

Accurate analysis of copy number variation at 16p11.2 in autism spectrum disorder and control cohorts

Using TaqMan® Copy Number Assays and the ViiA™ 7 Real-Time PCR System



Researchers from the Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada. From the left:
Guillermo Casallo, BSc, Research Technologist; Christian Marshall, PhD, Research Associate; Ali Mowjoodi, MSc, Research Technologist

Features	ViiA™ 7 Real-Time PCR System
Block configurations	96-well, Fast 96-well, 384-well (runs Fast or standard), TaqMan® Array Micro Fluidic Cards
Run time	30 minutes expected (Fast 96-well); 35 minutes (384-well)
Resolution	Down to 1.5-fold changes for singleplex reaction
Excitation source	OptiFlex™ System with halogen lamp
Detection channels	Decoupled—6 emission, 6 excitation
21 CFR p11 compliance module	Optional software module
Remote monitoring	Available to monitor up to 4 instruments in real time and the status of up to 15 instruments
Data export format	User configurable: *.xls, *.xlsx, *.txt, and 7900 formats, as well as the new MIQE-compliant RDML format



Introduction

Autism spectrum disorders (ASDs) are lifelong neurological conditions that affect a person's development and how their brain processes information. Autism is the prototypical form of the ASDs and is characterized by challenges in communication, social interaction, and learning, as well as by unusual behavior, interests, and activities. Collectively, ASDs are among the most common developmental disorders with an overall prevalence of about 1 in 150, and diagnosis in males is four times more common than in females. Moreover, the heritability of ASD is as high as 90%, making it the neuropsychiatric disorder most strongly influenced by genetics.

The International Autism Genome Project (AGP) consists of over 170 scientists from 50 academic centers and research institutions around the world. Dr. Stephen Scherer's Laboratory at the Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada is the Canadian lead for the AGP. The lab focuses on understanding the genomic architecture underlying the causes of ASD. Their recent discoveries have identified *de novo* copy number variations (CNVs) to be associated with ASD in greater than 10% of cases, and rare-inherited CNVs also likely to be significantly involved [1,2]. Based on trends in their empirical data, and that of others, their experiments focus on searching for rare but highly penetrant CNVs, compared with previous strategies that mainly looked for common low penetrance variants. To define a rare variant, it is essential to have accurate and reliable frequencies of CNVs from control cohorts.

One of the most significant CNV findings in ASD was highlighted in three studies published concurrently with identification of a ~600 kb microdeletion/microduplication of chromosome 16p11.2 associated with ~1% of cases [1,3,4]. These 16p11.2 CNVs were also observed in ASD cases with additional dysmorphology, and in non-ASD cases having developmental delay (DD) [4].

In their latest work, researchers from Dr. Scherer's lab have found that the 16p11.2 deletions are more penetrant (100% for either ASD or DD in their cohort) than the duplications (~50%) [5]. As such, they have not detected 16p11.2 deletions in several large control cohorts they genotyped using high-resolution SNP arrays. This is in contrast to Glessner et al. [6], who found both 16p11.2 duplications and deletions in control populations. Moreover, deletions of 16p11.2 have now been found to be associated with a penetrant form of obesity [7,8], a phenotype also observed in their cohort [5]. Smaller telomeric deletions associated with obesity that overlapped the gene *SH2B1*, which is known to be involved in leptin and insulin signaling, have also been identified [8]. A recent study by their lab found both rare gains and losses at *SH2B1* [2].

Because CNVs interrogated by microarray have a high false discovery rate, researchers from Dr. Scherer's lab wanted to screen ASD test samples and controls for 16p11.2 deletions and duplications to get an accurate estimation of variant frequencies. In this study, they used Applied Biosystems® TaqMan® Copy Number Assays and the Applied Biosystems® ViiA™ 7 Real-Time PCR System to obtain CNV frequencies for these loci.

Methods

Test samples and control cohorts

ASD test samples (n = 455) from a Canadian cohort that has been described previously [1] and controls (n = 455) from the Ontario Population Genomics Platform (OPGP) (The Centre for Applied Genomics) were used. Genomic DNA was isolated from whole blood or cell lines using standard protocols.

CNV genotyping

To screen for copy number changes within 16p11.2, predesigned TaqMan® Copy Number Assays targeting the genes *SEZ6L2* (Assay ID Hs02619064_cn) and *SH2B1* (Assay ID Hs01634955_cn) were run in duplex with TaqMan® Copy Number Reference Assay RNase P following the TaqMan® Copy Number Assays protocol. The samples were run on an Applied Biosystems® ViiA™ 7 Real-Time PCR System. The workflow is shown in Figure 1. Approximately 10 ng of DNA was used for each reaction and copy number was determined using CopyCaller™ Software. For each plate and assay, two negative (CN = 2) and two positive (CN = 1 and CN = 3) controls were used.

Results and discussion

An example of the copy number analysis results is shown in Figure 2. For all plates, the positive

and negative controls were called correctly, indicating good sensitivity for both copy number assays. In the 455 ASD test samples, no deletions of the gene targets were detected that previously went undetected.

For the OPGP controls, as expected, no deletions were detected in 455 test samples. However, one sample carrying a duplication at both gene loci and one sample containing a duplication of only *SH2B1* were detected in the control cohort indicating that, although rare, copy number gains in 16p11.2 do occur. Although the control's full phenotype is unknown, it does fit well with the observation that duplications at 16p11.2 are not as penetrant as deletions in ASD or DD cases. In 455 ASD test samples, two samples carrying deletions and no samples carrying duplications at *SH2B1* were detected; whereas in 455 OPGP controls, two samples containing duplications at *SH2B1* and no samples containing deletions were detected. Although copy number variation does exist at *SH2B1* at the same frequency in ASD test samples and controls, it is interesting to note that only deletions were found in the ASD cases and only duplications in the controls. Further phenotype measures, especially weight, are warranted as a follow up.

CopyCaller™ Software

CopyCaller™ Software was developed specifically for TaqMan® Copy Number Assay data analysis. This free, easy-to-use software utilizes a graphical interface that quickly calculates the raw and possible copy numbers for a set of samples in a run. It also estimates a confidence value for each copy number call, has outlier removal functionality, and algorithms that can be run with or without a calibrator sample.

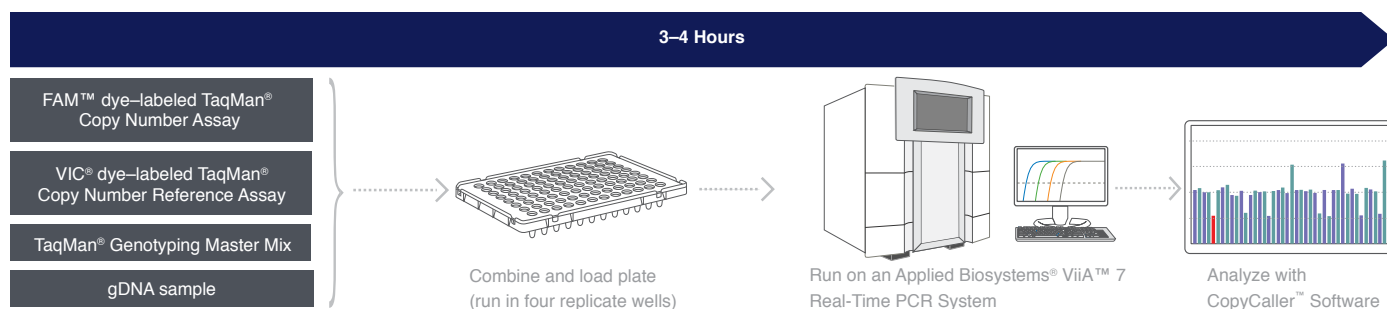


Figure 1. TaqMan® Copy Number Assay workflow.

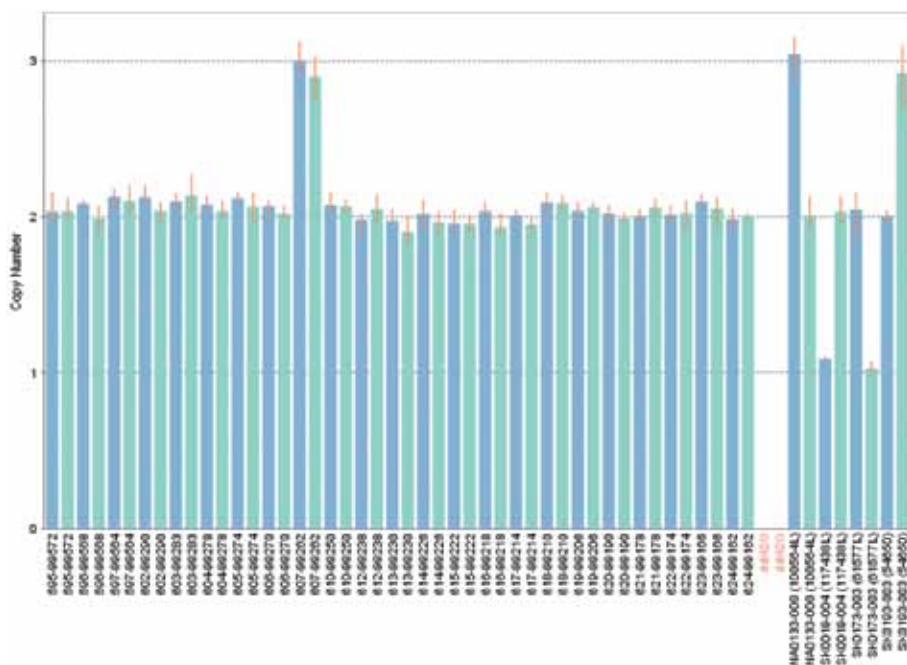


Figure 2. Copy number analysis of gene targets in cytoband 16p11.2. CopyCaller™ Software was used to determine the copy number of test and control samples by the relative quantitation method. Shown are samples from the OPKP control group run with TaqMan® Copy Numbers Assays targeting the *SEZ6L2* (blue bars) and *SH2B1* (green bars) genes, including one sample having a duplication of both genes. The right side of the panel shows “no template” and four sample controls containing either a duplication or deletion of the *SEZ6L2* or *SH2B1* genes. A control sample containing two copies of the targeted gene was used as the calibrator sample for the copy number analysis.

TaqMan® Copy Number Assay products

- Simplest method available to study copy number variation
- Pre-designed human and mouse assays for copy number analysis
 - Human: over 1.6 million assays available for genome-wide coverage
 - Mouse: over 180K assays available for gene exon coverage
- Assays available for common vector marker and reporter genes
- Custom Plus TaqMan® Copy Number Assays for user-defined human and mouse genomic targets
- Custom assays for other research targets of interest
- Reference assays for unique human and mouse genomic sequences

TaqMan® Assays are the gold standard for accurate target quantitation and an ideal validation tool for other platforms, including microarray follow-up studies. TaqMan® Copy Number Assays can also be used to screen research-specific targets and the workflow can be automated so that several hundred to thousands of samples can be processed in a single day.

Conclusion

TaqMan® Copy Number Assays, in conjunction with the ViiA™ 7 Real-Time PCR System, provided the necessary throughput and sensitivity to obtain accurate CNV frequencies at the loci tested. Guillermo Casallo, Research Technologist at the Centre for Applied Genomics at the Hospital for Sick Children says, “The ViiA™ 7 is an excellent machine. Combined with TaqMan® Copy Number Assays, you get results immediately and with high quality; CNV analysis made easy!”

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Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1.760.603.7200 | Toll Free in the USA 800.955.6288

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