yang, J. (2012) Five years of GWAS discovery. Am J Hum Genet 90: 7-24

Reviews:

Krueger, F., Kreck, B., Franke, A. and Andrews, S. R. (2012) DNA methylome analysis using short bisulfite sequencing data. Nat Methods 9: 145-151

Visscher, P. M., Brown, M. A., McCarthy, M. I. and Yang, J. (2012) Five years of GWAS discovery. Am J Hum Genet 90: 7-24

Cirulli, E. T. and Goldstein, D. B. (2010) Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 11: 415-425

Ku, C. S., Loy, E. Y., Pawitan, Y. and Chia, K. S. (2010) The pursuit of genome-wide association studies: where are we now? J Hum Genet 55: 195-206

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International Stroke Genetics, C., Wellcome Trust Case Control, C., Bellenguez, C., Bevan, S., Gschwendtner, A., et al. (2012) Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet 44: 328-333

Manning, A. K., Hivert, M. F., Scott, R. A., Grimsby, J. L., Bouatia-Naji, N., et al. (2012) A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet 44: 659-669

Sasayama, D., Hiraishi, A., Tatsumi, M., Kamijima, K., Ikeda, M., et al. (2012) Possible association of CUX1 gene polymorphisms with antidepressant response in major depressive disorder. Pharmacogenomics J

Sobrin, L., Ripke, S., Yu, Y., Fagerness, J., Bhangale, T. R., et al. (2012) Heritability and Genome-Wide Association Study to Assess Genetic Differences between Advanced Age-Related Macular Degeneration Subtypes. Ophthalmology 119:1874-85

Sun, L., Rommens, J. M., Corvol, H., Li, W., Li, X., et al. (2012) Multiple apical plasma membrane constituents are associated with susceptibility to meconium ileus in individuals with cystic fibrosis. Nat Genet 44: 562-569

Mitochondrial Disease

Mitochondrial diseases are caused by abnormal functioning of mitochondria. To date more than 200 different molecular defects have been described in patients with mitochondrial diseases.[23] These abnormalities may be the result of spontaneous or inherited mutations in the mitochondrial genome (mtDNA) or in nuclear genes that code for mitochondrial components. The mtDNA encodes only 13 proteins of the respiratory chain, while most of the estimated 1,500 mitochondrial proteins are nuclear-encoded. Mitochondrial deficiencies often affect multiple tissues leading to multi-system diseases that present with many phenotypic features.[24] Those characteristics make these diseases notoriously difficult to diagnose because of the multitude of candidate genes and the highly variable nature of the clinical presentation.[25]

Targeted NGS is a very effective approach to sequence mitochondrial genomes. This allows deep sequencing that can also detect low levels of heteroplasmy.

Reviews:

Chinnery, P. F., Elliott, H. R., Hudson, G., Samuels, D. C. and Relton, C. L. (2012) Epigenetics, epidemiology and mitochondrial DNA diseases. Int J Epidemiol 41: 177-187

References:

Calvo, S. E., Compton, A. G., Hershman, S. G., Lim, S. C., Lieber, D. S., et al. (2012) Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. Sci Transl Med 4: 118ra110

The authors performed "MitoExome" sequencing of the mitochondrial DNA (mtDNA) and exons of ~1000 nuclear genes encoding mitochondrial proteins. In 42 unrelated infants with clinical and biochemical evidence of mitochondrial oxidative phosphorylation disease, the investigators were able to establish firm diagnoses in 10 patients (24%) who had mutations in genes previously linked to disease. Thirteen patients (31%) had mutations in nuclear genes not previously linked to disease.

Illumina Technology: Genome Analyzer II exome sequencing with 76 bp paired-end reads.

Mayr, J. A., Haack, T. B., Graf, E., Zimmermann, F. A., Wieland, T., et al. (2012) Lack of the mitochondrial protein acylglycerol kinase causes Sengers syndrome. Am J Hum Genet 90: 314-320

Gunnarsdottir, E. D., Li, M., Bauchet, M., Finstermeier, K. and Stoneking, M. (2011) High-throughput sequencing of complete human mtDNA genomes from the Philippines. Genome Res 21: 1-11

Mayr, J. A., Zimmermann, F. A., Fauth, C., Bergheim, C., Meierhofer, D., et al. (2011) Lipoic acid synthetase deficiency causes neonatal-onset epilepsy, defective mitochondrial energy metabolism, and glycine elevation. Am J Hum Genet 89: 792-797

Pierson, T. M., Adams, D., Bonn, F., Martinelli, P., Cherukuri, P. F., et al. (2011) Whole-exome sequencing identifies homozygous AFG3L2 mutations in a spastic ataxia-neuropathy syndrome linked to mitochondrial m-AAA proteases. PLoS Genet 7: e1002325

[23] Chinnery, P. F., Elliott, H. R., Hudson, G., Samuels, D. C. and Relton, C. L. (2012) Epigenetics, epidemiology and mitochondrial DNA diseases. Int J Epidemiol 41: 177-187
[24] Scharfe, C., Lu, H. H., Neuenburg, J. K., Allen, E. A., Li, G. C., et al. (2009) Mapping gene associations in human mitochondria using clinical disease phenotypes. PLoS Comput Biol 5: e1000374
[25] Calvo, S. E., Compton, A. G., Hershman, S. G., Lim, S. C., Lieber, D. S., et al. (2012) Molecular diagnosis of infantile mitochondrial disease with targeted next-generation sequencing. Sci Transl Med 4: 118ra110

Heteroplasmy

Most eukaryotic cells contain many hundreds of mitochondria with hundreds of copies of mtDNA. Heteroplasmy occurs when mutations occur only in some copies while the remainder is unaffected. Heteroplasmy may play an important role mitochondrial diseases because it can modulate the severity of the diseases when only a fraction of the mitochondria is impacted. Deep coverage with NGS can readily detect even low levels of heteroplasmy. Extensive use of the technology has shown that heteroplasmy is much more common than previously appreciated.[26]

References:

Guo, Y., Cai, Q., Samuels, D. C., Ye, F., Long, J., et al. (2012) The use of next generation sequencing technology to study the effect of radiation therapy on mitochondrial DNA mutation. Mutat Res 744: 154-160

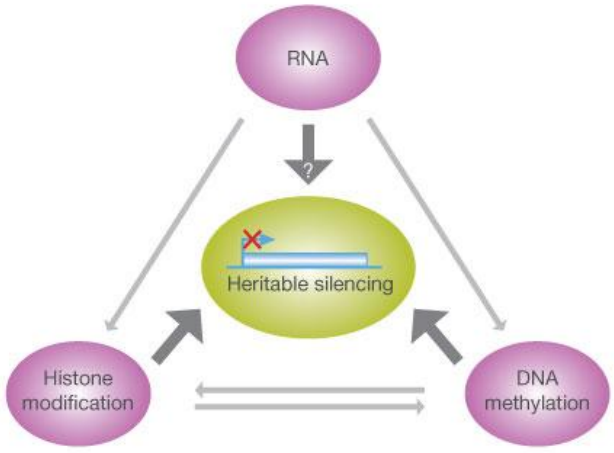
Grant, S. F., Glessner, J. T., Bradfield, J. P., Zhao, J., Tirone, J. E., et al. (2012) Lack of relationship between mitochondrial heteroplasmy or variation and childhood obesity. Int J Obes (Lond) 36: 80-83

Sondheimer, N., Glatz, C. E., Tirone, J. E., Deardorff, M. A., Krieger, A. M., et al. (2011) Neutral mitochondrial heteroplasmy and the influence of aging. Hum Mol Genet 20: 1653-1659

[26] He, Y., Wu, J., Dressman, D. C., Iacobuzio-Donahue, C., Markowitz, S. D., et al. (2010) Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. Nature 464: 610-614

Epigenetics and Imprinting Disease

Epigenetics refers to changes in the genome function, without changes in the sequence of the genome. Abnormalities in these epigenetic mechanisms have been linked to a wide range of diseases such as Prader–Willi syndrome, Angelman's syndrome, Rett syndrome, Rubinstein–Taybi syndrome and Coffin–Lowry syndrome.[27,28]



Interaction between RNA, histone modification, and DNA methylation in heritable silencing.

DNA methylation

The arrival of NGS technologies has led to a number of DNA methylome studies at a single base resolution.[29] DNA methylation occurs predominantly at CpG dinucleotides in the differentiated human genome. Embryonic stem cells may use different DNA methylation mechanisms in transcriptional regulation to maintain their pluripotency.[30]

Histone modifications

The development of ChIP-Seq with NGS enabled the first genome-wide mapping of histone modifications. This allowed the identification of activation marks such as mono-methylations of H3K27, H3K9, H4K20, H3K79, and H2BK.[31] The epigenetic control of expression for both PolI and PolII has been mapped.[32] These are examples of the information that can be obtained using this approach.

[27] Portela, A. and Esteller, M. (2010) Epigenetic modifications and human disease. Nat Biotechnol 28: 1057-1068
[28] Egger, G., Liang, G., Aparicio, A. and Jones, P. A. (2004) Epigenetics in human disease and prospects for epigenetic therapy. Nature 429: 457-463
[29] Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., et al. (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462: 315-322
[30] Lister, R., Pelizzola, M., Kida, Y. S., Hawkins, R. D., Nery, J. R., et al. (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471: 68-73
[31] Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., et al. (2007) High-resolution profiling of histone methylations in the human genome. Cell 129: 823-837
[32] Barski, A., Chepelev, I., Liko, D., Cuddapah, S., Fleming, A. B., et al. (2010) Pol II and its associated epigenetic marks are present at Pol III-transcribed noncoding RNA genes. Nat Struct Mol Biol 17: 629-634

Reviews:

Krueger, F., Kreck, B., Franke, A. and Andrews, S. R. (2012) DNA methylome analysis using short bisulfite sequencing data. Nat Methods 9: 145-151

Ku, C. S., Naidoo, N., Wu, M. and Soong, R. (2011) Studying the epigenome using next generation sequencing. J Med Genet 48: 721-730

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Jones, W. D., Dafou, D., McEntagart, M., Woollard, W. J., Elmslie, F. V., et al. (2012) De Novo Mutations in MLL Cause Wiedemann-Steiner Syndrome. Am J Hum Genet 91: 358-364

The authors identified de novo mutations in MLL in five of the six individuals with hypertrichosis cubiti. This condition is associated with short stature, intellectual disability, and a distinctive facial appearance, consistent with a diagnosis of Wiedemann-Steiner syndrome. MLL encodes a histone methyltransferase that regulates chromatin-mediated transcription through the catalysis of methylation of histone H3K4. Each of the five mutations is predicted to result in premature termination of the protein product.

Illumina Technology: HiSeq 2000 exome sequencing with 100 bp paired-end reads.

Simpson, M. A., Deshpande, C., Dafou, D., Vissers, L. E., Woollard, W. J., et al. (2012) De novo mutations of the gene encoding the histone acetyltransferase KAT6B cause Genitopatellar syndrome. Am J Hum Genet 90: 290-294

The authors found de novo mutations of KAT6B in five individuals with Genitopatellar Syndrome (GPS). KAT6B encodes a member of the MYST family of histone acetyltranferases. The authors demonstrate a reduced level of both histone H3 and H4 acetylation in patient-derived cells suggesting that dysregulation of histone acetylation is a direct functional consequence of GPS alleles.

Illumina Technology: Genome Analyzer$_{IIx.}$

Campeau, P. M., Kim, J. C., Lu, J. T., Schwartzentruber, J. A., Abdul-Rahman, O. A., et al. (2012) Mutations in KAT6B, encoding a histone acetyltransferase, cause Genitopatellar syndrome. Am J Hum Genet 90: 282-289

Freson, K., Izzi, B. and Van Geet, C. (2012) From genetics to epigenetics in platelet research. Thrombosis research 129: 325-329

Gordon, L., Joo, J. E., Powell, J. E., Ollikainen, M., Novakovic, B., et al. (2012) Neonatal DNA methylation profile in human twins is specified by a complex interplay between intrauterine environmental and genetic factors, subject to tissue-specific influence. Genome Res 22: 1395-1406

Li, J., Harris, R. A., Cheung, S. W., Coarfa, C., Jeong, M., et al. (2012) Genomic hypomethylation in the human germline associates with selective structural mutability in the human genome. PLoS Genet 8: e1002692

Meng, L., Person, R. E. and Beaudet, A. L. (2012) Ube3a-ATS is an atypical RNA polymerase II transcript that represses the paternal expression of Ube3a. Hum Mol Genet 21: 3001-3012

Radford, E. J., Isganaitis, E., Jimenez-Chillaron, J., Schroeder, J., Molla, M., et al. (2012) An unbiased assessment of the role of imprinted genes in an intergenerational model of developmental programming. PLoS Genet 8: e1002605

Teichroeb, J. H., Betts, D. H. and Vaziri, H. (2011) Suppression of the imprinted gene NNAT and X-chromosome gene activation in isogenic human iPS cells. PLoS ONE 6: e23436

Bell, C. G. and Beck, S. (2010) The epigenomic interface between genome and environment in common complex diseases. Brief Funct Genomics 9: 477-485

Kong, A., Steinthorsdottir, V., Masson, G., Thorleifsson, G., Sulem, P., et al. (2009) Parental origin of sequence variants associated with complex diseases. Nature 462: 868-874

Undiagnosed Genetic Disease

It is estimated that up to half of the patients tested currently receive no molecular diagnosis.[33] The remarkable success of the National Institute of Health (NIH) Undiagnosed Diseases Program, which has led to the diagnosis of 39 rare diseases and the identification of two new diseases, has demonstrated the utility of whole-genome and whole-exome sequencing in the clinic. Patients with undiagnosed genetic conditions tend to present with a wide range of clinical features and it is often necessary to consider each patient's genome individually, rather than looking for common disrupted genes in multiple cases with a similar phenotype. By using this approach Need and colleagues achieved a likely genetic diagnosis in six of 12 previously undiagnosed probands.[34] While many issues must still be addressed before next-generation can become a routine part of a clinical laboratory, the results so far indicate that it has the potential to become a powerful diagnostic tool for genetic diseases.

Step	Filter
1	homozygous (including hemizygous X variants) in the proband and never homozygous in the controls (recessive and X-linked variants)
2	heterozygous in the proband and absent in the parents and controls (putative de novo variants)
3	Two rare (MAF<0.03) variants in the proband that were not seen together in the parents or in any controls (compound heterozygotes).

The above table shows how variants were prioritized to identify highly penetrant genotypes that might account for each child's conditions. Need, A. C., et al. (2012) J Med Genet 49: 353-361

Reviews:

Raffan, E. and Semple, R. K. (2011) Next generation sequencing--implications for clinical practice. Br Med Bull 99: 53-71

This paper provides a general overview of some of the key NGS technologies. The authors go on to discuss areas of agreement and controversy between the new technologies and established clinical practices. They also outline issues that must be addressed before the new technologies are to become a mature part of the diagnostic repertoire. The review also includes a table of unexpected genetic defects that were discovered through NGS.

[33] Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., et al. (2010) Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 42: 30-35
[34] Need, A. C., Shashi, V., Hitomi, Y., Schoch, K., Shianna, K. V., et al. (2012) Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49: 353-361

References:

Need, A. C., Shashi, V., Hitomi, Y., Schoch, K., Shianna, K. V., et al. (2012) Clinical application of exome sequencing in undiagnosed genetic conditions. J Med Genet 49: 353-361

The authors report the results of a pilot program of whole-exome sequencing on 12 patients with unexplained and apparent genetic conditions, along with their unaffected parents. The patients previously tested negative in a micro-array based assay. This undertaking resulted in a likely genetic diagnosis in 6 of the 12 probands, including the identification of apparently causal mutations in four genes known to cause Mendelian disease. This study provides evidence that NGS can have high success rates in a clinical setting, but also highlights key challenges. It further suggests that the presentation of known Mendelian conditions may be considerably broader than currently recognized. The authors conclude that NGS should be strongly considered in all cases where a genetic condition is strongly suspected but traditional clinical genetic testing has proven negative. Furthermore, in some cases it is likely that NGS will prove faster and less expensive than the long diagnostic odyssey many families now endure.

Illumina Technology: HiSeq 2000.

Dias, C., Sincan, M., Cherukuri, P. F., Rupps, R., Huang, Y., et al. (2012) An analysis of exome sequencing for diagnostic testing of the genes associated with muscle disease and spastic paraplegia. Hum Mutat 33: 614-626

Leidenroth, A., Sorte, H. S., Gilfillan, G., Ehrlich, M., Lyle, R., et al. (2012) Diagnosis by sequencing: correction of misdiagnosis from FSHD2 to LGMD2A by whole-exome analysis. Eur J Hum Genet 20: 999-1003

Selmer, K. K., Gilfillan, G. D., Stromme, P., Lyle, R., Hughes, T., et al. (2012) A mild form of Mucopolysaccharidosis IIIB diagnosed with targeted next-generation sequencing of linked genomic regions. Eur J Hum Genet 20: 58-63

Maxmen, A. (2011) Exome sequencing deciphers rare diseases. Cell 144: 635-637

Reproductive Health

Carrier Screening

Carrier screening involves the identification of unaffected individuals who carry one copy of a dysfunctional gene for a disease that requires two dysfunctional copies for the disease to be expressed. Mendelian diseases account for approximately 20% of infant mortality and ~10% of pediatric hospitalizations.[35] Preconception screening, together with genetic counseling of carriers, has resulted in remarkable declines in the incidence of several severe recessive diseases such as Tay-Sachs disease.[36]

There are several approaches for carrier screening, from screening a small set of markers to whole exome sequencing. Bell et al. showed that sequencing a targeted set of 437 genes is a cost effective approach with excellent sensitivity and specificity.[37]

Reviews:

Jackson, L. and Pyeritz, R. E. (2011) Molecular technologies open new clinical genetic vistas. Sci Transl Med 3: 65ps62

Grody, W. W. (2011) Expanded carrier screening and the law of unintended consequences: from cystic fibrosis to fragile X. Genet Med 13: 996-997

References:

Johnston, J. J., Gropman, A. L., Sapp, J. C., Teer, J. K., Martin, J. M., et al. (2012) The phenotype of a germline mutation in PIGA: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. Am J Hum Genet 90: 295-300

This is a targeted resequencing study of a rare disease called paroxysmal nocturnal hemoglobinuria for all exons on the X chromosome. This rare disease was found in a single pedigree and the female carrier individual was subject to targeted resequencing screening.

Illumina Technology: Genome Analyzer 107x for the X exome.

Bell, C. J., Dinwiddie, D. L., Miller, N. A., Hateley, S. L., Ganusova, E. E., et al. (2011) Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 3: 65ra64

The authors report a preconception carrier screen for 448 severe recessive childhood diseases. The screen uses NGS sequencing of 7717 regions from 437 target genes. This approach yields a 160-fold average target coverage and mutation detection/genotyping, had ~95% sensitivity, and ~100% specificity for substitution, insertion/deletion, splicing, gross deletion mutations and SNPs. This targeted screen represents a cost-effective approach to screen for severe recessive childhood disorders.

Illumina Technology: Genome Analyzer$_{IIx}$ 50 bp reads and HiSeq 150 bp sequencing libraries.

[35] Kumar, P., Radhakrishnan, J., Chowdhary, M. A. and Giampietro, P. F. (2001) Prevalence and patterns of presentation of genetic disorders in a pediatric emergency department. Mayo Clin Proc 76: 777-783
[36] Kaback, M. M. (2000) Population-based genetic screening for reproductive counseling: the Tay-Sachs disease model. Eur J Pediatr 159 Suppl 3: S192-195
[37] Bell, C. J., Dinwiddie, D. L., Miller, N. A., Hateley, S. L., Ganusova, E. E., et al. (2011) Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 3: 65ra64

Bolton, K. L., Chenevix-Trench, G., Goh, C., Sadetzki, S., Ramus, S. J., et al. (2012) Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 307: 382-390

Prenatal Diagnostics

Prenatal diagnosis refers to testing for diseases or conditions in a fetus or embryo before it is born in order to detect birth defects. Traditionally, this has been done through invasive procedures such as amniocentesis. The observation that cell free DNA, present in the plasma of pregnant women, represents the complete genome of the fetus creates the opportunity for non-invasive genetic screens.[38,39] One of the first applications was the use of NGS to determine fetal chromosomal aneuploidy.[40,41] The initial findings were replicated in larger datasets and different cohorts.[42,43,44,45] It can be expected that this approach will extend beyond trisomy to other areas such as Rhesus disease.[46]

It is possible to non-invasively sequence the entire prenatal genome in the first and second trimester and in the absence of DNA from the father. Fan et al. Nature 487:320-4. 2012

[38] Lo, Y. M., Chan, K. C., Sun, H., Chen, E. Z., Jiang, P., et al. (2010) Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci Transl Med 2: 61ra91
[39] Fan, H. C., Blumenfeld, Y. J., Chitkara, U., Hudgins, L. and Quake, S. R. (2010) Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. Clin Chem 56: 1279-1286
[40] Chiu, R. W., Chan, K. C., Gao, Y., Lau, V. Y., Zheng, W., et al. (2008) Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc Natl Acad Sci U S A 105: 20458-20463
[41] Fan, H. C., Blumenfeld, Y. J., Chitkara, U., Hudgins, L. and Quake, S. R. (2008) Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. Proc Natl Acad Sci U S A 105: 16266-16271
[42] Ehrich, M., Deciu, C., Zwiefelhofer, T., Tynan, J. A., Cagasan, L., et al. (2011) Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am J Obstet Gynecol 204: 205 e201-211
[43] Chiu, R. W., Sun, H., Akolekar, R., Clouser, C., Lee, C., et al. (2010) Maternal plasma DNA analysis with massively parallel sequencing by ligation for noninvasive prenatal diagnosis of trisomy 21. Clin Chem 56: 459-463
[44] Fan, H. C. and Quake, S. R. (2010) Sensitivity of noninvasive prenatal detection of fetal aneuploidy from maternal plasma using shotgun sequencing is limited only by counting statistics. PLoS ONE 5: e10439
[45] Chu, T., Bunce, K., Hogge, W. A. and Peters, D. G. (2010) Statistical considerations for digital approaches to non-invasive fetal genotyping. Bioinformatics 26: 2863-2866
[46] Moise, K. J., Jr. (2008) Management of rhesus alloimmunization in pregnancy. Obstet Gynecol 112: 164-176

Reviews:

Chitty, L. S., Hill, M., White, H., Wright, D. and Morris, S. (2012) Noninvasive prenatal testing for aneuploidy-ready for prime time? Am J Obstet Gynecol 206: 269-275

Jackson, L. and Pyeritz, R. E. (2011) Molecular technologies open new clinical genetic vistas. Sci Transl Med 3: 65ps62

Evans, M. I. and Kilpatrick, M. (2010) Noninvasive prenatal diagnosis: 2010. Clin Lab Med 30: 655-665

Lee, C. (2010) The future of prenatal cytogenetic diagnostics: a personal perspective. Prenat Diagn 30: 706-709

Chiu, R. W., Cantor, C. R. and Lo, Y. M. (2009) Non-invasive prenatal diagnosis by single molecule counting technologies. Trends Genet 25: 324-331

References:

Fan, H. C., Gu, W., Wang, J., Blumenfeld, Y. J., El-Sayed, Y. Y., et al. (2012) Non-invasive prenatal measurement of the fetal genome. Nature 487: 320-324

The authors demonstrate that it is possible to non-invasively sequence the entire prenatal genome, in the first and second trimester and in the absence of DNA from the father. They also use exome sequencing to detect clinically relevant and deleterious alleles that were paternally inherited or had arisen as de novo germline mutations. This non-invasive sequencing of the fetal genome may ultimately facilitate the diagnosis of all inherited and de novo genetic diseases.

Illumina Technology: Whole-genome sequencing on a Genome Analyzer$_{II}$ and HiSeq 2000 to a depth of ~52.7X. Exome Sequencing on a HiSeq 2000 with 332, 344, and 930 million aligned reads for first, second, and third trimesters.

Kitzman, J. O., Snyder, M. W., Ventura, M., Lewis, A. P., Qiu, R., et al. (2012) Noninvasive whole-genome sequencing of a human fetus. Sci Transl Med 4: 137ra176

In this paper the authors reconstruct the whole-genome sequence of a human fetus using samples obtained non-invasively during the second trimester, including DNA from the pregnant mother, DNA from the father, and "cell-free" DNA from the pregnant mother's plasma. The key message of this paper is that new mutations in the genome of the fetus can be sensitively detected and triaged for validation.

Illumina Technology: HiSeq 2000 instruments (Illumina) using paired-end 101-bp reads with an index read of 9 bp.

Chiu, R. W., Akolekar, R., Zheng, Y. W., Leung, T. Y., Sun, H., et al. (2011) Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 342: c7401

This paper describes the feasibility testing of plasma DNA screening for fetal trisomy 21. The cohort consisted of 753 pregnant women at high risk for fetal trisomy, including 21 who underwent definitive diagnosis by full karyotyping and 86 who had a fetus with trisomy. The authors used an 8-plex and a 2-plex indexing protocol and found that the 2-plex indexing was superior. With the 2-plex protocol, trisomy 21 fetuses were detected at 100% sensitivity and 97.9% specificity, which resulted in a positive predictive value of 96.6% and negative predictive value of 100%. The authors conclude that, if referrals for amniocentesis or chorionic villus sampling were based on the sequencing test results, about 98% of the invasive diagnostic procedures could be avoided.

Illumina Technology: Genome Analyzer$_{II}$ and Genome Analyzer$_{IIx.}$

Sehnert, A. J., Rhees, B., Comstock, D., de Feo, E., Heilek, G., et al. (2011) Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem 57: 1042-1049

This paper describes a normalization method that minimizes the intra- and inter-run sequencing variation. The authors developed the algorithm on a training set of 71 samples with 26 abnormal karyotypes. The classification process was then evaluated on an independent test set of 48 samples with 27 abnormal karyotypes. They achieved 100% correct classification of T21 (13 of 13) and T18 (8 of 8) samples. They also discovered additional chromosomal abnormalities.

Illumina Technology: Genome Analyzer$_{IIX}$ 36 bp reads.

Palomaki, G. E., Kloza, E. M., Lambert-Messerlian, G. M., Haddow, J. E., Neveux, L. M., et al. (2011) DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 13: 913-920

To test the accuracy of non-invasive testing with NGS a study was designed with a cohort of 4664 pregnancies at high risk for Down syndrome. The internally validated, laboratory-developed test based on NGS was compared to fetal karyotyping in 212 Down syndrome and 1484 matched euploid pregnancies. In the blinded, nested case-control study, the Down syndrome detection rate was 98.6% (209/212), the false-positive rate was 0.20% (3/1471), and the testing failed in 13 pregnancies (0.8%). Taking into account the complexity of the test and the resources required, the authors conclude that the study supports offering NGS to women identified as being at high risk for Down syndrome. The turnaround time for 95% of patient results would be comparable with currently available cytogenetic analysis of amniotic fluid cells and chorionic villus sampling. The availability of NGS could also justify lowering serum/ultrasound screening cutoffs, resulting in a higher rate of Down syndrome detection.

Illumina Technology: HiSeq 2,000.

Dan, S., Chen, F., Choy, K. W., Jiang, F., Lin, J., et al. (2012) Prenatal detection of aneuploidy and imbalanced chromosomal arrangements by massively parallel sequencing. PLoS ONE 7: e27835

Jensen, T. J., Dzakula, Z., Deciu, C., van den Boom, D. and Ehrich, M. (2012) Detection of microdeletion 22q11.2 in a fetus by next-generation sequencing of maternal plasma. Clin Chem 58: 1148-1151[47]

[47] Baker, K. and Vorstman, J. A. (2012) Is there a core neuropsychiatric phenotype in 22q11.2 deletion syndrome? Curr Opin Neurol 25: 131-137

Liao, G. J., Lun, F. M., Zheng, Y. W., Chan, K. C., Leung, T. Y., et al. (2011) Targeted massively parallel sequencing of maternal plasma DNA permits efficient and unbiased detection of fetal alleles. Clin Chem 57: 92-101

Norton, M. E., Brar, H., Weiss, J., Karimi, A., Laurent, L. C., et al. (2012) Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol 207: 137 e131-138

Sparks, A. B., Struble, C. A., Wang, E. T., Song, K. and Oliphant, A. (2012) Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am J Obstet Gynecol 206: 319 e311-319

Sparks, A. B., Wang, E. T., Struble, C. A., Barrett, W., Stokowski, R., et al. (2012) Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn 32: 3-9

Chen, E. Z., Chiu, R. W., Sun, H., Akolekar, R., Chan, K. C., et al. (2011) Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PLoS ONE 6: e21791

Neonatal Diagnostics

Neonatal diagnosis refers to the special cases where there is a need to diagnose the condition of a newborn. Early diagnosis and intervention can significantly expand treatment options and improve outcomes. The following paper is an example of this approach.

Bonnefond, A., Durand, E., Sand, O., De Graeve, F., Gallina, S., et al. (2010) Molecular diagnosis of neonatal diabetes mellitus using next-generation sequencing of the whole exome. PLoS ONE 5: e13630

The molecular diagnosis of monogenic non-autoimmune neonatal diabetes mellitus (NDM) is critical for patient care, as patients carrying a mutation in KCNJ11 or ABCC8 can be treated by oral sulfonylurea drugs instead of insulin therapy. In this paper the authors evaluated the potential of whole-exome sequencing to diagnose a patient with permanent NDM for whom mutations in KCNJ11, ABCC8, and INS, as well as abnormalities in chromosome 6q24 had been previously excluded. The authors identified a novel non-synonymous mutation in ABCC8 (c.1455G>C/p.Q485H), which was subsequently confirmed by Sanger sequencing. They conclude that whole-genome exome sequencing is a cost-effective and rapid approach to identify mutations in NDM patients.

Illumina Technology: Genome Analyzer$_{IIx}$ with 76 bp paired-end reads and 65x coverage. Validated with the Illumina Human1M-Duo Array.

Types of Genetic Modifications

De Novo Mutations

One of the more surprising discoveries in the recent flood of genome sequences is that normal individuals carry between 20,000 and 40,000 variations in their exomes. In the 1000 Genomes Project, each person was found to carry approximately 250 to 300 loss-of-function variants in annotated genes and 50 to 100 variants previously implicated in genetic disorders. In addition there are approximately 10^{-8} de novo germline base substitutions per base pair per generation.[48] De novo mutations and microlesions are refractory to analysis by microarray-based methods and as a result, little was known about their frequency of occurrence or contribution to genetic disease until the advent of NGS.[49]

Exome sequencing accounts for the majority of papers that report de novo mutations. This approach is also cost-effective compared to the sequential testing involved in a typical diagnostic odyssey.

Among patients with the same disease, de novo mutations usually occur in multiple positions of a gene. These positions will not be represented on a microarray and will be missed by microarray analysis. Jones, W., et al. (2012) De Novo Mutations in MLL Cause Wiedemann-Steiner Syndrome. Am J Hum Genet 91: 358-364.[50]

Reviews:

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., et al. (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74: 285-299

Ku, C. S., Polychronakos, C., Tan, E. K., Naidoo, N., Pawitan, Y., et al. (2012) A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. Mol Psychiatry

[48] Genomes Project, C. (2010) A map of human genome variation from population-scale sequencing. Nature 467: 1061-1073
[49] Ku, C. S., Polychronakos, C., Tan, E. K., Naidoo, N., Pawitan, Y., et al. (2012) A new paradigm emerges from the study of de novo mutations in the context of neurodevelopmental disease. Mol Psychiatry
[50] Jones, W. D., Dafou, D., McEntagart, M., Woollard, W. J., Elmslie, F. V., et al. (2012) De Novo Mutations in MLL Cause Wiedemann-Steiner Syndrome. Am J Hum Genet 91: 358-364

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Heinzen, E. L., Swoboda, K. J., Hitomi, Y., Gurrieri, F., Nicole, S., et al. (2012) De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet 44: 1030-1034

The authors used exome sequencing of seven patients with Alternating Hemiplegia of Childhood (AHC) and their unaffected parents to identify de novo nonsynonymous mutations in ATP1A3 in all seven individuals. In a subsequent sequence analysis of 98 other patients with AHC, they found that ATP1A3 mutations were likely to be responsible for at least 74% of the cases. They also found one inherited mutation in a case of familial AHC. Notably, most AHC cases are caused by one of seven recurrent ATP1A3 mutations – one of which was observed in 36 patients. ATP1A3 mutations are known to cause rapid-onset dystonia-parkinsonism. In the case of AHC-causing mutations there were reductions in ATPase activity without affecting the level of protein expression.

Illumina Technology: Genome Analyzer$_{IIx}$ and HiSeq 2000 exome sequencing to an average coverage of 90-fold.

Jones, W. D., Dafou, D., McEntagart, M., Woollard, W. J., Elmslie, F. V., et al. (2012) De Novo Mutations in MLL Cause Wiedemann-Steiner Syndrome. Am J Hum Genet 91: 358-364

The authors identified de novo mutations in MLL in five of the six individuals with hypertrichosis cubiti associated with short stature, intellectual disability, and a distinctive facial appearance, consistent with a diagnosis of Wiedemann-Steiner syndrome. MLL encodes a histone methyltransferase that regulates chromatin-mediated transcription through the catalysis of methylation of histone H3K4. Each of the five mutations is predicted to result in premature termination of the protein product.

Illumina Technology: HiSeq 2000 exome sequencing with 100 bp paired-end reads.

Hood, R. L., Lines, M. A., Nikkel, S. M., Schwartzentruber, J., Beaulieu, C., et al. (2012) Mutations in SRCAP, encoding SNF2-related CREBBP activator protein, cause Floating-Harbor syndrome. Am J Hum Genet 90: 308-313

The authors identified heterozygous truncating mutations in SRCAP in five unrelated individuals with sporadic Floating-Harbor syndrome (FHS). Sanger sequencing identified mutations in SRCAP in eight more affected individuals. Mutations were de novo in all six instances in which parental DNA was available. SRCAP is an SNF2-related chromatin-remodeling factor that serves as a coactivator for CREB-binding protein (CREBBP, better known as CBP, the major cause of Rubinstein-Taybi syndrome (RTS)).

Illumina Technology: HiSeq exome sequencing with 35–40 Gbp of 100 bp paired-end reads

Kong, A., Frigge, M. L., Masson, G., Besenbacher, S., Sulem, P., et al. (2012) Rate of de novo mutations and the importance of father's age to disease risk. Nature 488: 471-475

The authors show that the diversity in the mutation rate of single nucleotide polymorphisms is dominated by the age of the father at conception of the child. Starting with an average de novo mutation rate of 1.20 x 10^{-8} per nucleotide per generation at age of 29.7, there is an increase of about two mutations per year. An exponential model estimates paternal mutations doubling every 16.5 years. This highlights the importance of the father's age in determining the risk of genetic diseases.

Illumina Technology: Genome Analyzer$_{IIx}$ and HiSeq 2000 whole-genome sequencing.

Lee, J. H., Huynh, M., Silhavy, J. L., Kim, S., Dixon-Salazar, T., et al. (2012) De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. Nat Genet 44: 941-945

O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., et al. (2012) Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature 485: 246-250

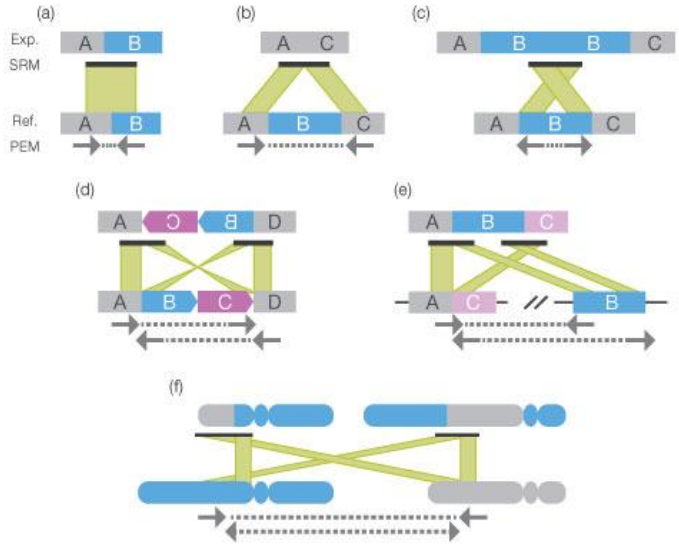
Riviere, J. B., Mirzaa, G. M., O'Roak, B. J., Beddaoui, M., Alcantara, D., et al. (2012) De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 44: 934-940

Structural Variants

Structural variants are remarkably common and complex[51] and can contribute to both inherited and de novo disease phenotypes.[52] Some complex variants exhibit profoundly complicated rearrangements between distinct loci from multiple chromosomes, whereas others involve more subtle alterations at a single locus.[53]

Copy number variations (CNVs) represent a significant source of genetic diversity and may be responsible for some of the missing heritability found in SNP-based studies. CNVs appear to be particularly important in neuronal diseases and have been shown to contribute to disease susceptibility for several neurobehavioral phenotypes, including autism spectrum disorders, mental retardation, and schizophrenia.[54]

Some notable successes have been achieved with array-based approaches, particularly with mapping CNVs. However, arrays cannot detect balanced translocations and fluorescence in situ hybridization (FISH) techniques are targeted with limited resolution. The true extent of balanced translocations in both healthy and diseased genomes was only discovered with the advent of NGS. Paired-end and mate-pair sequencing are particularly effective in mapping genomic rearrangements.[55]



Detecting canonical structural variation (SV) breakpoints through sequencing.

[51] Pang, A. W., MacDonald, J. R., Pinto, D., Wei, J., Rafiq, M. A., et al. (2010) Towards a comprehensive structural variation map of an individual human genome. Genome Biol 11: R52
[52] Zhang, F., Gu, W., Hurles, M. E. and Lupski, J. R. (2009) Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 10: 451-481
[53] Quinlan, A. R. and Hall, I. M. (2012) Characterizing complex structural variation in germline and somatic genomes. Trends Genet 28: 43-53
[54] Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., et al. (2010) Functional impact of global rare copy number variation in autism spectrum disorders. Nature 466: 368-372
[55] Quinlan, A. R. and Hall, I. M. (2012) Characterizing complex structural variation in germline and somatic genomes. Trends Genet 28: 43-53

(From previous page) *When DNA sequences are collected from an experimental (Exp.) genome and aligned to a reference (Ref.) genome, each structural variant class generates a distinct alignment pattern. The patterns observed for paired-end mapping (PEM) and splitread mapping (SRM) are illustrated when both genomes have identical structure (a), and cases where the experimental genome contains a deletion (b), a tandem duplication (c), an inversion (d), a transposon insertion (e) or a reciprocal translocation (f). PEM relies upon readpairs whose unsequenced portion (dotted lines) spans a SV breakpoint. When aligned to the reference genome, the alignment distance and orientation of such readpairs indicate the type of rearrangement that has occurred. Reads that map to the plus strand are shown as right-facing arrows. Reads that map to the negative strand are shown as leftward-facing arrows. Quinlan, A. R. and Hall I. M. (2012) Trends Genet 28: 43-53*

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Quinlan, A. R. and Hall, I. M. (2012) Characterizing complex structural variation in germline and somatic genomes. Trends Genet 28: 43-53

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Griswold, A. J., Ma, D., Cukier, H. N., Nations, L. D., Schmidt, M. A., et al. (2012) Evaluation of copy number variations reveals novel candidate genes in autism spectrum disorder-associated pathways. Hum Mol Genet 21: 3513-3523

This study was based on 813 unrelated Caucasian autism spectrum disorder (ASD) cases and 592 controls. In the ASD cases the authors found a significantly higher burden in the number and size of deletions, and the disruption of more genes. Of the deletions found, 18 were larger than 1 Mb and were detected exclusively in ASD cases. Some impacted genes also overlap with those found in other neurodevelopmental and neuropsychiatric diseases.

Illumina Technology: Human1M v1 DNA Analysis BeadChip and Human1M-Duo v3 DNA Analysis BeadChip processed with the Infinium II assay.

Luo, R., Sanders, S. J., Tian, Y., Voineagu, I., Huang, N., et al. (2012) Genome-wide Transcriptome Profiling Reveals the Functional Impact of Rare De Novo and Recurrent CNVs in Autism Spectrum Disorders. Am J Hum Genet 91: 38-55

This study provides evidence that pathogenic structural variants have a functional impact via transcriptome alterations in Autism Spectrum Disorders (ASDs). It demonstrates the utility of integrating gene expression with mutation data for the prioritization of genes disrupted by potentially pathogenic mutations. Because brain tissue is not available from most samples, the authors interrogated gene expression in lymphoblasts from 244 families with discordant siblings. They find that missexpressed genes cluster within the most pathogenic CNVs.

Illumina Technology: Whole Human Genome Array HumanRef-8 Expression BeadChips.

Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., et al. (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74: 285-299

Exome sequencing of 343 families, each with a single child on the autism spectrum and at least one unaffected sibling, reveal de novo small indels and point substitutions, which come mostly from the paternal line in an age-dependent manner.

Illumina Technology: HiSeq 2000 exome sequencing using paired-end 100 bp reads.

Amor, D. J., Burgess, T., Tan, T. Y. and Pertile, M. D. (2012) Questionable pathogenicity of FOXG1 duplication. Eur J Hum Genet 20: 595-596; author reply 596-597

Chow, M. L., Pramparo, T., Winn, M. E., Barnes, C. C., Li, H. R., et al. (2012) Age-dependent brain gene expression and copy number anomalies in autism suggest distinct pathological processes at young versus mature ages. PLoS Genet 8: e1002592

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Fernandez, T. V., Sanders, S. J., Yurkiewicz, I. R., Ercan-Sencicek, A. G., Kim, Y. S., et al. (2012) Rare copy number variants in tourette syndrome disrupt genes in histaminergic pathways and overlap with autism. Biol Psychiatry 71: 392-402

Hochstenbach, R., Poot, M., Nijman, I. J., Renkens, I., Duran, K. J., et al. (2012) Discovery of variants unmasked by hemizygous deletions. Eur J Hum Genet 20: 748-753

Holt, R., Sykes, N. H., Conceicao, I. C., Cazier, J. B., Anney, R. J., et al. (2012) CNVs leading to fusion transcripts in individuals with autism spectrum disorder. Eur J Hum Genet

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Li, J., Harris, R. A., Cheung, S. W., Coarfa, C., Jeong, M., et al. (2012) Genomic hypomethylation in the human germline associates with selective structural mutability in the human genome. PLoS Genet 8: e1002692

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Kirov, G., Pocklington, A. J., Holmans, P., Ivanov, D., Ikeda, M., et al. (2012) De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. Mol Psychiatry 17: 142-153

Liu, Y., Gibson, J., Wheeler, J., Kwee, L. C., Santiago-Turla, C. M., et al. (2011) GALC deletions increase the risk of primary open-angle glaucoma: the role of Mendelian variants in complex disease. PLoS ONE 6: e27134

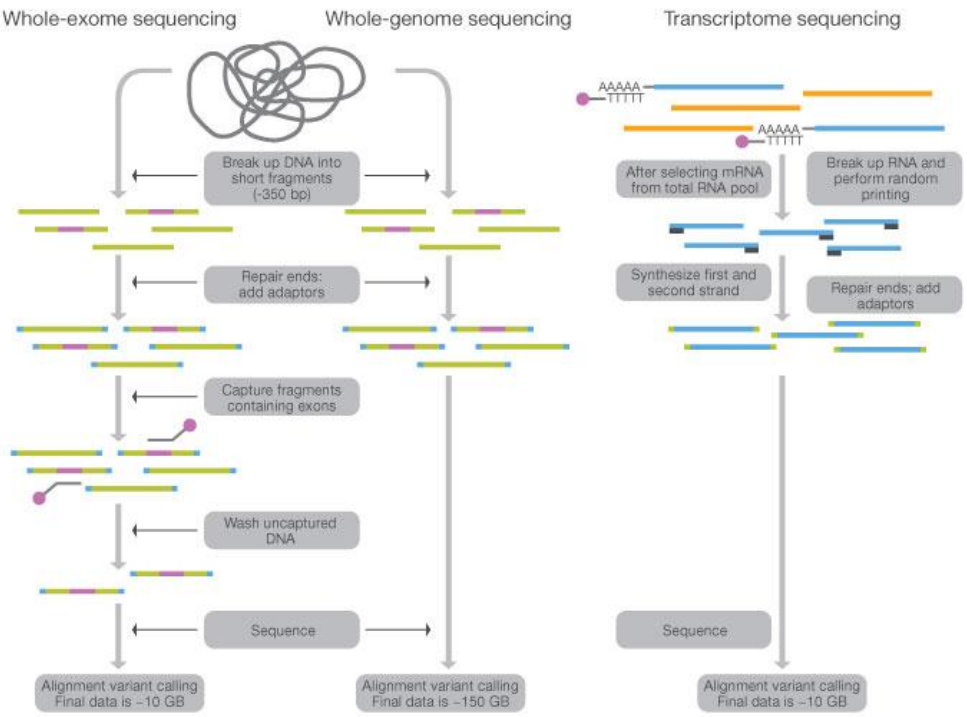
Salyakina, D., Cukier, H. N., Lee, J. M., Sacharow, S., Nations, L. D., et al. (2011) Copy number variants in extended autism spectrum disorder families reveal candidates potentially involved in autism risk. PLoS ONE 6: e26049

Veenma, D., Brosens, E., de Jong, E., van de Ven, C., Meeussen, C., et al. (2012) Copy number detection in discordant monozygotic twins of Congenital Diaphragmatic Hernia (CDH) and Esophageal Atresia (EA) cohorts. Eur J Hum Genet 20: 298-304

Types of Analysis

Genomic-Based Analysis

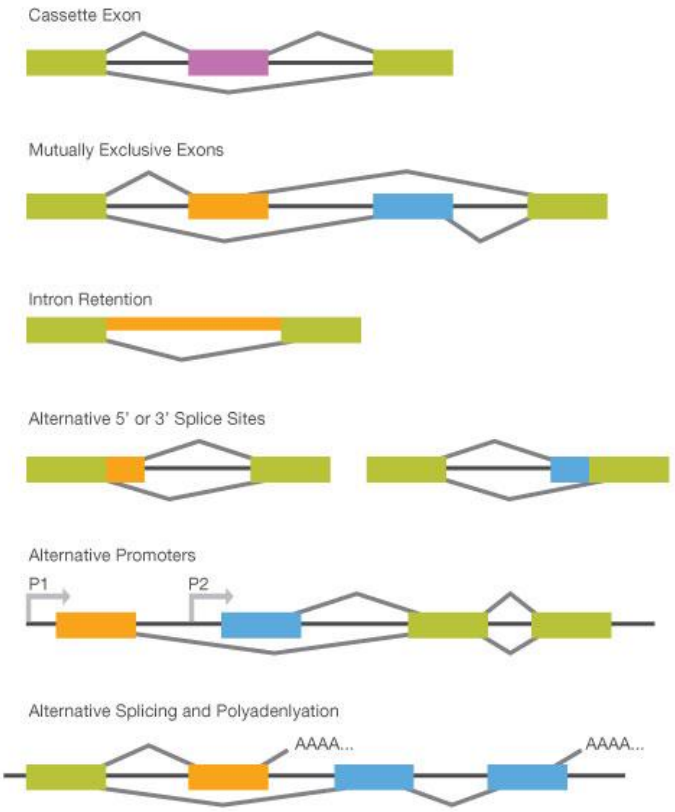
NGS provides a simple workflow that allows a variety of types of analyses, including whole-genome, whole-exome, transcriptome, ChIP-Seq, and epigenome sequencing.



Simplified workflows for whole-exome, whole-genome, and transcriptome sequencing. *The initial sample preparation is identical for both whole-exome and whole-genome sequencing. Genomic DNA is broken up into small fragments and sequence adaptors, which allow each fragment to be hybridized to the flowcell where the sequencing occurs, are added. Whole-exome sequencing protocols proceed with the hybridization of the fragments to probes that are complimentary to all the known exons in the genome, which are then captured while the remaining DNA is washed away, leaving a pool of fragments containing exons. Whole-genome sequencing requires no extra steps following the addition of adaptors and the library is ready to be sequenced at that point. For transcriptome sequencing, the procedure is identical to the other two protocols, with the exception of the initial stages of sample preparation. Here, it is customary to start with a pool of total RNA, from which mRNA is captured and then sheared and, finally, cDNA is synthesized. At this step, the preparation of the library and sequencing follows the same general procedures as for the two other protocols. Bras, J., et al. (2012). Nat Rev Neurosci 13:453-64*

Transcriptome Analysis

Expression analysis adds an additional level of information to help interpret the impact of genetic aberrations in genetic diseases. The expression of a mutated allele provides additional evidence that it could be a causative mutation.[56] Expression analysis is also a survey of the functionality of the genetic, epigenetic, and RNA processing machinery[57] and splice variants.[58] Sequencing of the RNA transcripts (RNA-Seq) is unaffected by changes in the RNA sequence and offers an objective tool to assess both the gene expression and modifications of the RNA.



Different types of alternative splicing. *Introns are represented by lines, and exons are represented by boxes. Promoters are indicated with broken arrows and polyadenylation sites with AAAA Mills, J. D. and Janitz M. (2012) Neurobiol Aging 33:1012 e11-24.*

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[58] Lines, M. A., Huang, L., Schwartzentruber, J., Douglas, S. L., Lynch, D. C., et al. (2012) Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly. Am J Hum Genet 90: 369-377

Reviews:

Costa, V., Aprile, M., Esposito, R. and Ciccodicola, A. (2012) RNA-Seq and human complex diseases: recent accomplishments and future perspectives. Eur J Hum Genet

Mills, J. D. and Janitz, M. (2012) Alternative splicing of mRNA in the molecular pathology of neurodegenerative diseases. Neurobiol Aging 33: 1012 e1011-1024

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Luo, R., Sanders, S. J., Tian, Y., Voineagu, I., Huang, N., et al. (2012) Genome-wide Transcriptome Profiling Reveals the Functional Impact of Rare De Novo and Recurrent CNVs in Autism Spectrum Disorders. Am J Hum Genet 91: 38-55

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Illumina Technology: Whole Human Genome Array HumanRef-8 Expression BeadChips.

Holt, R., Sykes, N. H., Conceicao, I. C., Cazier, J. B., Anney, R. J., et al. (2012) CNVs leading to fusion transcripts in individuals with autism spectrum disorder. Eur J Hum Genet

Li, G., Bahn, J. H., Lee, J. H., Peng, G., Chen, Z., Nelson, S. F., Xiao, X.; (2012) Identification of allele-specific alternative mRNA processing via transcriptome sequencing. Nucleic Acids Res 40: e104

Lines, M. A., Huang, L., Schwartzentruber, J., Douglas, S. L., Lynch, D. C., et al. (2012) Haploinsufficiency of a spliceosomal GTPase encoded by EFTUD2 causes mandibulofacial dysostosis with microcephaly. Am J Hum Genet 90: 369-377

Cytogenetics

Cytogenetics is a branch of genetics that studies the structure and function of DNA within the cell nucleus. It includes analysis of G-banded chromosomes (karyotyping), fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH). These techniques require considerable skill to execute and the resolution is limited. The use of array- and sequence-based mapping allows a greater degree of accuracy and will likely replace the traditional methods over time.[59]

Method	Advantage	Disadvantage
FISH	Well-established	Requires considerable skill. Needs primers to the area of interest. Limited resolution.
Array-based mapping	Inexpensive. Fast and can be automated. Does not require prior knowledge.	Cannot detect balanced translocations or rearrangements. Array could be population specific.
NGS	High resolution. No population bias. Does not require prior knowledge.	Requires more extensive data analysis.

Reviews:

Sato-Otsubo, A., Sanada, M. and Ogawa, S. (2012) Single-nucleotide polymorphism array karyotyping in clinical practice: where, when, and how? Seminars in oncology 39: 13-25

[59] Sato-Otsubo, A., Sanada, M. and Ogawa, S. (2012) Single-nucleotide polymorphism array karyotyping in clinical practice: where, when, and how? Seminars in oncology 39: 13-25

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Yang, Y., Wang, C., Wang, F., Zhu, L., Liu, H., et al. (2012) Novel chromosomal translocation t(11;9)(p15;p23) involving deletion and duplication of 9p in a girl associated with autism and mental retardation. Gene 502: 154-158

The authors describe four patients with rearrangements in p9. In all four cases the rearrangements are much more complex than originally indicated by routine cytogenetics. They find that precise mapping and full molecular characterization of the abnormal area in patients are key to better understanding phenotype-karyotype correlations that helps to identify candidate genes.

Illumina Technology: HumanCytoSNP-12 BeadChip.

Bystricka, D., Sarova, I., Zemanova, Z., Brezinova, J., Lizcova, L., et al. (2012) Recurrent chromosomal breakpoints in patients with myelodysplastic syndromes and complex karyotype versus fragile sites. Leuk Res 36: e125-127

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Pathway Analysis

A central goal of most genetic studies is to gain an understanding of the pathobiological mechanisms involved in the onset and pathology of a disease.[60] In pathway-based analysis the aim is to identify multiple associated genes that affect one biological pathway, yielding information not only on that particular pathway's involvement in the disease, but also suggesting other potential risk-conferring genes.[61] An example of this approach comes from the field of Alzheimer's disease, where it has been shown that there is a considerable overrepresentation of disease-associated genes in pathways related to cholesterol metabolism and the immune response in two large GWAS.[62] Our understanding of biological pathways and their interactions is still far from complete so these observations should be treated as hypotheses that can be tested more rigorously.

Reviews:

Bras, J., Guerreiro, R. and Hardy, J. (2012) Use of next-generation sequencing and other whole-genome strategies to dissect neurological disease. Nat Rev Neurosci 13: 453-464

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Chung, R. H. and Chen, Y. E. (2012) A two-stage random forest-based pathway analysis method. PLoS ONE 7: e36662

Fehringer, G., Liu, G., Briollais, L., Brennan, P., Amos, C. I., et al. (2012) Comparison of pathway analysis approaches using lung cancer GWAS data sets. PLoS ONE 7: e31816

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