Q&A of Workshop 05: Copy Number variant interpretation and classification

W05.1: "**How relevant CNVs can be missed**"

Q1: Did you used one CNV caller? Which one(s)?

A: We routinely use the CoNIFER algorithm to detect CNVs in exome data and in addition to that, ExomeDepth is predominantly used for the detection of homozygous exon losses as well as numerical chromosomal anomalies (aneuploidy).

Q2: Would you consider using the ACMG guidelines for CNV classification?

A: Yes, we do use the ACMG guidelines for CNV classification, but not for every CNV. Only if it is not clear whether it is a class 3 or 4 we like to use the ClinGen CNV Pathogenicity Calculator.

Q3: Case 1: did you do CNV analysis from exome data before the genome-wide array analysis?

A: Yes, extensive trio exome data analysis was done in this patient and her parents, first OMIM gene panel analysis and then subsequently exome wide analyse, both for nucleotide variants and CNVs.

Q4: For W05.1. For case 1, why CNV detection was not directly performed when WES was done? From what I understand, previously WES was done, but no pathogenic single point variant was identified and then exonic aCGH was done and the DEL and DUP in NXN were detected and thus also observed from WES data.

A: We did perform CNV in exome data analysis and the loss in the NXN gene was detected and called in the proband, but no 2nd mutation was detected and therefore this was loss was not reported in the two exome reports. The gain in the NXN gene was not called in the exome data, because this copy number gain was just below the threshold of the CNV calling.

Q5: I was wondering after CNV analysis using exome data, did you validate detected variants in mother and father with other techniques?

A: No, we did not validate these CNVs in the parents, because the loss in NXN was called in the exome data by the software and the gain appeared to be de novo in the proband. The proband (born in 1991) wanted to know whether she has a dominant or recessive form of Robinow syndrome, so no further testing was necessary in this family.

Q6: The third case can be solved by WGS, do you think other CNVs in other cases can be captured by WGS as well?

A: Absolutely! WGS is very suitable to detect CNVs and the breakpoints of the CNVs can be determined more accurately, at the nucleotide level. Moreover, CNVs smaller than 1,000 bp, which are not detectable by array or WES, are likely to be detected by WGS.

Q7: From your experience, which technique have high coverage rate and diagnostic yield in ID and developmental delay patients: A) WES B) WGS C) HD array

A: Currently, WES has a fairly high diagnostic yield of 30-40% in patients with neurodevelopmental disorders, if you perform both SNV and CNV analysis in the exome data. Nucleotide variants cannot be detected by array, which has a diagnostic yield of 10-15% in NDD patients. Based on clinical utility studies, the diagnostic yield of WGS is at least as high as that for WES, but with increasing knowledge on intergenic and intronic variants as well as various structural variants, either balanced or unbalanced, the diagnostic yield of WGS will increase for sure.

W05.2: "Don't forget about the X"

Q1: I don't understand the motivation behind doing MLPA since 80% of MECP2 positions have coverage >20x. This should motivate Sanger sequencing, not MLPA. Currently, even small CNVs can be detected in WES, however, MLPA is a much more reliable technique. I would understand better the MLPA motivated by the poor reliability of WES CNVs predictions.

A: In our lab at the time of this analysis, CNV detection was not yet possible/ offered in exome-data. Before, our lab used to Sanger sequence the MECP2 gene, and perform MLPA to cover for dels/dups. When going over to analysing all genes by exome-sequencing, but first without CNV detection, the decision was made to keep performing MLPA for this gene, because dels are quite frequent. I agree that you would need to perform subsequent Sanger sequencing to fully cover the gene because of the 80% coverage. But because the phenotype was not so typical for Rett, I decided to first look broader in the exome-data for another possible diagnosis. When nothing would have been found I would have requested Sanger sequencing for the MECP2 gene in another laboratory who does still offer Sanger sequencing for the gene.

Q2: Did you test for x-inactivation pattern in the mother since the Xq25 dup was maternally inherited? Did you confirm skewed x inactivation in the mother and daughter from the last case?

A: No, this was not performed. The family did not want further testing and there is only limited data on X-inactivation patterns in females with this syndrome (see below), thus making drawing substantial conclusions about the outcome of the test impossible. Otherwise, it would have been a good test to try to further gather prove that the duplication indeed is the cause of the phenotype in this girl.

According to the study by Carrel et al. and Cotton et al., the STAG2 gene is subject to X-chromosome inactivation in humans and thus, expressed only by the active X chromosome. Leroy et al (DOI: 10.1111/cge12567) in 2016 found moderate skewed x-inactivation in two carrier females who both had learning difficulties. They propose that the mosaic functional Xq25 disomy may explain the minor clinical features observed in the females. On the other hand, various skewed X-inactivation between tissues may account for the clinical variability of the syndrome in females. Kumar et al. in 2015 found slightly skewed X-inactivation (85:15) in a mother with borderline ID and schizophrenia. No data is known about the x-inactivation pattern in female carriers without a phenotype.

Q3: how can you explain a more severe phenotype in females when X-linked disorder?

A: Skewed x-inactivation in favour of the X-chromosome with the mutated gene in an X-linked disorder can cause more severe symptoms in females. On the other hand, skewing in favour of the normal copy of the gene can cause absence of a phenotype in an asymptomatic female carrier. Another factor that might also play a role can be the fact that for many syndromes there is variable expression, independent of x-inactivation. For example, in two brothers with the same X-linked syndrome, one can be more severely affected than the other because of variable expression. The same can also be true in girls.

Q4: Is the variant in HTT gene still considered for any clinical consequence in the individual or was it excluded due to mode of inheritance? Could it have any modifying effect?

A: for now, it is most likely that she is only a carrier of this syndrome (1 likely pathogenic variant in an autosomal recessive gene) because no second hit was found on the other allele in the exome data and XON array-data. But that can never be fully excluded with the present techniques. There is no evidence of a modifying effect of this variant on her phenotype at present. We will follow-up on the girl in the future to see if there are any unexplained phenotypic features.

W05.3: "New features for CNV classification in DECIPHER"

Q1: Is there any way to access Decipher data programmatically? Any API to interrogate programmatically genes and detected CNVs?

A: There is no API to programmatically interrogate the DECIPHER data, however, anyone can go to the DECIPHER website and browser the information. DECIPHER also shares the DECIPHER anonymized patient data in bulk (~40,000 records, phenotype and variant data) for research purposes or display, subject to an agreement. If you are interested in applying for access, please email contact@deciphergenomics.org.

Q2: To Julia. Great database. Can you use the CNV interpretation function in Decipher to assign ACMG criteria in patients were there is no consent for submission to Decipher?

A: It is not possible to use the ACMG/ClinGen pathogenicity interface without depositing the patient variant data to DECIPHER. However, depending on local regulations, patient consent is only required for open sharing. Anonymized patient data can be deposited into a private area in DECIPHER which only members of your centre (who are logged into DECIPHER) can access. This allows individuals to deposit patient data to DECIPHER and use the interpretation interfaces before explicit patient consent for open sharing is obtained. Once patient consent is attained this can be recorded in DECIPHER and then the record will be shared openly on the website. DECIPHER stores unshared data for two years, allowing adequate time for consent to be obtained. DECIPHER also supports consortium sharing, which is sharing between trusted partners (e.g. between UK National Health Service centres). Depending on local regulations, explicit patient consent may not be required to consortium share anonymized patient data.