Diagnostic Implications of Excessive Homozygosity Detected by SNP-Based Microarrays: Consanguinity, Uniparental Disomy, and Recessive Single-Gene Mutations

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KEYWORDS

- Microarray • Single nucleotide polymorphism
- Homozygosity • Autozygosity • Consanguinity
- Uniparental disomy

Single nucleotide polymorphism (SNP)-based microarray analysis provides detection of copy number variations (CNVs) as well as genotype information at multiple polymorphic loci throughout the genome. Although the clinical utility of CNV detection is well accepted,[1,2] information derived from SNP-based microarray analysis has only more recently been utilized in the constitutional cytogenetics laboratory setting.[3,4] In addition to specific genotype data, analysis of SNP allele patterns can provide (1) confirmation of CNV calls, (2) sensitivity for detection of mosaicism, and (3) detection of excessive homozygosity, which is the focus of this review.

Several different SNP-based microarray platforms are currently in clinical use, and this review illustrates similar data obtained from Affymetrix- and Illumina-based microarrays (Affymetrix Inc, Santa Clara, CA, USA; Illumina Inc, San Diego, CA, USA). It is generally not necessary to derive specific genotypes from the microarray data to view the SNP probes as present in a homozygous or heterozygous state. For both platforms, biallelic SNPs are denoted as "A" or "B." Relative allele distribution is typically provided by either allele difference plots or B-allele frequency plots (**Fig. 1**).

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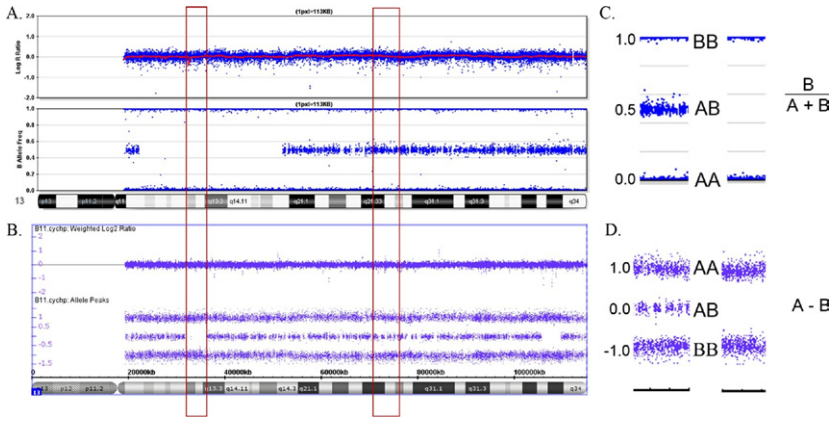


Fig. 1. Allele plots. *(A)* Screenshot of a chromosome 13 from a patient DNA sample run on the Illumina Quad610 array, and visualized in BeadStudio software. *Top*: Log R ratio plot shows a normal copy number state for the chromosome. *Bottom*: B-allele frequency plot shows a large region of homozygosity from bands 13q12.11 to 13q14.3. (*B*) Screenshot of a chromosome 13 from a patient DNA sample run on the Affymetrix CytoScan™ HD array and visualized in Affymetrix Chromosome Analysis Suite (ChAS) software. *Top*: Weighted $\log_2$ ratio plot shows a normal copy number state for the chromosome. *Bottom*: Allele difference plot shows 2 regions of homozygosity from bands 13q13.1 to q13.3 and 13q33.2 to q33.3. Panels *C* and *D* represent magnified views of the allele plots for each software, from the boxed regions in panels *A* and *B*. (*C*) B-allele frequency, as plotted in Illumina BeadStudio software. The Y-axis value is determined by the formula given on the right. Alleles are plotted as a frequency, determined by the number of B alleles compared with the total number of alleles (A+B). B-allele frequency of 1 indicates homozygosity for the B allele (2/2), a frequency of 0 indicates homozygosity for the A allele (0/2), and a frequency of 0.5 indicates a heterozygous genotype (1/2). (*D*) Allele difference, as plotted in Affymetrix Chromosome Analysis Suite (ChAS) software. The Y-axis value is determined by the formula given on the right. Alleles are plotted as the difference between the estimated number of A alleles and the estimated number of B alleles (A-B). Each allele corresponds to a value of 0.5. Values near 1 indicate homozygosity for the A allele (AA: [0.5+0.5] – [0] = 1), values near –1 indicate homozygosity for the B allele (BB: [0] – [0.5 + 0.5] = –1), and values near 0 indicate a heterozygous genotype (AB: [0.5] – [0.5] = 0).

Multiple terms are used to describe regions of homozygosity, and each conveys a slightly different meaning (eg, loss of heterozygosity, absence of heterozygosity, runs of homozygosity). Here we use the term *long-contiguous stretch of homozygosity* (LCSH) to describe an uninterrupted region of homozygous alleles with genomic copy number state of 2. The term *LCSH* excludes loss of heterozygosity caused by single copy deletions because these regions exist in a hemizygous state. Minimal thresholds for LCSH calls are generally set around 0.5 to 1 Mb in population genetic analyses[5–7] and more conservatively at 3 to 10 Mb in clinical analyses,[4](Kearney H, Kearney J, and Conlin L, personal communication).

Detection of excessive homozygosity, in and of itself, is not diagnostic of any underlying condition and may be clinically benign. The first step in assessing the clinical relevance of observed LCSH is to distinguish between excessive homozygosity found in multiple regions throughout the genome versus LCSH restricted to a single chromosome. When multiple LCSH regions are found throughout the genome, the findings are generally assumed to represent regions identical by descent (IBD),

Table 1
Correlation between percentage of LCSH and degree of parental relationship

Parental Relationship	Degree	Coefficient of Inbreeding (F)	LCSH (IBD) Predicted in Child (~%)[a]
Parent/child	First	0.25	25
Full siblings	First	0.25	25
Half siblings	Second	0.125	12.5
Uncle/niece or aunt/nephew	Second	0.125	12.5
Double first cousins	Second	0.125	12.5
Grandparent/grandchild	Second	0.125	12.5
First cousins	Third	0.0625	6
First cousins once removed	Fourth	0.03125	3
Second cousins	Fifth	0.015625	1.5
Third cousins	Seventh	0.0039062	< 0.5

[a] Assuming outbred population.

with the associated concerns for recessive disorders mapping to the homozygous intervals. When the genomic homozygosity is sufficiently excessive, the finding may trigger a suspicion for parental consanguinity or incest (**Table 1**; **Fig. 2**).[8] One or more regions of LCSH found only on a single chromosome can be a hallmark of uniparental disomy (UPD), either whole-chromosome UPD or segmental UPD,[3,4,9] and may warrant further clinical investigation, particularly when involving chromosomes associated with imprinted gene disorders (**Table 2**). Regardless of mechanism or size, all LCSH segments have the potential to harbor homozygous recessive mutations.

CONSANGUINITY

When LCSH is found distributed throughout the genome, this observation is presumed to represent homozygosity caused by inheritance of genomic regions of IBD. When the parents of a proband share a recent common ancestor, their union is defined as *consanguineous*. The closer the parental relationship, the greater the proportion of shared alleles and, therefore, the greater the risk of the child (proband) inheriting 2 copies of a deleterious gene mutation from his parents.[10–12] Clinical laboratories that perform SNP-based microarray analysis will encounter many cases of presumed parental consanguinity, occasionally with suspicion of abuse/incest because of the degree of consanguinity estimated (**Fig. 2**).[8]

Although this has no immediate clinical utility and represents a pursuit largely of academic or social/ethical/legal interest, an estimate of the total proportion of the LCSH in the genome can be used as a rough assessment of degree of parental relationship (see **Table 1**). A simple method to grossly estimate parental relationship is to add all homozygous regions greater than a defined threshold (eg, 3 Mb), excluding the sex chromosomes (because males are always hemizygous, and X chromosomes in females are often observed with increased LCSH because of more limited recombination). The total autosomal LCSH can then be divided by total autosomal length (2,867,733 kb for hg18) to estimate the percentage of IBD. This estimation can then be correlated with the predicted percentage of IBD for various degrees of relationship (see **Table 1**). It should be noted that this crude calculation is likely to represent an underestimate of the actual homozygous proportion given that

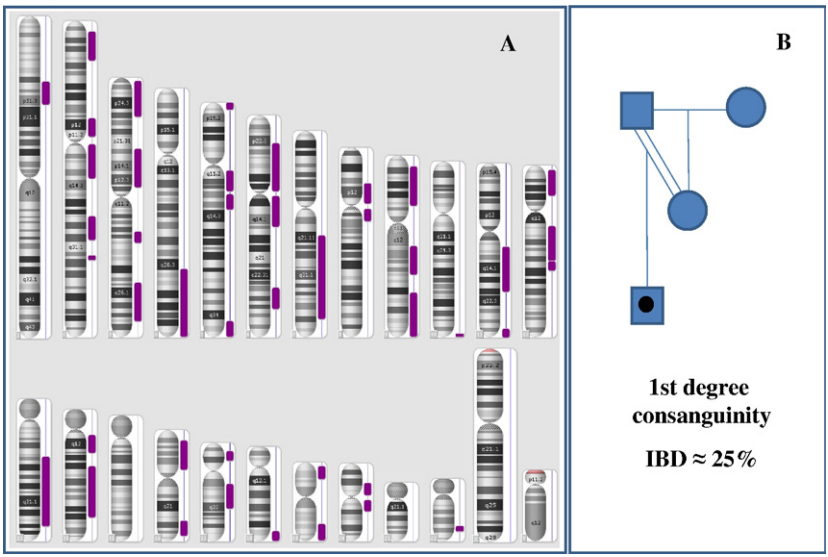


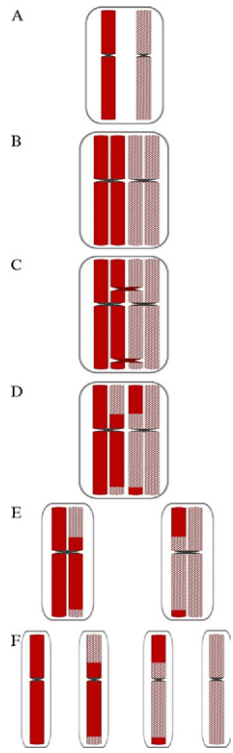
Fig. 2. LCSH patterns seen in consanguinity. *(A)* Male patient with LCSH on multiple chromosomes (Affymetrix Genome-Wide Human SNP 6.0 array data visualized in Affymetrix Chromosome Analysis Suite [ChAS] software). Note that the sex chromosomes are excluded in LCSH modeling. Calculations of total percentage IBD with different minimal LCSH thresholds in this case result in the following estimations: LCSH threshold ≥ 0.5 Mb: 868,341 kb (30% IBD); ≥ 1 Mb: 868,341 kb (30%); ≥ 3 Mb: 866,089 kb (30%); ≥ 5 Mb: 858,676 kb (30%); ≥ 10 Mb: 797,445 kb (28%); ≥ 15 Mb: 700,140 kb (24%), and ≥ 20 Mb: 577,279 kb (20%). These data and those of additional cases suggest that minimal LCSH thresholds between 0.5 and 10 Mb have negligible impact on estimation of percentage of IBD. These data are consistent with first degree consanguinity, as illustrated in panel B (and **Table 1**). (***B***) Pedigree illustrating first-degree consanguinity and predicted percentage IBD. The mother and father of the proband share 50% of their genome (because they are first-degree relatives). There is a one-half probability that the proband will inherit an identical genomic region from both of his parents, making the total estimate of the proband's percentage of IBD in this scenario roughly 25%. Note that other first-degree parental relationships (eg, full siblings) would also yield an estimated 25% IBD in the proband.

(1) only LCSH long enough to be detected by with the array platform/applied threshold will be included and (2) the denominator includes regions of the genome that may not be covered on the microarray (eg, acrocentric short arm and centromeric regions). Although in theory, the background level of population-specific homozygosity in any genome may artificially inflate this observation, in practice, this does not complicate the calculated percentage to any measurable degree, particularly if the threshold used to calculate total LCSH is greater than 1 Mb.[6,7]

Estimating percentage of IBD can be illustrative and suggestive of degree of parental relationship, but if this inference is made at all, it should be accompanied by an appropriate measure of uncertainty. Most importantly, this estimate cannot be taken as evidence of a specific parental relationship. Additionally, this inference assumes a standard 50% distribution of parental alleles in each meiotic division, and unpredictable recombination and chromosome segregation patterns may result in significant deviation from this distribution.[7] Furthermore, consanguinity is very common practice in some populations[13,14]; therefore, it is likely that individuals from

Table 2
UPD chromosomes associated with imprinting disorders

UPD Chromosome	Imprinted Locus	Maternal vs Paternal UPD	Associated Disorder	Major Clinical Features	Known/Proposed Dysregulated Gene(s)
6	q24	Paternal	Diabetes Mellitus, 6q24-related Transient Neonatal[52]	Intrauterine growth retardation, neonatal hyperglycemia	*PLAG1, HYMAI*
7	p11.2–p12 and q32.2	Maternal	Russell-Silver syndrome[53]	Intrauterine and postnatal growth retardation, triangular facies	*GRB10, MEST*
11	p15.5	Paternal (segmental)	Beckwith-Wiedemann syndrome[54]	Macrosomia, macroglossia, visceromegaly, omphalocele	*IGF2, H19, CDKN1C, KCNQ1, KCNQ1OT1*
		Maternal	Russell-Silver syndrome[55]	Intrauterine and postnatal growth retardation, triangular facies	*IGF2, H19, CDKN1C, KCNQ1, KCNQ1OT1*
14	q32.2	Maternal	Maternal UPD14 syndrome[56–58]	Precocious puberty, hypotonia, joint laxity	*RTL1, DLK1*
		Paternal	Paternal UPD14 syndrome[56–59]	Skeletal abnormalities, joint contractures, intellectual disability	*RTL1, DLK1*
15	q11–q13	Paternal	Angelman syndrome[60]	Severe intellectual disability, speech impairment	*UBE3A*
		Maternal	Prader-Willi syndrome[61]	Hypotonia, hypogonadism, obesity	*SNRPN, MKRN, MAGEL2, NDN, U5snoRNAs*
20	q13.3	Paternal	Pseudohypoparathyroidism type 1b[62,63]	Neonatal hyperbilirubinemia, parathyroid hormone resistance	*GNAS*



these populations share not just a single recent ancestor but also multiple common ancestors (eg, total genomic homozygosity near or exceeding that seen with first-degree consanguinity, yet the parents have a fairly distant relationship). The laboratory generally has limited or no information regarding the family/social/ethnic situation of the proband; therefore, inferences regarding suspected abuse involving a parent are generally poorly supported; any communications regarding suspicion of abuse should follow appropriate professional guidelines and take place under advisement of one's institutional legal/ethics consult. Currently, there are no professional guidelines for whether concerns for abuse/incest should be revealed after SNP-array analysis and, if so, under what circumstances, although guidance from the American College of Medical Genetics is forthcoming. Regardless of whether consanguinity is suspected, estimated, or even revealed, simply informing the referring physician of increased suspicion for recessive disorders has clinical utility (see Autozygosity Mapping section).

UNIPARENTAL DISOMY

Generally, when isolated LCSH (involving only a single chromosome) is detected, particularly when longer than 10 Mb,[4] uniparental disomy is considered a likely mechanism. UPD is defined as the inheritance of both homologues from a single parent.[15,16] Many excellent reviews have been devoted to UPD,[17–24] and there are informative Web-based resources available as well (Lier lab site, Jena University Hospital[25]; Morrison lab site, University of Otago[26]; Robinson lab site, University of

British Columbia[27]; Jirtle lab site, Duke University[28]). This review will, therefore, not cover the history, exhaustive mechanisms, or specific clinical features of UPD syndromes (see **Table 2** for summary), but will instead focus on the laboratory detection of UPD through SNP-based microarray analysis and appreciation of associated data complexities. Historically, UPD was only suspected when accompanied by a hallmark cytogenetic finding (mosaic trisomy, marker chromosome, or other structural rearrangement, such as a Robertsonian translocation), by clinical manifestation of a disorder of imprinting,[29] or finding homozygosity for a recessive allele with only a single carrier parent.[16] Through the use of SNP-based microarrays, clinical laboratories may now have serendipitous detection of unanticipated UPD events by recognition of hallmark patterns of homozygosity.[4] To appreciate the expected patterns of homozygosity encountered with UPD-involved chromosomes, it is useful to review the various mechanisms known to generate UPD. It is of fundamental importance to first appreciate the basic concepts of meiosis and meiotic recombination, which are summarized in **Fig. 3**.

There are 2 primary mechanisms by which UPD involving a whole chromosome may be generated: (1) trisomy rescue, the most frequently observed mechanism and (2) monosomy rescue.[23] Gamete complementation has also been proposed as a UPD mechanism,[15] but this is thought to be a very rare event. Additionally, segmental UPD (involving only part of a chromosome) may also be generated through somatic events.[17] Given that the majority of these mechanisms generate UPD with full or partial homozygosity of parental markers (**Figs. 4–6**), SNP-based microarrays are very useful for detecting LCSH patterns that may be predictive (but not diagnostic) of UPD.[3,4,9,24] Uniparental *isodisomy* refers to inheritance of 2 identical chromosomes from a single parent, whereas uniparental *heterodisomy* refers to inheritance of 2 homologous chromosomes from the same parent. Because of meiotic recombination, even UPD events involving an entire chromosome are usually not purely isodisomic or heterodisomic, but instead often have a mixture of both types of segments.

Trisomy Rescue

Zygotes with nonmosaic trisomy for most chromosomes are usually not compatible with life (exceptions are trisomies involving chromosomes 13, 18, 21, and sex chromosomes). Those zygotes that do survive have typically either lost the trisomy entirely, have experienced a structural reduction of the trisomic chromosome (usually conversion to a marker or ring chromosome), or are mosaic. These events are collectively known as *trisomy rescue*.[21] Restoration to euploid state can occur through mitotic nondisjunction, which produces a normal (euploid) daughter cell and an aneuploid daughter cell (with monosomy, which presumably does not survive) or by anaphase lag, in which one of the aneuploid chromosomes is not incorporated into the new-forming nucleus and is subsequently lost. By chance alone, two-thirds of the

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Fig. 3 Normal meiosis. *(A)* A pair of homologous chromosomes illustrated before chromosome replication; shading differences represent general heterozygosity. (*B*) Chromosome replication resulting in identical sister chromatids. (*C*) Recombination between nonsister chromatids (note that involvement of only 2 chromatids is depicted for clarity, yet crossover events may collectively involve 2, 3, or 4 chromatids). (*D*) Resulting genetic exchange from meiotic recombination. (*E*) First division (meiosis I), with separation of chromosome homologues into 2 daughter cells. (*F*) Second division (meiosis II), with each daughter cell (gamete or polar body) containing 1 chromatid.

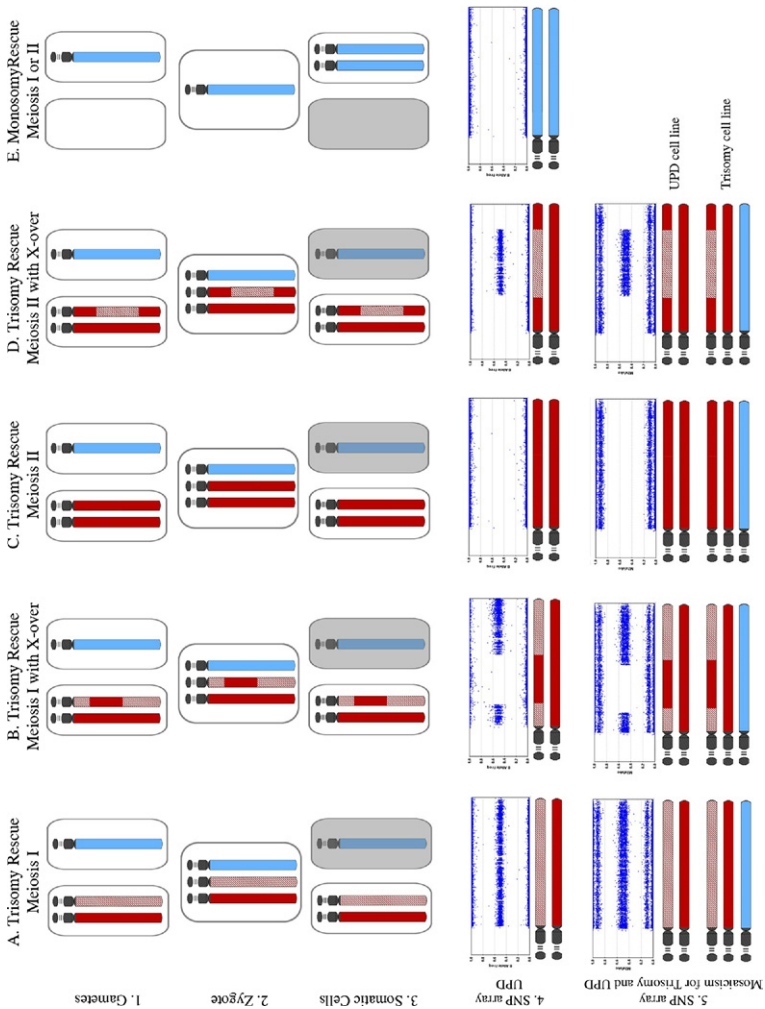


Fig. 4. Uniparental disomy resulting from meiotic errors. Columns *A*–*E* each represent 1 type of meiotic error, including (*A*) trisomy resulting from a meiosis I error without recombination and (*B*) with recombination, (*C*) a meiosis II error without recombination and (*D*) with recombination, and (*E*) a monosomy rescue resulting from either meiosis I or II error. Rows 1–3 model the chromosome involved in the uniparental disomy cells through various stages of development. Row 4 shows a theoretical allele plot for each of these scenarios as plotted in Illumina BeadStudio software. Row 5 shows a theoretical allele plot involving mosaicism for a uniparental disomy cell line and a trisomic cell line for each of the mechanisms. Predicted chromosomal compositions in the sampled tissue are illustrated below the allele plots. Plot A4 shows array data resulting from complete uniparental heterodisomy. This chromosome does not contain any LCSH and cannot be distinguished from a chromosome with normal biparental inheritance. Plots C4 and E4 show array data resulting from complete uniparental isodisomy; there are no heterozygous SNP alleles. These 2 plots are indistinguishable from one another; therefore, unless mosaicism is present (C5), the mechanism cannot be inferred. Plots B4 and D4 show chromosomes with mixed isodisomy and heterodisomy. The placement of the detected LCSH can be used to infer meiosis I errors (B4) from meiosis II errors (D4). Note that not all of the data modeled in this illustration represent confirmed cases of UPD; some of the data are modeled to illustrate concepts.

time a "rescue" event results in a disomic line with biparental inheritance, whereas one-third of the time UPD occurs. For topical relevance, the remainder of the discussion of trisomy rescue will presume that the rescue results in UPD. Depending on the origin of the trisomy (eg, meiosis I or II) and the stochastic arrangement of recombination events, the UPD chromosomes may be completely heterodisomic, completely isodisomic, or mixed hetero- and isodisomic (see **Fig. 4**). Importantly, the location of isodisomy (homozygosity) relative to any imprinted loci has no clinical relevance when considering UPD involving an entire chromosome. For example, as illustrated in **Fig. 5**, an individual with Prader-Willi syndrome caused by maternal UPD15 may have uniparental heterodisomy (heterozygous allele calls) across the critical region at 15q11.2–q13. This concept will be further supported in the following discussions of UPD mechanism.

First, consider trisomy rescue in the absence of recombination (see **Fig. 4**A). If the original segregation error occurred in meiosis I, the resulting gametes would either be nullisomic or contain both chromosome homologues (heterodisomic). Fertilization of the heterodisomic gamete followed by trisomy rescue to generate UPD would result in uniparental heterodisomy for the entire chromosome. This is a very important scenario to understand, given that this UPD event would not generate any regions of homozygosity detectable by SNP-based microarray yet would be pathogenic if involving an imprinted chromosome. If the initiating segregation error originated in meiosis II (see **Fig. 4**C), the resulting gametes would be normal, nullisomic, or contain 2 copies of identical chromosomes (isodisomic). Fertilization of the isodisomic gamete followed by trisomy rescue to generate UPD would result in uniparental isodisomy (homozygosity) for the entire chromosome and would be readily detectable by SNP-based microarray. When whole chromosome homozygosity is encountered, UPD can be reasonably presumed.

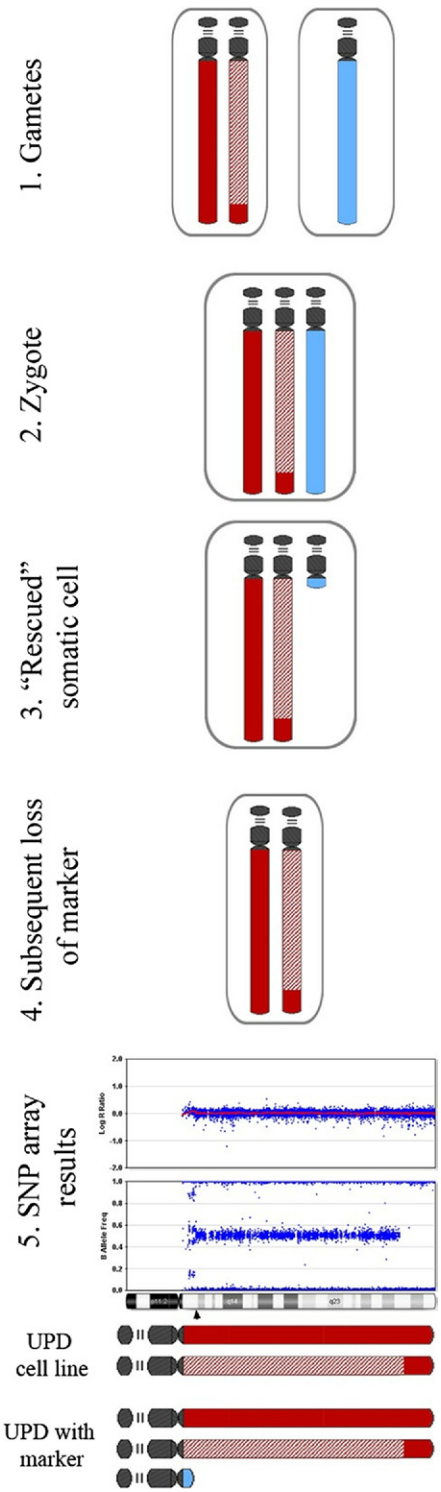
Now, let us consider trisomy rescue involving chromosomes with meiotic recombination events (crossing over) (see **Fig. 4**B, D). As before, a trisomic zygote can be corrected by a subsequent mitotic event (either nondisjunction or anaphase lag). Those rescued trisomies resulting from meiosis I errors will result in heterodisomy around the centromere, whereas those from meiosis II errors will result in isodisomy around the centromere. The remaining distribution of hetero- and isodisomy is dependent on the number and position of meiotic crossover events.

Monosomy Rescue

Zygotes with monosomy for a chromosome (other than the X chromosome) are not compatible with life and can be rescued only by duplication of the monosomic chromosome. This can occur through a mitotic nondisjunction, resulting in a daughter cell with nullisomy and a daughter cell with restored euploidy. This mechanism can only result in whole-chromosome uniparental isodisomy (see **Fig. 4**E).

Mitotic Errors of Chromosome Segregation to Generate UPD

In addition to uniparental disomy as a result of meiotic error, UPD can be a consequence of somatic events. When nondisjunction or anaphase lag occurs in a euploid somatic cell, the resulting daughter cells would be aneuploid. A second nondisjunction or anaphase lag could correct this event, resulting in a euploid cell again; however, this may result in daughter cells with incorrect distribution of the parental chromosomes, with UPD as a result (see **Fig. 6**B). This UPD would always be isodisomic for the entire chromosome (LKC, unpublished data).[30]



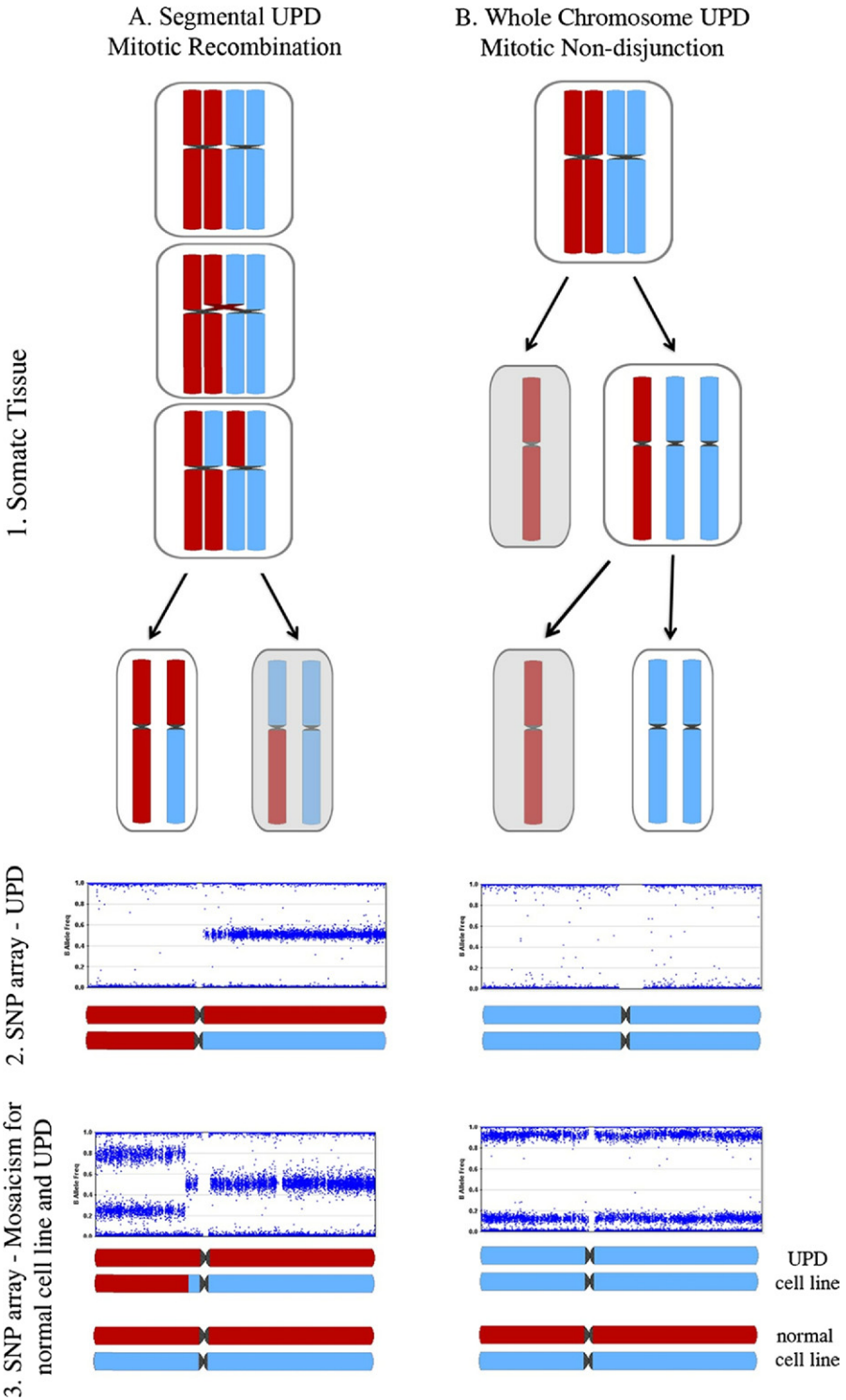
Segmental UPD

Segmental isodisomy, in which only a portion of a chromosome shows uniparental inheritance with the remainder of the chromosome showing biparental inheritance, can also occur somatically. One mechanism to generate segmental UPD is nonsister (homologous) chromatid exchange during mitosis (**Fig. 6**A). This would result in 2 daughter cells containing complementary segmental uniparental isodisomies. Because most cases of segmental UPD have only one abnormal cell line detected (reviewed in Kotzot[17]), one of the resulting daughter cells may not be compatible with survival. Alternatively, these events may represent chromosomal breakage and repair (break-induced replication) using the homologous chromosome as a template.[31] The resulting UPD from either mechanism (assuming a single break or exchange) would affect only one arm of the chromosome and should extend from the break/exchange to the telomere. Segmental UPD is unlikely if the homozygosity is interstitial or present at the termini of both arms, as these findings are not consistent with a single mitotic break/exchange event.[18] In rare cases, if 2 mitotic exchanges occurred, the segmental UPD would be interstitially located.[32] Because segmental UPD mechanisms generate only uniparental isodisomy, the location of the LCSH (isodisomy) relative to any imprinted loci should be considered.[28] In contrast to whole-chromosome UPD, only the loci involved in the isodisomic segment are at risk for imprinted disorders if segmental UPD is confirmed as the mechanism underlying the LCSH (see Follow-up testing to confirm UPD).

Whole-genome UPD

Chimeric individuals with whole-genome UPD have been reported in a handful of cases, with both maternal and paternal whole-genome UPD.[3,33–37] These individuals are chimeric for 2 cell lines: one cell line with normal biparental inheritance and one cell line with uniparental isodisomy of the entire genome. The presence of paternal whole-genome UPD (androgenetic chimerism) can result in the clinical presentation of known paternally imprinted disorders, such as Beckwith-Wiedemann syndrome, whereas the presence of maternal whole-genome UPD (parthenogenetic chimerism) can present with Prader-Willi or other maternally imprinted disorders (**Table 2**). Although several mechanisms have been hypothesized for these chimeric whole-genome UPD observations, the most plausible is endoreduplication of 1 parental genome before pronuclei fusion in the fertilized egg.[18,38]

Fig. 5. Trisomy rescue resulting in a mosaic marker chromosome and UPD. SNP-array data (Illumina Quad610 array, visualized in BeadStudio software) seen in a patient with Prader-Willi syndrome, with proposed mechanisms illustrated. (*1*) Gametes, with an ovum containing 2 chromosomes 15 as a result of a meiosis I error. (*2*) Zygote, with trisomy for chromosome 15. (*3*) A trisomy rescue event resulting in a small marker derived from the paternal chromosome 15. (*4*) Subsequent mosaic loss of the marker, resulting in whole-chromosome maternal UPD. (*5*) SNP array results show heterodisomy for the majority of the chromosome, with isodisomy only at the q terminus. A mosaic duplication is detected near the centromere, showing a pattern consistent with a mosaic marker. The imprinted region at 15q11.2 to q13 associated with Prader-Willi syndrome is indicated on the chromosome ideogram. Note that the marker chromosome does not contain this genomic region; therefore, both cell lines exhibit UPD of the relevant chromosomal region. Note also that the imprinted region is within a region of heterodisomy, but is fully uniparental (see also **Fig. 4**-A5).



Inferences Regarding Origin of UPD Derived from Detection of Mosaicism

Mosaicism for a trisomic cell line, a cell line with a marker/derivative chromosome, or a cell line with normal biparental inheritance is an additional line of evidence by which one may infer the origin of the UPD (see **Figs. 4–6**). If a trisomic cell line is present either in the same tissue as the UPD or in other tissues (such as placenta), the origin of the UPD is most likely a trisomy rescue (see **Fig. 4**A–D). Mosaicism involving a normal biparental cell line with a segmental UPD cell line or a whole chromosome uniparental isodisomy cell line most likely indicates a mitotic origin (see **Fig. 6**), although other more rare and esoteric chromosomal arrangements are possible.[17,18] SNP arrays have been used successfully to detect mosaicism for segmental UPD in cases of Beckwith-Wiedemann syndrome[3,38] and in revertant skin in patients with ichthyosis.[39]

The presence of homozygosity for an entire chromosome is most likely associated with a monosomy rescue (see **Fig. 4**E-4); however, chromosome segregation errors in meiosis II without the presence of crossovers (see **Fig. 4**C-4) and mitotic segregation errors (see **Fig. 6**B-2) may also result in uniparental isodisomy for the whole chromosome. The finding of mosaicism for a monosomic cell line (consistent with monosomy rescue), a trisomic cell line (consistent with trisomy rescue), or a normal biparental cell line (consistent with mitotic nondisjunction) may clarify the mechanism of the UPD event (see **Figs. 4E, 4**C-5, and **6**B-3). Note that the presence of a monosomic cell line in a monosomy rescue event would have to be ascertained by decreased log2 values across the chromosome (suggesting low-level monosomy), or traditional cytogenetic methods. SNP allele patterns would be unaffected in this scenario.

Follow-up Testing to Confirm UPD

As outlined previously, failure to detect LCSH by SNP microarray analysis does not exclude the possibility of UPD (see **Fig. 4**A). For this reason, SNP microarrays cannot be considered a diagnostic assay for UPD when analyzing the proband alone. Similarly, the finding of LCSH involving a single chromosome should not be considered diagnostic of UPD. Large isolated regions of LCSH (even those larger than 10 Mb) may still represent IBD with distant consanguinity. Regions of LSCH greater than 1 Mb have been found in outbred populations, typically attributed to recombination "cold spots" leading to SNP markers in linkage disequilibrium.[4,6,7] Additionally,

Fig. 6. Uniparental disomy resulting from mitotic errors. Row 1 illustrates a potential model of somatic events consistent with the SNP data shown. Row 2 shows patient allele plots (Illumina Quad610 array, visualized in BeadStudio software). Row 3 shows patient allele plots involving mosaicism for a UPD cell line and a normal cell line. Models for chromosome composition responsible for the data shown are illustrated below the allele plots. (*A*) Segmental uniparental disomy resulting from mitotic recombination. Homologous chromatid exchange during somatic development is illustrated with crossing over on the short arm of chromosome 7. The 2 potential daughter cells resulting from this exchange are shown; however, only one of these cell lines is detected in this individual (the shaded cell is not detected). Alternatively, the SNP data shown are consistent with break-induced replication at the site of exchange (not illustrated). (*B*) Mitotic nondisjunction resulting in whole chromosome isodisomy. Sequential mitotic nondisjunction and selection events can result in cells with isodisomy for an entire chromosome. Note that here only trisomy rescue is illustrated, but one could also model rescue of the monosomic cell line created by the first mitotic error.

structural elements such as translocation or inversion breakpoints are known to inhibit recombination[40]; therefore, it is possible that some families may be cosegregating very large (even >10 Mb) contiguous haplotypes surrounding these rare elements.

When an LCSH detected by SNP-based microarray suggests UPD, further molecular analysis is necessary for UPD confirmation and determination of parent of origin. LCSH involving a chromosome associated with an imprinting disorder (summarized in **Table 2**) should be strongly considered for UPD confirmatory testing, particularly when the clinical features of the patient are consistent with the syndrome of interest.

In principle, definitive UPD testing requires the demonstration of uniparental inheritance of multiple informative markers for the chromosome in question. Testing typically involves genotyping microsatellite polymorphisms distributed along the length of the putative UPD chromosome in the mother, father, and proband (trio analysis).[41,42] In cases of suspected UPD for imprinted chromosomes, specific diagnostic testing of methylation patterns for the corresponding imprinting disorder may also be available.[43]

Established clinical guidelines for *whole-chromosome* UPD molecular testing[44] require at least 2 fully informative markers to establish either uniparental or biparental inheritance of a chromosome. Informative markers distinguish maternal and paternal alleles such that the parent (or parents) of origin for a chromosome segment in the proband can be unambiguously determined. Because nonpaternity can confound UPD test results, it is recommended that parentage be established by demonstrating paternal inheritance of at least 1 marker on a non-UPD chromosome. Guidelines for the analysis of *segmental* UPD have not been established. Although numerous well-defined microsatellite markers exist, this technique may not be suited for detection of segmental UPD, given that the established (clinically validated) assay may not have sufficient markers within the specific region of isodisomy to confirm a small segmental UPD. Microarray-based SNP genotyping of trios has been used to detect both whole-chromosome and segmental UPD.[45,46] This option seems especially suited for follow-up of incidental LCSH findings detected by SNP-based microarray analysis, given that this analysis already genotypes thousands of SNPs distributed along the proband's putative UPD chromosome. Were it not cost prohibitive, this technique might be used to replace the relatively inexpensive microsatellite UPD analysis.

AUTOZYGOSITY MAPPING

Regardless of whether LCSH is present as a result of consanguinity, UPD, or undetermined origin, the potential for recessive disease may be significant. The term *autozygosity* refers to homozygosity of alleles that are identical by descent (inherited from a common ancestor), as contrasted with homozygosity identical by state (random inheritance). Autozygosity mapping has been used successfully to discover the genetic basis of numerous recessive disorders in consanguineous families.[47–49] Although it is generally outside the scope of a routine diagnostic laboratory investigation to pursue gene discovery efforts, autozygosity mapping restricted to known disease genes remains a feasible and useful diagnostic tool.[50,51] When the clinical indication for study is very specific and the differential diagnosis is limited to genetically well-characterized recessive disorders, mapping candidate genes to determine location relative to LCSH segments is relatively straightforward.[48,50] Finding a gene of interest in an LCSH, although certainly not diagnostic of a homozygous mutation in that gene, may help to prioritize follow-up testing for multigenic disorders. The utility of this exercise is illustrated in the following case (Kearney H , unpublished data). SNP microarray testing for a patient with a clinical

diagnosis of Usher syndrome (unknown type) revealed 4 regions of LCSH (each 3–10 Mb in length, totaling approximately 0.8% of the autosomal length). Given that Usher syndrome can result from mutations in multiple genes, all known disease-causing genes were investigated, and only one, *USH2A*, was found in an LCSH. This finding was followed by referral for *USH2A* gene sequencing, which resulted in successful identification of a homozygous pathogenic mutation in the patient (**Fig. 7**).

For cases of excessive homozygosity in which the genetic basis for the patient features is unclear, or in which limited phenotypic information is communicated to the laboratory, the referring physician can be provided with a list of candidate genes in the homozygous intervals. Further consideration of those genes associated with recessive disorders may prompt the physician to consider disorders that may not have

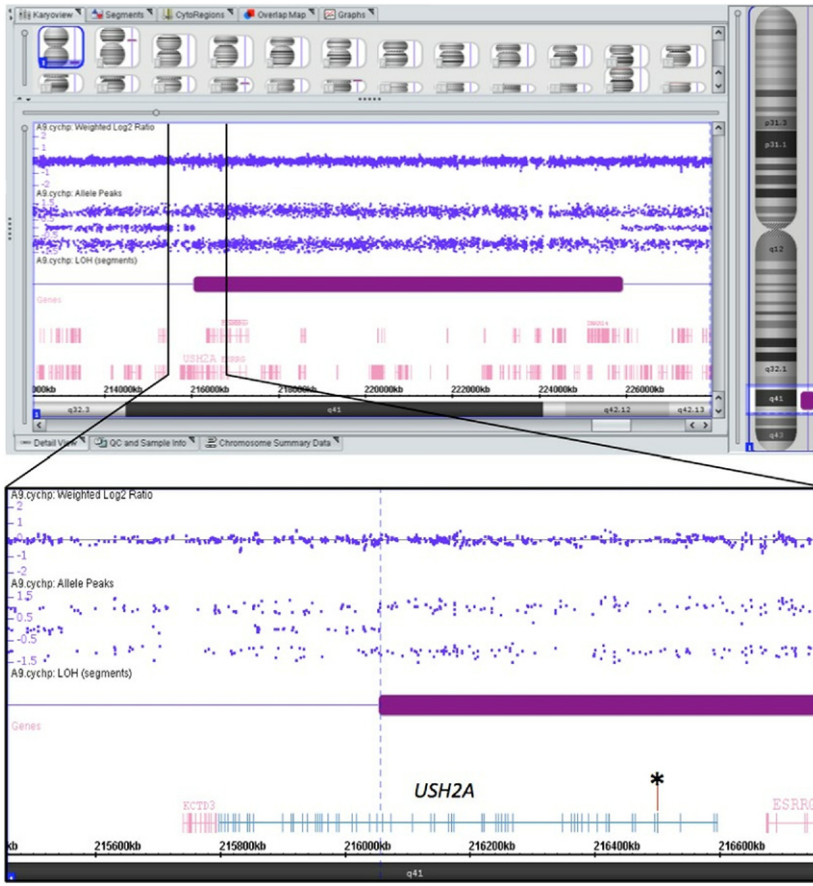


Fig. 7. Autozygosity mapping used to identify a *USH2A* homozygous mutation. *(A)* Screen shot of a 10-Mb LCSH (purple bar) on chromosome 1q seen in a patient with clinical features of Usher syndrome. This segment spans 85 genes, including *USH2A*, one of several known genes involved in the etiology of Usher syndrome. Vertical lines denote area of zoom highlighted in lower panel. (*B*) Zoomed view shows that the 1q LCSH spans only a portion of the *USH2A* gene (underscoring the utility of high-density SNP arrays for autozygosity mapping). Subsequent referral for *USH2A* gene sequencing identified a pathogenic homozygous recessive mutation (position of mutation shown as an *asterisk*).

been obvious during the initial clinical evaluation. Personal laboratory experience suggests that causative recessive gene mutations are detected through autozygosity mapping and sequence confirmation in approximately 10% of the cases reported of LCSH (Kearney and colleagues unpublished data).

SUMMARY

Given its widespread clinical use and high sensitivity for CNV detection, chromosomal microarray analysis has become an indispensible component of constitutional cytogenetic laboratory testing. SNP-based microarrays, with their added ability to detect genome-wide allele states, can also detect LCSH. Although a stand-alone finding of LCSH rarely results in a definitive diagnosis, it can trigger additional testing, potentially leading to a diagnosis of an imprinting syndrome or rare genetic recessive disorder not recognized in the original clinical differential. In addition, SNP data allow for inferences regarding complex cytogenetic mechanisms, furthering our understanding of uniparental disomy. As SNP-based arrays gain more widespread diagnostic use and more patient series are published, the clinical impact of LCSH detection will be more fully realized.

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