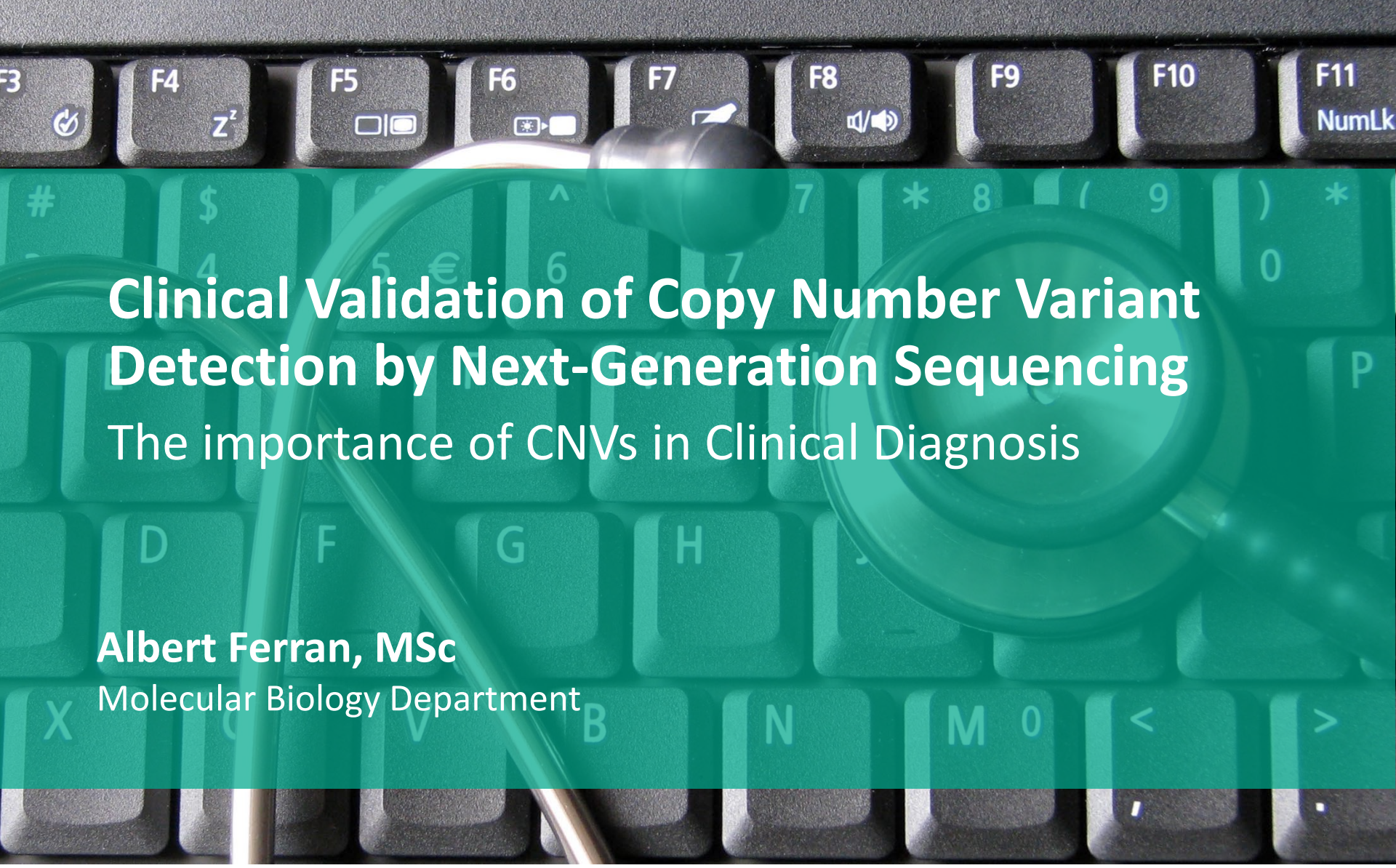

Clinical Validation of Copy Number Variant Detection by Next-Generation Sequencing

The importance of CNVs in Clinical Diagnosis

Albert Ferran, MSc
Molecular Biology Department

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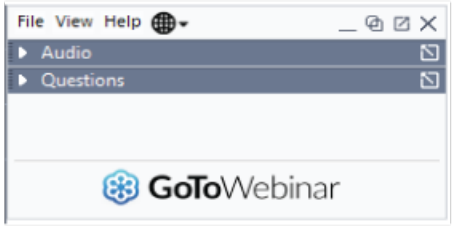

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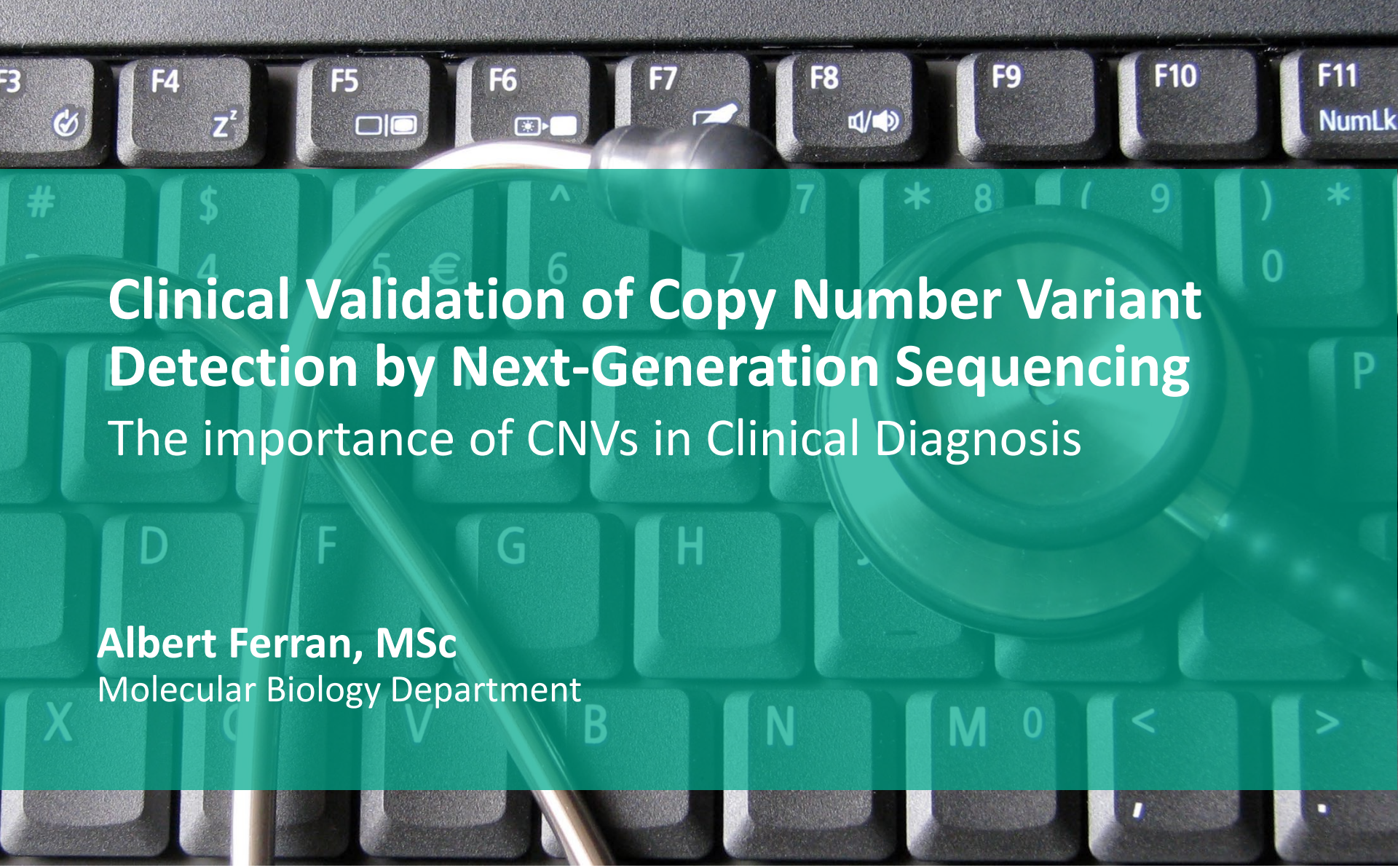

Clinical Validation of Copy Number Variant Detection by Next-Generation Sequencing

The importance of CNVs in Clinical Diagnosis

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Our **SERVICE**

We currently collaborate with >450 hospitals and >1,000 laboratories around the world.

Our passion is to develop new diagnostic tools that have a significant impact on the quality of life of patients and their families.

We review each case individually and propose to carry out the most appropriate genetic studies.

We are the largest genetics laboratory in Europe (15,000m2), always guaranteeing to carry out any genetic study at our own facilities and always using our own resources.

Our **RESPONSE TIMES**

<30 days for any genetic study

We carry out all kinds of genetic studies

- Hereditary genetic diseases
- Study of solid tumours and liquid biopsy
- Oncohematology
- Pharmacogenetics

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Our **TECHNOLOGY**

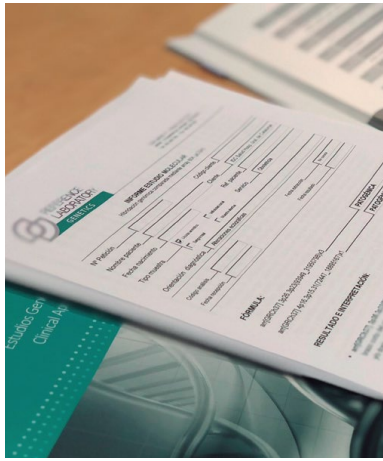
We have the best technology and the most advanced services

NGS, Sanger, MLPA, PCR, aCGH, FISH, cytogenetics, UPLC-tandem masses, RefLab Cloud®, RefLab Database® and support in bioinformatics tools

Our **REPORTS**

We make the difference

- The most complete, based on the guides of the ACMG, the ESHG and the EMQN
- The most conclusive: include interpretation of results and necessary recommendations



OUR **NGS PANELS**

They include all genes of clinical relevance and are constantly updated

- The customisation of any panel
- Study of CNVs. The detected variants are confirmed at no cost by MLPA
- The best coverages in the genes studied. We complement the study without cost by Sanger
- Fast and reliable results
- Possibility to analyze the samples of the parents next to the index patient (trio)

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1 CNVs in the clinical diagnosis

CNVs in the clinical diagnosis

What are CNVs?

- CNVs are a type of structural variants that have been defined as deletions or duplications greater than 1kb in size.

- They are an important factor in the population variability and we find them in most organisms.

- CNVs play an important role in clinical, population and evolutionary analysis.

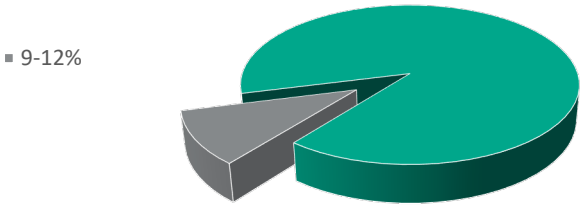
Seon-Hee Yim et al. 2015

CNVs in the clinical diagnosis

CNVs in Human Genome

- The molecular analysis of Copy Number Variants (CNVs) has been introduced into the routine of clinical diagnosis for about 15 years

- This has led to a greater knowledge of the effect and presence of CNVs in humans:
  - CNVs contribute to 9-12% of the diversity of the human genome.



  - Up to 1.2% difference has been detected when compared with the reference genome between individuals due to CNVs.
  - There is a large number of regions with population frequencies above 1%.
  - Nearly 100 genes have been discovered deleted in homozygosis without adverse phenotypic effects

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CNVs in the clinical diagnosis

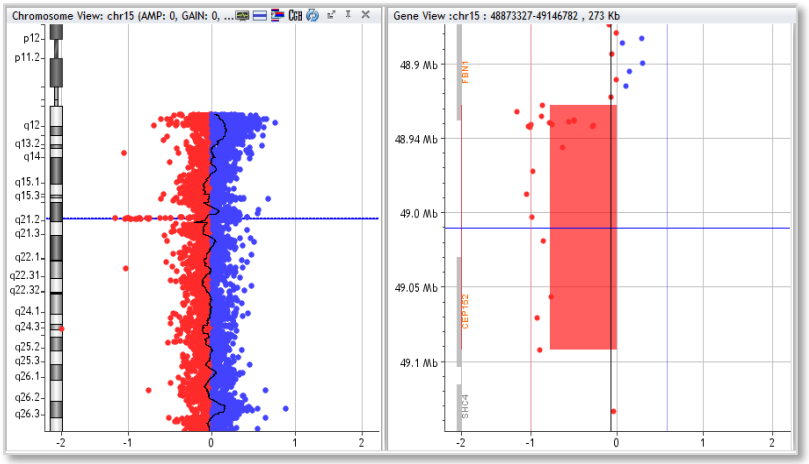
Pathogenicity of CNVs

- CNVs are an important source of pathogenic variants, although the impact of many is not yet known.

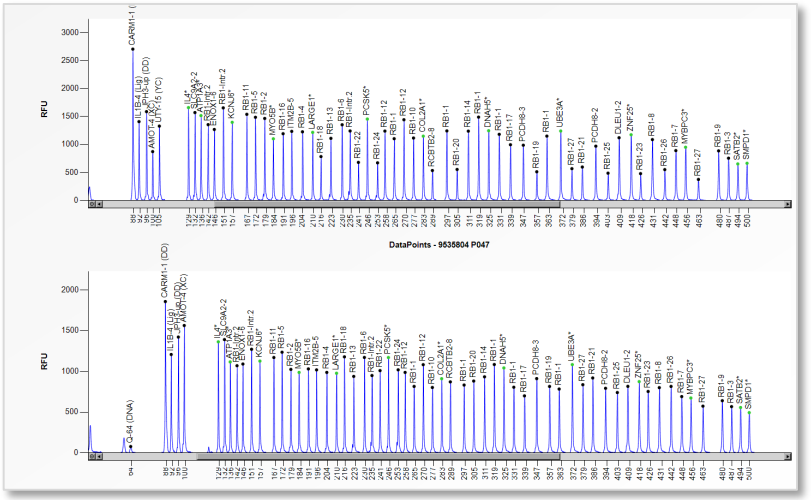
- Some examples of pathologies where CNVs are the main cause:
    - DiGeorge syndrome is a syndrome caused by the deletion of a small segment of chromosome 22.
    - Between 65% and 85% of patients with Dystrophinopathies are due to CNVs in the *DMD* gene.
    - CNVs in the *PMP22* gene produce different clinics, deletions generate Hereditary Pressure Sensitive Neuropathy, while duplications produce Charcot-Marie-Tooth type 1.
    - Pathogenic CNVs have been detected in 17% of patients with developmental delay of unknown etiology. An example would be the Koolen-De Vries syndrome, where we find that deletions in the *KANSL1* gene would explain 95% of the cases that present this syndrome.

CNVs in the clinical diagnosis

Clasical CNVs análisis techniques



ARRAY: Detection of CNVs in the whole genome, the detection sizes are very variable, but generally large. Depending on the number of probes in each region, it is standardised that at least 3 probes are needed for detection.



MLPA: It allows detection of a single exon, but it's very specific to a particular gene.

For the analysis of multiple genes you have to create panels *in house*.

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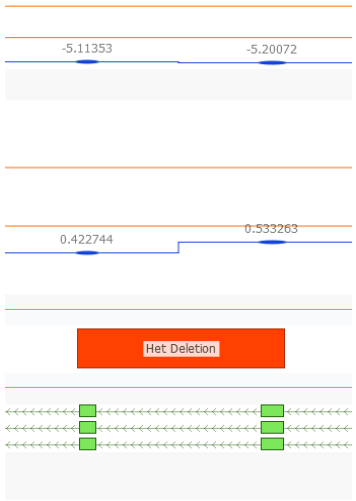
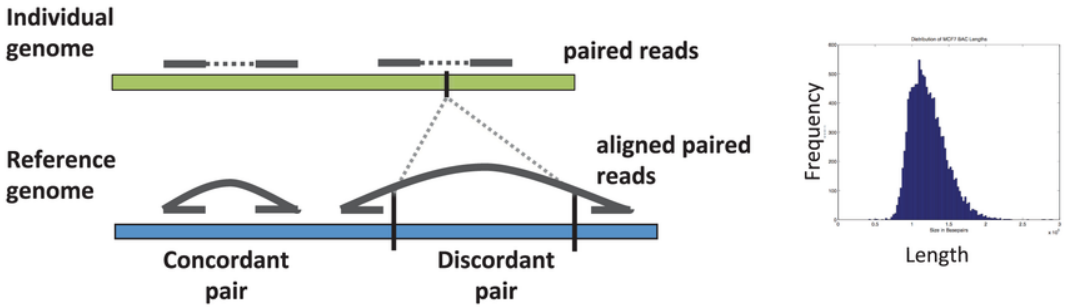
CNVs in the clinical diagnosis

Analysus of CNVs from NGS data

- The data of NGS have been used to make an alternative approach to the CNVs, allowing to analyze both SNVs and CNVs at the same time.
- This data allows detection from individual exons to complete chromosomes, depending on the probes that have the NGS kit.
- These analysis require specialised computational methods.
- High sensitivity.
- Generation of a high number of false positives -> Importance of database.

Depth of coverage	Paired-end mapping





Ben J Raphael (2012).

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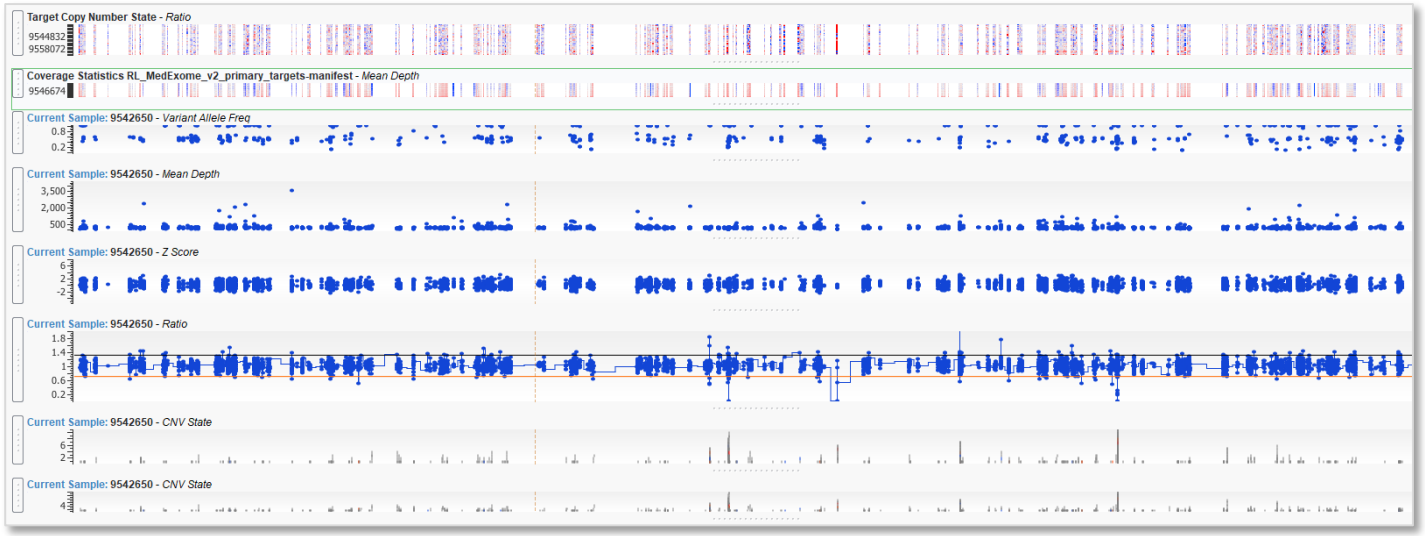
CNVs in Clinical Diagnosis

Analysis of CNVs from NGS data

- VarSeq is a software program with a very moldable architecture, allowing annotation and filtering for NGS data, adaptable to the criteria we see fit.
- Allows analysis of SNVs and CNVs within the same data set.
- The analysis of CNVs is carried out by the approaching of Depth of coverage.
- High sensitivity from regions of an exon to whole chromosomes.



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2 CNVs Validation

CNVs Validation

Our Validation

- Validation performed from 20+ samples with previously known CNVs.

  Deletions-duplications in:

  - One to multiple exons
  - Complete genes
  - Aneuploidies (two Trisomies of 21)

- Large number of false positives: low specificity

  For this reason it's a very important factor work with:

  - BBDD own controls (> 2000)
  - HPO / Clinical Diagnostics -> Work on targeted panels

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CNVs Validation

Our Validation

- Selection criteria for samples for the validation:

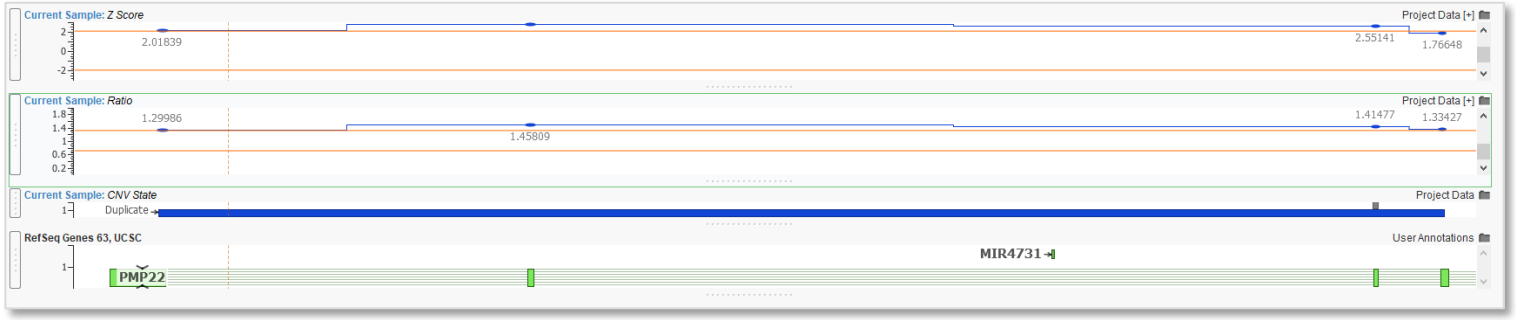
  - Different sizes of events

  - Any type of CNVs; Duplications and Deletions (homozygous and heterozygous)

  - Common pathologies: BRCA1 / BRCA2, F8, ...

  -Areas with many CNVs *: CFHR1-CFHR3 and PMP22

  *Very important to check the quality, since the high number of events affects the normalization



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CNVs Validation

Regions used in the validation

Regions used in the validation		
F8	Homozygous Deletion	e6
CFHR1-CFHR3		Complete
BRCA2	Heterozygous Deletion	e7
MSH2		e1
F8		e26
KCNH2		e12-e14
BRCA1		e18
BRCA1		e16-e20
CLCNKB	Duplication	Complete
PMP22		Complete
Chr21		Complete

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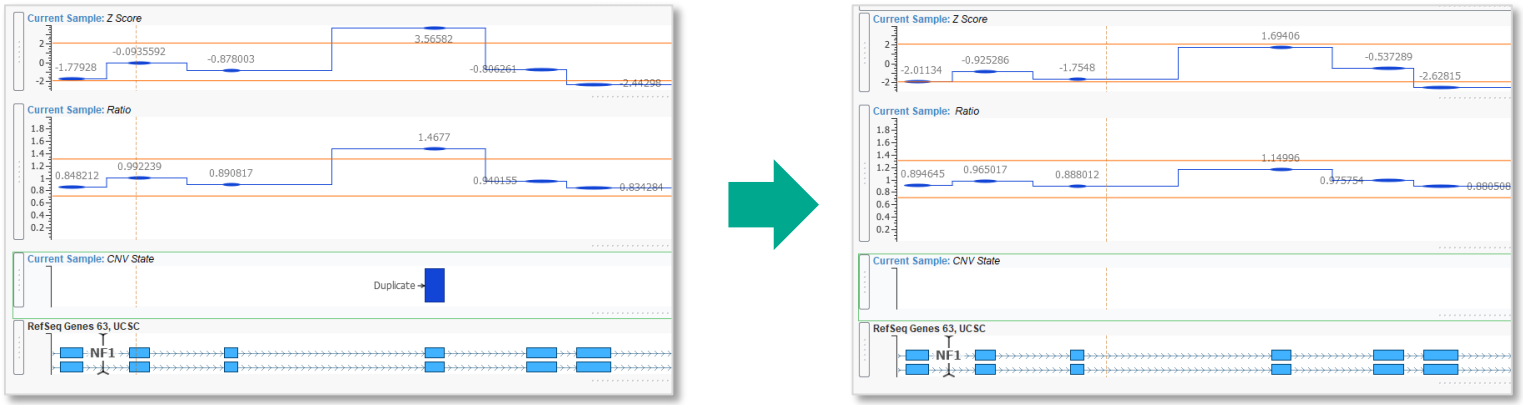
CNVs Validation

Importance of the database and controls

- To fight against low specificity, it is very important to have a high number of controls, to ensure accurate standardization.

  - Controls within the same run

  - Controls between runs



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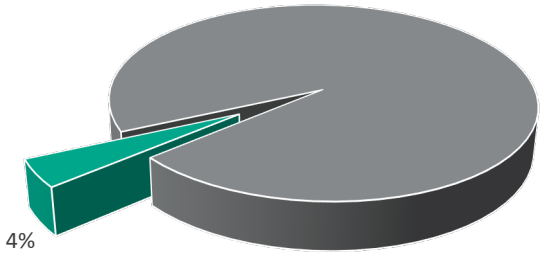
3 Clinical cases

Clinical cases



- Testing of CNVs on 275 samples without conclusive diagnosis in the analysis of SNVs in 2018.
- In 12 of the clinical analysis (4%), a CNV was detected that would explain the pathology.

Clinical cases		
TRPM6	Heterozygous Deletion	e8-e9
TRPM6		e30-e32
TSC2		e1-e30
MSH2		e1-e30
PKHD1		e51
FBN1		e2
RDX		e2
RDX	Homozygous Deletion	e2
KCNV2		Completo

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Clinical cases

- In the first quarter of 2019 we detected 4 positives. Of those, we would like to highlight the following two cases:

  - Complete duplication of BRCA1; detected in a cancer kit, does not have adjacent genes sequenced, we are pending confirmation of the region by a major panel / array.

  - Deletion of an exon in TNXB, gene that presents a pseudogene (explained in clinical cases).

- Possible introduction in Pharmacogenetics; Duplications in CYP2D6 produce rapid metabolizers.

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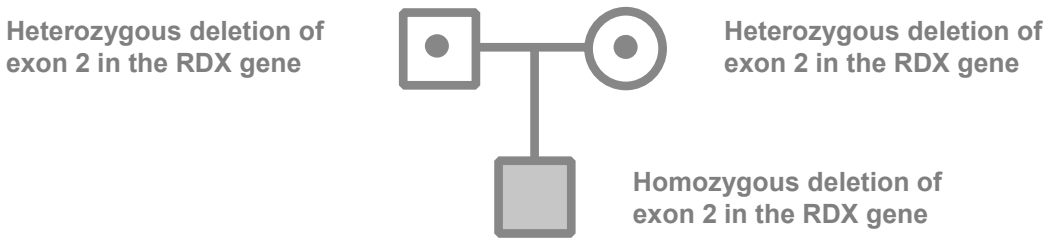
Clinical cases

Homozygous deletion on *RDX*

- Patient with suspected severe prelocutive bilateral sensorineural hearing loss. Patient presenting certain consanguinity.
- A Hereditary Recessive Hearing Loss panel of 39 genes is performed, which is inconclusive.
- Testing of CNVs where variant in homozygosis is detected in exon 2 of the *RDX* gene that would confirm the diagnostic hypothesis.
- Segregation is recommended in the parents and it is verified that both are carriers of the variant.



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Clinical cases

Homozygous deletion on RDX

- RDX is associated with Recessive Autosomal Type 24 Deafness
- It is the only CNV described in the literature on RDX, in an article in 2018, with a single patient with the same deletion in homozygosis in the Spanish population.

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Clinical cases

Homocygous deletion on RDX

- The segregation study confirmed that both parents were carriers of the same variant deletion. No commercial MLPA kit exist to prove it, so it was important for parents to be checked.

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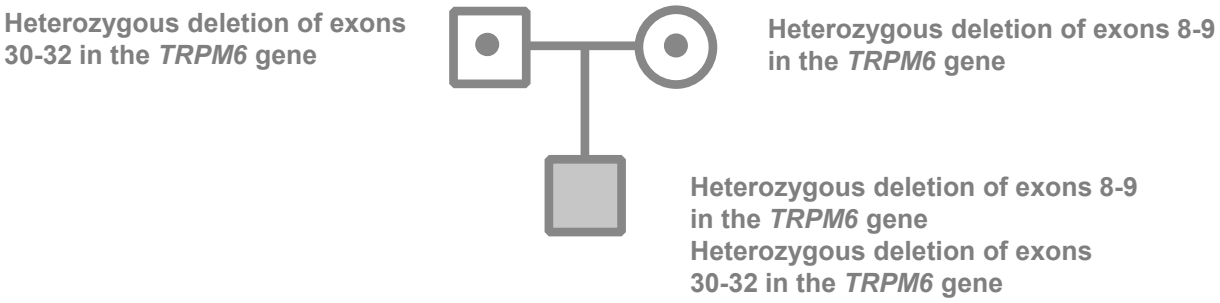
Clinical cases

Compound heterozigous deletion in TRPM6

- Compound heterozygous deletion in TRPM6 patient with chronic hypomagnesemia diagnosed in childhood, with secondary hypocalcemia. There are no other cases in the family.
- Hypomagnesemia with secondary hypocalcemia panel is performed in the TRPM6 gene that is inconclusive.
- Testing of CNVs where two deletions are detected in heterozygosis in the TRPM6 gene that could confirm the diagnostic hypothesis depending on the segregation.
- Segregation is performed in the parents and it is verified that both are carriers of the variant, confirming that in the index patient is in trans.



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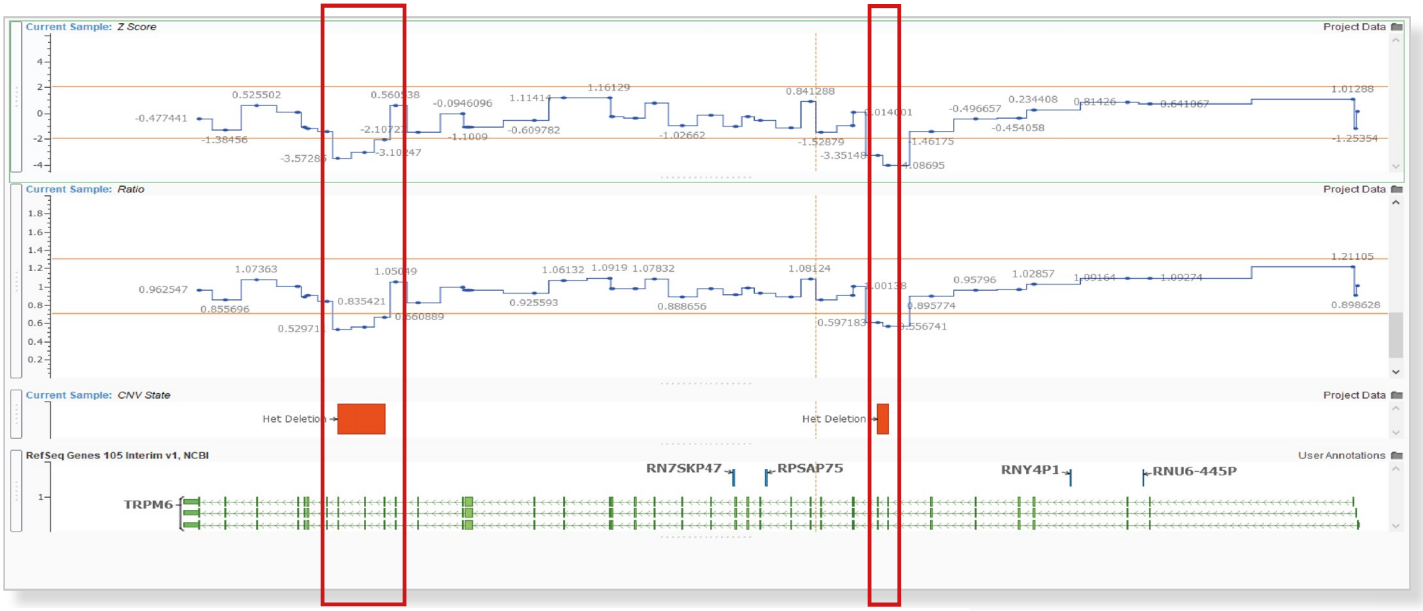
Clinical cases

Compound heterozigous deletion in TRPM6

- TRPM6 is associated with type 1 intestinal hypomagnesemia with autosomal recessive inheritance.
- Not described in clinical databases, but there are a number of deletions from other exons described as pathogenic.



Compound heterozigous deletion in TRPM6 (exons 8-9 / 30-32)

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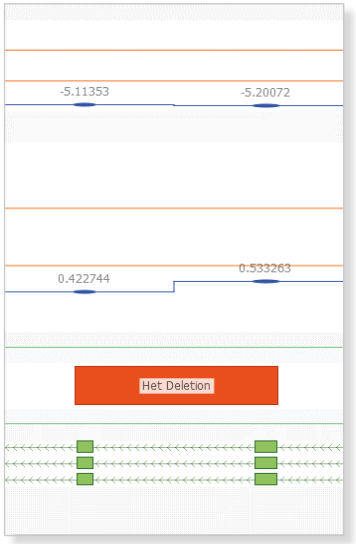
Clinical cases

Compound heterozigous deletion in TRPM6

- TRPM6 is associated with type 1 intestinal hypomagnesemia with autosomal recessive inheritance.
- Not described in clinical databases, but there are a number of deletions from other exons described as pathogenic.

Heterozigous deletion in TRPM6 (exons 30-32)



Heterozigous deletion in TRPM6 (exons 8-9)

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Clinical cases

Homocygous deletion on TNXB

- Patient with osteopenia and joint hypermobility of the skin.
- A panel of Ehler-Danlos Syndrome of 23 genes is performed, which is inconclusive.
- Realisation of CNVs where deletion is detected in homozygosis in the TNXB gene that would confirm the diagnostic hypothesis.

* This gene has an added difficulty because it has a pseudogene - the TNXA gene - but the detected exon is not homologous, being one of the few that can also be analysed by MLPA.

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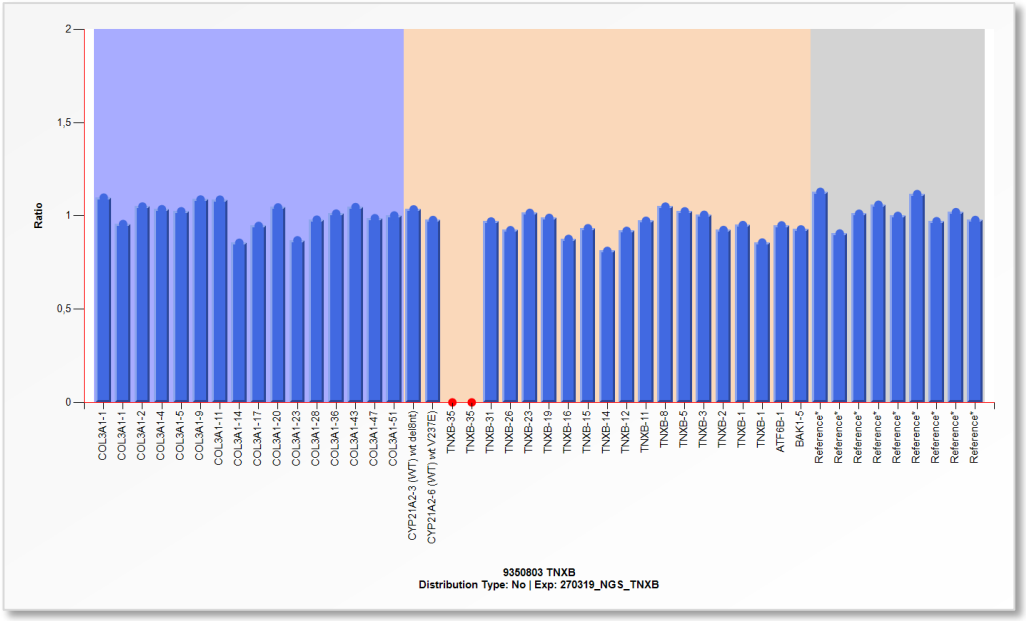
Clinical cases

Homocygous deletion on TNXB

• The TNXB gene is associated with the classical form of Ehler-Danlos type I with autosomal recessive inheritance.



* This gene has an added difficulty because it has a pseudogene - the TNXA gene - but the detected exon is not homologous, being one of the few that can also be analysed by MLPA.

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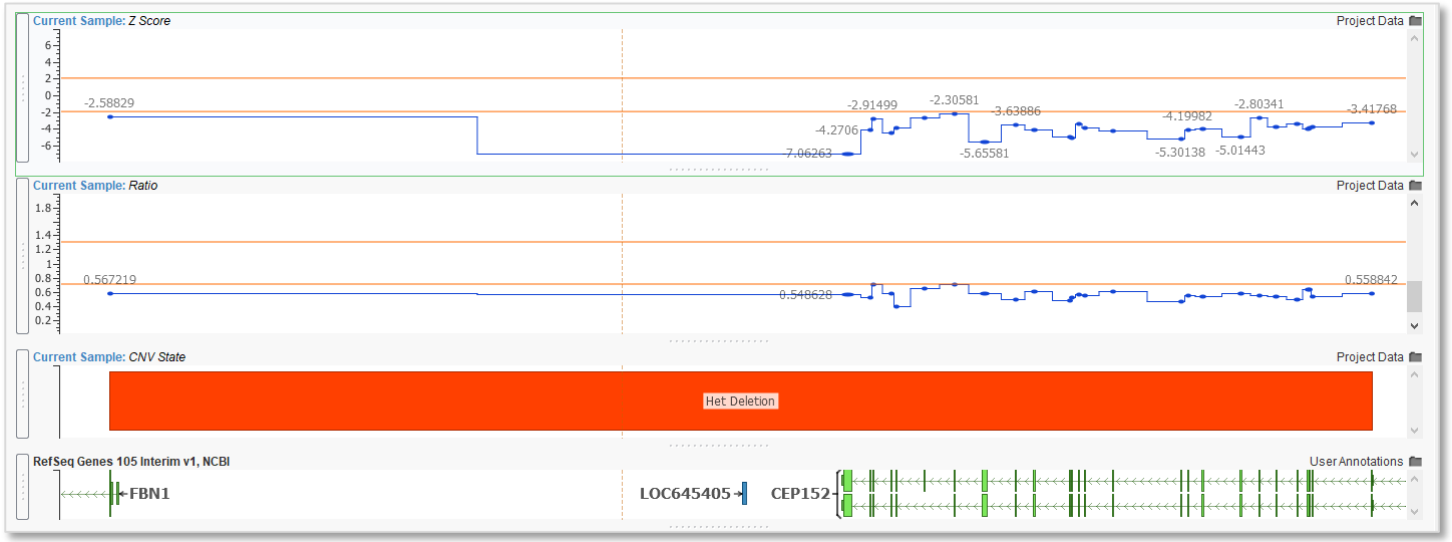
Clinical cases

Heterozygous deletion in FBN1

- Patient with suspected Marfan syndrome
- A Marfan Syndrome panel of 10 genes is performed, which is inconclusive.
- After one year we performed CNVs analysis where a heterozygous deletion is detected in exon 2 of the FBN1 gene that would confirm the diagnostic hypothesis. Additionally evidence that it also affects the CEP152 gene is detected.



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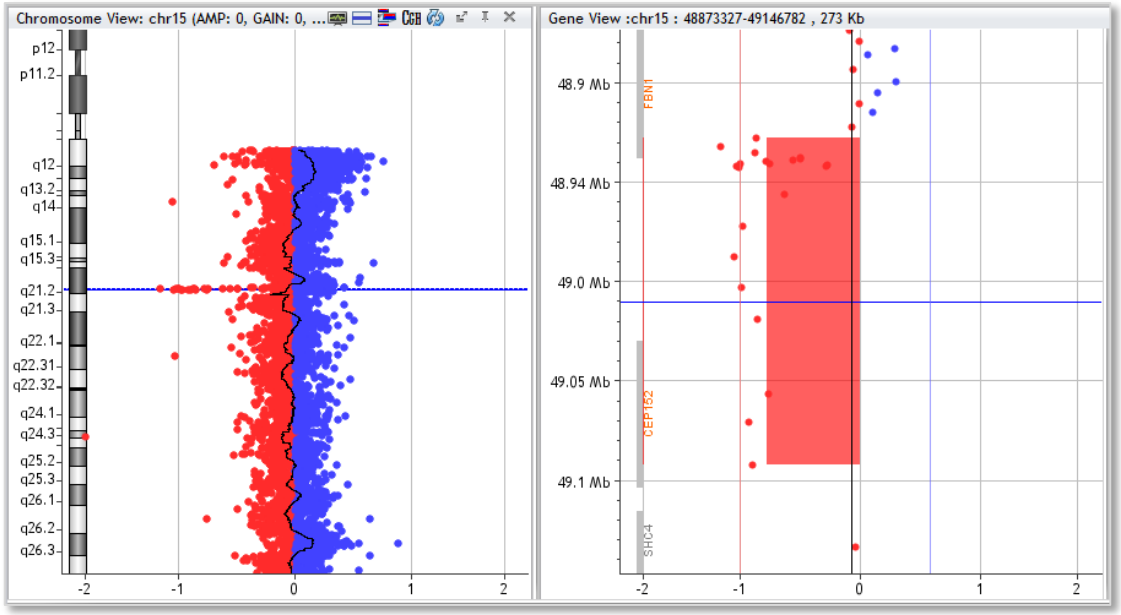
Clinical cases

Heterozygous deletion in FBN1

- FBN1 has been found associated with Marfan syndrome with autosomal dominant inheritance.
- In the bibliography we find multiple references of findings in patients with Marfan of the first exons as well as of adjacent genes.



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Clinical cases

Problems due to duplications in clinical diagnosis

- 46-year-old asymptomatic patient with multiple family history of breast cancer.
- In the bibliography we found multiple references of duplications as risk factors for breast cancer.
- The location of the duplication was not know, therefore it was reported as VOUS.

Where could the duplication be found ?

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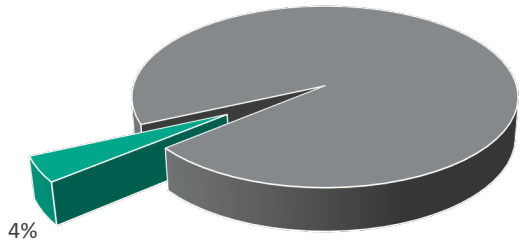
4 Conclusions

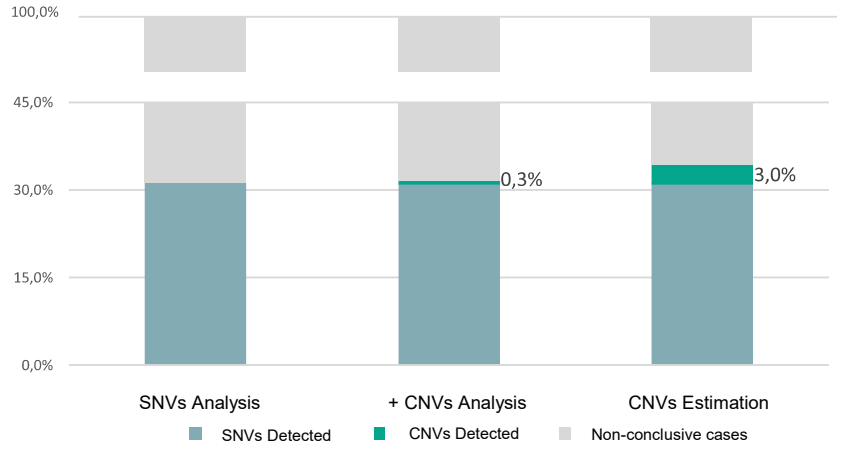
Conclusions

Diagnostic efficiency

Analysis of the Gross Data

- During 2018, in 4% of the samples without conclusive diagnosis in the analysis of SNVs, the cause was detected by CNVs (an increase in the diagnostic efficiency of 0.3% over the overall sample analysed).
- We estimate that if in all cases without diagnostic conclusion received an analysis of CNVs had been made, an increase of 3% in diagnostic efficiency would have been obtained.





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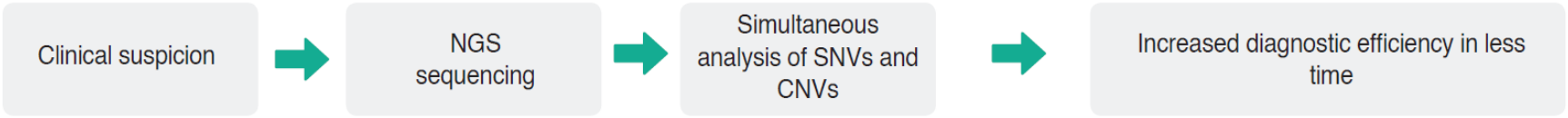
Conclusions

Diagnostic efficiency

Parallel analysis of SNVs and CNVs

- The results obtained confirm that the inclusion of the detection of CNVs by NGS in the routine of genetic diagnosis allows to increase the diagnostic efficiency offered.
- In the commented examples we have seen that not performing the analysis of SNVs and CNVs in parallel increased the time of the diagnostic conclusion in about 1-3 months.
- Therefore, we recommend carrying out the analysis of SNVs and CNVs in parallel.



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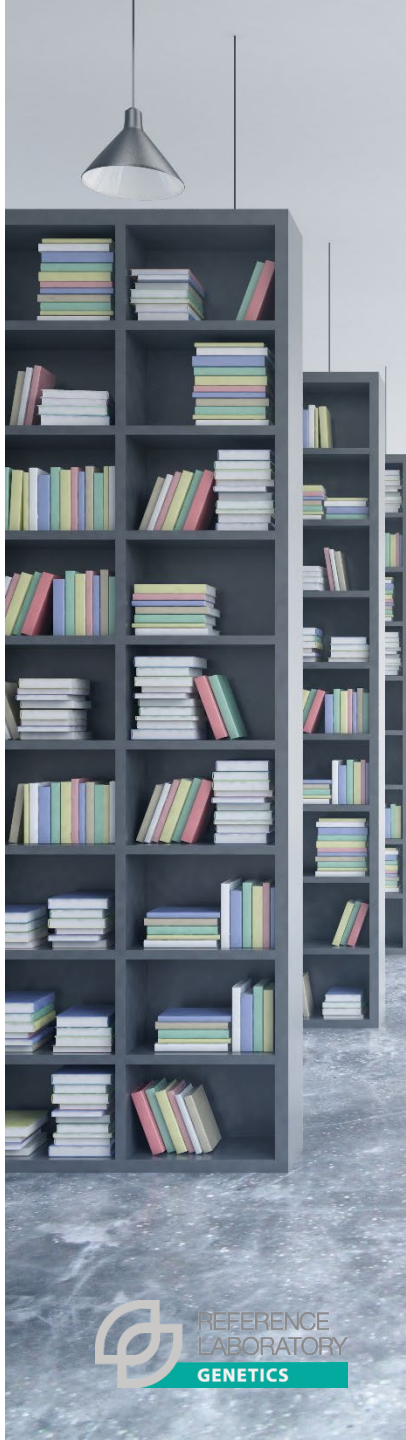

Thanks for your attention

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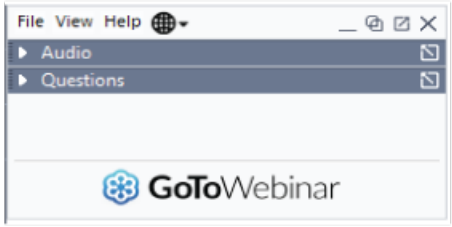

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