Next-generation sequencing for identifying new genes in rare genetic diseases: many challenges and a pinch of luck

Amélie Bonnefond1,2,3* and Philippe Froguel1,2,3,4,5*

Abstract
A report on the European Society of Human Genetics conference, held in Paris, France, June 8-11, 2013.

Keywords: Bioinformatics; genome sequencing; next-generation sequencing; prioritization of variants; rare disorder; syndrome; whole-exome sequencing

The European Society of Human Genetics is a non-profit organization aiming to promote research in basic and applied human and medical genetics. This year, there were almost 3,000 attendees at the annual meeting, which reflected state of the art technologies, innovations, novel strategies, counseling and applications in human and medical genetics. As expected, a plethora of genetic diseases was tackled: from very rare disorders (such as developmental syndromes, neuromuscular disorders, metabolic and mitochondrial disorders, dysmorphic syndromes, monogenic forms of epilepsies, retinal dystrophies), to more common diseases (such as cancer, autism, intellectual disability).

In this report, we focus on the several sessions, symposia and workshops that discussed next-generation sequencing (NGS) technologies and their contribution to the identification of new genetic etiologies of rare disorders. The current challenges of this technology are also discussed.

Success in finding new genetic etiologies in rare diseases through NGS
During the workshop on NGS in clinical practice, clinical geneticists from three European laboratories illustrated their diagnostic NGS approaches and results obtained so far. They mostly discussed generic whole-exome sequencing (WES) in severe syndromic cases, and two main filtering strategies for identifying the causal variant(s) were used in those cases: strategies focusing on de novo mutations (not observed in the parents) and strategies on homozygous mutations (in patients from consanguineous families), which have not been reported in public databases and are predicted to be damaging. Using this approach, Lissenka Vissers (Radboud University Nijmegen Medical Centre, The Netherlands) and collaborators identified a de novo mutation in the ARID1B gene, in a patient presenting with eczema, short stature, delayed development and intellectual disability. It is known that mutations in this gene cause Coffin-Siris syndrome, a disease with an autosomal recessive inheritance. Furthermore, the same group identified a de novo mutation in the PDHA1 gene, in a patient presenting with microencephaly, feeding problems, severe developmental delay (non-speech), behavior problems, intellectual disability, short stature, hirsutism and paraplegia. It is of note that before WES, the PDHA1 gene had been erroneously reported negative in the patient via Sanger sequencing.

Anita Rauch (Zürich University, Switzerland) and collaborators discussed a case presenting with Fraser-syndrome-like clinical features (including kidney and ureter agenesis). Four genes (including 100 exons) can be mutated in this syndrome. The assessment of these genes would have been too expensive and taken too much time using Sanger sequencing. Therefore, they used WES and found, after filters, a truncated variant and a substitution, both located in one of the four known genes: FRAS1.

They discussed another patient with intellectual disability, severe short stature and severe microencephaly, and born from consanguineous parents. They found a homozygous mutation of interest in the CRNP1 gene that is known to be mutated in Seckel syndrome. However, the overlap of phenotypes was incomplete. Interestingly, they found another homozygous mutation of interest in the SACS gene and suggested a compound phenotype that would explain all disorders of the patient.

Rauch and collaborators also investigated a third case presenting with macrosomia, severe hypotonia, hyperactivity, autistic features and postaxial hexadactyly. They found a de novo frameshift in OFD1 that causes oral-
facial-digital syndrome type 1 when mutated. However, the phenotypes were not overlapping. They subsequently looked for mosaic mutations in a candidate gene (that had just been published): PIK3CA. They successfully identified a mosaic substitution in this gene that was present in 16% of the saliva cells and in 7% of the blood cells.

In a concurrent session, Jean-Baptiste Rivière and collaborators (Laboratoire de Génétique Moléculaire, CHU, France) presented a study in which they performed WES in two patients (and their unaffected parents) with short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly and teething delay syndrome (SHORT) syndrome. By focusing on de novo mutations of interest, they found two different substitutions in PIK3R1. Subsequently, via Sanger sequencing, they found seven additional carriers of PIK3R1 mutations who presented with SHORT syndrome. Furthermore, they demonstrated the functionality of these mutations using fibroblasts derived from individuals with PIK3R1 mutations. Their findings highlighted the critical role of PIK3R1 in insulin action, and normal growth and development.

In the same session, Sian Ellard and collaborators (University of Exeter Medical School, UK) used a combination of genome sequencing and homozygosity mapping analysis in consanguineous pedigrees affected with neonatal diabetes linked to pancreatic agenesis. They found a shared run of homozygosity encompassing the PTF1A gene known to cause pancreatic agenesis when mutated. No mutations were found in the PTF1A gene. However, through genome sequencing in two probands, they identified a substitution located in a highly conserved 400 bp region. Through functional analyses, they showed that this region was actually an enhancer of PTF1A. Four additional mutations and one indel spanning the same region were identified in ten affected families. This study shows the potentially key role of non-coding regions in monogenic disorders.

Other NGS success stories in rare disorders were presented at the meeting. These successes were only found in monogenic diseases, especially recessive or de novo dominant disorders. NGS data in polygenic diseases have been very disappointing to date, and so it is clear that we still have many challenges to overcome in NGS.

Conclusion
To conclude, although many NGS success stories were reported during this European Society of Human Genetics meeting, these results were only found in monogenic diseases, especially recessive or de novo dominant disorders. NGS data in polygenic diseases have been very disappointing to date, and so it is clear that we still have many challenges to overcome in NGS.

Abbreviations
bp, base pair; NGS, next-generation sequencing; SHORT syndrome, short stature, hyperextensibility of joints and/or inguinal hernia, ocular depression, Rieger anomaly and teething delay syndrome; WES, whole-exome sequencing.

Competing interests
The authors declare that they have no competing interests.

Author details
1CNRS-UMR8199, Lille Pasteur Institute, Lille 59000, France. 2Lille II University, Lille 59000, France. 3European Genomic Institute for Diabetes (EGID), Lille 59000, France. 4Department of Genomics of Common Disease, School of Public Health, Imperial College London, Hammersmith Hospital, London W12 0NN, UK. 5Qatar Biomedical Research Institute (QBRI), Qatar Foundation, Doha, Qatar.

Published: 29 July 2013

Cite this article as: Bonnefond A, Froguel P. Next-generation sequencing for identifying new genes in rare genetic diseases: many challenges and a pinch of luck. Genome Biology 2013, 14:309.