Clinical exome sequencing

Patients with unexplained sporadic ID are subjected to 'trio'-exome sequencing to detect potentially pathogenic *de novo* (in dominant conditions) or inherited (in recessive conditions) mutations[1]. For familial ID or other inherited disorders, such as movement disorders, singleton sequencing of the patient is the usual approach. Clinical exome sequencing (both trio and singleton) is analyzed in a two-tiered approach. First, exome sequencing data are pre-filtered for mutations in the respective ID or movement disorder gene panels before manual inspection[2]. Second, if no causative mutations were identified in the first step and patients (or legal guardians) consented, the entire dataset was filtered for likely-causative mutations (which may or may not be in OMIM genes). Informed consent for clinical exome sequencing is obtained by clinical geneticists in accordance with local regulations and this procedure was approved by the medical ethics committee of the Radboud university medical center.

Exome sequencing was performed essentially as previously described [2]. Briefly, genomic DNA was fragmented and exome fragments were captured using the SureSelect^{XT} Human All Exon 50Mb Kit (Agilent). The fragments were amplified by emulsion PCR and subjected to sequencing on a SoliD 5500XL^{TM} (Life Technologies) or a HiSeq2000^{TM} machine (Illumina). Data from the 5500XL machine were analysed using Bioscope^{TM} (version 2.0, Life Technologies) and the Hiseq2000 data were analysed with BWA (read alignment,[3]) and GATK (variant calling,[4]) software packages. Variants were annotated using an in-house developed pipeline. Prioritization of variants was done by an in-house designed 'variant interface' and manual curation. Potentially causative variants were confirmed by Sanger sequencing.

1 de Ligt J, Willemsen MH, van Bon BW, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout AT, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BB, Brunner HG, Veltman JA, Vissers LE. Diagnostic exome sequencing in persons with severe intellectual disability. *N Engl J Med* 2012;**367**(20):1921-9.

2 Neveling K, Feenstra I, Gilissen C, Hoefsloot LH, Kamsteeg EJ, Mensenkamp AR, Rodenburg RJ, Yntema HG, Spruijt L, Vermeer S, Rinne T, van Gassen KL, Bodmer D, Lugtenberg D, de Reuver R, Buijsman W, Derks RC, Wieskamp N, van den Heuvel B, Ligtenberg MJ, Kremer H, Koolen DA, van de Warrenburg BP, Cremers FP, Marcelis CL, Smeitink JA, Wortmann SB, van Zelst-Stams WA, Veltman JA, Brunner HG, Scheffer H, Nelen MR. A post-hoc comparison of the utility of sanger sequencing and exome sequencing for the diagnosis of heterogeneous diseases. *Hum Mutat* 2013;**34**(12):1721-6.

3 Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010;**26**(5):589-95.

4 McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;**20**(9):1297-303.