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An efficient screening method for simultaneous detection of recurrent copy number variants associated with psychiatric disorders

Julio Rodriguez-Lopez [a,b], Noa Carrera [a,b,1], Manuel Arrojo [a,c], Jorge Amigo [a,d], Beatriz Sobrino [b], Mario Páramo [a,c], Eduardo Paz [a,c], Santiago Agra [a,c], Ramón Ramos-Ríos [a,c], Julio Brenlla [a,c], Ángel Carracedo [a,b,d], Javier Costas [a,*]

[a] Instituto de Investigación Sanitaria (IDIS) de Santiago de Compostela, Servizo Galego de Saúde (SERGAS), Santiago de Compostela, Spain
[b] Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain
[c] Servizo de Psiquiatría, Complexo Hospitalario Universitario de Santiago de Compostela, Servizo Galego de Saúde (SERGAS), Santiago de Compostela, Spain
[d] Grupo de Medicina Xenómica, Universidade de Santiago de Compostela, Santiago de Compostela, Spain

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ABSTRACT

Several recurrent copy number variants (CNVs) increasing risk to neuropsychiatric diseases have been identified in recent years. They show variable clinical expressivity, being associated with different disorders, and incomplete penetrance. However, due to its very low frequency, the full variety of clinical outcomes associated with each one of these CNVs is unknown. Current methods for detection of CNVs are labor intensive, expensive or not suitable for high throughput analysis. Quantitative interspecies competitive PCR linked to variant minisequencing and detection by mass-spectrometry may overcome these limitations. Here, we present two multiplex assays based on this method to screen for eleven psychiatric risk CNVs, such as 1q21, 16p11.2, 3q29, or 16p13.11 regions, among others. The assays were tested in our collection of 514 schizophrenia patients. Results were compared with MLPA at two CNVs. Additional positive results were confirmed by exome sequencing. A total of fourteen patients were CNV carriers. The method presents high sensitivity and specificity, showing its utility as a cheap, accurate, high throughput screening tool for recurrent CNVs. The method may be very useful for management of psychiatric patients as well as screening of different collections of samples to better identify the full spectrum of clinical variability.

1. Introduction

One of the most important recent discoveries in psychiatric genetics was the existence of several recurrent submicroscopic microduplications and microdeletions, known as copy number variants (CNVs), that confer risk to several different neurodevelopmental disorders such as autism spectrum disorders, schizophrenia, intellectual disability, or generalized epilepsy [1–3]. Kirov et al. [4] have estimated the overall penetrance of these CNVs for schizophrenia, autism spectrum disorders, developmental delay and congenital malformations ranging from 10.6% to 100%. Most of these CNVs are highly deleterious, being removed by purifying selection in less than 5 generations. Its frequency in populations is very low, generally less than 0.05%, established by an equilibrium between mutation rate and purifying selection [5].

Currently, there are at least 14 recurrent CNVs associated with schizophrenia risk, according to a recent meta-analysis [6]. Only one of them, the 22q11.2 deletion responsible for the velocardiofacial and diGeorge syndromes, was known for several years. The identification of these rare CNVs has been possible mainly due to technical improvements in hybridization-based technologies along the genome, and because of the high mutation/recurrence rate of these CNVs [7,8].

Analysis of different collections indicated that the combined frequency of these confirmed schizophrenia risk CNVs is around 2.5% of schizophrenic samples [6]. Several PCR-based approaches are available for detection of specific CNVs, such as multiplex-ligation dependent amplification (MLPA), quantitative real-time PCR (qPCR), multiplex amplicon quantification (MAQ), or invader assay, among others [9]. Unfortunately, each of these methods is labor intensive, requiring careful optimization of primers, probes and/or reaction conditions. In addition, some of them are relatively expensive and sensible to DNA quality. Therefore, there is an urgent need for the development of robust methods allowing the efficient, accurate, and cheap measurement of these CNVs in clinical samples with neurodevelopmental disorders.

One of the alternative methods is quantitative competitive PCR, a technique based on amplification of a test sequence in the presence of a known quantity of a competitor sequence that differs from the

* Corresponding author at: Instituto de Investigación Sanitaria (IDIS) de Santiago de Compostela, Hospital Clínico Universitario, edificio Consultas, andar -2, Grupo de xenética psiquiátrica, despacho 15, E-15706 Santiago de Compostela, Spain. Tel.: +34 981955452; fax: +34 981951473.
E-mail address: javier.costas.costas@sergas.es (J. Costas).
[1] Present address: Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Institute of Psychological Medicine and Clinical Neurosciences, Cardiff University, Cardiff CF24 4HQ, UK.

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test sequence by a single nucleotide. This method has the advantage of easy multiplexing. The use of chimpanzee DNA as a competitor precludes the need of synthetic DNA for each tested loci [10]. In this work, we present a quantitative interspecies competitive PCR (qicPCR) design, linked to minisequencing and variant detection by mass spectrometry, as a useful method to test for several recurrent CNVs involved in neurodevelopmental disorders at a reduced cost and high accuracy in large number of samples. We applied this method to our collection of schizophrenic patients, identifying an excess of these CNVs in comparison to expected population frequencies.

2. Material and methods

2.1. Samples

A total of 514 schizophrenic samples were included in the study. The samples are from the Santiago de Compostela healthcare area (Galicia, NW Spain) and meet the DSM-IV criteria for schizophrenia. All samples gave their written informed consent for this study. The study was performed in accordance with the latest version of the Declaration of Helsinki and has been approved by the Galician Ethical Committee for Clinical Research. Further details are presented in Carrera et al. [11].

Pan troglodytes DNA from the cell line EB176 (JC) was provided by the Health Protection Agency Culture Collection (UK). This sample corresponds to the chimpanzee used for the generation of the reference genome.

2.2. Selection of CNVs

CNVs for screening were selected from bibliography, mainly, the review of Malhotra and Sebat [3] and the meta-analysis of Levinsson et al. [2]. *VIPR2* region was selected based on Vacic et al. [12]. CNVs conferring higher risk to develop schizophrenia were prioritized in the group selected to carry out our study.

2.3. Quantitative interspecies competitive PCR

Identification of nucleotide positions with non-conserved nucleotides at human and chimpanzee reference sequences was done by inspection of the alignments of orthologous regions corresponding to the CNVs at the ENSEMBL web server (http://www.ensembl.org). Nucleotide positions were selected if there was no other difference in human–chimpanzee comparison in 100 bp at each edge. The positions were preferentially located in exons of different genes at each region. A minimum of five nucleotide positions per CNV region were chosen. These positions were used as input to design primers for PCR as well as for minisequencing using the Sequenom (Sequenom, Inc.; San Diego, California) MassArray Assay Design Suite v1.0 (https://seqpws1.sequenom.com/AssayDesignerSuite.html). Five ng of chimpanzee DNA and 5 ng of DNA from each human sample were subject to competitive PCR, following by detection of the amplification products by mass spectrometry using the Sequenom MassArray technology, according to manufacturer's instructions.

Analysis of results was based on the height of peaks corresponding to human or chimpanzee non-conserved nucleotides. First, quality

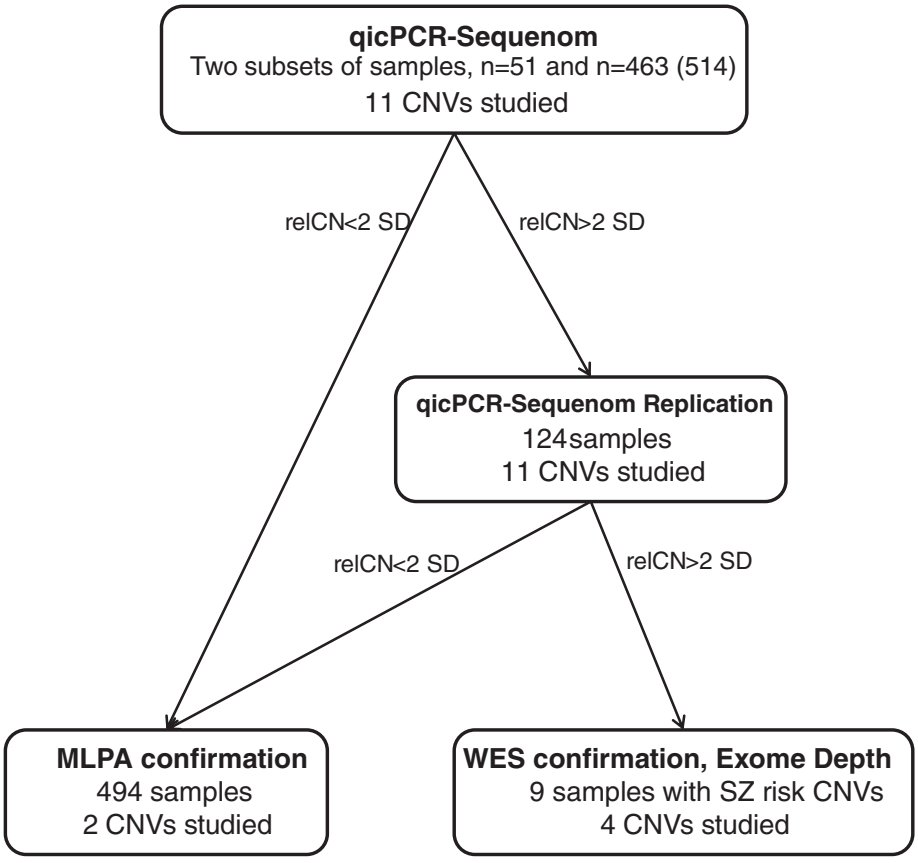

Fig. 1. Study design. A total of 514 samples were analyzed for the presence of 11 copy number variants (CNVs) by qicPCR using Sequenom MassArray. Those 14 samples with relCN > 2 SD from the mean (n = 124) were subject to a second round of analysis by qicPCR-Sequenom. Those samples whose relCN were again > 2 SD from mean were considered as positive results. Whole exome sequencing (WES) of the 9 positive samples with CNVs with established risk in schizophrenia (SZ), were used to confirm presence of CNVs. MLPA results for the whole sample were used as additional control for both positive as well as negative results at CNV regions 15q13.3 and 16p11.2.