Human Molecular Genetics, 2009, Vol. 18, No. 8 ***1377–1383***
doi:10.1093/hmg/ddp042
Advance Access published on February 3, 2009

Identification of familial and *de novo* microduplications of 22q11.21–q11.23 distal to the 22q11.21 microdeletion syndrome region

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Received September 25, 2008; Revised January 12, 2009; Accepted January 20, 2009

Deletions of the 22q11.2 region distal to the 22q11.21 microdeletion syndrome region have recently been described in individuals with mental retardation and congenital anomalies. Because these deletions are mediated by low-copy repeats (LCRs), located distal to the 22q11.21 DiGeorge/velocardiofacial microdeletion region, duplications are predicted to occur with a frequency equal to the deletion. However, few microduplications of this region have been reported. We report the identification of 18 individuals with microduplications of 22q11.21–q11.23. The duplication boundaries for all individuals are within LCRs distal to the DiGeorge/velocardiofacial microdeletion region. Clinical records for nine subjects reveal shared characteristics, but also several examples of contradicting clinical features (e.g. macrocephaly versus microcephaly and upslanting versus downslanting palpebral fissures). Of 12 cases for whom parental DNA samples were available for testing, one is *de novo* and 11 inherited the microduplication from a parent, three of whom reportedly have learning problems or developmental delay. The variable phenotypes and preponderance of familial cases obfuscate the clinical relevance of the molecular data and emphasize the need for careful parental assessments and clinical correlations.

INTRODUCTION

The instability of 22q11 has been demonstrated by the high frequency of pathological rearrangements of this region. Deletions of 22q11.21 are found in cases of Shprintzen/velocardiofacial syndrome (VCFS), DiGeorge syndrome (DGS) and conotruncal anomaly face syndrome, all of which are encompassed by the designation '22q11.21 microdeletion syndrome'. It is the most frequent microdeletion in humans, with an incidence of 1/4000 live births (1). The reciprocal duplication has also been reported (2–7). Other rearrangements associated with this region include partial tetrasomy of 22q11.1, associ-

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ated with cat eye syndrome, and partial trisomy associated with der(22) syndrome (8).

Low-copy repeats (LCRs) on 22q11 have been suggested to mediate non-allelic homologous recombination (NAHR), resulting in rearrangements of 22q (9). A similar mechanism has been proposed for Charcot–Marie–Tooth syndrome type 1A on 17p11.2 (10,11), Prader–Willi syndrome on 15q12 (12,13), Williams–Beuren syndrome on 7q11.23 (14) and Smith–Magenis syndrome on 17p11.2 (15). Eight LCRs have been characterized on 22q11 (16). Labeled LCR22A-H in order from proximal to distal, the LCR22s share 97–98% sequence identity within the duplicated modules shared among them (17); the sizes of the LCRs and the number, complexity, homology and orientation of shared sequences appear to correlate with the frequency of involvement in 22q11 deletions (16). Only one module is shared among 7/8 of the LCR22s: BCRL is present in one copy in LCR22A, C and E–H and in two copies in LCR22D (16). All three classes of rearrangements associated with 22q11.21 microdeletion syndrome occur within LCRs (18); the common 3 Mb deletion found in 87% of deleted individuals is mediated by LCR22A and LCR22D, the largest and most complex of the LCR22s. In addition, the sequences surrounding a common breakpoint localizing to LCR22B in the recurrent t(1;22), t(11;22) and t(17;22) show a proclivity for secondary-structure formation, suggesting a hotspot for rearrangement (19). Finally, the reciprocal duplication product of the 22q11.21 microdeletion has also been reported (2–7), further suggesting the role of NAHR in the mediation of rearrangements of 22q11.2.

The four distal LCR22s, LCR22E–H, are smaller than the proximal LCR22s, which may explain why they have been less commonly associated with chromosome rearrangements, despite the presence of duplicated modules in each (16). LCR22s E and H share a BCRL module with LCR22D, suggesting a substrate for NAHR-mediated rearrangements in distal 22q11.2. Deletions distal to 22q11.21 have been reported (20–24). In most cases, the proximal breakpoint lies within LCR22D, and the distal breakpoint lies within one of the distal LCR22s. Mikhail *et al*. (23) reported an individual with a deletion just telomeric to the 22q11.21 microdeletion region and encompassing the BCR gene at 22q11.23. Ben-Shachar *et al*. (22) reported six deletions distal to the ~3 Mb 22q11.21 microdeletion region, either a ~1.4 or ~2.1 Mb deletion flanked proximally by LCR22D and distally by LCR22E or LCR22F. Most deletions were detected in individuals with clinical features suggestive of DGS/VCFS, indicating that this abnormality would be more common in an unselected population. NAHR would predict that deletions and duplications of this region should occur with equal frequency.

Recently, Ou *et al*. (25) identified two microduplications distal to the 22q11.21 microdeletion region, both of which were flanked proximally by LCR22D. One individual was a 28-month-old female with a D-E duplication, developmental delay and dysmorphic features including triangular face, parietal and frontal bossing, broad forehead, upsweep of the front hairline, downslanting palpebral fissures, strabismus, hooded eyelids, large and protruding ears with preauricular pits, full nasal tip and smooth philtrum. Her father had the same duplication and normal development, but hooded eyelids and low posterior hairline. The second individual was a 4-day-old male with a D-F duplication, dysmorphic features and multiple congenital anomalies, including imperforate anus, hypoplastic left kidney, patent ductus arteriosus, patent foramen ovale and anomalous right subclavian artery. The inheritance of this second individual's microduplication was unknown.

Here, we report the clinical and molecular characterization of 18 individuals with duplications of 22q11.21–q11.23. Five of the duplications are flanked proximally by LCR22D; the remaining 13 duplications are flanked proximally by either LCR22E or LCR22F and are, to the best of our knowledge, the first reported microduplications of the region that do not involve LCR22s A–D. The variability of phenotypes among these individuals and the high rate of familial cases complicate genotype–phenotype correlations for this population.

RESULTS

We screened 22 096 consecutive individuals with idiopathic mental retardation and/or congenital anomalies, who were submitted to Signature Genomic Laboratories for analysis with the SignatureChip BAC microarray. Eighteen individuals had microduplications of 22q11.21–q11.23 (Fig. 1). To refine the breakpoints, we analyzed the microduplications of all individuals using either an Affymetrix 250K SNP oligonucleotide microarray (individuals 2, 3, 8, 9, 11, 12, 14) or a Signature Genomic Laboratories custom oligonucleotide microarray (individuals 1, 4–7, 10, 13, 15–18) (Fig. 1A–E). The LCRs flanking each individual's duplication are listed in Table 1.

For the 12 individuals with 22q11.23 microduplications for whom parental DNA was available for microarray analysis, one, subject 10, had a *de novo* abnormality. Eight individuals inherited the microduplication from an apparently normal parent, whereas three individuals inherited the microduplication from a reportedly abnormal parent, although clinical records were not available to us for these parents.

Clinical information was available for nine individuals with microduplications of 22q11.23. The clinical features varied among the individuals in our cohort, although developmental delay, seizures, heart defects, micro- or macrocephaly and hypotonia were identified in more than one individual (Table 2). Figure 1 shows facial features of eight individuals in our cohort.

DISCUSSION

We have identified 18 individuals with duplications of 22q11.21–q11.23. To the best of our knowledge, 13 of these individuals are the first reported with 22q11.2 duplications that do not involve LCR22s A–D; previously reported individuals had either the reciprocal product of the most common 22q11.21 microdeletion (2–7), larger duplications sharing the same proximal breakpoint but extending more distal than the 3 Mb 22q11.21 duplication (2), or duplications sharing their proximal breakpoints with the distal breakpoints of the common 22q11.21 microdeletion (25). All individuals in our cohort had duplication breakpoints that flanked or fell within previously characterized LCR22s; the proximal and distal breakpoints for individuals 1–6 and 8 flank or lie within LCR22E and LCR22H, respectively, whereas the proximal

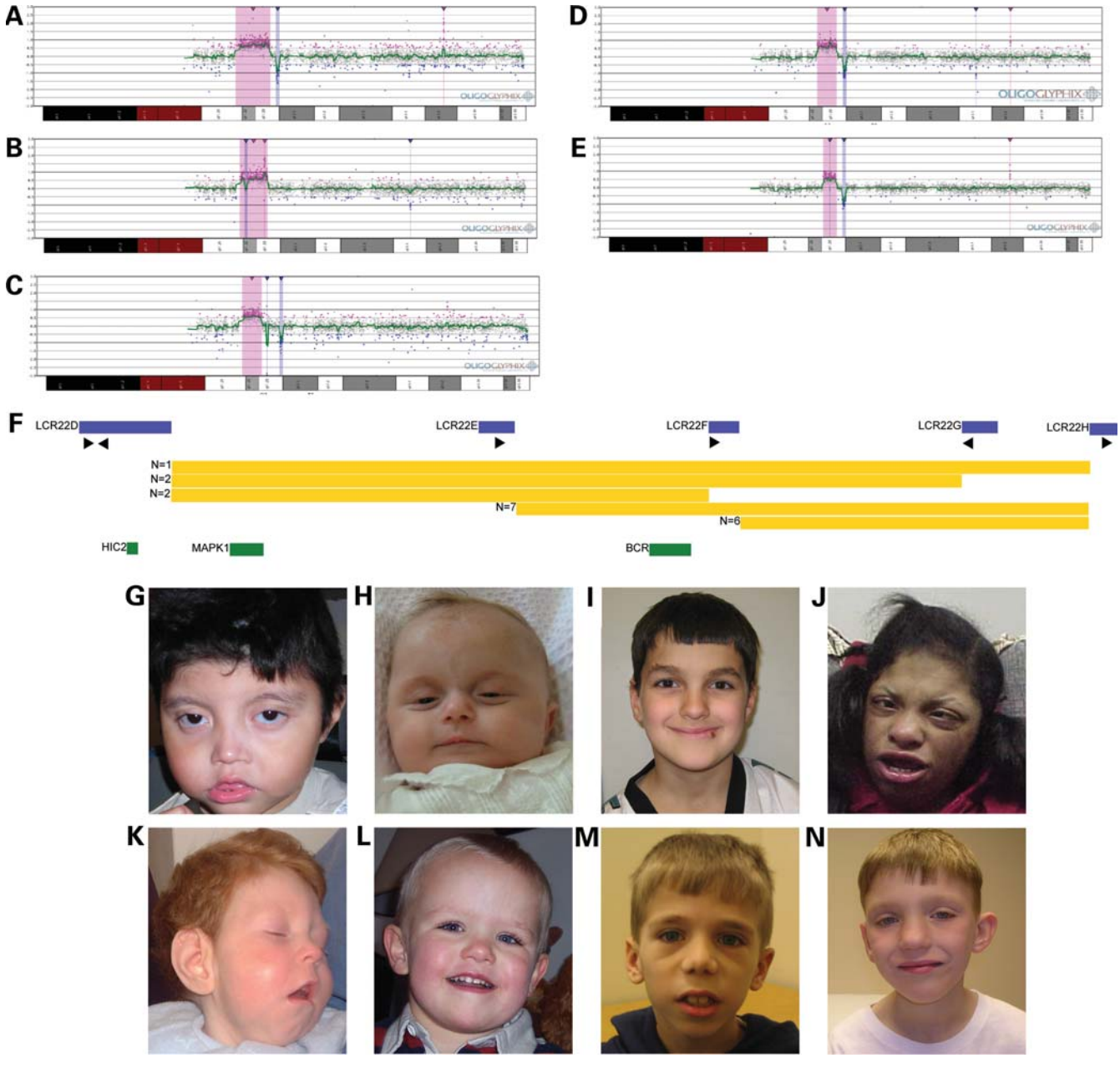

Figure 1. Analysis of individuals with duplications of 22q11.21–q11.23. (**A–E**) Representative Agilent 244K array profiles for (A) D–H duplication, (B) D–G duplication, (C) D–F duplication, (D) E–H duplication and (E) F–H duplication. For the microarray plots, clones are ordered on the x-axis according to physical mapping positions. (**F**) Schematic of the 22q11.21–q11.23 region with summary of the duplications identified in 18 individuals. The LCR22s D–H located in 22q11.2 are shown as blue boxes. The approximate locations and orientations of BCRL modules are shown as black arrowheads. Orange bars indicate duplication sizes identified in the study population. The locations of *HIC2*, *MAPK1* and *BCR* are shown. (**G–N**) Facial features of subjects (G) 2, (H) 3, (I) 8, (J) 9, (K) 11, (L) 12 (M) 14 and (N) 18.

and distal breakpoints for subjects 11–16 flank or lie within LCR22F and LCR22H, respectively. Five individuals had a proximal duplication breakpoint within LCR22D, the distal flanking LCR for the common 3 Mb deletion found in most 22q11.21 microdeletion individuals (18). The identification of E–H- and F–H-mediated duplications in our population supports the hypothesis by Shaikh *et al*. (16) that the orientation of the BCRL module may predict the substrates for NAHR (16); it has been suggested that LCRs in direct orientation mediate deletions and duplications (26). Based on orientation, BCRL-D1, BCRL-E, BCRL-F and BCRL-H are in one group and BCRL-D2 and BCRL-G are in the second group. Thus, it is not surprising that we have identified a large number of E–H and F–H duplications. Sequencing of the breakpoints would be necessary to determine which of the BCRL-Ds is involved in the D–F, D–G and D–H duplications in our population and whether the orientation of the BCRLs predicts the substrates for NAHR.

Table 1. Duplication breakpoints for individuals with microduplications of 22q11.21–q11.23

Subject	Band	LCR start/stop	Inheritance
1	q11.22–q11.23	E–H	Maternal
2	q11.22–q11.23	E–H	Maternal
3	q11.22–q11.23	E–H	Maternal
4	q11.22–q11.23	E–H	Paternal
5	q11.22–q11.23	E–H	Paternal
6	q11.22–q11.23	E–H	Paternal
7	q11.21–q11.23	D–G	N/A
8	q11.22–q11.23	E–H	N/A
9	q11.21–q11.23	D–G	N/A
10	q11.21–q11.23	D–F	*De novo*
11	q11.23	F–H	Maternal
12	q11.23	F–H	Maternal
13	q11.23	F–H	Paternal
14	q11.23	F–H	Paternal
15	q11.23	F–H	N/A
16	q11.23	F–H	N/A
17	q11.21–q11.23	D–F	N/A
18	q11.21–q11.23	D–H	Maternal

Previous reports suggest that there is no evidence of the presence of parental inversion polymorphisms that would predispose to rearrangements in the 22q11.21 microdeletion syndrome region (27). Because the inverted duplications in the proximal LCR22s A–D are the same as those in the distal LCR22s, it is unlikely that the inverted duplications would result in parental inversion polymorphisms of this distal region.

No clearly definable collection of clinical features is shared among the individuals in our study. The individuals' development varies from normal to profound developmental delay, with no speech or walking, and other neurological features vary from normal to profound hypotonia and/or severe seizures. Some contradicting features were present, with both macro- and microcephaly reported in our population. In addition, there appears to be no correlation between the severity of the individuals' clinical features and the size or location of their duplications. For example, subject 11, whose ~1.4 Mb duplication has breakpoints within LCR22s F and H, has severe developmental delay and severe hypotonia, whereas subject 18, whose ~3.6 Mb duplication, with breakpoints within LCR22s D and H, has mild dysmorphic features and behavioral problems but no developmental delay or hypotonia. The ascertainment of additional patients will be necessary to elucidate genotype/phenotype correlations further.

Only one individual, subject 10, for whom parental DNA samples were available for testing had a *de novo* abnormality. This individual had normal development, optic disc coloboma and mild dysmorphic features. Although abnormalities inherited from a clinically normal parent are usually considered benign copy-number variants (CNVs), parents of a child with an inherited chromosome abnormality may sometimes show mild variations of the child's phenotype; such instances have been reported for the 22q11.21 microdeletion syndrome, which has predominantly *de novo* occurrence (28). Three parents in our study who possessed the duplication were reported to have mild learning difficulties or developmental delay; the remaining parents were reportedly normal. The delays noted in parent and child may be related to each other, but unrelated to the duplication of 22q11.2. Alternatively, the delays noted in parent and child may have different etiologies. Careful clinical assessment of both child and parent is crucial to understanding the causative role, if any, of these duplications. Considering that parents may indeed have normal phenotypes, the presence of a genetic modifier—a combination of CNVs at the same or different loci, inherited from parents in whom the single variation was insufficient to cause disease—has been proposed for other syndromes with variable expressivity (29). Except for a deletion on 18q23 in subject 15, microarray analysis did not identify additional abnormalities in any individuals in our cohort. The variability of the phenotypes of the individuals with distal 22q11.2 microduplications in our study and the high rate of familial cases with reportedly normal parents with 22q11.2 microduplications complicate genotype–phenotype correlations for this population and emphasize the need for further studies as well as careful clinical assessment of both the diagnosed individual and the carrier parent.

MATERIALS AND METHODS

Subjects and controls

During the period encompassing March 2004 through June 2008, we screened 22 096 consecutive individuals with developmental disabilities, whose specimens were submitted to Signature Genomic Laboratories from the USA and abroad. For the individuals with 22q11.2 microduplications described here, informed consent was obtained to perform high-resolution molecular cytogenetic testing and to publish photographs.

Bacterial artificial chromosome microarray analysis

Microarray-based comparative genomic hybridization (array CGH) was performed with a bacterial artificial chromosome (BAC) microarray (the SignatureChip®; Signature Genomic Laboratories, Spokane, WA, USA) (30). The five versions of SignatureChip have increasing coverage of the genome. Version 1.0 was used from March 2004 until October 2004, version 2.0 until October 2005, version 3.0 until May 2006 and version 4.0 until November 2007. The SignatureChip Whole Genome (SignatureChipWG®) is currently in use in this laboratory. A comparison of the contents of versions 1.0-WG can be found at http://www.signaturegenomics.com/clone_list.html. Results were visualized using Signature Genomic Laboratories' laboratory-developed computer software program Genoglyphix (http://www.signaturegenomics.com/genoglyphix.html). Each BAC clone was fluorescence *in situ* hybridization (FISH) verified for its chromosomal location prior to microarray construction and validated for use in FISH to visualize chromosome abnormalities identified by the microarray. Clones that mismapped or cross-hybridized to multiple locations were not included on the microarray. Regions of known CNVs have been avoided when possible or replaced on subsequent versions of our microarray.

Microarray analysis was performed as previously described (30), with the following modifications: genomic DNA was extracted from peripheral blood using a Qiagen M48

Table 2. Summary of clinical features found in individuals with microduplications of 22q11.23

Patient no.	2	3	8	9	10	11	12	14	18
Sex	Male	Female	Male	Female	Male	Male	Male	Male	Male
Age	2 years 9 months	1 month	9 years 8 months	12 years 8 months	1 year 8 months	2 years 2 months	7 years 8 months	2 years 10 months	6 years 9 months
Duplication breakpoints	E–H	E–H	E–H	D–G	D–F	F–H	F–H	F–H	D–H
Inheritance	Maternal	Maternal	Unknown	Unknown	*De novo*	Maternal	Maternal	Unknown	Maternal
Development	No speech or walking	Normal	Slight DD	No speech or walking	Normal	Profound DD	Apraxia and dysarthria	Expressive language delays	
Dysmorphic features	Coarse facial features; macroglossia micrognathia	Deep-set eyes; posteriorly rotated ears; micrognathia	Right upslanting palpebral fissure; prognathism	Asymmetric pupils; upslanting palpebral fissures; thick ear helices; prominent antihelix; wide-space teeth; short philtrum; large lips	Wide, shallow nasal bridge; flat nose	Large ears; bitemporal narrowing; myopic facies; hypotelorism; epicanthal folds; upslanting palpebral fissures; micrognathia/ retrognathia	Epicanthal folds; periorbital fullness; long flat philtrum; high arched palate; micrognathia		Elongated face; hypertelorism; low-set ears; broad nasal bridge
Macrocephaly/ microcephaly	Microcephaly	Normal	Macrocephaly	Microcephaly	Normal	Microcephaly	Normal	Macrocephaly/ hydrocephalus	Normal
Neurologic	Seizures; profound hypotonia	Normal	ADHD	Possible seizures; hypotonia	Normal	Profound hypotonia	Mild hypotonia	Apraxia; low muscle tone	Possible seizures
Cardiovascular	Normal	Tricuspid regurgitation	Normal	VSD	Normal	Normal	Normal		Normal
Musculoskeletal	Contractures	Shallow sacral dimple		Scoliosis/brace and wheelchair; contractures; bilateral 3–4 fingers; syndactyly 2–3 toes	Contractures; dimples along lower spine and coccyx area; small tapering digits				Normal
Other		Redundant skin folds on neck; bilateral overlapping second and third toes; hypoplastic toenails			Coloboma; pale pigmentation	Abnormal genitalia	Undescended testes		Severe behavioral problems

ADHD, attention deficit/hyperactivity disorder; CNS, central nervous system; DD, developmental delay; N/A, not available; VSD, ventricular septal defect.

Biorobot-automated DNA extraction system. Purified genomic DNA was then sonicated and labeled with Alexaflour dyes 555 or 647 using a BioPrime Total DNA labeling kit (Invitrogen Corp., Carlsbad, CA, USA). Microarrays were hybridized as described previously (30) and washed using a Little Dipper automated microarray washing station (SciGene, Sunnyvale, CA, USA). Microarrays were scanned on an Axon 4000B scanner (Molecular Devices, Sunnyvale, CA, USA), and signal intensity ratios were analyzed as described (30) using a custom analysis and display interface (Genoglyphix®, Signature Genomic Laboratories).

Oligonucleotide array CGH

Whole-genome genotyping was performed using Affymetrix 250K Sty SNP arrays (Affymetrix, Santa Clara, CA, USA) for cases 2, 3, 8, 9, 11, 12, 14 to characterize the extent of the abnormalities. Copy number inferences were made using CNAG software based on the signal intensity of the probes and regions of homozygosity, as described previously (31).

Oligonucleotide-based microarray analysis was performed using a 244K-feature whole-genome microarray made for Signature Genomic Laboratories by Agilent Technologies (Santa Clara, CA, USA) for cases 1, 4–7, 10, 13, 15–18 to characterize the extent of the abnormalities. Genomic DNA labeling was performed as described for BAC arrays, whereas array hybridization and washing were performed as specified by the manufacturer (Agilent Technologies). Arrays were scanned using an Axon 4000B scanner (Molecular Devices) and analyzed using Agilent Feature Extraction software v9.5.1 and Agilent CGH Analytics software v3.5.14. Results were then displayed using custom oligonucleotide array CGH analysis software (Oligoglyphix™; Signature Genomic Laboratories).

FISH analysis

All abnormalities detected by array CGH were confirmed and visualized by metaphase or interphase FISH as published using one or more BAC clones determined to be abnormal by array CGH (32).

Genomic sequence analysis

LCRs located within 22q11.2 were identified using the annotated May 2006 assembly of the human genome on the UCSC genome browser (http://genome.ucsc.edu) and the Human Genome Segmental Duplication Database (http://projects.tcag.ca/humandup/). Copy-number variations within 22q11.2 were identified using the Database of Genomic Variants (http://projects.tcag.ca/variation/).

ACKNOWLEDGEMENTS

We thank Steve Byerly and Sara Minier (Signature Genomic Laboratories) for assistance with Figure 1.

Conflict of Interest statement. L.G.S. and B.A.B. sit on the Board of Directors of Signature Genomic Laboratories, LLC.

FUNDING

This work was partially supported by a grant from the National Institutes of Health [GM081519 to T.H.S].

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