Brief report

Homozygosity mapping with SNP arrays confirms 3p21 as a recessive locus for gray platelet syndrome and narrows the interval significantly

Shay Fabbro,1 Walter H. A. Kahr,2 Jesse Hinckley,1 Kai Wang,3 Jack Moseley,4 Gi-Yung Ryu,5 Brie Nixon,1 James G. White,6 Thomas Bair,7 Brian Schutte,8 and Jorge Di Paola1

1Department of Pediatrics, University of Colorado, Denver, CO; 2Department of Paediatrics, Division of Haematology/Oncology, Program in Cell Biology, Research Institute, Hospital for Sick Children, Toronto, ON; 3Department of Biostatistics, College of Public Health, University of Iowa, Iowa City, IA; 4Northern Oklahoma Resource Center, Enid, OK; 5Institute for Clinical and Translational Science at the University of Iowa, Iowa City, IA; 6Department of Laboratory Medicine, University of Minnesota, Minneapolis, MN; 7DNA Facility, University of Iowa, Iowa City, IA; and 8Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI

Gray platelet syndrome (GPS) is an inherited bleeding disorder characterized by thrombocytopenia and the absence of α-granules in platelets. Patients with GPS present with mild to moderate bleeding and many develop myelofibrosis. The genetic cause of GPS is unknown. We present 2 Native American families with a total of 5 affected persons and a single affected patient of Pakistani origin in which GPS appears to be inherited in an autosomal recessive manner. Homozygosity mapping using the Affymetrix 6.0 chips demonstrates that all 6 GPS-affected persons studied are homozygous for a 1.7-Mb region in 3p21. Linkage analysis confirmed the region with a logarithm of the odds score of 2.7. Data from our families enabled us to significantly decrease the size of the critical region for GPS from the previously reported 9.4-Mb region at 3p21. (Blood. 2011;117(12):3430-3434)

Introduction

Gray platelet syndrome (GPS) is a rare disorder characterized by mild to moderate thrombocytopenia and the presence of large platelets that lack α-granules.1,3 The diagnosis of GPS is usually confirmed by the absence of platelet α-granules as observed by electron microscopy.1,4-6 Patients with GPS exhibit mild to moderate bleeding, and many of them develop myelofibrosis later in life.3,7-10 There have been reports of rare autosomal dominant and X-linked variants of GPS; however, the majority of cases appear to be autosomal recessive. Although the genetic cause for GPS has yet to be determined, a recent report by Gunay-Aygun et al14 mapped the autosomal recessive GPS locus to a 9.4-Mb interval on 3p21.

In this report, we used homozygosity mapping and linkage analysis in 5 patients with GPS from 2 Native American families, and one person with GPS of Pakistani origin to localize the GPS locus to a 1.7-Mb region on chromosome 3 (3p21), which is significantly narrower than previously reported.14

Methods

Patients

A total of 16 persons were evaluated (5 affected and 11 unaffected) in 2 separate Native American families with GPS from the same settlement. One single patient of Pakistani origin was also included in the analysis (Figure 1). All patients and their families gave written, informed consent in accordance with institutional guidelines and the Declaration of Helsinki. Clinical and laboratory data included family and personal history of bleeding, physical examination, complete blood counts (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article), and optical and electron microscopy to document α-granule–deficient platelets. DNA was extracted from whole blood using the Gentra Puregene or QIAamp Blood Kit (QIAGEN). All patient studies were approved by the University of Colorado Denver Institutional Review Board.

Homozygosity mapping and linkage analyses

DNA from each of 11 unaffected and 5 affected persons was interrogated on the Genome-Wide Human SNP Array Version 6.0 (Affymetrix), which contains 906 600 single nucleotide polymorphisms (SNPs), using the Microarray Core facilities at University of Colorado Denver/Anschutz Medical Campus as previously described.15 The data were analyzed using the Genotyping Console Software (Affymetrix). Homozygosity and copy number variation analyses were performed on all samples using default settings. In addition to Genome-Wide Human SNP Array Version 6.0 SNPs, 21 microsatellite markers were also genotyped in the 3p21 region.

Two-point and multipoint nonparametric linkage analyses were performed using Genehunter.16 Parametric linkage analysis assuming a recessive model was performed. Marshfield genetic map files that come with easyLinkage17 were used for the analysis. Results are expressed as logarithm of the odds scores.

DNA sequencing

Sequencing of 11 candidate genes from the homozygous 1.7-Mb region was performed by standard Sanger sequencing as previously described (supplemental Table 2).

Targeted sequencing

A custom high-density oligonucleotide microarray (NimbleGen) was designed for the region spanning 3p21 for person III:9 from family number


The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2011 by The American Society of Hematology
3 (Figure 1), and the captured genomic region was sequenced using massively parallel sequencing as previously described.\textsuperscript{19} Specifically, the physical location of the captured region spans from bp 48003148 (rs13074973) to bp 50499562 (rs57998585) encompassing the 1.7-Mb region plus approximately 500,000 extra base pairs on each side (supplemental Data).

Results and discussion

Homozygosity mapping has been successfully used to identify genes responsible for recessive Mendelian disorders in consanguineous
families. In our report, although families 2 and 3 are not consanguineous, they are part of a Native American settlement with limited outbreeding. Therefore, we hypothesized a founder effect and performed homozygosity mapping, which is based on the premise that the disease phenotype is the result of the inheritance of 2 identical copies of the mutated gene. This approach is potentially more robust than traditional linkage because populations with limited outbreeding will provide higher statistical power than a collection of unrelated persons or nuclear families. Interestingly, person I:1 of family 2 is not of Native American descent, indicating potential compound heterozygosity for the locus. Initial analysis of the 6.0 Affymetrix chips identified 3 regions of homozygosity that all affected persons in the 3 families shared: 1q25.1 (171.8-172.3 Mb), 3p21 (48.4-50.1 Mb), and a large centromeric region of chromosome 16. A region of homozygosity was defined by a minimum of 50 homozygous consecutive SNPs.

Concomitant linkage analysis demonstrated a logarithm of the odds score of 2.7 at 3p21 (70.6 cM) for the gray platelet phenotype, and no linkage peaks greater than 1.5 were found elsewhere (supplemental Figure 3). Therefore, we focused all our efforts on the 3p21 region. The genotypes were reexamined in 3p21, and
homozygosity was confirmed for all affected persons in the region that spanned from bp 48462342 (rs9876781) to bp 50162794 (rs 2526397) (Figure 2). These data are consistent with the locus assignment of the GPS gene by linkage to 3p21 recently published by Gunay-Aygun et al.14 In their report, most of the affected persons exhibited a homozygous haplotype that extended from position 42663630 to 52036954 (9.4-Mb interval). Linkage and homozygosity mapping data from our 3 families allowed us to narrow this region to 1.7 Mb. This region contains a total of 74 genes and was carefully examined for obvious candidates. Candidate genes were chosen based on function and potential involvement in granule biogenesis (supplemental Table 2). Special emphasis was given to genes involved in vesicle trafficking, cytoskeleton organization, and signaling. A total of 11 candidate genes were sequenced, but no mutations were found (supplemental Table 3).

We then, in a nonbiased approach, decided to sequence the entire 1.7-Mb interval in person III:9, clearly affected with the GPS phenotype, using a NimbleGen microarray platform. No mutations were found in this region. Several intronic changes were noted, but the significance of those is not clear. No changes were noted in splice sites or within 50 bp from splice sites, and no sequence variations were found in the 3 intronic microRNAs encompassed within this region. Although the coverage was greater than 90%, we were not able to sequence many smaller regions within this interval. This could be related to the limitations that still characterize mutation detection by next-generation sequencing, including missed variant calls and the inability of identifying variants in coding and noncoding repeat sequences. It has been recently shown that specific sequence variants, such as segmental duplications and long repeats, are particularly subject to errors in high-throughput sequencing.23 Therefore, it is not completely surprising that a disease gene mutation was not found by us or other groups.

Gunay-Aygun et al used large-scale genome sequencing to sequence 165 protein-coding genes in a larger interval of 3p21.14 They successfully sequenced 69% of the region without finding any homozygous or compound heterozygous mutations. Combining our data with that of Gunay-Aygun et al,14 a total of 40 of 74 potential genes (within the 1.7-Mb interval) have now been fully sequenced by traditional Sanger sequencing. Interestingly, Gunay-Aygun et al14 reported difficulties in sequencing genes within this region. It is possible that the causative mutation for GPS is in a promoter region or in other regulatory regions that makes its identification more difficult. In summary, we have narrowed the region for the GPS locus from a 9.4-Mb interval to 1.7 Mb. Initial next-generation sequencing failed to identify a causative mutation. Improved whole genome sequencing techniques will probably allow for the discovery of the gene responsible for GPS. This could be relevant not only for the understanding of the disease but also to unveil unknown aspects of platelet α-granule biogenesis.

Acknowledgments

The authors thank all persons with GPS and their families who participated in this study and Jeffrey C. Murray for critical review of the manuscript.

This work was supported by the National Institutes of Health (R01 HL084086-01) and the Postle Family Chair in Pediatric Cancer and Blood Disorders (J.D.P.). W.H.A.K. was supported by the Canadian Institutes of Health Research (grant MOP-81208) and the Heart and Stroke Foundation of Ontario (Phase II Clinician Scientist Award CS 5982).

Authorship

Contribution: S.F. designed the study, performed experiments, analyzed data, and wrote the manuscript; W.H.A.K. designed the study, analyzed data, provided the patient of Pakistani origin, and edited the manuscript; J.H. performed initial mapping experiments; J.M. recruited families; K.W. performed linkage analysis and haplotype mapping; G.-Y.R. performed analysis of SNP arrays; B.N. performed microsatellite genotyping and SNP array genotyping; J.G.W. determined GPS phenotyping by electron microscopy; T.B. performed data analysis of massive parallel sequencing; B.S. designed the study, analyzed data, and edited the manuscript; and J.D.P. designed and supervised research, analyzed data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Jorge Di Paola, Department of Pediatrics, University of Colorado Denver, Mail Stop 8302, Bldg RC-1 North, 12800 E 19th Ave, PO Box 6511, Aurora, CO 80045; e-mail: jorge.dipaola@ucdenver.edu.

References

11. Baldini CL, De Candia E, Savoia A. Why the disorder induced by GATA1 Arg216Gln mutation should be called “X-linked thrombocytopenia with thalassemia” rather than “X-linked gray platelet syndrome.” Blood. 2007;110(7):2770-2771; author reply 2771.
15. McCarron SA, Kuruvilla FG, Kom JM, et al. Integrated detection and population-genetic analysis...


Homozygosity mapping with SNP arrays confirms 3p21 as a recessive locus for gray platelet syndrome and narrows the interval significantly

Shay Fabbro, Walter H. A. Kahr, Jesse Hinckley, Kai Wang, Jack Moseley, Gi-Yung Ryu, Brie Nixon, James G. White, Thomas Bair, Brian Schutte and Jorge Di Paola