## Title

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## Journal

Human mutation, 28(3)

## ISSN

1098-1004

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## Publication Date

2007-03-01

Peer reviewed

*RESEARCH ARTICLE*

# Deletion Mapping in Xp21 for Patients With Complex Glycerol Kinase Deficiency Using SNP Mapping Arrays

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*Communicated by Nancy Spinner*

**Infantile or complex glycerol kinase deficiency (cGKD) is a contiguous gene deletion syndrome caused by a loss of *GK* (MIM# 300474), along with its neighboring genes, Duchenne muscular dystrophy (*DMD*; MIM# 300377) and/or Nuclear Receptor Subfamily 0, Group B, Member 1 (*NR0B1*; MIM# 300473). Patients with cGKD present with glyceroluria and hyperglycerolemia in association with DMD and/or adrenal hypoplasia congenita (AHC). The purpose of these investigations was to determine whether the Affymetrix GeneChip Mapping Array (SNP chip) could be utilized to detect and map breakpoints in patients with cGKD. Genomic DNAs from several primary lymphoblastoid cell lines from patients with cGKD were analyzed on the Affymetrix platform. The Affymetrix SNP chip is a high-density oligonucleotide array that allows a standardized, parallel interrogation of thousands of SNPs across the entire genome (except for the Y chromosome). Analysis of the array features' hybridization intensities enabled clear delineation of the patient deletions with a high degree of confidence. Many of these patient deletions had been mapped by PCR and their breakpoints confirmed by sequencing. This study demonstrates the utility of the Affymetrix Mapping GeneChips for molecular cytogenetic analysis, beyond the SNP genotyping for which the arrays were initially designed. With one out of 160 live births (approximately 25,000 U.S. neonates annually) reported to have cytogenetic disorders, we envision a significant need for such a standardized platform to carry out rapid, high-throughput, genomic analyses for molecular cytogenetics applications. Hum Mutat 0, 1–8, 2006. Published 2006 Wiley-Liss, Inc.[†]**

KEY WORDS: contiguous gene syndrome; deletion mapping array; glycerol kinase deficiency; molecular cytogenetics; SNP mapping array

## INTRODUCTION

Glycerol kinase deficiency (GKD) is an inborn error of metabolism that was initially described by McCabe et al. [1977]; affected individuals manifest hyperglycerolemia and glyceroluria. GKD is an X-linked recessive disorder that results from mutation of the glycerol kinase (*GK*; MIM# 300474) gene [McCabe, 2001]. *GK* catalyzes the phosphorylation of glycerol to glycerol 3-phosphate in an ATP-dependent reaction.

The *GK* gene has been mapped to Xp21 and lies in a region containing the following genes: (Xpter) . . . *IL1RAPL1* (MIM# 300206) – *MAGE* cluster (MIM#s 300097, 300152, 300153) – *NROB1* (MIM# 300473) – *GK* – *DMD* (MIM# 300377) . . . (Xcen) [McCabe, 2001]. Two testis-specific genes, *GK2* (MIM# 137028) and *GK3*, are located at 4q13 and 4q32 and two pseudogenes are located at 1q41 and Xq23 [Sargent et al., 1994]. *GK* was originally described with 19 exons [Sjarif et al., 1998] and two alternately spliced exons were subsequently reported, bringing the total to 21 exons [Sargent et al., 1994]. The genomic locus is approximately 50 kb, and the major isoform mRNA is 2,581 bp and encodes a 553–amino acid protein [Sargent et al., 1994].

GKD presents with diverse phenotypes and is categorized into three different clinical presentations [McCabe, 2001]:

1. Infantile or complex GKD (cGKD), the most common form of *GK* mutation, is a contiguous gene syndrome caused by a microdeletion of *GK* and its neighboring genes, Duchenne

The Supplementary Material referred to in this article can be accessed at http://www.interscience.wiley.com/jpages/1059-7794/suppmat.

Received 20 March 2006; accepted revised manuscript 15 August 2006.

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Grant sponsor: National Institute of Child Health and Human Development; Grant number: RO1 HD 22563.

DOI 10.1002/humu.20424

Published online in Wiley InterScience (www.interscience.wiley.com).

muscular dystrophy (*DMD*) and/or Nuclear Receptor Subfamily 0, Group B, Member 1 (*NR0B1*), the causative gene for adrenal hypoplasia congenita (AHC). Patients present with hyperglycerolemia and glyceroluria, along with DMD and/or AHC.
2. Juvenile or symptomatic GKD results from point mutations in *GK* that cause episodic vomiting, acidemia, and central nervous system (CNS) deterioration, including stupor and coma.
3. Adult or asymptomatic GKD, also resulting from point mutations, is a benign form detected incidentally with pseudohypertriglyceridemia.

The severity of symptomatic GKD varies greatly and does not seem to be correlated with enzyme activity [Dipple et al., 2001; Dipple and McCabe, 2000]. We have postulated that modifier genes and multiple functions of the same protein (moonlighting activities) may contribute to the lack of a simple genotype–phenotype relationship [Sriram et al., 2005].

We have collected lymphoblastoid cell lines (LCLs) from patients with GKD, including a number with cGKD. Many of these patients' deletions have been mapped by PCR of sequence-tagged sites (STSs) [Zhang et al., 2004].

We hypothesized that it would be possible to utilize Affymetrix Gene Chips (Affymetrix, Santa Clara, CA; http://affymetrix.com) for copy number estimation by comparing the intensities for each SNP relative to a control. Affymetrix 10K GeneChips (SNP Chips) contain 11,555 SNPs covering the entire human genome, except the Y chromosome. The median physical distance between SNPs is about 105 kb, and the average distance is about 210 kb. To be able to achieve a >90% call rate with 99.96% reproducibility, each SNP has 40 different 25-bp oligonucleotides tiled on the slide, each with variations of perfect matches, mismatches, and flanking sequences (per Affymetrix). The Affymetrix 10K GeneChip contains multiple markers in Xp21 that may be deleted in cGKD. With the 100K GeneChip, the coverage increases approximately 10-fold.

This study was undertaken to determine if the Affymetrix SNP Chip would be applicable to mapping the deletions in patients with cGKD.

## MATERIALS AND METHODS

### DNA Application and SNP Chip Analysis

Following the Affymetrix protocol for the 10K and 100K human Mapping GeneChips and reagents, we sought to map the breakpoints of the deletions in 12 cGKD patient cell lines. We grew the patients' lymphoblastoid cell lines and harvested and purified the DNA using commercially available kits and reagents. Each patient's genomic DNA was then subjected to restriction digestion with *XbaI* (New England Biolabs, Ipswich, MA; www.neb.com/nebecomm) for 2 hr and the enzyme was deactivated by heating to 70°C for 20 minutes. Next, proprietary adaptor sequences (Affymetrix) containing *XbaI* compatible ends and primer binding sites were ligated to the digested genomic DNA fragments using T4 DNA ligase (New England Biolabs) at 16°C for 2 hr and heat inactivated at 70°C for 20 minutes. A single-primer PCR amplification was performed for 35 cycles with an annealing temperature of 59°C using AmpliTaq Gold (Applied Biosystems, Foster City, CA; www.appliedbiosystems.com). The products were purified using QIAquick PCR Purification spin columns (Qiagen, Valencia, CA; www1.qiagen.com), quantitated by spectrophotometry and qualitatively checked by agarose gel electrophoresis. Each sample was fragmented by DNase I (Affymetrix) at 37°C for 30 minutes, again checked by gel electrophoresis, and end-labeled using terminal deoxynucleotidyl transferase (Affymetrix) for 2 hr at 37°C. The samples were then added to hybridization buffer, denatured at 95°C for 10 minutes, and hybridized to the Affymetrix SNP Chip for 18 hr at 48°C. Finally, the SNP Chips were washed and stained with streptavidin (Pierce, Rockford, IL; www.piercenet.com), biotinylated goat anti-streptavidin antibody (Vector Laboratories, Burlingame, CA; www.vectorlabs.com), and streptavidin phycoerythrin (Molecular Probes, Carlsbad, CA). The arrays were scanned in the UCLA Microarray Core Facility and analyzed using GCOS and GDAS Affymetrix software and copy number was determined using the Chromosome Copy Number Analysis Tool (Affymetrix).

### Statistics

The probability that a stretch of n "No Calls" would happen by chance was determined to be (1 – the average call rate)$^{n}$ [De Veaux et al., 2004]. Normalized copy number in the patients' deleted regions was calculated by setting the average X chromosome copy number to 1 (or 2 in the case of the females) by dividing the average copy number within the deletion by the average copy number of the X chromosome(s) for each patient.

### Controls

The data on controls were obtained from experimental results on four patients' with no known cytogenetic abnormality on their X chromosome.

## RESULTS

### 10K SNP Chip

Deletions in the DNA from these 12 patients were identified by two methods: 1) the strings of Affymetrix "No Call" genotypes, and 2) low perfect match–minus mismatch (PM-MM) intensity readings.

The PM-MM model corrects for spurious nonspecific binding by subtracting the average oligonucleotide intensity for the mismatches from the average element intensity of the matching sequences. With the PM-MM method negative relative intensity readings may be observed due to background subtraction. The deletions were unique in each patient (Supplementary Tables S1 and S2; available online at http://www.interscience.wiley.com/jpages/1059-7794/suppmat) and were reproducible when DNA from the same patient was tested in duplicate (Supplementary Fig. S1; Patient 13155-1).

Mapping relative PM-MM intensity across a patient's X chromosome clearly indicated the location of the deleted segment by its low intensity (Fig. 1). The variability in PM-MM intensities may correlate with the binding affinities of the SNP sequences. In the deleted regions, the PM-MM intensities were variable but were uniformly diminished relative to the nondeleted regions (Table 1).

The genomic locations of the "No Call" genotypes were distributed randomly in the normal controls and in the nondeleted regions of our patient's genomic DNAs. Analysis of the genotype call percentages for the SNPs on the array and of the SNPs within each deletion showed that the average call percents declined from an overall average of 94.19 to 4.92% in the region of the deletions (Supplementary Fig. S1). The fact that the call percentage in the unaffected controls remained at 94% indicated that the decreased call frequency in the deleted regions of the patients was due to the deletion and not to a defect in the SNP features on the chips in the region. The higher call rate (28.6%) in Patient 3122-1 (Supplementary Fig. S1) resulted from the fact that he had a small deletion that spanned only seven SNPs, and therefore two spurious calls resulted in a relatively increased call percentage. The results on the DNA from Patient 3122-1 illustrated the usefulness of the combined approach of "No Calls" and PM-MM intensity in

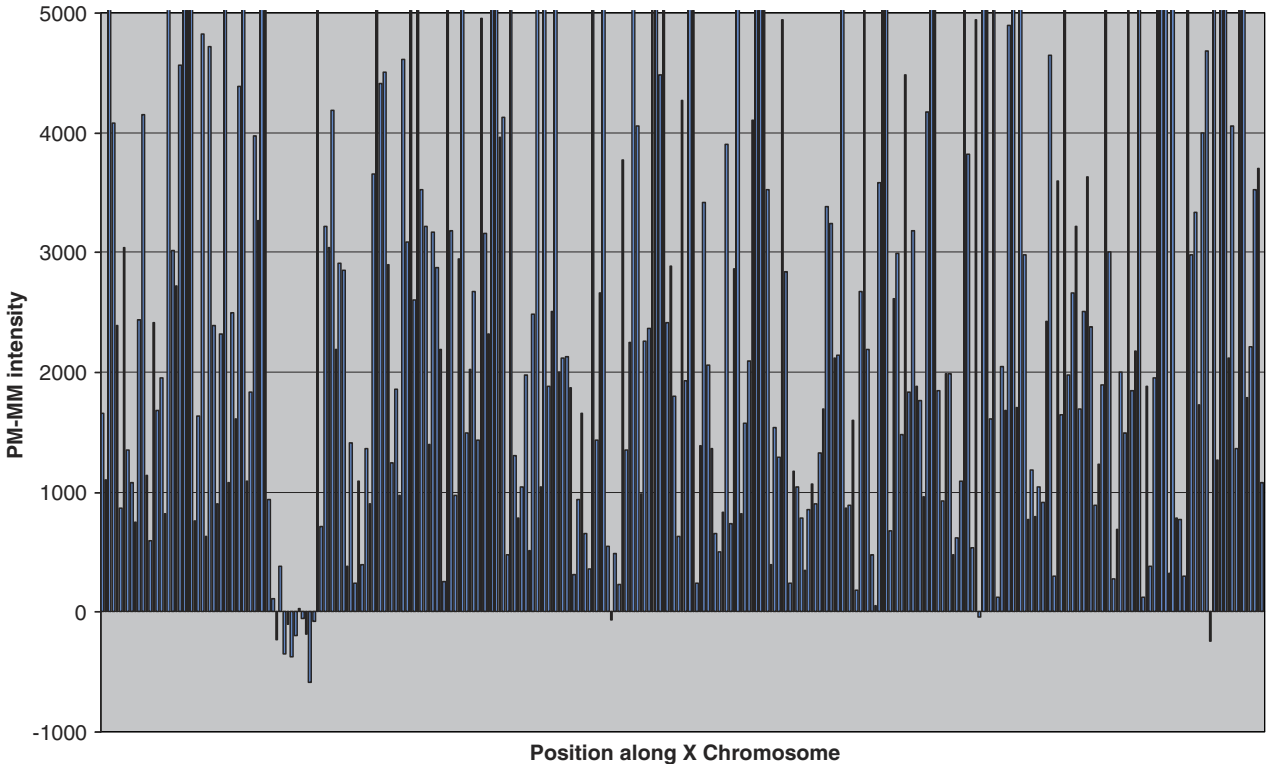


FIGURE 1. **Patient 17219-1 deletion detection by SNP intensity. The PM-MM SNP intensity, calculated by subtracting the average intensity of the oligonucleotides that had a mismatched base from the average intensity of the oligonucleotides that correspond to the perfect sequence, was graphed relative to the X chromosome physical position (bp) of the SNPs. Despite considerable variation, the deleted region in Xp21 was clearly recognized by the consecutive low PM-MM intensities. Negative readings occurred where background was greater than signal intensity. Bars extending above 0 PM-MM intensity indicate presence of oligonucleotides, while bars extending below 0 PM-MM intensity indicate absence of oligonucleotides.**

TABLE 1. **Summary of Call Percentages and Intensities in Each of the Patients, and the Normal Male and Female Controls**

| | Entire genome | | | With deletion | | |
|---|---|---|---|---|---|---|
| **Sample** | **"No calls"** | **Call %** | **Average intensity** | **"No calls"** | **Call %** | **Average intensity** |
| **Patient 381-1** | **921** | **92.03** | **3362.5** | **19** | **5** | **–43.4** |
| **Patient 1187-1** | **782** | **93.24** | **4750.8** | **16** | **5.88** | **–145.6** |
| **Patient 1618-1** | **1001** | **91.34** | **4374.8** | **10** | **9.09** | **–118.3** |
| **Patient 3122-1** | **856** | **92.6** | **5065.4** | **5** | **28.6** | **–140.5** |
| **Patient 4512-1** | **535** | **95.37** | **4126.5** | **5** | **0** | **–151.0** |
| **Patient 13155-1** | **355** | **96.93** | **7686.6** | **11** | **0** | **63.0** |
| **Patient 13155-1 #2** | **339** | **97.07** | **7566.5** | **10** | **9.09** | **100.7** |
| **Patient 13518-1** | **563** | **95.13** | **4674.2** | **8** | **0** | **–88.6** |
| **Patient 16118-1** | **857** | **92.59** | **4907.2** | **24** | **0** | **–30.5** |
| **Patient 17219-1** | **387** | **96.65** | **5808.2** | **13** | **0** | **–53.9** |
| **Patient 19112-1** | **888** | **92.32** | **3445.4** | **34** | **0** | **–29.1** |
| **Patient 23189-1** | **542** | **95.31** | **7167.4** | **12** | **0** | **–59.5** |
| **Patient 131518-1** | **706** | **93.89** | **5888.5** | **15** | **6.25** | **–118.2** |
| **Average** | **676.0** | **94.152** | **5443.3** | **19.6** | **1.25** | **–62.7** |
| **Normal male** | **643** | **94.44** | **3908.0** | **3** | **94** | **3707.3** |
| **Normal female** | **457** | **96.05** | **11761.8** | **3** | **94** | **6497.7** |

determining the robustness of the data at this location. The average PM-MM intensity in the deleted region of Patient 3122-1 was –140.5 vs. 5065.4 for the entire array, and the intensities of the two called SNPs were also low (144.7 and –109.8). The probability of getting seven "No Calls" in a row by chance was calculated to be $2 \times 10^{-9}$. The average Affymetrix PM-MM intensities of all the SNPs on the array varied in each patient sample (Supplementary Fig. S2), but within the deleted region of each of our patients, the average PM-MM intensity was markedly diminished. This ablation of signal was evident in each of the patient cell lines, but not in the controls.

By comparing the ratio of SNP intensities between the patients and reference controls, we estimated copy number. Representative data from our determination of copy number along the X chromosome (Supplementary Fig. S3) clearly indicated the deleted region in Xp21. Patient 13155-1's calculated copy number average

TABLE 2. Average Calculated Copy Number for the Entire X Chromosome and in the Regions of the Deletions in the Normal Male and Female Controls and the cGKD Patients*

| Sample | Average X copy number | Average deletion copy number | Normalized deletion copy number |
|---|---|---|---|
| Normal male | 1.20 | 1.18 | 0.98 |
| Normal male | 1.29 | 1.26 | 0.98 |
| Normal female | 2.07 | 2.02 | 1.95 |
| Normal female | 1.98 | 2.05 | 2.07 |
| Patient 381-1 | 1.26 | 0.66 | 0.52 |
| Patient 1187-1 | 1.22 | 0.42 | 0.34 |
| Patient 1618-1 | 1.28 | 0.49 | 0.38 |
| Patient 3122-1 | 1.23 | 0.50 | 0.41 |
| Patient 4512-1 | 1.26 | 0.24 | 0.19 |
| Patient 13155-1 | 1.17 | 0.40 | 0.34 |
| Patient 13155-1 #2 | 1.17 | 0.37 | 0.32 |
| Patient 13518-1 | 1.20 | 0.40 | 0.33 |
| Patient 16118-1 | 1.22 | 0.61 | 0.50 |
| Patient 17219-1 | 1.18 | 0.36 | 0.31 |
| Patient 19112-1 | 1.20 | 0.55 | 0.46 |
| Patient 23189-1 | 1.20 | 0.32 | 0.27 |
| Patient 131518-1 | 1.22 | 0.71 | 0.58 |

*Mean cGKD = 0.385.

for his X chromosome was 1.17, while the average calculated copy number within his deletion was 0.40. (The same patient's DNA analyzed on the 100K SNP array yielded a average calculated copy number of 1.16 for his X Chromosome and 0.28 with the deleted region.) The summary of each of the patients' and controls' copy number determinations is provided in Table 2, and the mean normalized copy number was 0.38 with a range of 0.19–0.58. The data on controls were obtained from experimental results on four patients with no known cytogenetic abnormality on their X chromosome (DiGeorge patients).

By mapping the deleted SNPs using the UCSC Genome Browser (www.genome.UCSC.edu), we were able to determine the minimum and maximum sizes of the deletions in the patients' cell lines (Fig. 2). The breakpoints of the deletions were between the last present (maximum) and first absent (minimum) SNP. These breakpoints confirmed previously performed breakpoint sequencing results in the patients for whom these data were available (Zhang Y-H, Ho JC, Huang B-L, McCabe LL, August G, Kern I, Morris M, McCabe ERB, unpublished results).

We compared individual SNP intensities telomeric, within, and centromeric of Xp21 *GK* to other *GK* SNPs (Supplementary Fig. S4). The reduction of Xp21 *GK* specifically (SNP_A-1512978, SNP_A-1515463, and SNP_A-1507601) and not of either the testis-specific *GK2* (SNP_A-1510447 and SNP_A-1511576) intensities or the *GK3* (SNP_A-1515104 and SNP_A-1512733) genes on chromosome 4 indicated the specificity of the SNP hybridization. The high intensity (5222.5) of SNP_A-1512978 in Patient 4512-1 confirmed that the deletion breakpoint was between this SNP and SNP_A-1515463.

### 100K SNP Chip

While our 10K SNP Chip results succeeded in detecting deletions in our GKD patient samples, the location of the deletion breakpoints could not be precisely determined (Fig. 2). In the course of these investigations Affymetrix released a 100K SNP Chip with 10 times the number of SNPs. Our successful results with the 10K SNP array suggested that we should attempt to use the 100K SNP Chip to take advantage of the greater resolution offered by more SNPs in the vicinity of our patients' deletions to determine precisely the location of their breakpoints. We mapped denser SNP coverage of the 100K SNP Chip with 75 SNPs in Xp21.2 (UCSC Genome Browser).

Our finer mapping results allowed us to confirm the patients' deletions and validate our 10K results. The 100K SNP chip contained a sufficient number of SNPs to permit us to vary the bin size used to determine the global copy number. In the representative analysis (Supplementary Fig. S5), increasing the bin size resulted in reduced noise in the data, allowing us to eliminate false deletions caused by spurious low SNP intensities, but at the expense of clearly defined deletion breakpoints. Conversely, using a smaller bin size allowed us to map the patients' deletion endpoints with accuracy.

The confidence we could assign to our copy number determination also assisted in detecting true deletions and their endpoints. The copy number and associated probability was calculated for Patient 13155-1 using copy number analysis tool (CNAT) for each SNP along the X chromosome (Fig. 3). The deleted region was clearly indicated by the reduction in copy number and P-value. By including the associated probability in the analysis of the calculated copy number, the number of false-positive deletions was reduced and more refined deletion mapping was possible.

## DISCUSSION

We demonstrated that SNP arrays can be utilized to detect X chromosomal deletions responsible for cGKD in affected males. The mean normalized X chromosomal copy number was approximately two in females, approximately one in males and $<0.4$ in the regions of the cGKD deletions. The observation that signal intensity remained detectable in these patients was not due to mRNAs responsible for transcription of other GK isoforms and remains undetermined at this time.

The first microarrays were spotted with cDNA to monitor gene expression levels. Pollack et al. [1999] attempted to utilize these arrays for copy number analysis. However, this technique had many shortfalls. The microarray was not sufficiently sensitive to detect deletions due to the individual features having homology between paralogous genes and lacking intervening sequences. Additionally, the genomic representation was limited to only "gene rich" regions, and, most importantly, copy number did not correlate well with hybridization strength.

While the comparative genomic hybridization (CGH) technique has several limitations, it provides more specific breakpoint delineation, and is less labor-intensive and locus-specific than fluorescence in situ hybridization (FISH). The most significant problem for array CGH, using large clones, such as BAC clones, for its arranged features, is high background noise. The relatively low signal to noise ratio results from several factors, including imperfect spotting and cross-hybridization. Despite the use of copious amounts of cot-1 DNA to suppress nonspecific hybridization, large genomic clones contain many common repeats that lead to spurious binding and background [Kooperberg et al., 2002]. Furthermore, the large clones limit the spatial resolution needed to map smaller deletions accurately [Ren et al., 2005]. We therefore sought to utilize another array methodology to improve the detection sensitivity and resolution of molecular cytogenetic analyses.

As the potential for this technology became more apparent, several BAC and cosmid clone arrays were created to detect

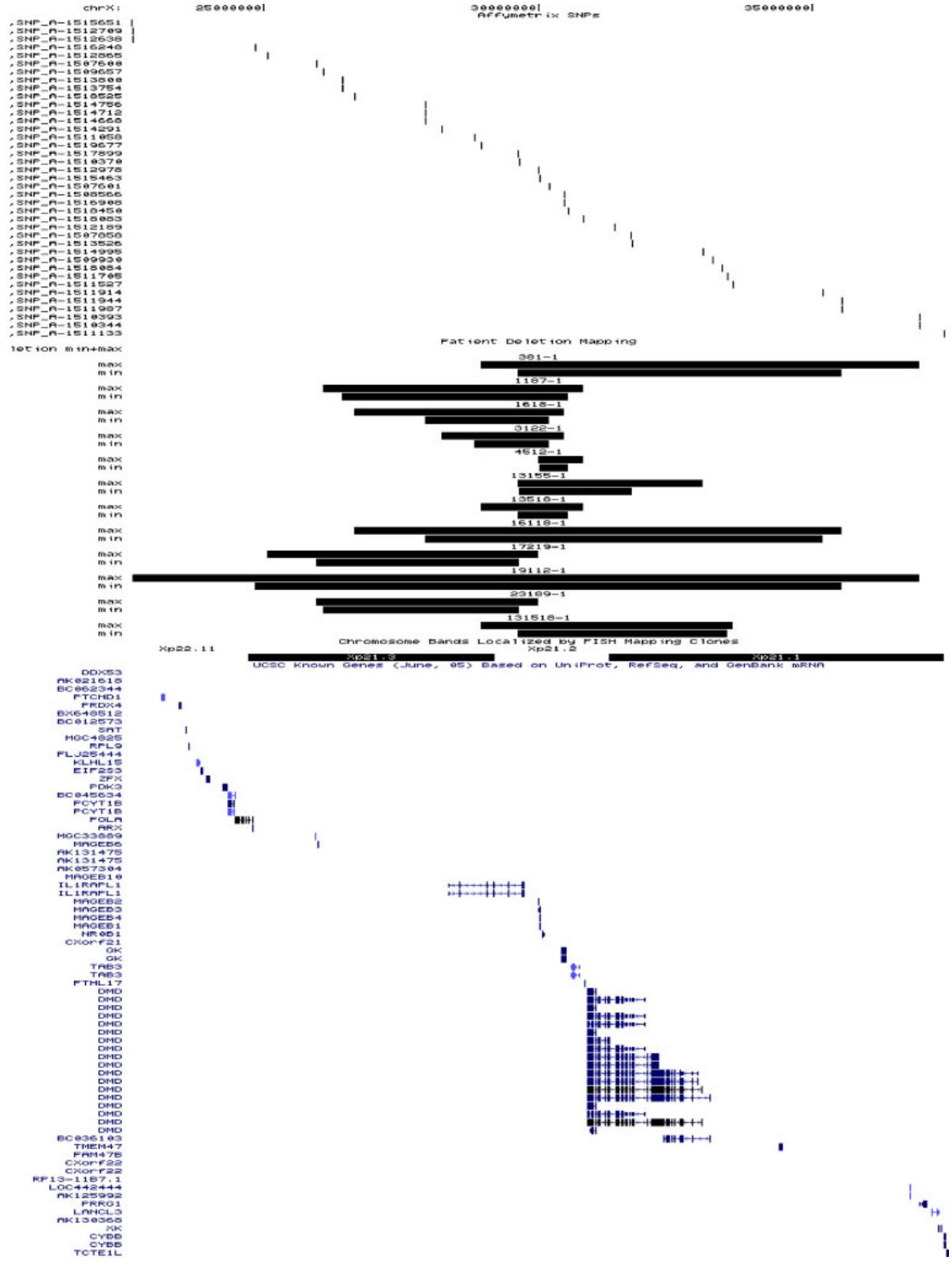


FIGURE 2. **Patient deletions mapped using SNP locations and the UCSC Genome Browser. The patients' deleted genomic regions were represented by black bars. The minimum and maximum size of the deletions were determined by the locations of the last present and the first deleted SNPs (shown above black bars), respectively. The genes in this region are shown below the black bars. The data compared well with breakpoint sequencing results in patients for whom these data were available (Zhang Y-H, Ho JC, Huang B-L, McCabe LL, August G, Kern I, Morris M, McCabe ERB, unpublished results) and validated the use of the SNP Chip for molecular cytogenetic analysis.**


FIGURE 3. **Copy number and probabilities of copy number determinations for Patient 13155-1. Copy number was calculated using CNAT and plotted light (gray) for each SNP along the X chromosome. The associated probability was graphed (darker gray) below. The deleted region was clearly indicated by the reduction in copy number and P-value. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]**

cytogenetic changes [Ishkanian et al., 2004]. As one of the major difficulties was background noise due to cross-hybridization to common repeats in the clones, an array was spotted with repeat-free, nonredundant DNA [Tanabe et al., 2003]. While this was a more successful approach, it was costly and labor-intensive to PCR-amplify thousands of fragments, and, more importantly, it was limited in the number of genomic regions to which it could be applied. In an effort to increase the specificity and resolution of array CGH with genomic clones Lucito et al. [2003] designed an array using 70-mer oligonucleotides.

Copy number estimation with Affymetrix SNP Chips offers an attractive alternative to spotted arrays that contain BACs or cosmids. The SNP Chips contain a proprietary array of 25-mer oligos in quartets of perfectly matching sequence (PM) and sequence that had a single basepair mismatch (MM) for each DNA strand (Affymetrix). The shorter sequence and ability to subtract cross-hybridization theoretically should provide improved signal-to-noise ratio and greater mapping resolution. We demonstrate that it is possible to detect gene loss in patients with cGKD using SNP chips. Since the arrays were designed for genotyping, the output string of "No Call" reading was anticipated in the regions of deleted DNA. But such a result could also occur due to high background or a poorly hybridizing sequence surrounding the SNP and resulting in low signal. The purpose of a quantifiable, more direct method to detect deletions was to look for regions with low PM-MM intensity readings. Our data indicate excellent concordance of low PM-MM intensity readings with the "No Call" readings within previously identified deletions.

Normalized SNP intensity readings can immediately highlight a region of deleted DNA, but there still is considerable variation of intensity levels. By standardizing against control intensity at each SNP in determining copy number, the noise is greatly reduced since the variability of each SNP's PM-MM intensities is normalized relative to its feature's unique binding affinity for that particular sequence of DNA. The smallest deletion that we detected was about 0.5 MB (Patient 4512-1), but the detection limit varies greatly depending on the density of SNPs in the region of the deletion. Unfortunately for SNP array CGH, most deletion breakpoints are in areas of low copy number repeats that provide a substrate for recombination [Stankiewicz and Lupski, 2002] and this is also true for cGKD (Zhang Y-H, Ho JC, Huang B-L, McCabe LL, August G, Kern I, Morris M, McCabe ERB, unpublished results). We therefore tried a higher resolution array for some of our samples to validate these results and to determine more precisely the patient's breakpoints. The concurrent analysis of copy number and associated P-value, as well as breakpoint sequencing (Zhang Y-H, Ho JC, Huang B-L, McCabe LL, August G, Kern I, Morris M, McCabe ERB, unpublished results) provided additional evidence that these deletions were accurately mapped.

The SNP Chip–based method suffers from the inability to detect balanced translocations or inversions. As in all array CGH techniques, if genomic content is present in another genomic region it still can bind to the array elements. In other words, physical position cannot be determined by array CGH, only the absence or presence of DNA in the genome. Despite this limitation, SNP-based arrays have been utilized to detect genomic aberrations [Zhou et al., 2004]. SNPs have been used to detect regions of loss of heterozygosity [Mei et al., 2000]. Affymetrix platforms have been used to detect copy number changes in cancer and other acquired cytogenetic abnormalities as well as constitu-

tional and congenital abnormalities, and copy-number polymorphisms [Huang et al., 2004; Wong et al., 2004; Slater et al., 2005; Zhao et al., 2005; Ming et al., 2006; Wirtenberger et al., 2006].

Many feel that microarray-based molecular cytogenetic approaches will replace more traditional chromosome visualization methods over the next decade [Shaffer and Bejjani, 2004; Smeets, 2004]. It is anticipated that these newer methods will identify an increased proportion of patients who will have subtle molecular cytogenetic abnormalities compared with current approaches [Hwang et al., 2005; Lapierre et al., 1998; Oostlander et al., 2004]. The number of individuals with cytogenetic disorders recognized by the current methods are quite impressive: 1 in 160 live births (approximately 25,000 U.S. neonates annually); approximately 2% of prenatal diagnoses in women 35 years or older; and 50% of all first trimester spontaneous abortions [Nussbaum et al., 2001]. Indications for ordering a chromosome analysis include infertility and cancer, indicating a significant opportunity for the appropriate microarray platform.

The method we describe permits molecular cytogenetic analyses on DNA without requiring intact chromosomes for karyotype or FISH analysis. Since DNA can be analyzed in newborn screening dried blood specimens [McCabe et al., 1987; Jinks et al., 1989; Bhardwaj et al., 2003], this SNP Chip–based approach would permit newborn screening for cytogenetic disorders if this approach passes rigorous validation processes, including comparisons with FISH analysis or quantitative PCR, and if the cost can be reduced. Such a program would require considerable discussion of the ethical and social issues, however, before it could be implemented.

In conclusion, the Affymetrix SNP Chip can be utilized for the detection of genomic deletions in patient cell lines with cGKD. Our work provides proof of principle that the SNP chip can be used for molecular cytogenetic analysis beyond the SNP genotyping for which the arrays were initially designed. We envision the use of such a platform for rapid, high-throughput, genomic analysis for molecular cytogenetic applications in the future.

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