Go Short, Go Long

Marcel Nelen, PhD
X-omics festival 2021

RGTC – Radboud Genome Technology Centre - a clinical focus

Go Short - Short TAT , Short Reads

Goal

In-house rapidWES / smMIP – high pressure workflow

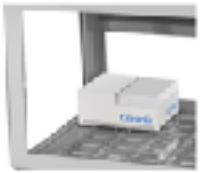
automated TWIST enrichment rapidWES workflow in 2020

Consult → Admini-stration → DNA isolation → Library preparation → Sequencing → Bio-informatics → Inter-pretation → Reporting

Automated Library Preparation

96-wells pippeting head

On-deck thermocycler



On-deck Covaris

Two rapidWES runs every week

Current Capacity

4 * 48 samples week

TAT: max. 14 days

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Human Genetics Nijmegen

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JAMA | Preliminary Communication

Presence of Genetic Variants Among Young Men With Severe COVID-19

Caspar I. van der Made, MD; Annet Simons, PhD; Janneke Schuurs-Hoeijmakers, MD, PhD; Guus van den Heuvel, MD; Tuomo Mantere, PhD; Simone Kersten, MSc; Rosanne C. van Deuren, MSc; Marloes Steehouwer, BSc; Simon V. van Reijmersdal, BSc; Martin Jaeger, PhD; Tom Hofste, BSc; Galuh Astuti, PhD; Jordi Corominas Galbany, PhD; Vyne van der Schoot, MD, PhD; Hans van der Hoeven, MD, PhD; Wanda Hagmolen of ten Have, MD, PhD; Eva Klijn, MD, PhD; Catrien van den Meer, MD; Jeroen Fiddelaers, MD; Quirijn de Mast, MD, PhD; Chantal P. Bleeker-Rovers, MD, PhD; Leo A. B. Joosten, PhD; Helger G. Yntema, PhD; Christian Gilissen, PhD; Marcel Nelen, PhD; Jos W. M. van der Meer, MD, PhD; Han G. Brunner, MD, PhD; Mihai G. Netea, MD, PhD; Frank L. van de Veerdonk, MD, PhD; Alexander Hoischen, PhD

PRENATAL DIAGNOSIS

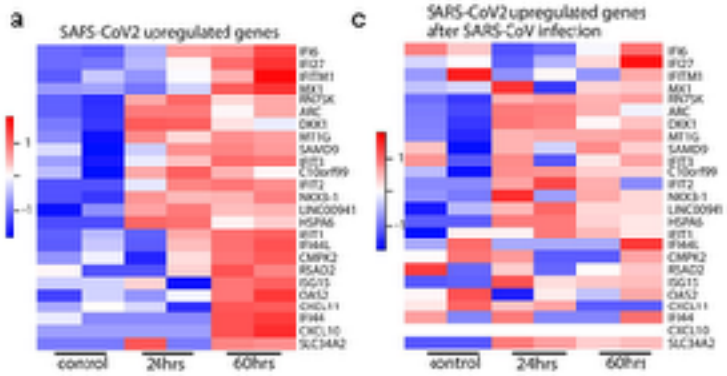
ORIGINAL ARTICLE | Open Access

Rapid whole exome sequencing in pregnancies to identify the underlying genetic cause in fetuses with congenital anomalies detected by ultrasound imaging

Chantal Deden, Kornelia Neveling, Dimitra Zafeiropopoulou, Christian Gilissen, Rolph Pfundt, Tuula Rinne, Nicole de Leeuw, Brigitte Faas, Thatjana Gardeitchik, Suzanne C. E. H. Sallevelt, Aimee Paulussen, Servi J. C. Stevens, Esther Sikkel, Mariet W. Elting, Merel C. van Maarle, Karin E. M. Diderich, Nicole Corsten-Janssen, Klaske D. Lichtenbelt, Guus Lachmeijer, Lisenka E. L. M. Vissers, Helger G. Yntema, Marcel Nelen, Ilse Feenstra, Wendy A. G. van Zelst-Stams ... See fewer authors

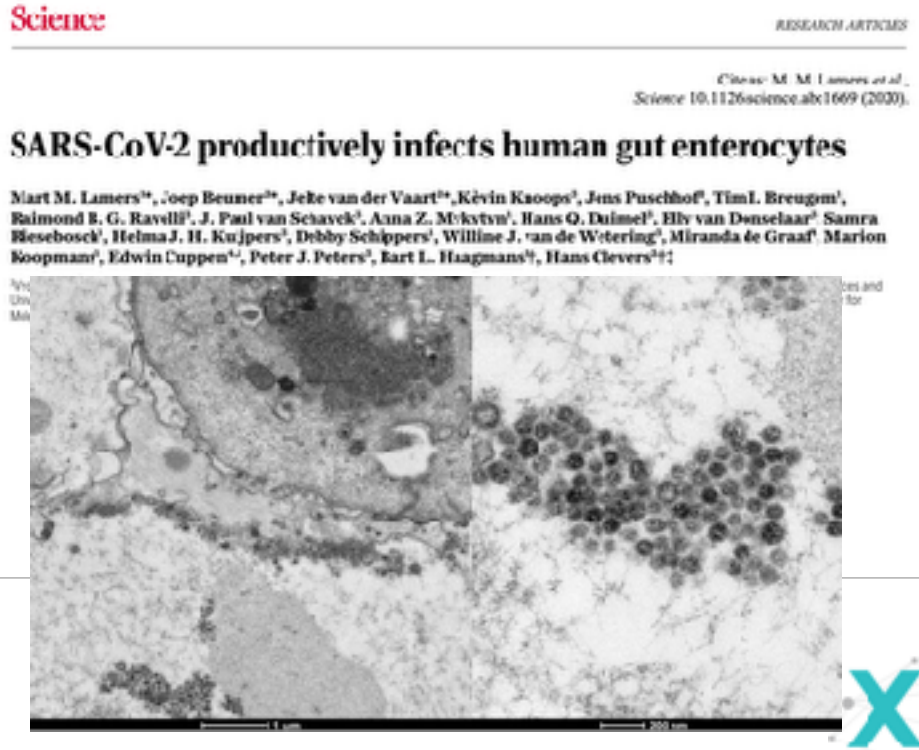
Cellular demonstrator and COVid-19

- Infection model of intestinal organoids
- single cell transcriptomics to demonstrate infection response
- conception experiments half-way March, publication May 1st
- Key: Availability of required technology platform (including X-omics) and expertise and collaboration



b

	ID	Name	pValue
1	GO:0051607	defense response to virus	3.568E-14
2	GO:0009615	response to virus	2.325E-13
3	GO:0071357	cellular response to type I interferon	2.687E-8
4	GO:0060337	type I interferon signaling pathway	2.687E-8
5	GO:0034340	response to type I interferon	4.515E-8


Science

RESEARCH ARTICLES

Science 10.1126/science.abc1669 (2020).

SARS-CoV-2 productively infects human gut enterocytes

Mart M. Lamers[1*], Joep Beumer[2*], Jelte van der Vaart[2*], Kèvin Knoops[3], Jens Puschhof[2], Tim I. Breugem[1], Raimond B. G. Ravelli[3], J. Paul van Schayck[3], Anna Z. Mykytyn[1], Hans Q. Duimel[2], Elly van Donselaar[2], Samra Hesebosch[1], Helma J. H. Kuijpers[1], Debby Schippers[1], Willine J. van de Wetering[2], Miranda de Graaf[1], Marion Koopmans[1], Edwin Cuppen[4,5], Peter J. Peters[1], Bart L. Haagmans[1†], Hans Clevers[2†‡]

Go Short

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the WGS-first project

Neurodevelopmental disorders

150 patient-parent trios, from every day clinical practice

Standard pathway

- Array
- WES & CNV

-Diagnosis?

'WGS first'pathway

- WGS

- Diagnosis?

Compare diagnostic yield

Prospective parallel design

WGS in parallel to routine tract

Can WGS replace WES and arrays as first line test?

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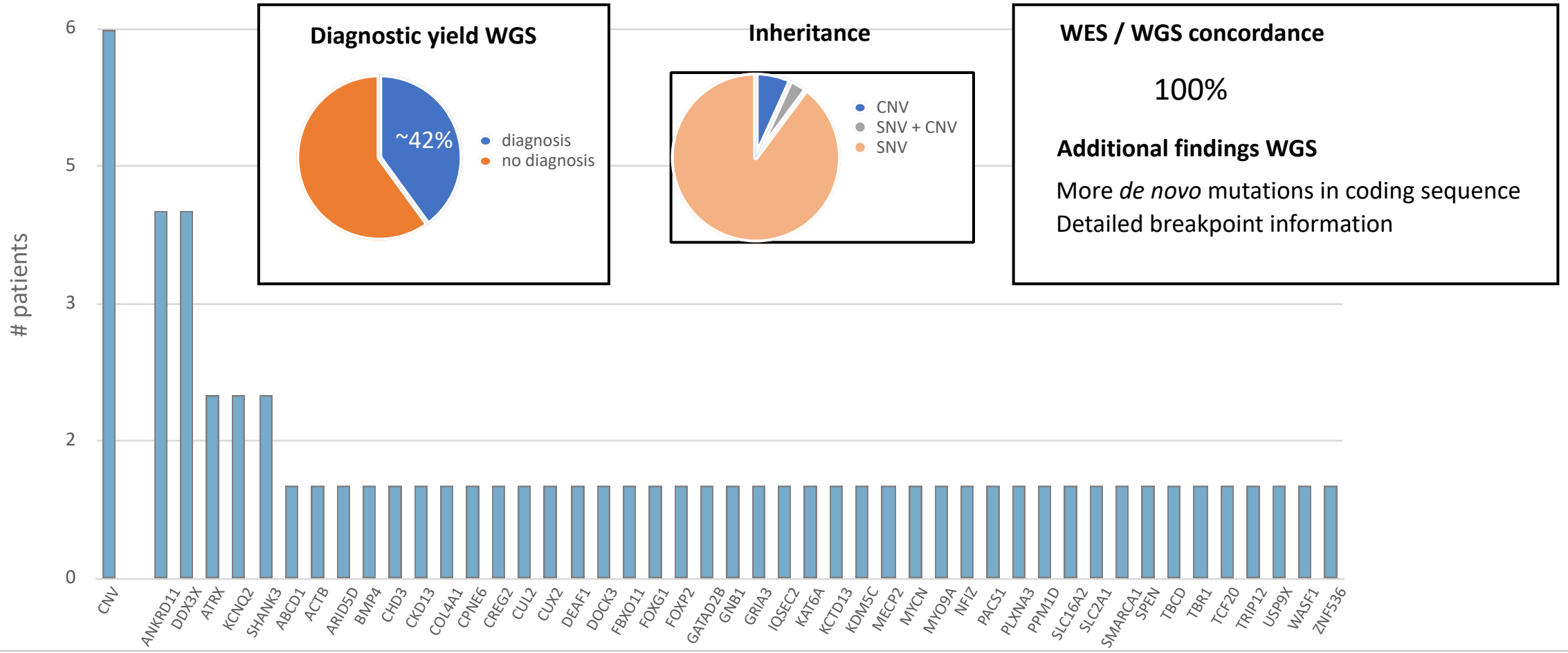
Go Short - WGS can reliably replace WES/array



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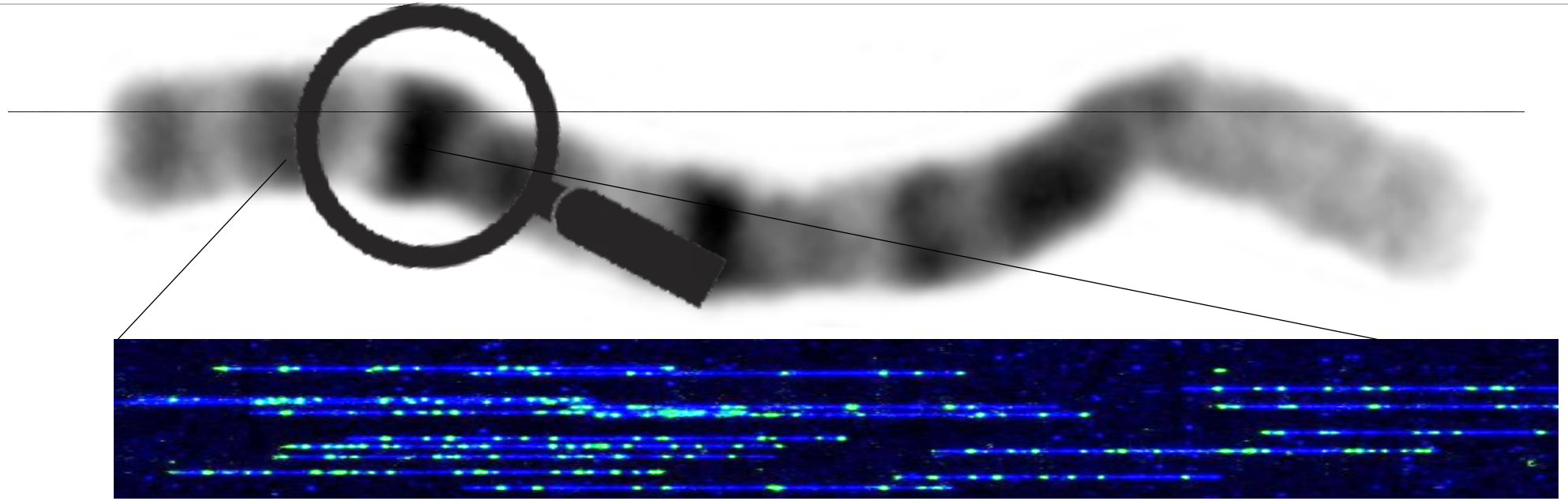
Go Long – Next Generation Cytogenetics



Cytogenetics with 500,000 ‘bands’

~10,000x improved sensitivity!

- Genomewide analysis
- Positional information
- Single molecule resolution

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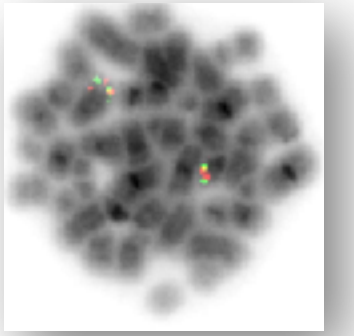
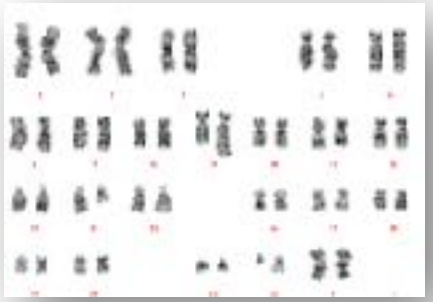
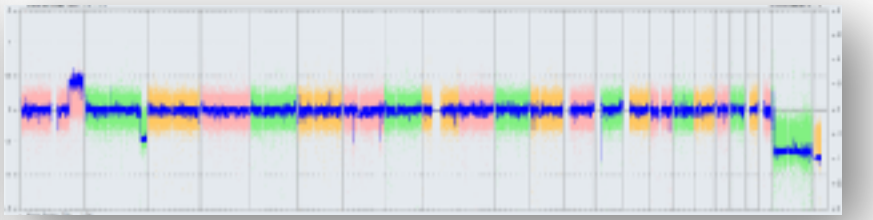
2 years of optical genome mapping

- **85 samples with constitutional cytogenetic aberrations**

  100% concordance

- **52 leukemia samples** compared to the routine work-up

  100% conconcordance if variant is >10%

- In addition: test unsolved rare disease cases: Identify disease causing SVs: **~70 samples**: **5/20 projects 'solved'**

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Go Long - Ongoing long-read SMRT efforts

- >500 PacBio HiFi genomes (1 SMRT cell per sample, main focus SVs) **unsolved rare diseases** (SOLVE-RD): www.solve-rd.eu
- 10 HiFi **unsolved patient-parent trios** 30x coverage in collaboration with PacBio
- **Clinical LR-amplicon based sequencing** (5-20kb) since 2020
  - mtDNA , HLA (5kb amplicons), OPN1, PMS2, vWF, PKD1,
- “de novo“ WGS -projects of various animal and plant species

Go Long - long read sequencing

European Journal of Human Genetics (2021) 29:637–648
https://doi.org/10.1038/s41431-020-00770-0

ESHG

ARTICLE

Long-read trio sequencing of individuals with unsolved intellectual disability

Marc Pauper[1] · Erdi Kucuk[1,2] · Aaron M. Wenger[3] · Shreyasee Chakraborty[3] · Primo Baybayan[3] · Michael Kwint[1] · Bart van der Sanden[1,4] · Marcel R. Nelen[1] · Ronny Derks[1] · Han G. Brunner[1,2,5] · Alexander Hoischen[1,2,6] · Lisenka E. L. M. Vissers[1,4] · Christian Gilissen[1,2]

First long-read genome sequencing (LR-WGS) patient-parent trio study published. Use of LR-WGS enables identification of up to 50,000 structural variants per genome, of which 60% remains hidden when applying standard SR-WGS.

A multi-platform reference for somatic structural variation detection

Jose Espejo Valle-Inclan, Nicolle J.M. Besselink, Ewart de Bruijn, Daniel L. Cameron, Jana Ebler, Joachim Kutzera, Stef van Lieshout, Tobias Marschall, Marcel Nelen, Andy Wing Chun Pang, Peter Priestley, Ivo Renkens, Margaretha G.M. Roemer, Markus J. van Roosmalen, Aaron M. Wenger, Bauke Ylstra, Remond J.A. Fijneman, Wigard P. Kloosterman, Edwin Cuppen

doi: https://doi.org/10.1101/2020.10.15.340497

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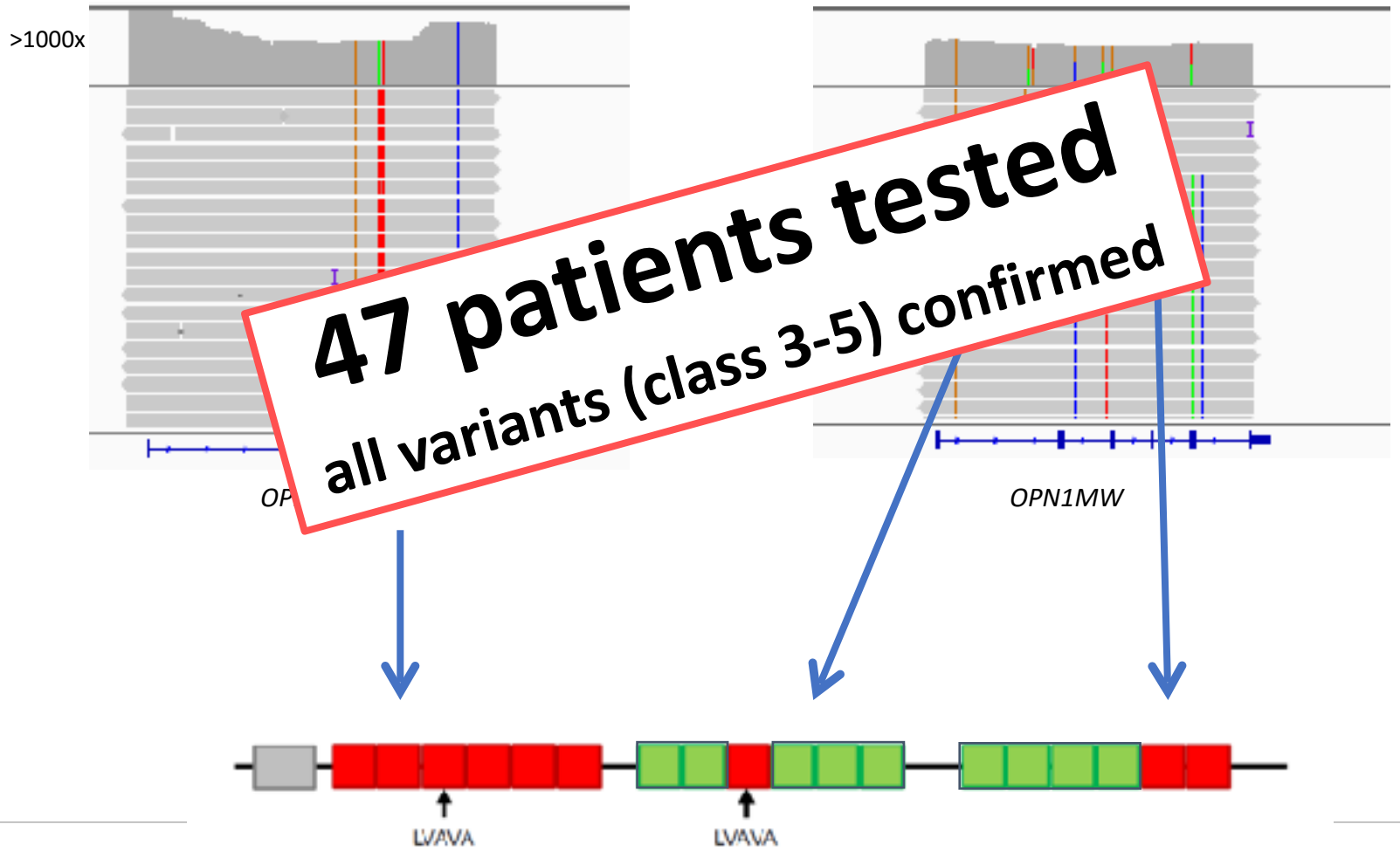
Go Long - OPN1: interpretable by 16 kb reads



- 98% homology, in tandem

nearly impossible with short reads

➢ LR-PCR, shearing,
short read sequencing
MLPA

> analysis very difficult

Go Short, Go Long

- **Short read WES / WGS**
  - Standardized high thoughput generic workflows
  - Sophisticated analysis available
  - Solves up to 60-70% of the rare disease
- **OGM**
  - Ease of use (no bioinformatics / HPC needed)
  - Sequence-context independent for all large SVs (>500bp), bridge repeats (SegDups)
  - Coverage up to 1500x (same price as 100x) → Low level mosaic aberrations
- **HiFi 30x LR-WGS**
  - Best quality & most comprehensive genomes to date
  - Phasing of variants based on assembly
  - Identify hidden variants on all levels.

Konny Neveling

Lisenka Vissers

RGTC

Alex Hoischen

Christian Gillissen

Maastricht UMC+

Human Genetics Nijmegen

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