European Journal of Medical Genetics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Rare copy number variants identified in prune belly syndrome

Nansi S. Boghossian[a,b,*], Robert J. Sicko[c], Andreas Giannakou[b], Aggeliki Dimopoulos[b], Michele Caggana[c], Michael Y. Tsai[d], Edwina H. Yeung[b], Nathan Pankratz[d], Benjamin R. Cole[d], Paul A. Romitti[e], Marilyn L. Browne[f,g], Ruzong Fan[h], Aiyi Liu[b], Denise M. Kay[c], James L. Mills[b]

[a] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC, United States
[b] Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, United States
[c] Division of Genetics, Wadsworth Center, Department of Health, Albany, NY, United States
[d] Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN, United States
[e] Department of Epidemiology, College of Public Health, The University of Iowa, Iowa City, IA, United States
[f] New York State Department of Health, Congenital Malformations Registry, Albany, NY, United States
[g] University at Albany School of Public Health, Rensselaer, NY, United States
[h] Department of Biostatistics, Bioinformatics, and Biomathematics, Georgetown University Medical Center (GUMC), Washington, DC, United States

ARTICLE INFO

Keywords:
Prune belly syndrome
Eagle-barrett syndrome
Copy number variant
Abdominal wall musculature

ABSTRACT

Prune belly syndrome (PBS), also known as Eagle-Barrett syndrome, is a rare congenital disorder characterized by absence or hypoplasia of the abdominal wall musculature, urinary tract anomalies, and cryptorchidism in males. The etiology of PBS is largely unresolved, but genetic factors are implicated given its recurrence in families. We examined cases of PBS to identify novel pathogenic copy number variants (CNVs). A total of 34 cases (30 males and 4 females) with PBS identified from all live births in New York State (1998–2005) were genotyped using Illumina HumanOmni2.5 microarrays. CNVs were prioritized if they were absent from in-house controls, encompassed ≥10 consecutive probes, were ≥20 Kb in size, had ≤20% overlap with common variants in population reference controls, and had ≤20% overlap with any variant previously detected in other birth defect phenotypes screened in our laboratory. We identified 17 candidate autosomal CNVs; 10 cases each had one CNV and four cases each had two CNVs. The CNVs included a 158 Kb duplication at 4q22 that overlaps the *BMPR1B* gene; duplications of different sizes carried by two cases in the intron of *STIM1* gene; a 67 Kb duplication 202 Kb downstream of the *NOG* gene, and a 1.34 Mb deletion including the *MYOCD* gene. The identified rare CNVs spanned genes involved in mesodermal, muscle, and urinary tract development and differentiation, which might help in elucidating the genetic contribution to PBS. We did not have parental DNA and cannot identify whether these CNVs were de novo or inherited. Further research on these CNVs, particularly *BMP* signaling is warranted to elucidate the pathogenesis of PBS.

1. Introduction

Prune belly syndrome (PBS) is a rare congenital disorder characterized by absence or hypoplasia of the abdominal wall musculature, urinary tract anomalies, and cryptorchidism in males (Lloyd et al., 2013; Seidel et al., 2015). A wide variability in disease severity exists with some patients also experiencing other associated defects including pulmonary hypoplasia, renal hypoplasia, cardiac defects, imperforate anus, and intestinal malrotation (Jennings, 2000). PBS profoundly affects a child's physical, emotional, social, and school functioning (Arlen et al., 2016). The estimated prevalence of PBS in the United States is 3.8 per 100,000 live-births (Routh et al., 2010) with a higher occurrence in males than in females (5:1 ratio) (Druschel, 1995).

The etiology of PBS remains largely unresolved. Mesenchymal developmental defects have been suggested as the underlying defect in PBS (Stephens and Gupta, 1994; Straub and Spranger, 1981). Although PBS often presents as a sporadic condition, familial cases of PBS (Balaji et al., 2000; Ramasamy et al., 2005), as well as occurrence with chromosomal defects (Amacker et al., 1986; Fryns et al., 1991), suggest a genetic contribution. Specifically, PBS has been associated with

Abbreviations: BAF, B allele frequency; BPA, British Pediatric Association; CMR, Congenital Malformations Registry; CNV, copy number variant; DGV, Database of Genomic Variants; LRR, Log R ratio; NYS, New York State; PBS, prune belly syndrome; qPCR, quantitative real-time PCR; SGA, small for gestational age; SNP, single nucleotide polymorphism

* Corresponding author. Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, 915 Greene Street Rm #447, Columbia, SC 29208, United States.
E-mail address: nboghoss@mailbox.sc.edu (N.S. Boghossian).

https://doi.org/10.1016/j.ejmg.2017.11.008
Received 10 August 2017; Received in revised form 31 October 2017; Accepted 21 November 2017
1769-7212/ © 2017 Elsevier Masson SAS. All rights reserved.

chromosomal anomalies including trisomy 21 (Amacker et al., 1986) and large deletions in the long arm of chromosome 6 (Fryns et al., 1991). Additionally, there have been several reports showing that PBS occurs in both twin and non-twin siblings, as well as in successive generations (Balaji et al., 2000; Ramasamy et al., 2005). A study from a national database also noted a twofold higher proportion of PBS among blacks compared to the general population (Routh et al., 2010). Previous studies have also reported a gene deletion of the hepatocyte nuclear factor-1β gene in PBS cases (Haeri et al., 2010; Murray et al., 2008).

Studies examining PBS gene defects have been limited. To our knowledge, no study has systematically screened the genome of PBS cases for copy number variants (CNVs). We aimed to identify CNVs in PBS patients.

2. Materials and methods

2.1. Cases

The New York State (NYS) Congenital Malformations Registry (CMR) mandates reporting of major structural birth defects identified within the first two years of life. Hospitals enter birth defect descriptions as text fields which are then coded using the expanded British Pediatric Association (BPA) coding system. To identify PBS cases, we conducted a population-based review searching for a BPA code corresponding to congenital PBS (BPA code 756.720) in the NYS CMR database. We also searched the text field for 'prune belly syndrome' and 'Eagle-Barrett syndrome' to ensure maximum case capture. In total, we identified 38 PBS cases with archived newborn screening dried blood spots from among 2,023,083 live-births occurring in NYS from January 1, 1998 through December 31, 2005. We excluded those with a known genetic syndrome (Turner syndrome (n = 1) and Beckwith-Wiedemann syndrome (n = 1)) and those where the BPA code and the clinical narrative were inconsistent (n = 2). A total of 34 cases were studied; 18 with isolated PBS and 16 with PBS and other associated defects. We classified as isolated PBS cases those with other genitourinary defects that could have occurred secondarily to the primary PBS defects, such as renal dysplasia, hydronephrosis, ureteropelvic junction obstruction, renal pelvic obstruction, ureteral dilation/obstruction, and cryptorchism. The non-isolated PBS cases had other major birth defects such as gastrointestinal and heart defects.

Demographic and clinical characteristics of mothers and cases were extracted from NYS vital records and compared with a random sample of NYS live-births (n = 7683). Statistical analyses were performed using t-tests or Fisher's exact tests, where applicable with a p-value < 0.05 used for statistical significance. All cases were de-identified by removing any personally identifying data and assigning a random identification number prior to genotyping and analysis. This study was approved by the NYS Department of Health Institutional Review Board (NYS IRB #07-007) and the National Institutes of Health Office of Human Subjects Research Protection (OHSRP#3687).

2.2. Genotyping

DNA was extracted from two 3-mm dried bloodspot punches using a lab-developed method (Saavedra-Matiz et al., 2013). Genotyping was performed at the University of Minnesota using IlluminaHumanOmni2.5-8_v1 bead arrays and the Infinium HD assay protocol. Data were analyzed with Illumina GenomeStudio v2011.1 with a genotype no-call threshold set at < 0.15. In total, 34 PBS samples were genotyped concurrently with 140 cases with other unrelated phenotypes, three technical controls, and one HapMap control (in duplicate). Genotype clustering was based on the data generated in this project. Clusters were reviewed and cleaned based on Illumina's Infinium Genotyping Data Analysis Technical Note (Illumina, 2014). A total of 2,278,660 autosomal probes, 55,207 probes on chromosome X, and 2560 probes on chromosome Y were included in the CNV analysis. For autosomal single nucleotide polymorphisms (SNPs), the average PBS sample SNP call rate ± SD (range) was 99.6% ± 0.7% (98.2–99.9%) and the mean log R ratio deviation was 0.133 ± 0.041 (0.096–0.287). After cleaning, SNP genotype reproducibility (based on two duplicates included among the 174 samples genotyped) was 100%.

2.3. CNV calling and annotation

Autosomal CNVs were called using PennCNV v2011/05/03 (Wang et al., 2007) and Illumina's cnvPartition algorithm v3.1.6. For both algorithms, data were GC-wave adjusted, and the minimum number of probes required for a CNV call was three. The confidence threshold for CNV calling was set to the default value of 10 for PennCNV and 35 for cnvPartition. Sex chromosome CNVs were called using PennCNV after recomputing Log R ratio (LRR) and B allele frequency (BAF) values using sex-specific centroids. Median values for R and theta were computed for each marker on the X and Y chromosomes in males and females separately and then applied using in-house software that implement the standard formulas (Peiffer et al., 2006) to generate new LRR and BAF values. These new values were then fed into PennCNV as "autosomal" probes using custom sex-specific population frequency of B allele (.pfb) and GC content (.gcmodel) files. The PennCNV function clean_cnv.pl was run with default parameters to merge adjacent CNV calls. Autosomal CNV call files were annotated using custom C++ programs as previously (Rigler et al., 2015) to compare concordance between CNV calling algorithms, count the number of cases and controls carrying overlapping CNVs in the current study, determine overlap with an in-house database of CNVs generated from cases and controls of other unrelated defects, determine overlap with the Database of Genomic Variants archive (DGV2), and identify intersecting transcripts and genes (Iafrate et al., 2004). Transcripts included full-length coding transcripts and full-length non-coding transcripts with a well characterized biotype downloaded from GENCODE (version 19, accessed via UCSC genome browser May 2014) (Harrow et al., 2012). Genes were defined as those included in the Consensus Coding Sequence project (CCDS; release 15, accessed via UCSC genome browser June 2014) (Pruitt et al., 2009). Each sex chromosome CNV call was manually reviewed and annotated.

2.4. CNV selection and prioritization

We prioritized CNVs that were not detected in our in-house controls (i.e. PBS CNVs of the same type and with the same predicted breakpoints), encompassed a minimum of 10 consecutive SNP probes, were at least 20 Kb in size, had ≤20% overlap with common variants in HapMap (Altshuler et al., 2010) and Children's Hospital of Philadelphia (CHOP) (Shaikh et al., 2009) CNV datasets, and had ≤20% overlap with any variant previously detected in other birth defect phenotypes screened in our laboratory. We uploaded CNVs meeting these requirements to the DGV2 genome browser (release data 2014-10-16 version), using build37/hg19 coordinates, and examined them for overlap with known CNVs.

2.5. CNV validation

Seventeen autosomal CNVs were selected for validation studies using two to four quantitative real-time PCR (qPCR) TaqMan assays (Applied Biosystems, Carlsbad, CA) per CNV region. Genomic DNA was extracted from one 3-mm dried blood spot, diluted 1:10 in water, and amplified using TaqMan Environmental Master Mix (ABI) in 5 μl reaction volumes. A fragment of the RNaseP H1 RNA gene was co-amplified and used as an internal control (TaqMan Copy Number Reference Assay, ABI). Assays were run in quadruplicate on either an ABI 7900HT or an ABI QuantStudio. CopyCaller software v2.0 (ABI) was used to analyze the real-time data using relative quantitation (2-

Download English Version:

https://daneshyari.com/en/article/8644292

Download Persian Version:

https://daneshyari.com/article/8644292

Daneshyari.com