Published in final edited form as:

Am J Med Genet C Semin Med Genet. 2018 June ; 178(2): 175–186. doi:10.1002/ajmg.c.31622.

Cytogenetics and Holoprosencephaly: A Chromosomal Microarray Study of 222 Individuals with Holoprosencephaly

Tommy Hu^{1,*}, Paul Kruszka^{1,*}, Ariel F. Martinez¹, Jeffrey E. Ming², Emily K. Shabason^{1,3},
Manu S. Raam^{1,4,5}, Tamim H. Shaikh⁶, Daniel E. Pineda-Alvarez^{1,7}, and Maximilian
Muenke^{1,**}

¹Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

²Division of Human Genetics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

⁶Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

Abstract

Holoprosencephaly (HPE), a common developmental forebrain malformation, is characterized by failure of the cerebrum to completely divide into left and right hemispheres. The etiology of HPE is heterogeneous and a number of environmental and genetic factors have been identified. Cytogenetically visible alterations occur in 25% to 45% of HPE patients and cytogenetic techniques have long been used to study copy number variants (CNV) in this disorder. The karyotype approach initially demonstrated several recurrent chromosomal anomalies, which led to the identification of HPE-specific loci and, eventually, several major HPE genes. More recently, higher-resolution cytogenetic techniques such as subtelomeric multiplex ligation-dependent probe amplification (MLPA) and chromosomal microarray have been used to analyze chromosomal anomalies. By using chromosomal microarray, we sought to identify submicroscopic chromosomal deletions and duplications in patients with HPE. In an analysis of 222 individuals with HPE, a deletion or duplication was detected in 107 individuals. Of these 107 individuals, 23 (21%) had variants that were classified as pathogenic or likely pathogenic by board-certified medical geneticists. We identified multiple patients with deletions in established HPE loci as well as three patients with deletions encompassed by 6q12–q14.3, a CNV previously reported by Bendavid et al. In addition, we identified a new locus, 16p13.2, that warrants further investigation for HPE association. Incidentally, we also found a case of Potocki-Lupski Syndrome, a case of Phelan-McDermid Syndrome, and multiple cases of 22q11.2 deletion syndrome within our cohort. These data confirm the genetically heterogeneous nature of HPE, and also demonstrate clinical utility of chromosomal microarray in diagnosing patients affected by HPE.

**Corresponding author: mamuenke@mail.nih.gov.

³Present address: Division of Developmental and Behavioral Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA, USA

⁴Present address: Shriners for Children Medical Center, Pasadena, CA, USA

⁵Present address: Children's Hospital Los Angeles, Los Angeles, CA, USA

⁷Present address: Invitae Corporation, San Francisco, CA, USA

*Co-first authors

CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

Keywords

Holoprosencephaly; copy number variant; CNV; chromosomal microarray; array CGH

INTRODUCTION

Holoprosencephaly (HPE) is the most common congenital anomaly of the developing forebrain in humans, occurring in approximately 1:250 conceptuses (Matsunaga and Shiota, 1977). HPE is characterized by failure of the cerebrum to form fully distinct right and left hemispheres, resulting in partial or complete fusion of the cerebral cortex and underlying structures across the midline. Classic HPE can present with cognitive impairments, seizures, and distinct craniofacial abnormalities such as cyclopia (one eye), synophthalmia (two fused eyes), and proboscis. Less severe forms of HPE, often referred to as microform HPE, may present with no cognitive impairment and milder craniofacial abnormalities such as microcephaly, hypotelorism, cleft lip and/or palate, and a single maxillary central incisor (Muenke and Beachy, 2001; Solomon et al., 2010).

HPE is etiologically heterogeneous, and a number of both environmental and genetic causes have been identified (Muenke and Beachy, 2000; Ming and Muenke, 2002). Familial inheritance of HPE can occur as a part of multiple-malformation syndromes, inherited in either an autosomal dominant or autosomal recessive manner, such as Smith-Lemli-Opitz syndrome (reviewed in this issue; Kruszka and Muenke, 2018), or can be inherited in an isolated manner (Stokes et al., 2018; Kruszka et al., 2015). Furthermore, cytogenetic anomalies, such as trisomy 13 and trisomy 18, can cause HPE (Roessler and Muenke, 1998; Kruszka and Muenke, 2018). In general, cytogenetic anomalies are estimated to be present in 25% to 45% of individuals with HPE (Croen et al., 1996; Olsen et al., 1997; Bullen et al., 2001).

Analysis of cytogenetic anomalies has long been used in the diagnosis and study of HPE. An association between *SIX3* and HPE was first suggested by our group (Muenke et al., 1988) after a deletion in 2p21 in a patient with HPE was identified through high-resolution analysis of GTG-banded chromosomes. At the time, we also began to recognize specific patterns of chromosomal anomalies associated with HPE (Figure 1; Muenke et al., 1988). Detection of recurrent chromosomal anomalies by karyotype eventually led to the identification of 12 HPE candidate loci (Roessler and Muenke, 1998). Molecular studies of these regions helped identify four major causative genes in HPE, including *SHH* at 7q36 (OMIM 600725) (Belloni et al., 1996; Roessler et al., 1996), *ZIC2* at 13q32 (OMIM 603073) (Brown et al., 1998), *TGIF1* at 18p11.3 (OMIM 602630) (Gripp et al., 2000), and *SIX3* at 2p21 (OMIM 603714) (Wallis et al., 1999).

Sequence variants in these four major genes, however, only account for approximately 25% of genetic cases of HPE after excluding for chromosomal anomalies (Dubourg et al., 2007). Further studies on chromosomal anomalies (Ming and Muenke, 2002) helped reveal more candidate HPE loci (Figure 2) and led to the establishment of the SHH signaling pathway as a hallmark in HPE pathogenicity: *PTCH1* at 9q22 (OMIM 601309) (Ming et al., 2002), *GLI2* at 2q14 (OMIM 165230) (Roessler et al., 2003), and *DISP1* at 1q41 (OMIM 607502)

(Roessler et al., 2009a). Coinciding with these discoveries was the identification of two contributory genes in the NODAL/TGF- β pathway: *TDGF1* at 3p21.31 (OMIM 187395) (de la Cruz et al., 2002) and *FOXH1* at 8q24.3 (OMIM 603621) (Roessler et al., 2008).

With the advent of technologies such as subtelomeric multiplex ligation-dependent probe amplification (MLPA), which is capable of greater resolution than traditional karyotype analysis (Schouten et al., 2002), groups such as Bendavid et al. were able to identify candidate HPE loci in novel subtelomeric regions not previously reported by karyotype analysis. Bendavid et al. screened 181 patients with HPE using MLPA and found six patients with chromosomal anomalies in novel regions, including two cases each with a 1p deletion, and one each with a 5q deletion associated with a 17q gain, an 18q deletion associated with a Xq gain, a 15q subcentromeric gain, and a 22q deletion, respectively (Bendavid et al., 2007). However, subtelomeric MLPA has important limitations, such as the extrapolation of copy-number alterations of a relatively small probe to a rather large subtelomeric region. As consequence, these CNVs detected by this MLPA assay may not represent the minimal critical region for such subtelomeric disorders. To date, no novel HPE genes have been identified in these regions.

Following up on their work, Bendavid et al. used microarray-based comparative genomic hybridization (array CGH), which combines the principles of traditional comparative genomic hybridization with the use of microarrays, allowing for the detection of chromosomal CNVs on a genome-wide and high-resolution scale, also known as chromosomal microarray (CMA), to study a cohort of 111 HPE patients with normal karyotypes. Within this cohort, Bendavid et al. identified 19 patients with *de novo* gains and/or losses, some of whom had deletions in previously identified HPE loci, such as 21q22.3 (*HPE1*) and 14q13 (*HPE8*). However, they also identified deletions in new candidate loci not previously associated with HPE, including one patient with a deletion including 1p31.3-p31.1, one with a 6q12-q14.3 deletion, four patients with a 10p12.1 deletion, one with a 19pter deletion, and one with a Xp11.23-p11.22 deletion (Bendavid et al., 2009). Given the lack of follow-up studies on these rather large regions and the complex genetic heterogeneity of HPE, further investigation of these loci is required to determine their clinical significance. In this study, we evaluated 222 patients with HPE for copy number variants (CNV) using array CGH to potentially corroborate past findings by other groups and identify new HPE loci.

MATERIALS AND METHODS

Patients

We studied 222 pediatric and adult patients with HPE spectrum ascertained through our institutional review board approved research protocols (ClinicalTrials.gov: NCT00088426), with appropriate informed consent. Details of the clinical evaluations were provided by the referring physicians, including a standardized checklist, genetics consultation notes, neurological imaging results, and other records, when available. All patients underwent molecular testing by Sanger sequencing for the four main HPE genes as previously described (Roessler et al, 2009b; Pineda-Alvarez et al., 2010). Results of the four-gene screening, when positive, are presented in the Supplementary Tables I and II.

Chromosomal Microarrays

Patients were screened for copy number variants (CNVs) through one of three different CMA designs. Patients were recruited at various points over the span of a few years. Assignment of a patient to a given CMA design was dependent on the time of recruitment and available technology, as opposed to his or her specific phenotype or previous test results. Group 1, which only consists of two patients, was evaluated with chromosomal microarray as previously described (Stokes et al., 2018). Group 2 was screened using SurePrint G3 Custom Microarrays with 180K probes distributed throughout the genome, with denser coverage over regions of clinical interest and chromosome X (Agilent Technologies, Inc.). It should be noted that, within this group of patients, 8 patients with known deletions in 18p were intentionally included. Five hundred ng of patient genomic DNA was labeled with Cyanine-5dUTP (Cy5) (Enzo Life Sciences, Inc.), and 500 ng of gender matched control DNA (Human Genomic DNA, Promega) was labeled with Cyanine-3dUTP (Cy3) (Enzo Life Sciences, Inc.). The DNA samples were then blocked, hybridized onto the microarray slides, cleaned and scanned with a DNA Microarray Scanner with SureScan High-Resolution Technology (Agilent Technologies, Inc.) at 3 μ m, double-pass, as recommended by the manufacturers. The array images were extracted using Feature Extraction Software Version 10.7.3.1 (Agilent Technologies, Inc.) and the analysis was performed in Genomic Workbench Standard Edition Version 5.0.14 for array CGH (Agilent Technologies, Inc.). Copy numbers were considered under the following parameters: loss of copy if four or more consecutive probes had a Log_2 of the ratio of the normalized intensity of Cy5 over Cy3 equal to or less than -1 , and gain of copy if four or more consecutive probes had a Log_2 of the ratio of the normalized intensity of Cy5 over Cy3 equal to or greater than 0.58 .

Group 3 was screened using the Affymetrix GeneChip 500K arrays (Affymetrix, Santa Clara, CA, USA). Post-hybridization, the microarrays were processed in the Affymetrix GeneChip Fluidics Station 450 and resultant image (.CEL file) was analyzed with the Affymetrix GeneChip® Genotyping Analysis Software (GTTYPE). The .CEL files were further analyzed using the Partek Genomics Suite (PGS) (Partek Inc, St. Louis, MO) software for copy number detection. Log_2 Ratios (LR) and Allelic Ratio (AR) generated by Partek software were subsequently exported for downstream copy number alteration (CNA) detection and analysis. Call rate and the standard deviation of the LR values were used as quality control measures for CNA detection purposes. Data sets with LR standard deviations above 0.35 were deemed noisy and were excluded from the study. Chromosomes were segmented based on LRs using the Circular Binary Segmentation (CBS) algorithm implemented in the R statistical package module DNACopy 1.7. For autosomal chromosomes, we used threshold of acceptance values of -2 , -0.3 and 0.25 for the mean LRs of potential homozygous deletions, hemizygous deletions, and duplications, respectively. The initial inclusion criteria for potential pathogenicity were deletions larger than 200kb and duplications larger than 500kb and did not have overlap with CNVs in control data sets (including the Database of Genomic Variants, Human Genomic Structural Variations Project, and the Children's Hospital of Philadelphia CNV Database).

Interpretation of CNVs

The CNVs generated for all groups were evaluated for clinical relevance by a board-certified medical geneticists (D.E.P., J.E.M., P.K.) according to the standards and guidelines established by the American College of Medical Genetics (Kearney et al., 2011; South et al., 2013) and the framework for assessing dosage sensitivity established by the International Standards for Cytogenomic Arrays Consortium (Riggs et al., 2012).

RESULTS

We screened 222 patients with HPE and identified copy number variants in 107 individuals (48%) ranging in size from 2 kb to 58 Mb (Supplementary Tables I and II). However, a large portion of these variants are likely benign, benign, or are variants of unknown significance (VUS). Out of the 107 patients who had clinically significant CNVs, only 23 patients (21%) had variants that would be reported as pathogenic or likely pathogenic in a clinical setting (Table II). As expected, 18 these patients exhibited deletions in well-established HPE loci, including three cases with a deletion including *SIX3*, one case with a deletion including *SHH*, 11 cases with a deletion in *TGIF1* (8 of which were previously identified and intentionally included in our study as positive controls), one case with a deletion in *ZIC2*, one case with a deletion in *GLI2*, and one case with a deletion in *DISP1*. Also of note, three patients exhibited deletions encompassed by a deletion found earlier by Bendavid et al., 6q12–q14.3 (Bendavid et al., 2009). In addition to these findings, we discovered a deletion in 16p13.2, a region that we believe warrants further investigation.

DISCUSSION

We examined 222 individuals within the HPE spectrum using chromosomal microarray. CNVs were found in six established HPE loci, including *SIX3* (3), *SHH* (1), *TGIF1* (11), *ZIC2* (1), *GLI2* (1) and *DISP1* (1). Two of the patients with deletions encompassing *SIX3* (Stokes et al., 2018) and eight of the patients with deletions encompassing *TGIF1* (Kauvar et al., 2011) have previously been reported. The two patients with deletions in *SIX3* were recruited to our study before the deletions were discovered. However, the eight patients with deletions in *TGIF1* reported by Kauvar et al. had known deletions in 18p when they were recruited. These patients were included in the present study to ascertain the specific breakpoints in their deletions and to determine whether their deletions encompassed *TWSG1*, which, at the time, was considered a candidate HPE gene. As Kauvar et al. later concluded, however, sequence alterations in *TWSG1* are not a common direct cause for HPE (Kauvar et al., 2011).

While the inclusion of these eight patients skews our results by increasing the percentage of patients with pathogenic or likely pathogenic deletions from 14% (15/107) to 21% (23/107) and artificially inflates the incidence of *TGIF1* deletions within our study population, loss of function variants in *TGIF1*, as well as in *SHH*, *ZIC2*, and *SIX3*, are, nevertheless, widely recognized causes of HPE (reviewed in this issue in Roessler et al., 2018) and our present study supports this. There is less data, however, to support the association between HPE and deletions in *GLI2* and *DISP1*. Rather, there has been increasing evidence that variants in *GLI2* lead to a set of phenotypes, predominated by pituitary anomalies, polydactyly, and

subtle facial features, separate from HPE (Bear et al., 2014; Kruszka and Muenke, 2018). Similarly, a study by Jun et al. characterized a patient with a 183kb deletion encompassing *DISP1* that presented with no midline defects, brain abnormalities, or facial dysmorphism, which calls into question the role of *DISP1* as a causative gene in HPE (Jun et al., 2013). Our results seem to support the association between *GLI2* and *DISP1* and HPE; however, it should also be taken into consideration that the patients with deletions in *GLI2* and *DISP1* in our cohort were reported as having HPE findings, but due to lack of follow-up we were unable to determine the specific HPE type and phenotypic presentation.

Our cohort also contained three individuals with similar deletions to one reported by Bendavid et al., specifically 6q12–q14.3 (arr[hg18] 6q12q14.3(69,637,936–86,100,038)x1). The 16.9 Mb deletion reported by Bendavid et al. encompasses the three we discovered in our cohort which included only part of 6q13 or 6q13–q14.1 and were all less than 2 Mb in size (Bendavid et al., 2009). Deletions in the proximal region of 6q have been associated with a phenotypic spectrum, including intellectual disability, upslanted palpebral fissures with epicanthal folds, a short nose with broad nasal tip, anteverted nares, long philtrum, and thin upper lip, that closely overlaps with the HPE spectrum (Becker et al., 2012). Catena et al., however, have suggested that deletions in this region could be related Toriello-Carey syndrome, key features of which includes postnatal growth failure, hypotonia, developmental delay, a short neck, characteristic facial anomalies such as hypertelorism, blepharophimosis, abnormal ears, small nose, full cheeks, thin lips, micrognathia, and abnormal corpus callosum (Catena et al., 2017; Toriello et al., 2003). Unfortunately, the patient presented by Bendavid et al. was only described as having “minor signs” of HPE and the patients with proximal 6q deletions in our cohort exhibited microform HPE or “HPE findings”, which makes it more difficult to evaluate the possibility that these patients were misdiagnosed. Regardless of the syndromic diagnosis, little correlation has been found between specific genes in the proximal 6q region and the phenotypes mentioned thus far.

In addition to the aforementioned copy number variants (CNVs), we identified a new locus of interest within our cohort. We observed one case with a deletion in 16p13.2 encompassing *GRIN2A* (OMIM 138253). Interestingly, this patient also has a deletion in 6q13, which we do not believe contributes to his phenotypic presentation. A study by Reutlinger et al. presented patients with deletions spanning *GRIN2A* who had choanal stenosis, intellectual disability, and seizures (Reutlinger et al., 2010). Although *GRIN2A* is primarily associated with epilepsy, the patients presented by Reutlinger et al. display phenotypic features that fall within the spectrum of HPE (Endele et al., 2010). This data, however, is still speculative and we cannot draw any conclusions as to the association between this locus and HPE until further studies, such as genotype-phenotype correlations and functional studies, are conducted.

Two individuals were found to have CNVs consistent with recognized syndromes previously not associated with HPE. One patient exhibited a *de novo* 3.9 Mb duplication at 17q11.2 encompassing *RAI1*, a recurrent CNV seen in Potocki-Lupski Syndrome. This syndrome typically presents with intellectual disability, autism spectrum disorder, infantile hypotonia, structural cardiovascular anomalies, and mild and nonspecific dysmorphic facial features, such as micrognathia and down-slanting palpebral fissures (Potocki et al., 2007; Neira-

Fresneda & Potocki, 2015). Our patient, on the other hand, presented with semilobar HPE, microcephaly, hypertelorism, bilateral cleft lip, midline cleft palate, mild seizures, and developmental delay. No known cases of patients with both Potocki-Lupski syndrome and HPE have been reported in the literature and no other molecular findings associated with HPE was found in our patient; however, this patient may have had an unknown pathogenic change in a gene involved in the pathogenesis of HPE. The other patient exhibited a *de novo* 4.7 Mb deletion at 22q13.31-q13.33 encompassing *SHANK3*, *ACR*, and *RABL2B*. Heterozygous variants in *SHANK3* are associated with Phelan-McDermid syndrome, which presents with a wide range of signs and symptoms, including infantile hypotonia, absent to severely delayed speech, intellectual disability, large fleshy hands, dysplastic toenails, and, less commonly, kidney anomalies and agenesis of the corpus callosum (Phelan et al., 2010). Our patient presented with hypoplasia of the anterior corpus callosum, absent septum pellucidum, developmental delay, and kidney anomalies, all of which actually fall within the spectrum of Phelan-McDermid syndrome. Given that this patient had no other molecular findings associated with HPE, we believe that this could have likely been a case of clinical misdiagnosis due to the presence of the hypoplastic corpus callosum. More importantly, this finding raises the point that Phelan-McDermid syndrome should be included in the list of differential diagnoses when assessing patients with brain anomalies for HPE.

While chromosomal microarray continues to be a powerful tool in the study and diagnosis of fetal anomalies (South et al., 2013), advances in next-generation sequencing (NGS) technology has led to its increased use in the discovery of both sequence variants and CNVs that underlie phenotype and disease. The NGS approach allows for higher coverage and resolution, more accurate estimation of copy numbers, and higher capability of identifying novel CNVs (Alkan, Coe, and Eichler, 2011; Meyerson, Gabriel, and Getz, 2010). Several groups such as Dubourg et al. have already begun using NGS to detect sequence variants in HPE and, as a result, were able to determine the frequency at which variants in genes in the fibroblast growth factor (FGF) family occur in HPE, confirming previous work by group such as ours (Dubourg et al., 2016; Arauz et al., 2010).

In summary, we screened 222 patients with HPE with CMA and found 23 (10%) individuals to have pathogenic or likely pathogenic variants, supporting the importance of CMA in the workup of HPE. We also provide a large database of CNVs (supplementary tables I and II) in individuals with HPE that will be used as a reference by clinicians and laboratories for years to come. As sequencing technology improves, especially with CNV calls being made from NGS, we expect continued refinement of CNVs in HPE.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all the patients and their families for their participation. We also thank Dr. Oscar Cano, MD, for his input to this manuscript. This work was supported by the Division of Intramural Research of the National Institutes of Health (Bethesda, MD) and by the National Institute of Dental and Craniofacial Research of the National Institutes of Health under award number RO1DE015874. This research was also made possible through the NIH Medical Research Scholars Program, a public-private partnership supported jointly by the NIH and generous contributions to

the Foundation for the NIH from the Doris Duke Charitable Foundation, the American Association for Dental Research, the Colgate-Palmolive Company, Genentech, Elsevier, and other private donors.

References

- Alkan C, Coe BP, Eichler EE. Genome structural variation discovery and genotyping. *Nature Reviews Genetics*. 2011; 12:363–376.
- Arauz RF, Solomon BD, Pineda-Alvarez DE, Gropman AL, Parsons JA, Roessler E, Muenke M. A Hypomorphic Allele in the FGF8 Gene Contributes to Holoprosencephaly and Is Allelic to Gonadotropin-Releasing Hormone Deficiency in Humans. *Molecular Syndromology*. 2010; 1(2): 59–66. [PubMed: 21045958]
- Bear KA, Solomon BD, Antonini S, Arnhold IJ, França MM, Gerkes EH, Grange DK, Hadley DW, Jääskeläinen J, Paulo SS, Rump P, Stratakis CA, Thompson EM, Willis M, Winder TL, Jorge AA, Roessler E, Muenke M. Pathogenic mutations in GLI2 cause a specific phenotype that is distinct from holoprosencephaly. *Journal of Medical Genetics*. 2014; 51(6):413–8. [PubMed: 24744436]
- Becker K, Di Donato N, Holder-Espinasse M, Andrieux J, Cuisset JM, Vallée L, Plessis G, Jean N, Delobel B, Thuresson AC, Annerén G, Ravn K, Tümer Z, Tinschert S, Schrock E, Jönch AE, Hackmann K. De novo microdeletions of chromosome 6q14.1-q14.3 and 6q12.1-q14.1 in two patients with intellectual disability - further delineation of the 6q14 microdeletion syndrome and review of the literature. *European Journal of Medical Genetics*. 2012; 55(8–9):490–7. [PubMed: 22561202]
- Belloni E, Muenke M, Roessler E, Traverso G, Siegel-Bartelt J, Frumkin A, Mitchell HF, Donis-Keller H, Helms C, Hing AV, Heng HH, Koop B, Martindale D, Rommens JM, Tsui LC, Scherer SW. Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly. *Nature Genetics*. 1996; 14:353–356. [PubMed: 8896571]
- Bendavid C, Dubourg C, Pasquier L, Gicquel I, Le Gallou S, Mottier S, Durou MR, Henry C, Odent S, David V. MLPA screening reveals novel subtelomeric rearrangements in holoprosencephaly. *Human Mutation*. 2007; 28:1189–1197. [PubMed: 17683084]
- Bendavid C, Rochard L, Dubourg C, Seguin J, Gicquel I, Pasquier L, Vigneron J, Laquerrière A, Marcorelles P, Jeanne-Pasquier C, Rouleau C, Jaillard S, Mosser J, Odent S, David V. Array-CGH analysis indicates a high prevalence of genomic rearrangements in holoprosencephaly: an updated map of candidate loci. *Human Mutation*. 2009; 30(8):1175–82. [PubMed: 19431187]
- Bendavid C, Dupe V, Rochard L, Gicquel I, Dubourg C, David V. Holoprosencephaly: An update on cytogenetic abnormalities. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2010; 154C:86–92.
- Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D. A chromosomal deletion map of human malformations. *American Journal of Human Genetics*. 1998; 63:1153–1159. [PubMed: 9758599]
- Brown SA, Warburton D, Brown LY, Yu CY, Roeder ER, Stengel-Rutkowski S, Hennekam RC, Muenke M. Holoprosencephaly due to mutations in ZIC2, a homologue of Drosophila odd-paired. *Nature Genetics*. 1998; 20:180–183. [PubMed: 9771712]
- Bullen PJ, Rankin JM, Robson SC. Investigation of the epidemiology and prenatal diagnosis of holoprosencephaly in the North of England. *American Journal of Obstetrics and Gynecology*. 2001; 184:1256–1262. [PubMed: 11349198]
- Catena S, Aracena M, Pizarro Ó, Espinoza K, Lay-Son G. Proximal Deletion of 6q Overlapping with Toriello-Carey Facial Phenotype: Prenatal Findings, Clinical Course, Differential Diagnosis, and Review. *Molecular Syndromology*. 2017; 9(1):15–21. [PubMed: 29456478]
- Croen LA, Shaw GM, Lammer EJ. Holoprosencephaly: Epidemiologic and clinical characteristics of a California population. *American Journal of Medical Genetics*. 1996; 64:465–472. [PubMed: 8862623]
- Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. *Orphanet Journal of Rare Diseases*. 2007; 2:8. [PubMed: 17274816]
- Dubourg C, Carré W, Hamdi-Rozé H, Mouden C, Roume J, Abdelmajid B, Amram D, Baumann C, Chassaing N, Coubes C, Faivre-Olivier L, Ginglinger E, Gonzales M, Levy-Mozziconacci A, Lynch SA, Naudion S, Pasquier L, Poidvin A, Prieur F, Sarda P, Toutain A, Dupé V, Akloul L,

- Odent S, de Tayrac M, David V. Mutational Spectrum in Holoprosencephaly Shows That FGF is a New Major Signaling Pathway. *Human Mutation*. 2016; 37(12):1329–1339. [PubMed: 27363716]
- Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I, Milh M, Kortüm F, Fritsch A, Pientka FK, Hellenbroich Y, Kalscheuer VM, Kohlhase J, Moog U, Rappold G, Rauch A, Ropers HH, von Spiczak S, Tönnies H, Villeneuve N, Villard L, Zabel B, Zenker M, Laube B, Reis A, Wiczorek D, Van Maldergem L, Kutsche K. Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. *Nature Genetics*. 2010; 42(11):1021–6. [PubMed: 20890276]
- Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Van Vooren S, Moreau Y, Pettett RM, Carter NP. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *American Journal of Human Genetics*. 2009; 84(4):524–33. [PubMed: 19344873]
- Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, Handsaker RE, McCarroll SA, O'Donovan MC, Owen MJ. Discovery and Statistical Genotyping of Copy-Number Variation from Whole-Exome Sequencing Depth. *American Journal of Human Genetics*. 2012; 91:597–607. [PubMed: 23040492]
- Gripp KW, Wotton D, Edwards MC, Roessler E, Ades L, Meinecke P, Richieri-Costa A, Zackai EH, Massague J, Muenke M, Elledge SJ. Mutations in TGIF cause holoprosencephaly and link NODAL signaling to human neural axis determination. *Nature Genetics*. 2000; 25:205–208. [PubMed: 10835638]
- Jensen M, Kooy RF, Simon TJ, Reyniers E, Girirajan S, Tassone F. A higher rare CNV burden in the genetic background potentially contributes to intellectual disability phenotypes in 22q11.2 deletion syndrome. *European Journal of Medical Genetics*. 2018; 61(4):209–212. [PubMed: 29191496]
- Jun KR, Hur YJ, Lee JN, Kim HR, Shin JH, Oh SH, Lee JY, Seo EJ. Clinical characterization of DISP1 haploinsufficiency: A case report. *European Journal of Medical Genetics*. 2013; 56(6):309–13. [PubMed: 23542665]
- Kauvar EF, Hu P, Pineda-Alvarez DE, Solomon BD, Dutra A, Pak E, Blessing B, Proud V, Shanske AL, Stevens CA, Rosenfeld JA, Shaffer LG, Roessler E, Muenke M. Minimal evidence for a direct involvement of twisted gastrulation homolog 1 (TWSG1) gene in human holoprosencephaly. *Molecular Genetics and Metabolism*. 2011; 102(4):470–80. [PubMed: 21227728]
- Kamnasaran D, Chen CP, Devriendt K, Mehta L, Cox DW. Defining a holoprosencephaly locus on human chromosome 14q13 and characterization of potential candidate genes. *Genomics*. 2005; 85:608–621. [PubMed: 15820313]
- Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST. Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genetics in Medicine*. 2011; 13(7):680–5. [PubMed: 21681106]
- Kruszka P, Hart RA, Hadley DW, Muenke M, Habal MB. Expanding the phenotypic expression of Sonic Hedgehog mutations beyond holoprosencephaly. *Journal of Craniofacial Surgery*. 2015; 26(1):3–5. [PubMed: 25569381]
- Kruszka P, Muenke M. Syndromes Associated with Holoprosencephaly. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2018 this issue.
- Matsunaga E, Shiota K. Holoprosencephaly in human embryos: epidemiologic studies of 150 cases. *Teratology*. 1977; 16:261–272. [PubMed: 594909]
- Meuwissen ME, Halley DJ, Smit LS, Lequin MH, Cobben JM, de Coo R, van Harssel J, Sallevelt S, Woldringh G, van der Knaap MS, de Vries LS, Mancini GM. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. *Genetics in Medicine*. 2015; 17(11):843–53. [PubMed: 25719457]
- Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nature Reviews Genetics*. 2010; 11:685–696.
- Mikhail FM, Burnside RD, Rush B, Ibrahim J, Godshalk R, Rutledge SL, Robin NH, Descartes MD, Carroll AJ. The recurrent distal 22q11.2 microdeletions are often de novo and do not represent a single clinical entity: a proposed categorization system. *Genetics in Medicine*. 2014; 16(1):92–100. [PubMed: 23765049]

- Ming JE, Kaupas ME, Roessler E, Brunner HG, Golabi M, Tekin M, Stratton RF, Sujansky E, Bale SJ, Muenke M. Mutations in *PATCHED-1*, the receptor for *SONIC HEDGEHOG*, are associated with holoprosencephaly. *Human Genetics*. 2002; 110:297–301. [PubMed: 11941477]
- Ming JE, Muenke M. Multiple hits during early embryonic development: digenic diseases and holoprosencephaly. *American Journal of Human Genetics*. 2002; 71:1017–1032. [PubMed: 12395298]
- Muenke M, Beachy PA. Genetics of ventral forebrain development and holoprosencephaly. *Current Opinion in Genetics & Development*. 2000; 10:262–269. [PubMed: 10826992]
- Muenke M, Beachy PA. Holoprosencephaly. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors *The metabolic & molecular bases of inherited disease*. 8. New York, NY: McGraw-Hill; 2001. 6203–6230.
- Münke M, Emanuel BS, Zackai EH. Holoprosencephaly: association with interstitial deletion of 2p and review of the cytogenetic literature. *Am J Med Genet*. 1988; 30:929–38. [PubMed: 3055987]
- Neira-Fresneda J, Potocki L. Neurodevelopmental disorders associated with abnormal gene dosage: Smith-Magenis and Potocki-Lupski Syndromes. *Journal of Pediatric Genetics*. 2015; 4:159–67. [PubMed: 27617127]
- Olsen CL, Hughes JP, Youngblood LG, Sharpe-Stimac M. Epidemiology of holoprosencephaly and phenotypic characteristics of affected children: New York State, 1984–1989. *American Journal of Medical Genetics*. 1997; 73:217–226. [PubMed: 9409876]
- Pineda-Alvarez DE, Dubourg C, David V, Roessler E, Muenke M. Current recommendations for the molecular evaluation of newly diagnosed holoprosencephaly patients. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2010; 154C(1):93–101.
- Phelan MC, Stapleton GA, Rogers RC. Deletion 22q13 syndrome: Phelan-McDermid syndrome. In: Cassidy SB, Allanson JE, editors *The Management of Genetic Syndromes*. 3. Hoboken, NJ: John Wiley & Sons Inc; 2010. 285–297.
- Potocki L, Bi W, Treadwell-Deering D, Carvalho CMB, Eifert A, Friedman EM, Glaze D, Krull K, Lee JA, Lewis RA, Mendoza-Londono R, Robbins-Furman P, Shaw C, Shi X, Weissenberger G, Withers M, Yatsenko SA, Zackai EH, Stankiewicz P, Lupski JR. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism Phenotype. *American Journal of Human Genetics*. 2007; 80:633–49. [PubMed: 17357070]
- Reutlinger C, Helbig I, Gawelczyk B, Subero JI, Tönnies H, Muhle H, Finsterwalder K, Vermeer S, Pfundt R, Sperner J, Stefanova I, Gillessen-Kaesbach G, von Spiczak S, van Baalen A, Boor R, Siebert R, Stephani U, Caliebe A. Deletions in 16p13 including *GRIN2A* in patients with intellectual disability, various dysmorphic features, and seizure disorders of the rolandic region. *Epilepsia*. 2010; 51(9):1870–3. [PubMed: 20384727]
- Riggs ER, Church DM, Hanson K, Horner VL, Kaminsky EB, Kuhn RM, Wain KE, Williams ES, Aradhya S, Kearney HM, Ledbetter DH, South ST, Thorland EC, Martin CL. Towards an evidence-based process for the clinical interpretation of copy number variation. *Clinical Genetics*. 2012; 81(5):403–12. [PubMed: 22097934]
- Roessler E, Belloni E, Gaudenz K, Jay P, Berta P, Scherer SW, Tsui LC, Muenke M. Mutations in the human *Sonic Hedgehog* gene cause holoprosencephaly. *Nature Genetics*. 1996; 14:357–360. [PubMed: 8896572]
- Roessler E, Hu P, Muenke M. Holoprosencephaly in the genomics era. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2018 this issue.
- Roessler E, Muenke M. Holoprosencephaly: A paradigm for the complex genetics of brain development. *Journal of Inherited Metabolic Disease*. 1998; 21:481–497. [PubMed: 9728329]
- Roessler E, Mittaz L, Du Y, Scott HS, Chang J, Rossier C, Guipponi M, Matsuda SP, Muenke M, Antonarakis SE. Structure of the human *Lanosterol synthase* gene and its analysis as a candidate for holoprosencephaly (HPE1). *Human Genetics*. 1999; 105:489–495. [PubMed: 10598817]
- Roessler E, Du YZ, Mullor JL, Casas E, Allen WP, Gillessen-Kaesbach G, Roeder ER, Ming JE, Ruiz i Altaba A, Muenke M. Loss-of-function mutations in the human *GLI2* gene are associated with pituitary anomalies and holoprosencephaly-like features. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:13424–13429. [PubMed: 14581620]

- Roessler E, Ouspenskaia MV, Karkera JD, Velez JI, Kantipong A, Lacbawan F, Bowers P, Belmont JW, Towbin JA, Goldmuntz E, Feldman B, Muenke M. Reduced NODAL signaling strength via mutation of several pathway members including FOXL1 is linked to human heart defects and holoprosencephaly. *American Journal of Human Genetics*. 2008; 83:18–29. [PubMed: 18538293]
- Roessler E, Ma Y, Ouspenskaia MV, Lacbawan F, Bendavid C, Dubourg C, Beachy PA, Muenke M. Truncating loss-of-function mutations of DISP1 contribute to holoprosencephaly-like microform features in humans. *Human Genetics*. 2009a; 125:393–400. [PubMed: 19184110]
- Roessler E, El-Jaick KB, Dubourg C, Vélez JI, Solomon BD, Pineda-Alvarez DE, Lacbawan F, Zhou N, Ouspenskaia M, Paulussen A, Smeets HJ, Hehr U, Bendavid C, Bale S, Odent S, David V, Muenke M. The mutational spectrum of holoprosencephaly-associated changes within the SHH gene in humans predicts loss-of-function through either key structural alterations of the ligand or its altered synthesis. *Human Mutation*. 2009b; 30(10):E921–35. [PubMed: 19603532]
- Schouten JP, McElgunn CJ, Waaijer R, Zwiijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Research*. 2002; 30(12):e57. [PubMed: 12060695]
- Solomon BD, Mercier S, Vélez JI, Pineda-Alvarez DE, Wyllie A, Zhou N, Dubourg C, David V, Odent S, Roessler E, Muenke M. Analysis of genotype–phenotype correlations in human holoprosencephaly. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2010; 154C:133–141.
- South ST, Lee C, Lamb AN, Higgins AW, Kearney HM. Working Group for the American College of Medical Genetics, Genomics Laboratory Quality Assurance Committee. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genetics in Medicine*. 2013; 15(11):901–9. [PubMed: 24071793]
- Stokes B, Berger SI, Hall BA, Weiss K, Martinez AF, Hadley DW, Murdock DR, Ramanathan S, Clark RD, Roessler E, Kruszka P, Muenke M. SIX3 deletions and incomplete penetrance in families affected by holoprosencephaly. *Congenital Anomalies (Kyoto)*. 2018; 58(1):29–32.
- Toriello HV, Carey JC, Addor MC, Allen W, Burke L, Chun N, Dobyns W, Elias E, Gallagher R, Hordijk R, Hoyne G, Irons M, Jewett T, LeMerrer M, Lubinsky M, Martin R, McDonald-McGinn D, Neumann L, Newman W, Pauli R, Seaver L, Tsai A, Wargowsky D, Williams M, Zackai E. Toriello-Carey syndrome: delineation and review. *American Journal of Medical Genetics Part A*. 2003; 123A(1):84–90. [PubMed: 14556252]
- Tyshchenko N, Lurie I, Schinzel A. Chromosomal map of human brain malformations. *Human Genetics*. 2008; 124:73–80. [PubMed: 18563447]
- Wallis DE, Roessler E, Hehr U, Nanni L, Wiltshire T, Richieri-Costa A, Gillessen-Kaesbach G, Zackai EH, Rommens J, Muenke M. Mutations in the homeodomain of the human SIX3 gene cause holoprosencephaly. *Nature Genetics*. 1999; 22:196–198. [PubMed: 10369266]
- Wang W, Wei Z, Lam TW, Wang J. Next generation sequencing has lower sequence coverage and poorer SNP-detection capability in the regulatory regions. *Scientific Reports*. 2011; 1:55. [PubMed: 22355574]

Biographies

Tommy Hu is a medical student at the Penn State College of Medicine (Hershey, PA) and is currently working in the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD) as part of a research fellowship through the NIH Medical Research Scholars Program. His current work in Dr. Muenke's laboratory is focused on the genetics of holoprosencephaly and craniosynostosis.

Dr. Paul Kruszka, MD, MPH, is a board-certified family physician and clinical geneticist at the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD). His research interests include early embryonic errors in brain, craniofacial, and heart development.

Dr. Ariel F. Martinez, PhD, is a research biologist at the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD) with eight years of experience working on human developmental diseases, including holoprosencephaly (HPE). He is an Active Candidate of the American Board of Medical Genetics and Genomics in the specialty of Clinical Molecular Genetics and Genomics. His current work in Dr. Muenke's laboratory involves genetics testing of patients with HPE, where he serves as CLIA Laboratory Supervisor. His research is focused on understanding the molecular mechanisms underlying HPE pathogenicity.

Dr. Jeffrey E. Ming, MD, PhD, is a board-certified pediatrician and medical geneticist and is an Adjunct Assistant Professor of Pediatrics at the Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine (Philadelphia, PA). His research has focused on genetic multiple congenital anomaly syndromes.

Dr. Emily K. Shabason, MD, is currently a pediatrician in the Division of Developmental and Behavioral Pediatrics at Children's Hospital of Philadelphia (Philadelphia, PA). She was formerly an HHMI-NIH Research Scholar and conducted research in the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD).

Dr. Manu S. Raam, MD, is a board-certified pediatrician at Shriners for Children Medical Center and Children's Hospital Los Angeles. He was formerly an HHMI-NIH Research Scholar and conducted research in the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD). His clinical and research interests include osteogenesis imperfecta, spina bifida, craniosynostosis, holoprosencephaly, VACTERL association, sudden infant death syndrome (SIDS), and general preventive pediatrics.

Dr. Tamim H. Shaikh, PhD, is a Professor of Pediatrics and Biochemistry and Molecular Genetics at the University of Colorado Denver School of Medicine (Aurora, CO). He has over 20 years of experience in the discovery of the genetic mutations underlying multiple congenital anomaly syndromes and has discovered several novel genetic mutations including copy number variations (CNVs) associated with pediatric disorders. In addition to gene discovery, his current research includes functional analysis of the detected mutations using model organisms including zebrafish and mouse.

Dr. Daniel Pineda-Alvarez, MD, is a Colombian trained medical graduate and is board-certified in Clinical Molecular Genetics and Clinical Cytogenetics. He is currently the Medical Director of Oncology at Invitae (San Francisco, CA) and formerly trained as both a Clinical Molecular Genetics fellow and a Clinical Cytogenetics fellow in the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health (Bethesda, MD).

Dr. Maximilian Muenke, MD, is a board-certified medical geneticist and the Branch Chief of the Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD. He has worked on the genetics of HPE for the past 25

years and his group has discovered numerous genes contributing to HPE, including the first and most well-known gene, Sonic Hedgehog. His other research interests include cardiac disease, craniofacial malformation syndromes, attention-deficit-hyperactivity disorder (ADHD), and the genetics of diverse populations.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Correlation between chromosome abnormalities and the holoprosencephaly spectrum.

Hatched bars on the left side of chromosomes represent deletions, full bars on the right, duplications. Thin bars indicate individual case reports, whereas thick bars represent more than three reports. Ring chromosomes are represented by hatched bars on the terminal ends (Münke, Emanuel, and Zackai, 1988).

Figure 2.

Chromosomal anomalies in holoprosencephaly. At least 12 human chromosomal regions have been implicated in the pathogenesis of holoprosencephaly on the basis of structural anomalies. With the exception of one region on 3p, which is duplicated, all other chromosomal regions shown here are deleted in holoprosencephaly. At the time of publication, four genes had been identified by positional candidate gene approach and two, dispatched (DISP) and HNF3β, had been proposed as additional holoprosencephaly genes (modified from Ming and Muenke, 2002).

Figure 3. Region of deletion in the patient with a deletion in 16q13.2. The only gene this region covers is *GRIN2A*, specifically from exon 4 to 14 (ENSG00000183454).

Table I

Notable chromosomal anomalies found in 222 patients with HPE.

Previously identified (OMIM)			
Chromosome	Human locus (OMIM)	Human gene (identified)	Cases identified in present study
21q22.3	HPE1		0
2p21	HPE2	<i>SIX3</i>	3
7q36	HPE3	<i>SHH</i>	1
18p11.3	HPE4	<i>TGIF1</i>	11
13q32	HPE5	<i>ZIC2</i>	1
2q37.1-p37.3	HPE6		0
9q22.3	HPE7	<i>PTCH1</i>	0
14q13	HPE8		0
2q14	HPE9	<i>GLI2</i>	1
1q42	HPE10	<i>DISP1</i>	1
11q24.2	HPE11	<i>CDON</i>	0

Previously identified (literature)		
Chromosome	Reference	Cases identified in present study
6q13	(Bendavid et al., 2009)	3

Novel findings		
Chromosome	Potential gene at locus involved with HPE	Cases identified in present study
16p13.2	<i>GRIN2A</i>	1

Table II

Summary of patients with pathogenic or likely pathogenic variants and patients with variants in 6p13–14.1 and 16p13.2. (GRCh37/hg19)

Patient	HPE Phenotype	Age at Testing (years)	Sex	CMA Design	Anomalies	Start	Stop	Size (Mb)	Interpretation
3	Unspecified	12	M	SurePrint G3 Custom Microarrays	1) del(6)(p22.2) 2) dup(7)(p21.1) 3) dup(7)(p12.3) 4) del(18)(p11.31)	1) 26257226 2) 17893152 3) 47678216 4) 3457318	1) 26364664 2) 18465683 3) 47811742 4) 3462531	1) 0.1 2) 0.6 3) 0.1 4) 5 kb	1) VUS 2) VUS 3) VUS 4) Likely pathogenic; includes last exon of <i>TGIF1</i>
5	Semilobar	3	F	SurePrint G3 Custom Microarrays	dup(17)(p11.2)	16662464	20523734	3.9	Pathogenic; Potocki-Lupski
7	Alobar	Fetus	F	SurePrint G3 Custom Microarrays	del(18)(p11.32p11.31)	2243436	4501916	2.2	Pathogenic; includes <i>TGIF1</i>
8	Lobar	1	F	SurePrint G3 Custom Microarrays	1) dup(4)(p16.3p16.1) 2) del(7)(q36.1q36.3)	1) 43578 2) 150469844	1) 6947103 2) 159128663	1) 7.0 2) 9.0	Pathogenic; unbalanced translocation between 4p and 7q; 4p16.1 duplication is consistent with 4p15 duplication syndrome (PMID: 7684190) and 7q36.1 deletion encompasses <i>SHH</i>
12 *	Alobar	Fetus	M	SurePrint G3 Custom Microarrays	1) del(18)(p11.32p11.21) 2) del(Y)(q11.21q12)	1) 79754 2) 13140000	1) 13659732 2) 59343615	1) 13.6 2) 46.5	1) Pathogenic 2) Pathogenic; azoospermia
16 *	Alobar	Fetus	M	SurePrint G3 Custom Microarrays	1) del(18)(p11.32p11.21) 2) dup(18)(q11.2q23)	1) 128819 2) 20275853	1) 15390683 2) 78016181	1) 15.3 2) 59.5	Pathogenic; chromosome 18 ring
18	Hypoplasia of corpus callosum and absence of the septum pellucidum	1.5	F	SurePrint G3 Custom Microarrays	del(22)(q13.31q13.33)	46577970	51244566	6.3	Pathogenic; Phelan-McDermid syndrome
19 *	SMCI, no holoprosencephaly but has developmental delay	7	F	SurePrint G3 Custom Microarrays	1) dup(9)(q34.12) 2) del(18)(p11.32p11.21)	1) 133687312 2) 58826	1) 133962538 2) 15175151	1) 0.3 2) 15.1	1) VUS 2) Pathogenic; includes <i>TGIF1</i>
21	Unspecified	NA	NA	SurePrint G3 Custom Microarrays	1) del(18)(p11.32p11.21) 2) del(18)(q22.3q23) 3) dup(19)(p13.11)	1) 20001 2) 67783601 3) 19096432	1) 15175151 2) 78016181 3) 19207211	1) 15.2 2) 12.1 3) 0.1	1) & 2) Pathogenic; chromosome 18 ring 3) VUS
23 *	Unspecified	20	F	SurePrint G3 Custom Microarrays	del(18)(p11.32p11.21)	128151	15175151	15.0	Pathogenic; includes <i>TGIF1</i>
28 *	Unspecified	6	M	SurePrint G3 Custom Microarrays	del(18)(p11.32p11.31)	79754	5236552	5.2	Pathogenic; includes <i>TGIF1</i>
29 *	Unspecified	8 months	F	SurePrint G3 Custom Microarrays	1) del(18)(p11.32p11.31) 2) dup(18)(p11.32p11.21)	1) 14316 2) 56383	1) 3648271 2) 15175151	1) 3.6 2) 15.1	1) Pathogenic; includes <i>TGIF1</i> 2) Pathogenic; idic(18)
31 *	Congenital nasal pyriform aperture stenosis, absence of the upper labial frenulum	6	M	SurePrint G3 Custom Microarrays	del(18)(p11.31)	3204026	4980239	1.8	Pathogenic; includes <i>TGIF1</i>
32 *	Microcephaly, developmental delay, SMCI	5	M	SurePrint G3 Custom Microarrays	1) del(18)(p11.32p11.31) 2) del(18)(q23)	1) 79813 2) 74807064	1) 4032544 2) 78012829	1) 4.0 2) 3.2	Pathogenic; chromosome 18 ring
42	Semilobar	2	M	SurePrint G3 Custom Microarrays	del(2)(p16.3)	51187367	51275353	88.0 kb	Pathogenic; heterozygous carriers of deletions of <i>NRXN1</i> have susceptibility for neurodevelopmental disorders (PMID: 20162629, 18057082, 20468056)
47	Alobar	7 months	F	SurePrint G3 Custom Microarrays	1) del(2)(p21) 2) dup(2)(q12.2q12.3)	1) 45030525 2) 107458905	1) 45565704 2) 109174146	1) 0.5 2) 1.7	1) Pathogenic; deletion of <i>SLX3</i> 2) VUS

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Patient	HPE Phenotype	Age at Testing (years)	Sex	CMA Design	Anomalies	Start	Stop	Size (Mb)	Interpretation
49	Microform	4 months	F	Affymetrix GeneChip 500K arrays	3) dup(15)(q13.3q14)	3) 33077876	3) 34868531	3) 1.8	3) VUS
					1) dup(15)(q11.2)	1) 20935077	1) 22212114	1) 1.3	1) Benign
					2) del(6)(q13)	2) 72674265	2) 73237197	2) 0.6	2) VUS
					3) del(3)(q25.32)	3) 157901734	3) 158326570	3) 0.4	3) Benign
					4) del(8)(p23.1)	4) 12002717	4) 12402941	4) 0.4	4) Benign
					1) dup(12)(q11.1q12)	1) 34296804	1) 38706942	1) 4.4	1) Benign; centromeric
					2) del(4)(q31.3)	2) 151245454	2) 152141399	2) 0.9	2) VUS; includes <i>MAB2/L2</i> ; Microphthalmia/coloboma and skeletal dysplasia syndrome
					3) del(6)(q13)	3) 74514153	3) 74852508	3) 0.3	3) Benign
60	Microcephaly	NA	M	Affymetrix GeneChip 500K arrays	4) del(16)(p13.2)	4) 9833154	4) 10102969	4) 0.3	4) VUS; includes <i>GRIN2A</i>
78	Unspecified	NA	M	Affymetrix GeneChip 500K arrays	1) dup(19)(p13.11)	1) 17795360	1) 19889148	1) 2.1	1) VUS
					2) del(17)(q21.31)	2) 44246624	2) 44789285	2) 0.5	2) Likely pathogenic; contains <i>KANSL1</i> (Koolen-De Vries syndrome)
					3) del(7)(q34)	3) 141747071	3) 142191578	3) 0.4	3) Benign
81	Unspecified	NA	F	Affymetrix GeneChip 500K arrays	del(1)(q41)	222707392	224061069	1.4	Likely pathogenic; includes <i>D/SP1</i>
83	Unspecified	NA	M	Affymetrix GeneChip 500K arrays	1) del(16)(p13.3)	1) 91010	1) 3376243	1) 3.3	1) VUS
					2) del(2)(q14.2)	2) 120942349	2) 121737198	2) 0.8	2) Likely pathogenic; includes <i>GLI2</i>
					3) del(9)(q21.33)	3) 88699276	3) 89288340	3) 0.6	3) Benign
					4) del(16)(q23.1)	4) 77272582	4) 77683296	4) 0.4	4) Benign
					5) del(4)(q35.1)	5) 185245610	5) 185589739	5) 0.3	5) Benign
87	Unspecified	NA	F	Affymetrix GeneChip 500K arrays	1) del(13)(q32.3q33.3)	1) 100201640	1) 107066606	1) 6.9	1) Pathogenic; contains <i>ZIC2</i>
					2) del(3)(q22.2)	2) 133711729	2) 134044068	2) 0.3	2) Benign
88	Unspecified	NA	M	Affymetrix GeneChip 500K arrays	1) dup(6)(p21.1)	1) 43879843	1) 45888073	1) 2.0	1) VUS
					2) del(6)(q13q14.1)	2) 75449648	2) 76680444	2) 1.2	2) VUS
					3) del(14)(q11.2)	3) 22393148	3) 23069853	3) 0.7	3) Benign
					4) del(8)(q12.1)	4) 59686309	4) 60321600	4) 0.6	4) Benign
98	Unspecified	NA	NA		del(6)(q25.3q27)	158132992	170905977	12.8	Likely pathogenic, given 12.8 Mb size
106**	Lobar	6 months	M	Illumina HumanExome BeadChip-12v1_A	del(2)(p21)	41782340	45300029	3.5	Pathogenic; deletion of <i>STX3</i>
107**	Alobar	Fetus	M	Illumina HumanExome BeadChip-12v1_A	del(2)(p21)	45037497	45298668	0.3	Pathogenic; deletion of <i>STX3</i>

* Previously reported by Kauvar et al. (2011).

** Previously reported by Stokes et al. (2018).