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Original article

Interstitial microduplication of Xp22.31: Causative of intellectual disability or benign copy number variant?

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ABSTRACT

The use of comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) arrays has dramatically altered the approach to identification of genetic alterations that can explain intellectual disability and /or congenital anomalies. However, the discovery of numerous copy number changes with benign or unknown clinical significance has made interpretation problematic. Submicroscopic duplication of Xp22.31 has been reported as either a possible cause of intellectual disability and/or developmental delay or a benign variant. Here we report 29 individuals with the microduplication found as part of microarray analysis of 7793 samples submitted to an international group of 13 clinical laboratories. The referral reasons varied and included developmental delay, intellectual disability, autism, dysmorphic features and/or multiple congenital anomalies. The size of the Xp22.31 duplication varied between 149 kb and 1.74 Mb and included the steroid sulfatase (*STS*) gene with the male to female ratio of 0.7. Duplication within this segment is seen at a frequency of 0.15% in a healthy control population, whereas a frequency of 0.37% was observed in our cohort of individuals with abnormal phenotypes. We present a detailed comparison of the breakpoints, inheritance, X-inactivation and clinical phenotype in our cohort and a review of the literature for a total of 41 patients. To date, this report is the largest compilation of clinical and array data regarding the microduplication of Xp22.31 and will serve to broaden the knowledge of regions involving copy number variation (CNV).

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1. Introduction

Intellectual disability (ID) of variable severity has a population prevalence of 1–3% with a male to female ratio of 1.4 for moderate to severe and 1.9 for mild ID [23,31,32]. ID can be classified as syndromic when it includes other phenotypic abnormalities or non-syndromic when ID is the only feature. However, in some cases, both types of ID can be caused by the same genetic abnormality. The high genetic heterogeneity, especially for non-syndromic ID, makes finding the cause(s) a difficult task. The disproportionate ratio of affected males suggests a significant contribution of X-linked genes in regulating neurocognitive development [41]. However, excluding *FMR1* of fragile X syndrome, it was estimated that X-linked mental retardation (XLMR) genes account for only 10–16% of moderate to severe ID in males and therefore, cannot fully explain the excess of affected males [32]. Mutations in 76 genes [25] as well as deletions and duplications on the X chromosome have been described in association with XLMR.

X-Linked ichthyosis (XLI) is due to deficiency of the steroid sulfatase (*STS*) gene transcript located at Xp22.31. Deletion of this gene and adjacent regions is found in 90% of the affected individuals and it occurs due to recombination between low copy repeats (LCRs) located near the *VCX* genes that flank *STS* [13]. Some individuals with XLI also present with ID [13,18] and in addition, recent data showed an association between *STS* deficiency and susceptibility to attention deficit hyperactivity disorder (ADHD), autism and social communication deficits [19]. The common Xp22.31 deletion is approximately 1.5 Mb in size and is located from 6.3 to 7.9 Mb on chromosome X [13]. Deletion of *VCX3A* has been reported in association with abnormal neurocognitive phenotype [13,15,18]. However, this association was not found by others [11,26,30].

Several authors [4,18,21,27,33,34,38–40,42,43] and investigators from many clinical laboratories (personal communication) have observed a submicroscopic duplication of Xp22.31 that involves the *STS* gene, the counterpart of the deletion causing XLI. These patients all presented an abnormal phenotype which could be directly related to the genomic imbalance or simply reflect bias of ascertainment. Indeed, some authors interpreted this duplication as a normal variant [4,38], whereas others classified it as pathogenic [17,33,39,42,43], and still others were unclear about its clinical significance [27,34]. The fact that the duplication tended to be inherited from a normal parent in most cases seems to favor the interpretation of a benign variant. However, the phenotype of a genomic disorder can be variable due to a number of genetic mechanisms such as incomplete penetrance, variable expressivity and skewed X-inactivation. A benign variant might also behave differently in different populations or different genomic backgrounds and could cause a pathological phenotype under different conditions.

In an attempt to elucidate the significance of the microduplication Xp22.31, we present data from 13 clinical centers from five countries. These centers utilized whole genome BAC, oligonucleotide, or SNP arrays as diagnostic tools for individuals with an abnormal phenotype. Here we describe the molecular boundaries, inheritance, X-inactivation pattern and presenting phenotypes of 29 cases/families with the microduplication detected amongst 7793 referred individuals. We also reviewed literature data and compared it with our own to compile the largest and most comprehensive evaluation of the significance of dup Xp22.31 to date.

2. Materials and methods

2.1. Patient samples

A total of 7793 individuals were referred for microarray analysis with a variety of indications including developmental delay (DD), ID, autism, dysmorphic features and multiple congenital anomalies. These individuals were examined in 13 clinical centers from five countries and microarray analysis was performed in each center's respective cytogenetic/molecular laboratory. The laboratories and countries included in this report were: one in Australia, one in France, two in Germany, five in the United Kingdom, and four in the United States. Clinical evaluation was performed by clinicians from the referring institutions.

2.2. Chromosome, FISH and microarray analysis

Chromosome analysis was performed in peripheral blood lymphocyte cultures according to established protocols. FISH testing utilized BAC probes mapped within the Xp22.31 region (BlueGnome, Cambridge, UK) or the STS probe (Abbott Molecular/Vysis, Des Plaines, IL, USA). Both G-banded karyotype and array analyses were performed for most of the patients. Fluorescence in situ hybridization (FISH) was utilized to confirm the duplication in the probands and to evaluate parental inheritance.

Microarray was performed using one of four different platforms: BAC based high resolution array composed of 3600 targets (CytoChip v1.1) or 4200 targets (CytoChip v2.0 or 2.0.1) (BlueGnome, UK); oligonucleotide array comprised of 244,000 probes (Agilent, USA); SNP array with 250,000 targets (Affymetrix, USA); and SNP array with 610,000 targets (Illumina, USA). Genomic DNA labeling and hybridization were performed following each of the manufacturers' instructions. Data were analyzed with different software depending on the platform utilized: BlueFuse for the CytoChip arrays, CGH analytics for the Agilent oligonucleotide, Affymetrix Genotyping Console v2.1-for the Affymetrix SNP array and BeadStudio for the Illumina SNP array. Each laboratory utilized its own standards for interpretation of CNVs, but in general, an observed change was interpreted as non-pathogenic if it had been previously well documented in databases cataloging benign CNVs, such as the Database of Genomic Variants, and/or laboratory internal databases.

2.3. X-Inactivation analysis

An assay to determine X chromosome inactivation was performed in individuals with the microduplication when DNA samples were available and included: five female patients, eight carrier mothers and one family including sister, mother and grandmother. All DNA samples tested were derived from peripheral blood cells. Samples were examined by testing the androgen-receptor gene for assessment of the methylation status (modification of the HUMARA assay) [2]. 250 ng genomic DNA was double-digested overnight with 20 units of HhaI and 20 units of HpaII (New England BioLabs, USA). Mock digestion with no enzyme was set up for each sample. Restriction enzymes were subsequently inactivated by heating the samples at 65 °C for 20 min. 50 ng digested or mock-digested DNA was then amplified with PCR primers as previously described [2]. PCR products were separated and detected by capillary gel electrophoresis using the 3100 Genetic Analyzer (ABI PRISM, USA) and analyzed using Genescan 3.7. The X-inactivation ratio in heterozygotes was calculated as previously described [37]. A sample was considered to have skewed X-inactivation if the same X chromosome was inactivated in at least 80% of the cells. Samples with a single homozygous peak were considered not informative.

3. Results

Among 7793 patients referred for microarray testing, 3202 were analyzed by BAC array, 2600 by oligonucleotide array, and 1991 by