



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Myers, KA;Bennett, MF;Chow, CW;Carden, SM;Mandelstam, SA;Bahlo, M;Scheffer, IE

Title:

Mosaic uniparental disomy results in GM1 gangliosidosis with normal enzyme assay

Date:

2018-01-01

Citation:

Myers, K. A., Bennett, M. F., Chow, C. W., Carden, S. M., Mandelstam, S. A., Bahlo, M. & Scheffer, I. E. (2018). Mosaic uniparental disomy results in GM1 gangliosidosis with normal enzyme assay. *American Journal of Medical Genetics Part A*, 176 (1), pp.230-234. <https://doi.org/10.1002/ajmg.a.38549>.

Persistent Link:

<https://hdl.handle.net/11343/293901>

Clinical Report

Mosaic Uniparental Disomy results in GM1 Gangliosidosis with Normal Enzyme Assay¹

Kenneth A. Myers 0000-0001-7831-4593 0000-0001-7831-4593¹, Mark F. Bennett^{1,2,3}, Chung W. Chow^{4,5}, Susan M. Carden^{5,6},
Simone A. Mandelstam^{5,7,8}, Melanie Bahlo^{2,3,9}, Ingrid E. Scheffer^{1,5,8}

¹Epilepsy Research Centre; Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria, Australia; ²The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia; ³Department of Medical Biology, The University of Melbourne, Parkville, Victoria, Australia; ⁴Department of Anatomical Pathology, Royal Children's Hospital, Parkville, Victoria, Australia; ⁵Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria, Australia; ⁶The Royal Victorian Eye and Ear Hospital, East Melbourne, Victoria, Australia; ⁷Department of Radiology, The University of Melbourne, Parkville, Victoria, Australia; ⁸The Florey Institute of Neuroscience and Mental Health, Heidelberg, Victoria, Australia; and ⁹Department of Mathematics and Statistics, The University of Melbourne, Parkville, Victoria, Australia

Address Correspondence to: Ingrid Scheffer, Level 2, Melbourne Brain Centre, Austin Health, 245 Burgundy St., Heidelberg, Victoria, 3084, Australia; e-mail: scheffer@unimelb.edu.au, Phone +61-3-9035-7112; Fax +61-3-9496-2291

Acknowledgements: The study was supported by grants from the National Health and Medical Research Council.

Conflict of Interests: KAM has received a travel grant from Zynerva. MB receives research support from the NHMRC, NZ HRC, NIH, March of Dimes, CureKids NZ, Lowy Medical Research Institute, The Walter and Eliza Hall Institute of Medical Research, Murdoch Children's Research Institute, and the Australian Institute of Tropical Health and Medicine. IES serves on the editorial boards of *Neurology*® and *Epileptic Disorders*; may accrue future revenue on a pending patent re: Therapeutic compound; has received speaker honoraria from Athena Diagnostics, UCB, GSK, Eisai, and Transgenomics; has received scientific advisory board honoraria from Nutricia and GSK, has received funding for travel from Athena Diagnostics, UCB, and GSK; and receives/has received research support from the NHMRC, ARC, NIH, Health Research Council of New Zealand, March of Dimes, the Weizmann Institute, CURE, US Department of Defense, and the Perpetual Charitable Trustees. The other authors have indicated they have no financial relationships relevant to this article to disclose.

¹ This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:[10.1002/ajmga.38549](https://doi.org/10.1002/ajmga.38549)

Abstract

Inherited metabolic disorders are traditionally diagnosed using broad and expensive panels of screening tests, often including invasive skin and muscle biopsy. Proponents of next generation genetic sequencing have argued that replacing these screening panels with whole exome sequencing (WES) would save money. Here, we present a complex patient in whom WES allowed diagnosis of GM1 gangliosidosis, caused by homozygous *GLB1* mutations, resulting in β -galactosidase deficiency. A 10-year-old girl had progressive neurologic deterioration, macular cherry-red spot and cornea verticillata. She had marked clinical improvement with initiation of the ketogenic diet. Comparative genomic hybridization microarray showed mosaic chromosome 3 paternal uniparental disomy (UPD). GM1 gangliosidosis was suspected, however β -galactosidase assay was normal. Trio WES identified a paternally-inherited pathogenic splice-site *GLB1* mutation (c.75+2dupT). The girl had GM1 gangliosidosis; however, enzymatic testing in blood was normal, presumably compensated for by non-UPD cells. Severe neurologic dysfunction occurred due to disruptive effects of UPD brain cells.

Key words: GM1 gangliosidosis, Ketogenic diet, Lysosomal disorders, GLB1, Whole exome sequencing, Uniparental disomy, Mosaic, Cherry-red spot, Cornea verticillata, Skin biopsy

Introduction

Clinical genetic testing has been revolutionized by the development and increasing affordability of high throughput molecular technologies. Whole exome sequencing (WES) allows clinicians to screen for mutations in the protein-coding regions of all ~20,000 genes carried by human beings. Point mutations can be detected by WES while comparative genomic hybridization (CGH) microarray is more traditionally used to identify copy number variants and regions of homozygosity.

One appealing application for WES is in patients suspected to have complex disorders such as inherited disorders of metabolism. These diseases can be very challenging to diagnose, often requiring a large number of screening biochemical tests. In patients with intellectual disability, early WES can be a more cost-effective approach than traditional diagnostic pathways [Monroe and others 2016], though this may not be the case for patients with clinical features that point to a specific category of metabolic disease. In addition to cost issues, traditional diagnostic approaches often require biopsy of skin, muscle or other tissues, procedures which are invasive and potentially painful.

One metabolic disease gene is *GLB1*, homozygous mutations of which result in impaired β -galactosidase function. A lysosomal storage disorder results, with two distinct possible phenotypes, mucopolysaccharidosis type IVB and GM1 gangliosidosis [Caciotti and others 2011]. The latter is characterized by progressive neurological deterioration, progressive brain atrophy, cardiac and skeletal abnormalities, and ocular findings which may include corneal clouding and oculomotor dyspraxia [Caciotti and others 2011]. There are infantile, late-infantile and juvenile forms of GM1 gangliosidosis, which vary in severity and rate of deterioration;

macular cherry red spot is a feature usually unique to the infantile-onset form [Caciotti and others 2011].

We describe a girl with GM1 gangliosidosis due to mosaic paternal uniparental disomy (UPD) of chromosome 3. Due to mosaicism, lysosomal enzyme testing was normal, and the diagnosis was not made until the results of WES determined the underlying molecular defect. We highlight that next generation sequencing can solve the molecular basis of metabolic disease in the setting of complicated genetic pathology.

Clinical Data

A 10-year-old girl had epilepsy, severe intellectual disability and progressive neurological decline. Epilepsy was diagnosed at three years of age, initially involving 10-30 second focal impaired awareness seizures occurring up to five times per day. At four years, she developed twice-weekly “drop attacks” which were focal impaired awareness seizures involving eye deviation with loss of awareness for several seconds, a sudden loss of tone, with continued staring and eye deviation; these events lasted approximately 15 seconds. Tonic seizures developed soon after, involving an aura of fear and agitation, followed by tonic stiffening of the upper limbs and manual automatisms. Myoclonic seizures also developed at four years, initially seen every two days but progressively worsening to become near continuous. Seizures were refractory to clobazam, valproate, lamotrigine, and topiramate; levetiracetam showed some benefit. The ketogenic diet elicited the most dramatic effect, with marked improvements in both myoclonus and gait stability, and seizure frequency decreasing from 1-3 per day to 1-3 per week.

Electroencephalography (EEG) studies showed diffuse background slowing and frontally predominant generalized spike-wave activity that was at times near continuous at 2-3 Hz.

Early developmental milestones were normal, with plateauing in the second year of life. She did not walk independently until 23 months and language plateaued after she began putting two words together late in the second year of life. Autism spectrum disorder was diagnosed at 3.5 years. From four to eight years of age, she regressed with progressively worsening unsteadiness and myoclonus, and becoming non-verbal by eight years. Neuropsychologic assessment at five years estimated her full-scale IQ at 35-50. There was no known family history of febrile seizures or intellectual disability. She was the only child of unrelated parents and lived with her mother. She had minimal contact with her father, who was of Finnish descent and reported to have some obsessional features.

General examination showed no evidence of hepatosplenomegaly or cardiac dysfunction. Neurologic examination was significant for mild spasticity, more prominent in the lower limbs, and brisk deep tendon reflexes. Ophthalmologic examination at five years showed cornea verticillata and a faint macular cherry-red spot. Both of these signs improved so that neither was apparent at nine years of age. Her vision and ocular motility appeared to be normal throughout. She had precocious puberty; at eight years she had measurable estradiol and marginally elevated gonadotropins, and bone age was 2-3 years advanced, so gonadotropin-releasing hormone antagonist therapy (leuprorelin acetate) was initiated.

Brain magnetic resonance imaging (MRI) studies at four and six years of age showed diffuse atrophy with white matter abnormalities (Figure 1A-B), with slight interval worsening of atrophy between the scans. Magnetic resonance spectroscopy was normal. Fluorodeoxyglucose positron emission tomography demonstrated diffuse hypometabolism involving both hemispheres (Figure 1C). CGH microarray from a blood sample revealed mosaic paternal UPD of the entirety of chromosome 3. Lysosomal enzyme testing on peripheral leukocytes, including β -galactosidase (1.2 nmol/min/mg protein; laboratory reference range 1.0-6.0), was normal. Serum lactate, very long chain fatty acids and acylcarnitine profile were normal, as were urine glycosaminoglycans. Cerebrospinal fluid glucose, lactate and neurotransmitters were normal. Nerve conduction testing and electromyography were normal. Skin biopsy showed a prominent increase of lysosomal bodies in Schwann cells and neuritic processes (Figure 1D); however, less storage material was seen in endothelial cells and pericytes when compared to a patient with non-mosaic autosomal recessive GM1 gangliosidosis (Figure 1E).

Trio WES was undertaken, and allele variant frequencies were plotted (Figure 2). This approach confirmed the mosaic paternal UPD, estimated at ~45% of cells in blood. With normal biparental inheritance, mean variant allele frequency should be ~0.5 for heterozygous variants since one copy of the variant allele is inherited from one parent. However, chromosome 3 shows two populations of variant allele frequency due to the mosaic UPD; one with mean allele frequency ~0.33 (maternal) and the other ~0.67 (paternal). If the patient had non-mosaic UPD (i.e. all cells affected), we would see a variant allele frequency of 1.0.

Separate variant filtering algorithms were developed to identify potentially pathogenic variants inherited in a *de novo* heterozygous, recessive, compound heterozygous, and paternal UPD pattern. No pathogenic candidates were identified when filtering for variants inherited in a homozygous or *de novo* heterozygous manner. Filtering for compound heterozygotes revealed that both parents carried *GLB1* splice region variants. The maternal variant (NM000404.3:c.1069-4A>G; rs781267265) is present in heterozygous form in the Genome Aggregation Database (gnomAD) 17 times, and is of uncertain significance. The paternal variant (NM000404.3:c.75+2dupT; rs587776525) is an insertion present in heterozygous form in gnomAD 57 times, which has been reported as pathogenic in GM1 gangliosidosis [Chakraborty and others 1994; Morrone and others 1994]. There are no homozygotes for either variant in gnomAD.

Discussion

The girl had clinical features of a neurodegenerative disease consistent with GM1 gangliosidosis including cherry-red spot, cornea verticillata, refractory epilepsy and progressively worsening myoclonus. Further supportive evidence came from brain atrophy and white matter abnormalities on MRI, and increased lysosomal bodies observed on skin biopsy [Tome and Fardeau 1976]. However, this diagnosis was initially considered ruled out after β -galactosidase testing was normal.

The girl's age of onset and early clinical features are most consistent with Type II, late-infantile onset form of GM1 gangliosidosis [Regier 0000-0001-7525-0088 and Tiffet 1993]. In addition, her pattern of MRI abnormality including progressively worsening generalized cerebral atrophy

and deep white matter abnormalities is that most commonly seen in the late-infantile form [Regier and others 2016]. Her mutation was previously reported in the more severe infantile onset form of GM1 gangliosidosis [Morrone and others 1994] in a patient who died at 14 months. However, at 10 years of age our patient is already outside the usual life expectancy for those with the late-infantile form and is generally doing very well, with some clinical features, such as the ocular findings, actually resolving [Regier and Tiffit 1993]. This relatively good clinical course may be explained in part by her mosaic state; however, the ketogenic diet may also have improved her outcome.

This patient's underlying molecular genetics are complicated; she has mosaicism (~45% of cells in blood) for paternal UPD of chromosome 3. Since her father carries a previously reported pathogenic splice site mutation in *GLB1* [Chakraborty and others 1994; Morrone and others 1994], she is mosaic for this homozygous mutation. We hypothesize that her normal β -galactosidase testing occurred because of the mosaicism; i.e., her blood may have enough cells without the homozygous mutation to produce a normal enzymatic assay. Mosaicism may also explain the unusual corneal whorls seen, when GM1 gangliosidosis is classically associated with more uniform corneal opacities [Emery and others 1971]. Her severe neurologic phenotype may occur because the fraction of metabolically abnormal cells in her brain are sufficient to produce severe, global cerebral dysfunction. In addition, the percentage mosaicism of UPD may be greater in the brain than in blood. Repeating the enzyme assay in another tissue (e.g. skin fibroblasts) would clarify this issue, but such testing cannot be ethically performed now that she has a molecular diagnosis.

These findings emphasize the potential of WES to aid in diagnosis of inherited disorders of metabolism [Pierson and others 2012]. GM1 gangliosidosis has been previously reported in a case of non-mosaic UPD [King and others 2014]; however, β -galactosidase activity was markedly decreased in that case, allowing for easy confirmation of the diagnosis. Our patient illustrates that WES can identify inherited disorders of metabolism, even when functional metabolic testing is normal. We were, however, fortunate that our patient had a previously published mutation, as confirming diagnosis is much more challenging when faced with variants of uncertain significance.

UPD is diagnosed in $\sim 1/400$ cases referred for clinical WES [Yang and others 2014], but this study demonstrates that mosaic UPD has a recognizable signature when trio WES is analyzed with a focus on variant (non-reference) allele frequency. CGH microarray remains the preferred test for identifying and quantifying regions of homozygosity; however, utilizing allele frequency plots of WES data is a reasonable screening tool for patients who have WES prior to CGH microarray. Recently described techniques using both read depth data and allele frequency have improved the efficiency and accuracy of copy number variant detection with WES, and could also be applied to improve homozygosity identification [Glessner and others 2015].

In summary, this case demonstrates that mosaic UPD can result in severe genetic metabolic disease with normal blood enzymatic assay. In such cases, WES may enable a molecular diagnosis. Use of allele frequency plots in WES analysis is a useful means of screening for regions of homozygosity in patients who have not already had CGH microarray.

Figure Legends

Figure 1 – Neuroimaging and Skin Biopsy: (A) Axial T1-weighted MRI shows diffuse atrophy, most prominent in the temporal lobes (arrows). (B) Axial T2-weighted MRI demonstrates white matter hyperintensity in periventricular and deep lobar regions (arrows). (C) Fluorodeoxyglucose positron emission tomography shows diffuse, bi-hemispheric hypometabolism. (D) In our patient, increased lysosomal bodies are seen in the Schwann cells and neuritic processes (thick arrow). A similar pattern is seen in a 15-month-old girl with non-mosaic GM1 gangliosidosis (E); however, more storage material is observed in endothelial cells (thick arrow) and pericytes (thin arrow).

Figure 2 – Variant allele frequency across all chromosomes: In all but one chromosome, variant allele frequency clusters around 0.5 for heterozygous variants and 1.0 for homozygous variants. Chromosome 3 (red arrow) is markedly different, with clusters around 0.3 and 0.7. This latter pattern occurs due to the patient's mosaicism for chromosome 3 uniparental isodisomy.

References

- Caciotti A, Garman SC, Rivera-Colon Y, Procopio E, Catarzi S, Ferri L, Guido C, Martelli P, Parini R, Antuzzi D, Battini R, Sibilio M, Simonati A, Fontana E, Salviati A, Akinci G, Cereda C, Dionisi-Vici C, Deodato F, d'Amico A, d'Azzo A, Bertini E, Filocamo M, Scarpa M, di Rocco M, Tiffit CJ, Ciani F, Gasperini S, Pasquini E, Guerrini R, Donati MA, Morrone A. 2011. GM1 gangliosidosis and Morquio B disease: an update on genetic alterations and clinical findings. *Biochim Biophys Acta* 1812(7):782-790.
- Chakraborty S, Rafi MA, Wenger DA. 1994. Mutations in the lysosomal beta-galactosidase gene that cause the adult form of GM1 gangliosidosis. *Am J Hum Genet* 54(6):1004-1013.
- Emery JM, Green WR, Wyllie RG, Howell RR. 1971. GM1-gangliosidosis. Ocular and pathological manifestations. *Arch Ophthalmol* 85(2):177-187.
- Glessner JT, Li J, Liu Y, Tian L, Thomas KA, Golhar R, Desai A, Chang B, Chang X, Hakonarson H. 2015. Penncnv-Exomeseq: Genotype Improves Copy Number Variant Detection in Exome Sequencing. *Austin J Proteomics Bioinform & Genomics* 2(1):1009.
- King JE, Dexter A, Gadi I, Zvereff V, Martin M, Bloom M, Vanderver A, Pizzino A, Schmidt JL. 2014. Maternal uniparental isodisomy causing autosomal recessive GM1 gangliosidosis: a clinical report. *J Genet Couns* 23(5):734-741.
- Monroe GR, Frederix GW, Savelberg SM, de Vries TI, Duran KJ, van der Smagt JJ, Terhal PA, van Hasselt PM, Kroes HY, Verhoeven-Duif NM, Nijman IJ, Carbo EC, van Gassen KL, Knoers NV, Hovels AM, van Haelst MM, Visser G, van Haften G. 2016. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. *Genet Med* 18(9):949-956.

- Morrone A, Morreau H, Zhou XY, Zammarchi E, Kleijer WJ, Galjaard H, d'Azzo A. 1994. Insertion of a T next to the donor splice site of intron 1 causes aberrantly spliced mRNA in a case of infantile GM1-gangliosidosis. *Hum Mutat* 3(2):112-120.
- Pierson TM, Adams DA, Markello T, Golas G, Yang S, Sincan M, Simeonov DR, Fuentes Fajardo K, Hansen NF, Cherukuri PF, Cruz P, Teer JK, Mullikin JC, Program NCS, Boerkoel CF, Gahl WA, Tifft CJ. 2012. Exome sequencing as a diagnostic tool in a case of undiagnosed juvenile-onset GM1-gangliosidosis. *Neurology* 79(2):123-126.
- Regier DS, Kwon HJ, Johnston J, Golas G, Yang S, Wiggs E, Latour Y, Thomas S, Portner C, Adams D, Vezina G, Baker EH, Tifft CJ. 2016. MRI/MRS as a surrogate marker for clinical progression in GM1 gangliosidosis. *Am J Med Genet A* 170(3):634-644.
- Regier DS, Tifft CJ. 1993. GLB1-Related Disorders. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Ledbetter N, Mefford HC, Smith RJH, Stephens K, editors. *GeneReviews(R)*. Seattle (WA).
- Tome FM, Fardeau M. 1976. Ultrastructural study of a muscle biopsy in a case of GM1 gangliosidosis type I. *Pathol Eur* 11(1):15-25.
- Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, Veeraraghavan N, Hawes A, Chiang T, Leduc M, Beuten J, Zhang J, He W, Scull J, Willis A, Landsverk M, Craigen WJ, Bekheirnia MR, Stray-Pedersen A, Liu P, Wen S, Alcaraz W, Cui H, Walkiewicz M, Reid J, Bainbridge M, Patel A, Boerwinkle E, Beaudet AL, Lupski JR, Plon SE, Gibbs RA, Eng CM. 2014. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA* 312(18):1870-1879.

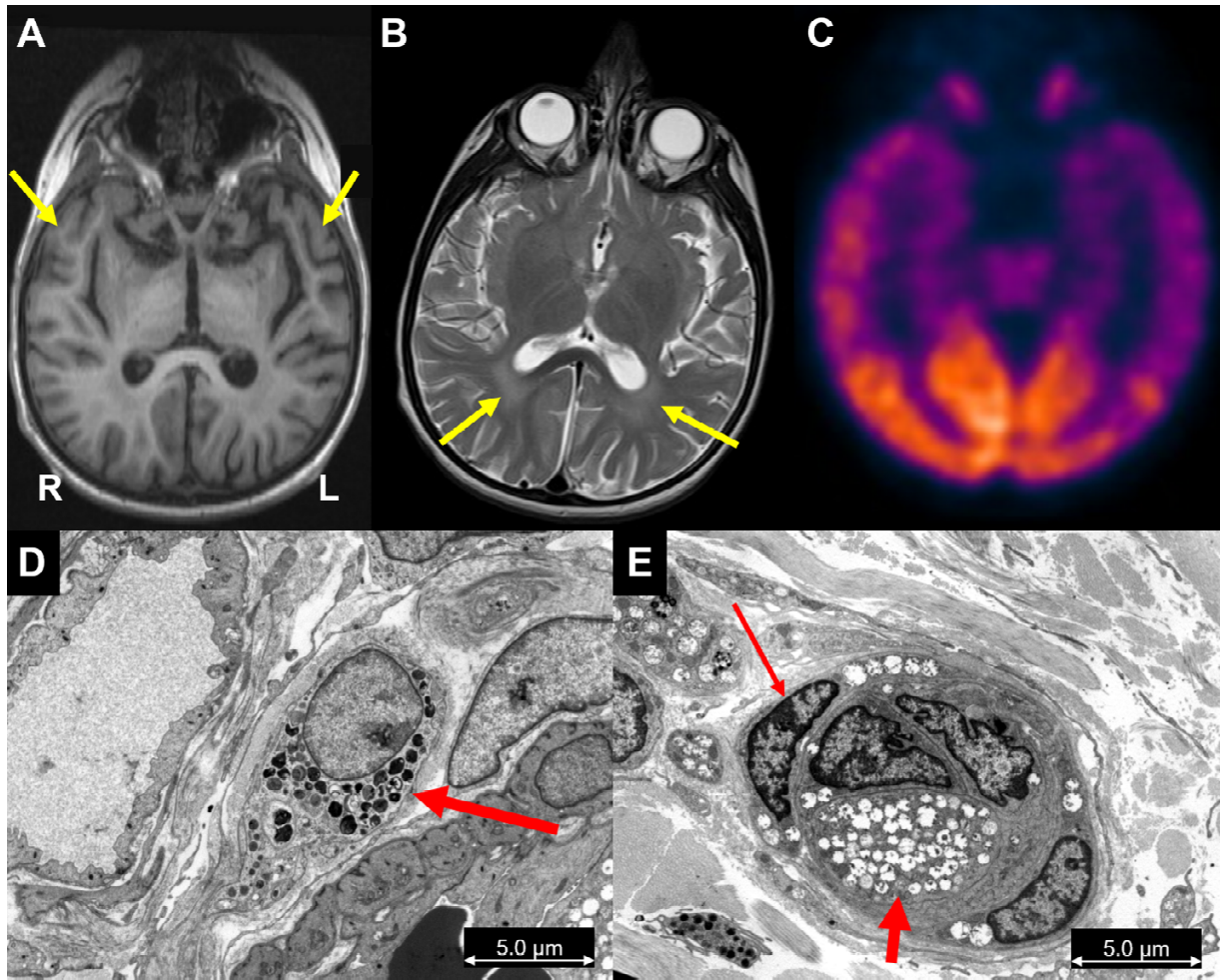


Figure 1

Author Mail

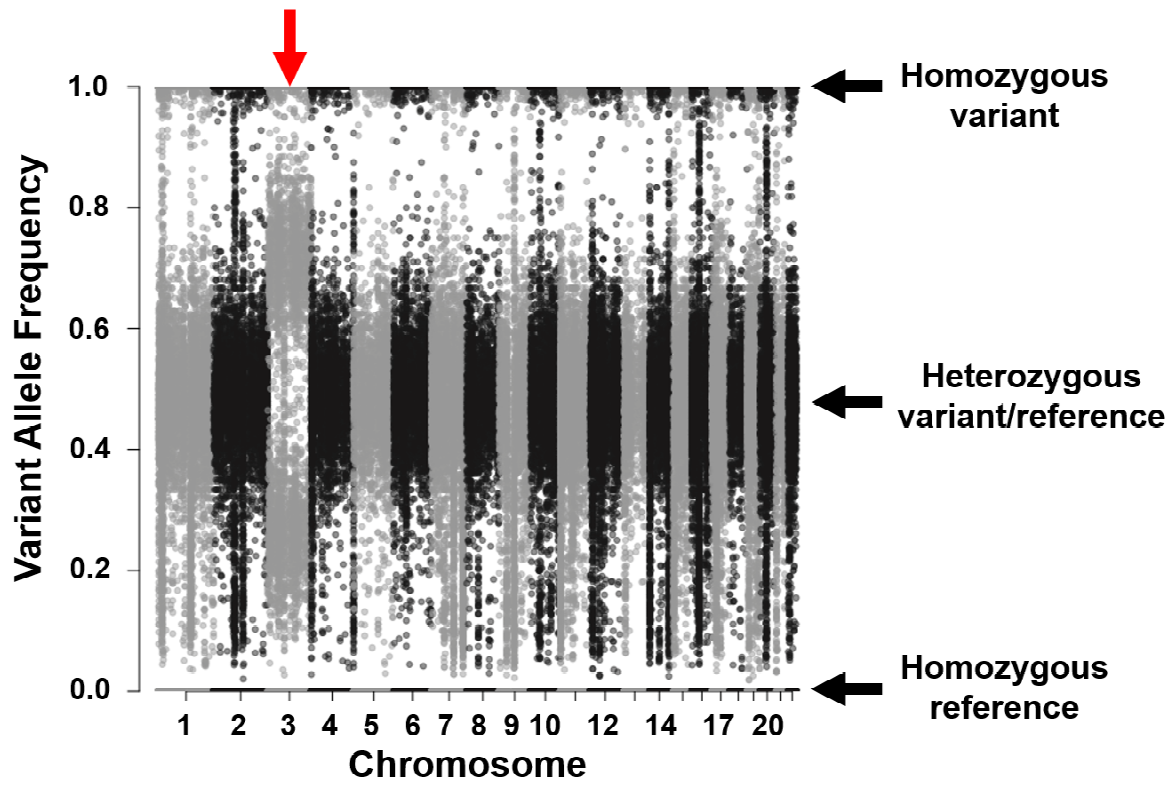


Figure 2

Author Manuscript