Introduction

• Structural brain malformations result from disruptions in normal brain development and are often associated with developmental and neurological symptoms such as intellectual disability, epilepsy, and movement disorders.
• Recent advances in neuroimaging have enabled better classification systems for the various types of structural brain malformations, aiding in the identification of the genetic etiology for many of these disorders, including cortical malformations, pontocerebellar hypoplasia, and Joubert syndrome.
• To date, over 100 genes have been reported in association with brain malformations. These genetic brain malformation disorders demonstrate significant clinical and genetic heterogeneity and are inherited in an autosomal dominant, autosomal recessive, or X-linked manner.

Objectives

• Describe the diagnostic yield of multi-gene panels for brain malformation disorders
• Recognize the phenotypic indications most likely to yield a positive result by multi-gene panel testing for brain malformation disorders
• List genes commonly associated with different brain malformation syndromes
• Recognize the clinical importance of uncommon molecular findings, such as mosaicism and gene deletions

Methods

• Using next generation sequencing and exon-level array CGH, genetic testing was performed on one of the four brain malformation panels in over 300 patients with various brain malformations. The four panels are comprehensive brain malformations panel (44 genes), cortical brain malformations panel (24 genes), pontocerebellar hypoplasia panel (7 genes) and Joubert syndrome and related disorders panel (13 genes).
• Using genomic DNA from the submitted specimen, the coding regions and splice junctions of targeted genes were enriched by microdroplet PCR (RainDance Technologies) and sequenced simultaneously by massively parallel (NextGen) sequencing on an Illumina platform with single-end reads. Capillary sequencing was used to confirm all potentially pathogenic variants and to obtain sequence for regions where fewer than 15 reads were achieved by NextGen sequencing.
• Concurrent deletion/duplication testing was performed for target genes in the panels using exon-level oligo array CGH. Data analysis was performed using gene-specific filtering. Confirmation of copy number changes was performed by MLPA, qPCR, or repeat array CGH analysis.
• Positive genetic diagnosis: one or two pathogenic or likely pathogenic variants in a single gene, depending on the mode of inheritance of the disorder.
• Criteria used to assess the pathogenicity of a variant: frequency in general population, publication, in silico predictions, functional evidence, variant location, De novo or segregating with phenotype (parental studies). All data were analyzed prior to the implementation of the updated ACMG Standards and Guidelines for the classification of sequence variants.

Results
Results Cont.

- A positive genetic diagnosis was established in 46 patients (15%). The diagnostic yield varied greatly by test indication, ranging from 36% for patients who had Joubert panel testing (13 gene panel) to 7% for non-specific test indications (44 gene comprehensive panel). (Figure 1)

- Mosaicism in blood was detected in two male patients, both of whom had pathogenic missense variants in the DCX gene. Mosaicism was detected by NextGen (term defined in Methods) sequencing at 44% and 9% in these patients, and confirmed by Sanger sequencing. (Figure 2)

- Among all pathogenic variants identified missense variants were the most common type (36.7%). Approximately 7% of pathogenic variants were gene deletions, ranging in size from a single exon to the entire gene (2kb to 89kb).

- Most positive results for brain malformation panels were identified in genes associated with X-linked disorders (40%). 30% were in genes associated with AD disorders, 30% were in genes associated with AR disorders. The latter included patients who were homozygous (29%) or compound heterozygous (71%).

- Approximately half of all positive cases tested for cortical brain malformation panel had a form of lissencephaly.

- Majority of pathogenic variants are in well-established genes for lissencephaly (PAFH1B1 and DCX), periventricular nodular heterotopia (FLNA), Joubert syndrome (AH1, CC2D2A, CEP290, and TMEM67), and cerebellar hypoplasia (CASK and TSEN54).

Conclusions

- The average diagnostic yield of brain malformation panels is 15% with highest diagnostic yield of 36% for the Joubert syndrome panel.

- The majority of positive cases were due to pathogenic variants in the following genes commonly associated with brain malformations: PAFH1B1, DCX, FLNA, AH1, CC2D2A, CEP290, TMEM67, CASK and TSEN54.

- Missense variants were the most common type of identified pathogenic variants.

- Our results show that multi-gene panels including deletion/duplication analysis are an effective diagnostic approach for patients with brain malformations, with a high yield for patients with suspected Joubert syndrome.