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Mutations in *NBEAL2*, encoding a BEACH protein, cause gray platelet syndrome

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AUTHOR CONTRIBUTIONS

Designed experiments: W.H.A.K., A.S.W., J.H. and J.D.P. Performed research, analyzed data: W.H.A.K., A.S.W., J.D.P., J.H., L.L., H.S., H.C., J.W.R., F.G.P., D.U., S.F., B.N., R.G., M.S., K.W., G.-Y.R., S.M.J., B.C.S., J.M., N.B.L. and J.P. Wrote and edited manuscript: W.H.A.K., A.S.W., J.D.P., J.P., N.B.L., J.H., J.W.R. and F.G.P.

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Abstract

Next-generation RNA sequence analysis of platelets from an individual with autosomal recessive gray platelet syndrome (GPS, MIM139090) detected abnormal transcript reads, including intron retention, mapping to *NBEAL2* (encoding neurobeachin-like 2). Genomic DNA sequencing confirmed mutations in *NBEAL2* as the genetic cause of GPS. *NBEAL2* encodes a protein containing a BEACH domain that is predicted to be involved in vesicular trafficking and may be critical for the development of platelet α -granules.

GPS is characterized by variable thrombocytopenia and large platelets that lack α -granules but have normal dense (δ) granules, lysosomes, mitochondria and peroxisomes¹. Genome-wide linkage analysis has mapped the GPS locus to within a 9.4-megabase region on chromosome 3p21 (refs. 2,3) containing 197 protein coding genes³, of which 69 have been completely or partially sequenced^{2,3}. Our analysis led us to target a 2.7-megabase region in three families affected with GPS, two of Native American and one of Pakistani ancestry. Microscopic analysis of GPS platelets confirmed the absence of α -granules and characterized their abnormal size, shape and protein distribution (**Supplementary Fig. 1**).

As platelets have a diverse repertoire of mRNAs⁴, we reasoned that GPS-associated aberrations or deficiencies in gene expression might be detected by examining platelet transcript expression patterns. We performed focused next-generation genome-wide RNA sequencing (RNA-seq) of platelet mRNA from an individual with GPS to look for atypical expression of transcripts derived from genes located within the target region that are suspected to be involved in cellular vesicular trafficking. We found an abnormal distribution of reads mapping to *NBEAL2*; specifically, *NBEAL2* transcripts isolated from GPS platelets showed retention of introns (**Fig. 1**), indicating abnormal pre-mRNA processing.

Sequencing of *NBEAL2* in genomic DNA from the same individual detected a splice variant at position 1,029 (c.1029+1G>A) at the exon-intron 9 boundary (family 1, **Fig. 2**). This mutation seems to prevent recognition of the 3' splice site in exon 9, causing the retention of intron 9 in the mRNA and introducing three in-frame premature stop codons within 80 nucleotides of the putative splice site. This mutation was also identified in RNA-seq reads spanning the exon-intron 9 junction (**Supplementary Fig. 2**). We found a second mutation consisting of a five-base pair duplication of bases 4,371–4,375 in exon 28 (c.4371_4375dupCGTGG), which introduces an in-frame premature stop codon (TGA, nucleotides 4,496–4,499) 120 bases downstream. The duplication was also detected in the RNA-seq reads (data not shown). The compound heterozygous mutations were inherited from the parents (family 1, **Fig. 2**).

Sequencing of genomic *NBEAL2* in two other affected families identified a homozygous nonsense mutation at position 1,820 of exon 13 (c.1820G>A) that introduced a stop codon (Trp607X) in three affected individuals of family 2 (**Fig. 2**) and segregated with disease status (parents were heterozygous for the mutation). In family 3 (**Fig. 2**), all affected individuals showed heterozygosity for the 1820G>A mutation, as did the mother who is

from the same settlement as family 2. As this family has a parent originating outside the settlement, we searched for a compound heterozygosity that could explain the phenotype and identified a one-base pair duplication of position 5,413 (c.5413dupG) in exon 33. This mutation was present only in the father and his two affected children, and the single-base frameshift introduced a TGA stop codon 172 bases downstream (bases 5,586–5,588).

Mutations in the three families are summarized in **Supplementary Table 1** and **Supplementary Figure 3**.

Premature stop codons caused by nonsense mutations, frameshifts or splice-site alterations are known to have deleterious effects on gene expression, including nonsense-mediated mRNA decay and, to a lesser extent, protein truncation⁵. Thus, our results indicate that *NBEAL2* mutations are the main cause of autosomal recessive GPS.

NBEAL2 encodes a polypeptide of 2,754 amino acids with a predicted molecular weight of 302.5 kDa that shows similarity to the product of the *LYST* gene (encoding lysosomal trafficking regulator), which is mutated in Chediak-Higashi syndrome (CHS; MIM214500)⁶. CHS is characterized by a deficiency of platelet δ -granules⁷. Domain analysis (**Supplementary Figs. 4 and 5**) showed that, like *LYST*, *NBEAL2* contains a concavalin A-like lectin domain⁸, a pleckstrin homology domain that is predicted to function in recruiting proteins to cellular membranes⁹, a BEACH (beige and CHS) domain¹⁰ and WD40 repeats, which have been implicated in a variety of cellular functions¹¹. Phylogenetic analysis indicated that *NBEAL2* and its close homolog *NBEAL1* are members of an ancient family of BEACH domain-containing proteins related to neurobeachin (which is expressed in neurons) and LRBA (lipopolysaccharide-responsive and beige-like anchor protein, which is involved in LPS-responsive vesicle trafficking) but distinct from *LYST* (**Supplementary Fig. 6**). Our analysis indicates that during the emergence of animals, the ancestral *NBEAL* gene underwent duplication to produce the neurobeachin/LRBA and *NBEAL* subfamilies, whereas a more recent duplication in the craniate (vertebrate) lineage produced the *NBEAL1* and *NBEAL2* subfamilies.

LYST mutations predict that the *LYST* protein is involved in trafficking among lysosomes and lysosome-related organelles (platelet-granules) that interact with different partners¹². Other BEACH domain-containing proteins have been linked to cellular vesicular trafficking, including Bph1, the product of the *S. cerevisiae* homolog of *LYST*¹³, LysB in *Dictyostelium*¹⁴ and *bchs* in *Drosophila*¹⁵. Thus, it is reasonable to suspect that, like *LYST* and other BEACH domain-containing proteins, *NBEAL2* is involved in vesicular trafficking. Our results indicate that the emergence of the *NBEAL2* gene family in the vertebrate lineage might have been key to the evolution of platelet α -granules as unique specialized organelles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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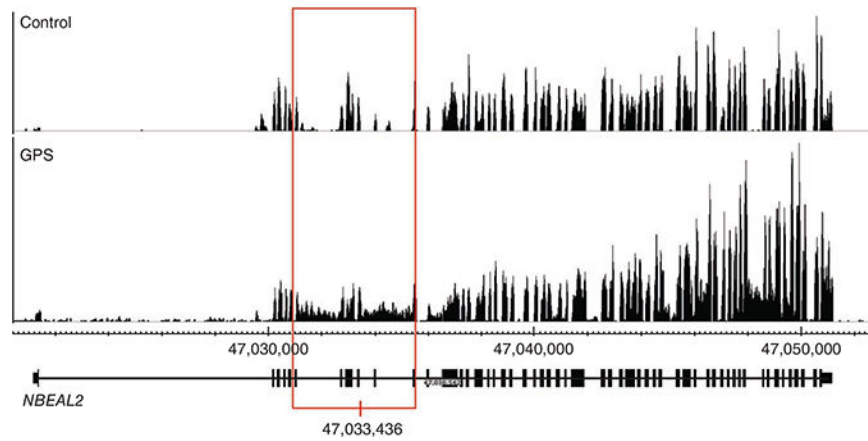
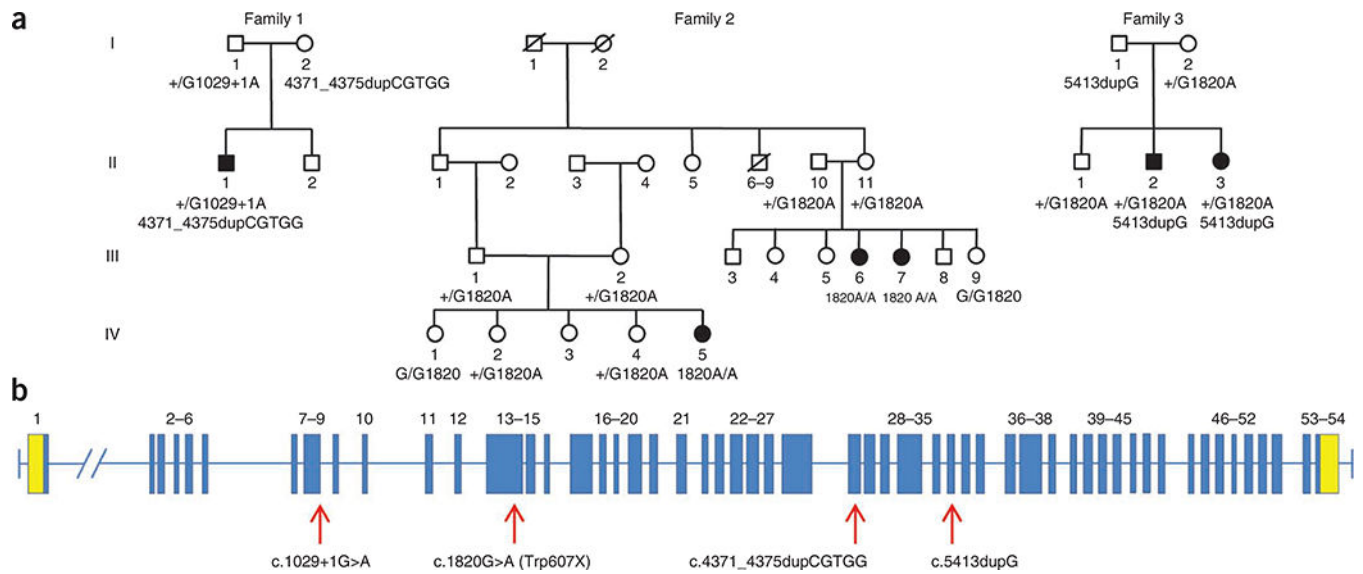


Figure 1. Abnormal sequence reads in *NBEAL2* transcripts observed in platelet RNA from an individual with GPS. Snapshots shown from Integrated Genome Browser (IGB) for *NBEAL2* transcripts expressed in platelets isolated from (top) a healthy donor and (bottom) an individual with GPS. Bar heights represent relative numbers of 50-base pair reads spanning *NBEAL2*; Refseq gene annotation for *NBEAL2* is shown at the bottom. The red box outlines a region where introns are abnormally retained in *NBEAL2* transcripts from platelets from the individual with GPS. See **Supplementary Methods** for details.

**Figure 2.**

Mutation analysis of *NBEAL2*. **(a)** Pedigrees for three GPS-affected families with mutation status shown beneath symbols for each individual. **(b)** Schematic of *NBEAL2*, which is composed of 54 exons encoding untranslated regions (yellow) and protein-coding sequences (blue; the BEACH domain is encoded by exons 37–45). Red arrows indicate the locations of mutations in the *NBEAL2* genomic sequence found in individuals with GPS (chromatograms are shown in **Supplementary Fig. 3**).