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Establishing an Array CGH Platform for Molecular Diagnosis of Saudi Patients with Intellectual Disability

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Abstract: Genomic instability that results in a net gain or loss of genetic material is an obvious feature of genetic disorders such as Mental Retardation (MR) that are associated with Copy Number Variations (CNVs) and structural chromosomal abnormalities. It is crucial to identify fragile genomic regions and the genes contained in them to specifically diagnose genetic disorders. Conventional karyotyping and Fluorescent *in situ* Hybridisation (FISH) are commonly used techniques for detecting such abnormalities. However, each technique has its own limitations. Karyotyping can detect microscopic rearrangements as small as approximately five Mega bases (Mb). FISH can locate the position of specific DNA sequences on a chromosome but it relies on a single target or prior knowledge of the region under investigation. Comparative Genomic Hybridisation (CGH) is a technique used to identify CNVs on a genome-wide scale. However, traditional CGH which uses a metaphase chromosome spread is limited by lower resolution and a sensitivity of approximately 5-10 Mb. High resolution CGH, commonly known as array CGH (aCGH) was developed to overcome such limitations by substituting a hybridisation target with a genomic segment spotted on an array format. To establish and validate aCGH as an advanced technique for detecting known and novel cryptic genetic changes in selected Saudi patients with idiopathic mental retardation, dysmorphic features and/or malformations. A high-resolution 2×105 K Agilent microarray scanner was used to perform aCGH on genomic DNA (gDNA) obtained from blood samples of two Saudi female patients, aged 3 and 14 years who showed clinical features resembling Angelman Syndrome (AS) and William's Syndrome (WS), respectively. The Aberration Detection Method 2 (ADM-2) algorithm with a sensitivity threshold of 6.0 was used for data analysis. In the AS patient (patient 1), the aCGH results revealed no deletions in chromosome 15 but smaller non-specific interstitial deletions were observed in chromosomes 4, 6 and 17 in addition to amplifications in chromosomes 3, 8, 11, 14, 16, 17 and 19. In the WS patient (patient 2), the expected deletion was detected in chromosome 7. However, other non-specific interstitials deletions were observed in chromosomes 7 and 15 and amplifications in chromosomes 14 and 22 were also observed. When the aCGH results of these patients were compared with FISH data from the Diagnostic Genomic Medicine Unit (DGMU) of King Abdulaziz University (KAU), the researchers observed a high concordance between the two methods with respect to chromosomes 15 and 7; no deletion was observed by FISH in chromosome 15 of patient 1 and a deletion in chromosome 7 was found in patient 2. High resolution aCGH and FISH techniques demonstrated a high degree of correlation with aCGH resulting in a wider spectrum of CNCs. This increased spectrum may ameliorate the prognosis of mental retardation in large cohorts of patients. Therefore, the researchers recommend using aCGH extensively as a routine diagnostic platform for screening patients with intellectual disabilities in Western Saudi Arabia.

Key words: Chromosome, patients, disabilities, platform, diagnostic, Saudi Arabia

INTRODUCTION

Mental Retardation (MR) is one of the most frequently encountered and most distressing childhood disabilities in industrialised and developing countries, affecting 2-3% of the global population (Dailyd *et al*., 2000). The prevalence of moderate to severe mental retardation MR disorders ranges from 14-20% (Brandenburg *et al*., 1990). The etiological factors underlying the development of MR also vary across different populations. In Saudi Arabia, the prevalence of MR per 1000 children is 8.9 (El-Hazmi *et al*., 2003).

Although, exacerbated by multiple environmental factors including birth trauma, parasitic infections, embryopathies and head injuries, the fundamental underlying causes of childhood MR remain chromosomal

aberrations arising from complex genetic defects. Thus, the disease becomes symptomatic well before adulthood and has a lasting effect on the development and progression of the affected child. The essential features of mentally retarded children include substantially below average general intellectual functioning, delayed expressive language, absence of analytical thinking, lack of social behaviour and delays in developing adaptive behaviours such as self-care skills (Ferrari, 2009). These shortcomings are mainly due to their inability to understand and retain information as reflected by their impaired intelligence. Based upon these findings, MR has recently been renamed as intellectual disability (Schalock *et al*., 2007).

Traditionally, intellectual functioning has been measured by an Intelligence Quotient (IQ) test. Based on the IQ score, MR can be further classified as borderline, mild, moderate, severe and profound (Ferrari, 2009). Generally, an IQ score ≤70 is considered to be significantly below average intellectual functioning. However, IQ tests are now treated with a certain degree of flexibility that may permit some people with IQ scores <70 from an MR diagnosis. This exclusion tends to occur if there are no significant defects in adaptive functions such as social skills, communication and daily living capabilities. The Terman-Binet test is now routinely used in conjunction with the IQ test to measure intellectual ability based on expressive language, vocabulary, numerical reasoning, memory, motor speed and analytical approach.

The genetics of MR: Chromosomal aberrations resulting in a net gain or loss of genetic material represent a major genetic cause of MR. According to an OMIM database, approximately 282 human genes are known to cause this disorder. Fragile X is the most common cause of inherited MR with an incidence of approximately 1 in 4000 males and 1 in 7000 females (Chelly and Mandel, 2001; Rejeb *et al*., 2011). Fragile X is characterised by varying degrees of MR, dysmorphic facial features, behavioural disturbances, developmental delay, autism and macro-orchidism in males. Mutations in X-linked genes are likely to account for more males than females being severely affected by MR.

Numerical and structural chromosomal aberrations are the most common identifiable cause of developmental delay related to congenital MR. The estimated percentage of cases in which an etiologic diagnosis can be established has been found to be between 40 and 60% (Anonymous, 1996; Curry *et al*., 1997). In this case, there is either a change in the normal structure or a variation in the number of chromosomes that often results in phenotypic symptoms. Analysis of the available epidemiological data has indicated that hereditary disorders and congenital malformations are rapidly becoming a major public health problem in the Middle East. A relatively large proportion of children with serious chromosomal disorders have been reported to be born to older mothers in this region (16-19% are born to mothers who are 35 years old or older) (Hamamy and Alwan, 1994). Complex Chromosomal Rearrangements (CCRs) are defined as structural chromosomal rearrangements with at least three breakpoints and exchange of genetic material between two or more chromosomes (Rosenberg *et al*., 2005; American Academy of Pediatrics Committee on Genetics, 1994). A common example of CCR is Trisomy 21. Studies have shown that the risk of having a child born with down syndrome increases from 1 in 600 births among mothers below age 30 to 1 in 50 births for mothers over 40 (Hook, 1992). Other chromosomal abnormalities that cause severe forms of MR include aneusomies which lead to multiple congenital anomalies and trisomies involving abnormalities in chromosomes 13, 18 and 21 (Hassold *et al*., 1995). The gain or loss of 5-10 Mb of DNA almost inevitably leads to developmental abnormalities during embryogenesis. Subtelomeric rearrangements and telomere deletions including microdeletions or duplications, represent a significant fraction of the unexplained MR (Rooms *et al*., 2004).

Microdeletion syndromes: Micro deletions are usually 4 kb or less in size and encompass multiple genes, all of which may contribute to the phenotype (Sharp *et al*., 2008). Microdeletions occurring in regions of the chromosome where there is a repetitive DNA sequence tends to have similar breakpoints. Di-george syndrome (velocardiofacial syndrome) is characterised by a microdeletion in chromosome 22q11 (Tonelli *et al*., 2007) and Prader-Willi Syndrome (PWS) is characterised by a microdeletion in chromosome 15q11-13 (Cassidy and Driscoll, 2009).

Angelman Syndrome (AS) which is associated with neurological symptoms that include ataxia, jerky limb movement, abnormal gait, absence of speech and severe MR was first identified in 1965 by Harold Angelman (Angelman, 1965). Although, AS infants appear normal, the syndrome manifests itself in feeding problems that typically arise during the 1st 2 months of life. Clinically, the condition is marked by developmental delay, communication deficits and difficulties in walking and standing for extended periods. This genetic disorder involves a microdeletion leading to the absence or inactivity of a certain group of genes that control ubiquitin, a protein present on chromosome 15q11-13. The abnormal chromosome is typically inherited but some cases seem to be caused by spontaneous genetic mutations. William's Syndrome (WS) is caused by a

microdeletion in chromosome 7q31. In 1961, it was identified as a distinct condition that causes severe MR (Williams *et al*., 1961; Donnai and Karmiloff-Smith, 2000). This condition has no affiliation with a specific socioeconomic group and it is equally present in males and females of all races. The common facial features among WS children are subtle but distinct: A tiny, upturned nose; a wide and full mouth; a small chin and distended skin around the eyes. The physical and facial features are so, subtle that it usually takes a trained eye to spot them at infancy. However, the symptoms become obvious with increasing age.

Laboratory based testing for MR: Karyotyping to obtain a complete chromosome profile is used as the first step in testing for generalised MR in cytogenetic laboratories. In 1971, Giemsa and Trypsin-based chromosome banding was introduced (Seabright, 1971). Since then, karyotyping has assisted in detecting structural aberrations such as deletions, duplications, translocations and inversions in patient samples. Improvements in banding resolutions and cell culture techniques have enabled observing 550-850 bands per haploid set for routine metaphase spreads (Yunis, 1976). As a direct consequence of these refinements, even smaller (approximately 5 Mb) chromosome imbalances are now microscopically detectable (Ahn *et al*., 2010; Manolakos *et al*., 2010).

Fluorescence *In Situ* Hybridisation (FISH) merges traditional cytogenetic methods with molecular technologies (Jobanputra *et al*., 2001; Ravnan *et al*., 2006). This method allows detecting submicroscopic deletions in MR patients. Chromosome sequence-specific FISH probes have been developed. They are used to detect submicroscopic deletions which allows investigating many microdeletion syndromes such as PWS, AS and WS.

Both karyotyping and FISH involve several non-automated, time-consuming, cumbersome steps that limit their throughput and demand greater manpower. Although, these techniques are highly diagnostic, their utilisation remains limited.

Comparative Genomic Hybridisation (CGH) can be used to overcome these limitations. Kallioniemi *et al*. (1992) first reported that CGH can detect and map changes in the copy number of DNA sequences (Kallioniemi *et al*., 1992). CGH is a versatile platform that can uncover known and novel chromosomal aberrations throughout the genome (Levy *et al*., 1998). Furthermore, high resolution array CGH (aCGH), a more refined platform has contributed to detecting genome-wide submicroscopic CNVs and has provided comprehensive coverage of all subtelomeric regions (Levy *et al*., 1998; Pinkel *et al*., 1998). The aCGH offers a flexible, high resolution format with potential for customisation. Several different oligonucleotide arrays, ranging from 25-85 oligomers in length are available. Some of these oligomers have been adapted from genome-wide SNP-based oligonucleotide markers and others have been created from a library of probes that span the genome and can be constructed to have extremely high resolution (Pinkel *et al*., 1998).

The aCGH has the advantage of being able to scan the entire genome in a single hybridisation. Differentially labelled test and reference gDNA samples are co-hybridised to metaphase chromosomes with high concentrations of unlabelled Cot-1 which blocks the repetitive sequences. Hybridisation of the two samples is measured as fluorescent intensity and these values are used to give an indication of the copy number for a particular chromosome region which is expressed as the ratio of the test sample to reference sample of approximately one. The regions where there is an additional copy or amplification in the test sample have strong fluorescent signals and the deleted regions have less-fluorescent signals (Shaikh, 2007). Since, its introduction, aCGH applications have proven useful for detecting large unidentified amplifications of genomic sequences in cancer and tumour cytogenetics (Albertson and Pinkel, 2003; Kallioniemi, 2008; Andre *et al*., 2009) and aCGH is also useful for prenatal diagnosis and *in vitro* fertilisation (Le Caignec *et al*., 2005; Sher *et al*., 2009).

Other advantages of this technology include detecting chromosomal mosaicisms that would otherwise be missed by conventional cytogenetic analysis (Ballif *et al*., 2006; Cheung *et al*., 2007). As with other clinical diagnostic methods however, aCGH has certain limitations. As this technology is only able to detect CNVs relative to other DNA regions within the same sample, it is unable to identify balanced rearrangements such as translocations and inversions. Thus, aCGH cannot be used to detect polyploidy. Furthermore, platforms that cover the entire genome at high resolution are expensive tools and have a higher risk of detecting insignificant and irrelevant genomic imbalances.

MATERIALS AND METHODS

Sample collection, gDNA extraction, quantification and quality assessment: Whole blood (3-4 mL) was collected in lavender-top tubes containing EDTA from two female patients (3 and 14 years old) at the DGMU of KAU after an informed written consent from their parents. Based upon their phenotypic features and previous karyotyping

and FISH results, the 3 years old patient (patient 1) was being treated for possible AS and the 14 years old patient (patient 2) was diagnosed with WS. Reference samples were also collected from four healthy male volunteers and these samples were pooled and divided into two fresh tubes.

Genomic DNA (gDNA) was extracted from all sample tubes using a Qiagen DNeasy™ Blood and Tissue mini column kit according to manufacturer's instructions. The extracted gDNA was checked for concentration and purity as both of these criteria are crucial for a successful aCGH assay. A Nanodrop 2000c (Thermo scientific) was used to ensure that the extracted gDNA was free from carbohydrates, proteins and traces of organic solvents including guanidinium isothiocyanate, alcohol and phenol. The Nanodrop also measured the integrity of the gDNA which showed minimal degradation. The gDNA with A_{260}/A_{280} and A_{260}/A_{230} ratios of 1.8-2.0 and >2, respectively was considered to be high-quality usable gDNA. The sample concentrations were recorded as ng μL^{-1}.

Microarray CGH protocols

The gDNA fluorescent labelling and purification: A reference gDNA sample (200 ng) was labelled with Cyanine 3-UTP (Cy3) and patient samples with an equal amount of gDNA were labelled with Cyanine 5-UTP (Cy5). The samples were labelled in independent Eppendorf tubes using the Agilent gDNA enzymatic labelling master mix which consisted of a reaction buffer, dNTPs, fluorescent probes and Exo-Klenow. Both the patient samples were prepared in duplicate to ensure reliability of the outcomes. The labelling process was performed by heating the samples at 37°C for 2 h on a thermocycler followed by spin vortexing. The samples were then stored on ice until the purification step.

The post-labelling purification of the samples was performed by washing the samples twice using a molecular grade 1×TE buffer (pH 8.0) and Amicon Ultracell-30 kDa membrane filter columns. The flow through was discarded after each wash and the purified labelled gDNA was collected after the final step of inverting and spinning the columns in clean 1.5 mL Eppendorf tubes.

To equate the gDNA concentrations, 1×TE was added to each sample to bring the total volume of each sample to 41 μL as recommended for the 2×105 K array CGH format. The A_{260}/A_{280} absorbance was re-measured for all of the samples to ensure equal gDNA concentrations. Each of the treated gDNA patient samples was mixed with an equal volume of reference gDNA to bring the total volume of mixed sample in each tube to 82 μL.

Sample hybridisation: The crucial hybridisation step began with preparing a master mix using the Agilent Oligo aCGH hybridisation kit. The master mix was prepared by sequential addition of human Cot-1 DNA followed by 10× blocking agent and a 2×Oligo aCGH hybridisation solution. The mix was added to each tube and was followed by spin vortex to allow thorough mixing. All of the tubes were then transferred to a thermo cycler to allow denaturation at 95°C for 3 min followed by incubation at 37°C for 30 min.

A clean gasket was loaded onto the Agilent SureHyb chamber base according to the manufacturer's protocol and 245 μL of each sample mixture was carefully dispensed into the gasket well in a drag and dispense manner. The 2×105 K microarray slide was then placed with the active side facing downwards and in contact with the gasket well. The sandwich chamber was properly aligned, covered with a SureHyb chamber and secured by clamps. The assembled chamber was subjected to a hybridisation oven at 65°C and a rotation rack adjusted to 20 rpm for 40 h.

Post-hybridisation wash and microarray scanning: Procedure B in the manufacturer's protocol for using the stabilisation and drying solution was employed. Fresh Oligo aCGH wash buffer 1 and Oligo aCGH wash buffer 2 were used to protect the Cy5 which is susceptible to degradation by ozone. A slide-staining dish, slide rack, magnetic stirrer bar, heated plate and acetonitrile were used to carefully wash the microarray slide after separating it from the gasket chamber while minimising atmospheric exposure. The dried array was then scanned using the Agilent microarray scanner bundle.

Microarray data analysis and interpretation: The data were extracted using the Feature Extraction software V.9.5.3.1 and were analysed using the Genomics Workbench software. The analysis was performed using the ADM-2 algorithm with a sensitivity threshold of 6.0 and a moving average window of 0.2 Mb. Variations with less than three consecutive probes and a log ratio <0.1 were excluded. The hybridisation quality was assessed using the feature extraction Quality Control (QC) report which sets the derivative log 2 ratio spectrum to be <0.3. The annotations were based on the human genome build 18.

RESULTS AND DISCUSSION

Samples from the Saudi female patients with unexplained mental retardation and features suggestive of chromosome anomalies were analysed by aCGH. The patients initially visited the DGMU for cytogenetic

diagnosis of clinical features resembling AS and WS. Both the patients showed normal karyotypes using conventional karyotyping procedures.

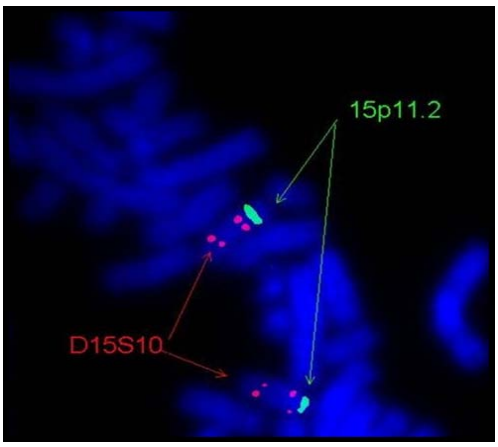

Fig. 1: FISH analysis of the proband in patient 1 using a fluorescent probe (labelled red). AS could not be confirmed due to the absence of a deletion at the critical p11.2 breakage region on chromosome 15

Patient 1: This patient was a 3 years old female with clinical features resembling AS who was submitted to DGMU/CEGMR on suspicion of a chromosome 15 deletion.

No characteristic deletion at the expected p11.2 region had been found in 100 metaphase and interphase analyses performed by the FISH technique using probes for the SNRPN and D15S10 loci (Fig. 1). The FISH result reported for this patient was: Ish 15 q11.2 (SNRP×2) (D15S10×2). The aCGH analysis of this sample and its duplicate using the 2×105 Agilent array format did not reveal any deletions in chromosome 15 (Fig. 2) as should have been the case in AS. Nevertheless, the aCGH confirmed and validated the FISH finding.

However, some non-specific chromosomal aberrations were also observed in other chromosomes. In chromosomes 4, 6 and 17, these aberrations appeared as interstitial deletions in which loss of the patient's gDNA was observed (Fig. 3). Moreover, non-specific interstitial amplification in various regions of chromosomes 3, 8, 11, 14, 16, 17 and 19 were also observed. An example of such amplification in chromosome 17 is shown in Fig. 4. Data analysis using the Genome Workbench software (Agilent)

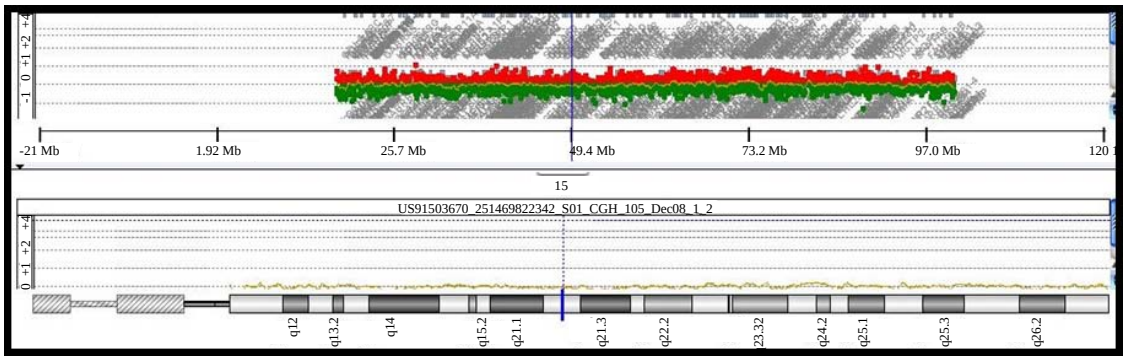

Fig. 2: The aCGH profile of chromosome 15 for patient 1 showing balanced Cy3 and Cy5 signals representing equal hybridisation in the reference and patient sample mix

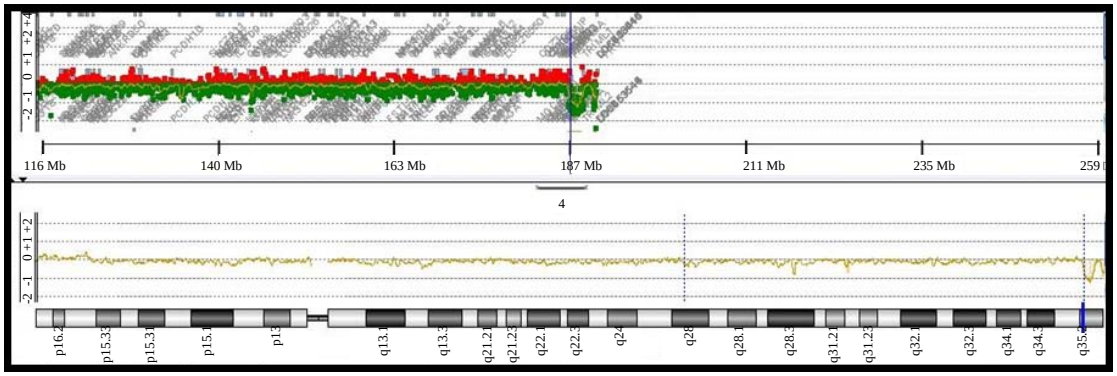

Fig. 3: The aCGH profile for patient 1 showing a decrease in the Cy5 signal corresponding to a loss of gDNA in the patient sample due to interstitial deletion in chromosome 4

Table 1: The comprehensive genome-wide data for patient 1 showing the deletion and amplification levels

Chr	Cytoband	Start	Stop	Probes	Amplification	Deletion	Gene involved
Chr3	q26.1	163997028	1.64E+08	4	0.821483	0	-
Chr3	q27.1	185366510	1.86E+08	33	0.410519	0	DVL3, AP2M1
Chr4	q31.22	145061838	1.45E+08	7	0	-0.6684	GYPB
Chr4	q35.2	187504814	1.89E+08	71	0	-0.9006	MTNR1A, FAT1
Chr4	q35.2	190823273	1.91E+08	7	0	-0.8595	-
Chr6	p21.32	32595202	32633891	3	0	-1.7171	HLA-DRB5, HLA-DRB6
Chr8	p11.23	39356395	39499952	7	1.247605	0	ADAM5P, ADAM3A
Chr11	p15.5-p15.4	186766	3635036	191	0.32836	0	ODF3, BET1L
Chr14	q32.33	104962036	1.05E+08	21	0.624072	0	MTA1, CRIP2
Chr16	q22.1	68710077	68751590	3	1.075632	0	PDPR
Chr17	q21.31-q21.32	41706670	42049740	4	0	-0.793	LRRC37A, ARL17
Chr17	q25.1-q25.3	71355077	78653860	341	0.773838	0	WBP2, TRIM47
Chr19	p13.3-p13.11	258517	19633833	982	0.325128	0	MIER2, THEG

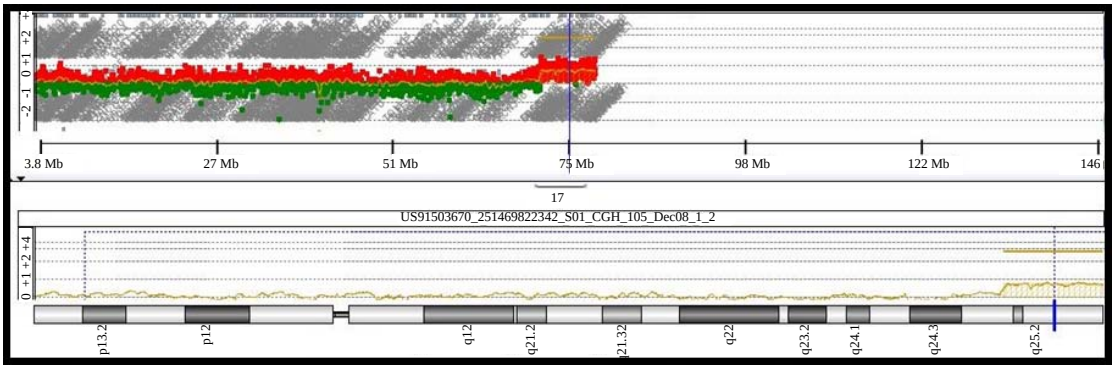

Fig. 4: The aCGH profile of chromosome 17 for patient 1 showing a gain in the Cy5 signal due to the non-specific interstitial amplification of gDNA

revealed a number of genes that spanned the regions of these chromosomal aberrations in patient 1 and this data is shown in Table 1. The duplicate samples produced the same data with minor differences in signal values.

Patient 2: This patient was a 14 years old female with clinical features resembling WS who was submitted to DGMU/CEGMR for molecular diagnosis. The karyotyping and FISH were performed at DGMU/CEGMR. Based on 20 metaphase analyses using standard cytogenetics, a female karyotype with a derivative of chromosome 19 resulting from a t (18;19) translocation was found. The karyotype was 45, XX, der (19) t (18;19) (q11.1;p13.3) del (10) (q11.11). Based on 50 metaphase and interphase analyses using the FISH technique and an LSI William's syndrome region probe, a deletion was observed in chromosome 7 (Fig. 5). The FISH result was Ish 7q11.23 (ELN×2, Limk1×2 and D7S613×2).

The aCGH analysis of this sample and its duplicate using the 2×105 Agilent array format revealed a deletion in chromosome 7 (Fig. 6) which was expected in this selected case of WS. However, this finding not only confirmed and validated the FISH result but also revealed some non-specific aberrations in other chromosomes.

The aCGH profile clearly pointed to deletions in chromosomes 6, 7, 9, 14, 15 and 19 where loss of the Cy5

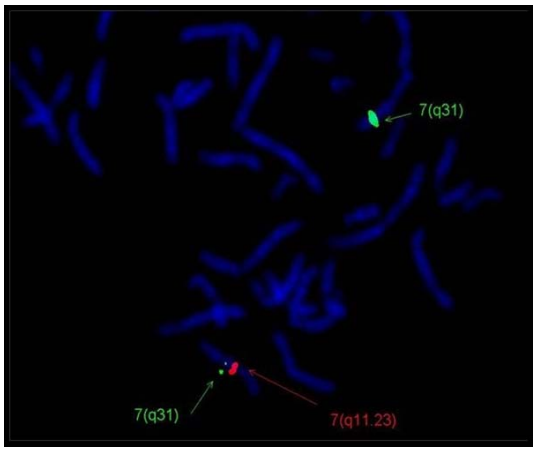

Fig. 5: The FISH analysis of the proband for patient 2 using a fluorescent probe (labelled red). WS was confirmed by the detection of a breakage point at the critical q31 region in chromosome 7

probe was observed. An example of a non-specific interstitial deletion that occurred in chromosome 15 is shown in Fig. 7. Non-specific amplifications in chromosomes 1, 3, 11, 12, 14 and 22 were also observed. An example of a non-specific interstitial amplification that

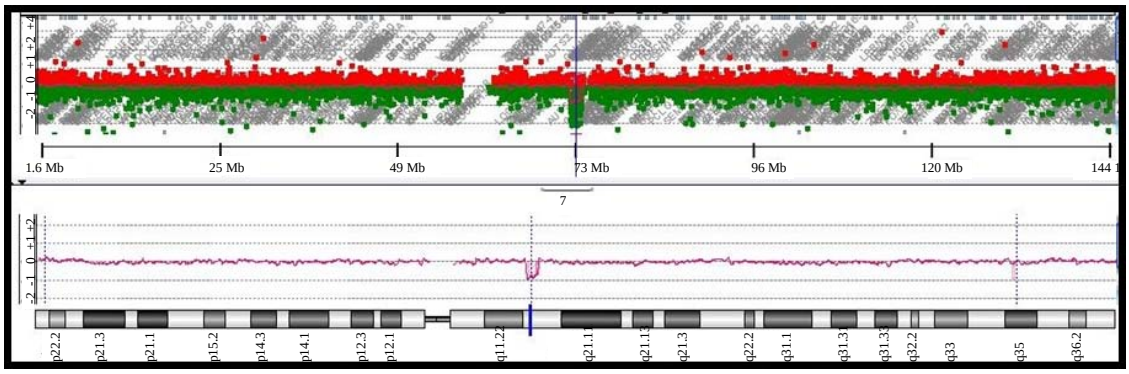

Fig. 6: The aCGH profile of chromosome 7 for patient 2 showing loss of the Cy5 signal due to deletion of the gDNA in the critical region of the patient sample

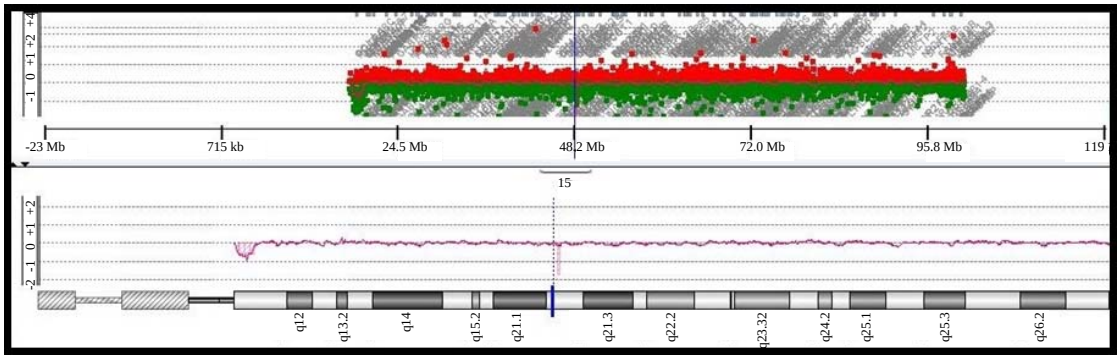

Fig. 7: The aCGH profile of chromosome 15 for patient 2 showing a non-specific interstitial deletion

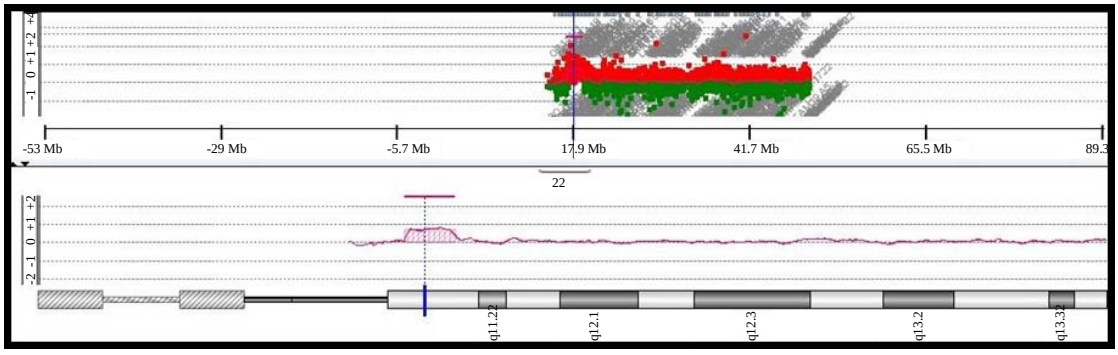

Fig. 8: TheCGH profile of chromosome 22 for patient 2 showing gain in the Cy5 signal due to gDNA amplification

occurred in chromosome 22 of patient 2 is shown in Fig. 8. Data analysis using the Genome Workbench software (Agilent) revealed a number of genes that spanned the regions of these chromosomal aberrations in patient 2. This data is shown in Table 2.

Deletion and duplication syndromes represent recurrent chromosomal abnormalities that are associated with distinct phenotypes. Only identifying similar genomic imbalances with a recognisable phenotype can help clarify the role of these genomic changes in causing the specific clinical features that ultimately define a genetic syndrome. There have been improvements in the clinical interpretation of genomic aCGH results from individuals with rare genetic disorders which has led to the rapid characterisation of new genomic syndromes. The list of novel syndromes caused

Table 2: The comprehensive genome-wide data for patient 2 showing the level of deletions and amplifications

Chr	Cytoband	Start	Stop	Probes	Amplification	Deletion	GeneInvolved
Chr1	p36.33	862319	1556626	117	0.368994	0	SAMD11, NOC2L
Chr1	q21.3	150822873	150853058	6	1.394593	0	LCE3C, LCE3B
Chr1	q44	246822638	246859020	6	0.885928	0	OR2T10, OR2T11
Chr3	q29	196830070	197006478	17	0.512623	0	SDHALP2, MUC20
Chr6	p12.1	55959274	56047133	7	0	-1.00689	COL21A1
Chr7	q11.23	72364314	73780404	176	0	-0.67931	TRIM50, FKBP6
Chr7	q11.23	73567628	73640067	12	0	-0.11797	GTF2IRD1, GTF2IRD1
Chr7	q35	143582664	143584550	3	0	-0.93082	-
Chr9	p13.1	39141496	39244529	5	0	-0.82147	CNTNAP3
Chr11	p15.5-p15.4	263086	3610640	392	0.224666	0	NLRP6, ATHL1
Chr12	p13.31	9446482	9613274	6	0.877463	0	DDX12
Chr14	q11.2	19216169	19490830	31	0.854559	0	OR4Q3, OR4M1
Chr14	q11.2	21635228	22034904	49	0	-0.3629	-
Chr15	q11.2	18790389	20250086	67	0	-0.61047	LOC727832, GOLGA8C
Chr15	q21.2	48665658	48678946	3	0	-1.66617	TRPM7
Chr19	p12	22045505	22143143	8	0	-1.07904	ZNF257
Chr22	q11.21	17041524	19396082	257	0.713969	0	GGT3P, DGCR6

Table 3: The novel deletion syndromes classified by aCGH

Condition	Size	Array	Clinical features or main findings
15q13.3	1.5 Mb	WG, targeted to segmental dup: 2007 BACs	Mild dysmorphic features, MR and seizures
15q24	1.7-3.9 Mb	Targeted Nimble Gen (147 bp)	FTT, microcephaly, digital abnormalities, hypospadias and loose connective tissue
16p11.2-p12.12	7.1-8.7 Mb	Clinical aCGH (Signature Chip); Nimble Gen; Affymetrix 250 K SNP	DD and DF (Flat faces, down slanting palpebral fissures, low-set and malformed ears and eye anomalies
21q22.12	Minimal overlapping del.: 0.7 Mb	Clinical aCGH (Baylor, V.5, 6.3); Agilent 244 K	Syndromic thrombocytopaenia, acute myelogenous leukaemia, FTT, DD
17q21.31	600 kb	Tiling WG: 32,477 BAC clones	Moderate MR, hypotonia and DF (Ptosis, blepharophimosis, abnormal ears, tubular nose, long columella and a broad chin)

CNVs that have been detected using aCGH is continuously growing (Table 3) (Koolen *et al*., 2006; Ballif *et al*., 2007; Sharp *et al*., 2007; Shinawi and Cheung, 2008). In the study, the researchers aimed to establish and validate high resolution aCGH technology and to assess its potential as a diagnostic tool for Saudi mental retardation cases. The study was also intended to detect known and novel CNVs and to compare the sensitivity, specificity, reproducibility and resolution of the aCGH and FISH techniques. Selected samples of young Saudi females suffering from mental retardation (one patient was suspected of having AS and the other had a confirmed WS diagnosis) were used for this purpose. AS and WS are characterised by deletions in chromosome 15 and chromosome 7, respectively. As expected, the aCGH results revealed a deletion in chromosome 7 in the WS patient (patient 2) (Fig. 6). This result was in complete agreement with the FISH data obtained at DGMU/CEGMR which found a deletion in chromosome 7 using specific probes for the critical q31 chromosome region (Fig. 5). This data indicated that the FISH and aCGH techniques are compatible with each other when identifying deletions in chromosome 7. The reliability and reproducibility of the two techniques were comparable although, aCGH had much better resolution. Moreover, deletions and amplifications in other chromosomes were observed by the aCGH. The sample for patient 1 also showed comparable results between the FISH and aCGH techniques with an overall negative result (Fig. 2). This study demonstrated that genome-wide scans can reveal new chromosomal aberrations that have not been previously associated with disorders such as AS and WS. The aCGH maps DNA CNVs to positions in the genome by detecting the gain and loss of gDNA in different disease states. New and established microdeletion/ duplication syndromes can be diagnosed by aCGH. These observations are supported by an enormous amount of existing evidence from the literature. In microduplication syndromes, segmental duplications mediate genomic rearrangements that are responsible for many of the well-known microdeletion syndromes (Lupski, 1998). A majority of aCGH studies have reported the number of deletions to be greater than the number of duplications, potentially reflecting an ascertainment bias caused by a milder phenotype in the syndromes involving duplications (Shinawi *et al*., 2008). Some studies have revealed that the long arm of chromosome 15, essentially the 15q11.2-q14 Prader-Willi Syndrome/Angelman Critical Region (PWS/ACR) is highly susceptible to clinically important genomic rearrangements including interstitial deletions, duplications and triplications (Ungaro *et al*., 2001; Horsthemke and Wagstaff, 2008). The duplications

reported to date usually share two proximal breakage points (BPs, BP1 and BP2), one distal breakage point (BP3) and include the PWS/ACR. More distal BPs (BP4 and BP5) are involved in large inversion duplications and intrachromosomal triplications (Ungaro *et al*., 2001; Vialard *et al*., 2003). The frequency of 15q11-q13 interstitial duplications is estimated at 1 in 600 individuals and is associated with developmental delay (Thomas *et al*., 2003). Similar to the case of most solid tumours, it is well known that MBC progression to malignancy is the result of a multistep process in which genomic DNA alteration plays an important role in gene expression of key downstream cellular processes. The application of DNA microarray technology to conventional CGH technologies allows reliable high-resolution mapping of unbalanced genetic alterations such as DNA gains and losses, to be achieved (Wolf *et al*., 2004). Moreover, identifying the impact of DNA CNVs on gene expression allows new insights for detecting new diagnostic and/or prognostic markers (Ballif *et al*., 2006; Mangia *et al*., 2008).

CONCLUSION

The researchers were prompted to conduct this study by the several advantages that aCGH has compared to other conventional cytogenetic and molecular cytogenetic methods. This study confirmed the following characteristics of the aCGH technique: High resolution, high throughput, robustness, simplicity, high reproducibility and precise mapping of aberrations. Only a few micrograms of gDNA were required for whole-genome amplification and CNV screening in the MR samples. In addition, various other interstitial deletions and amplifications were detected that normally cannot be identified by metaphase or even interphase FISH analyses. This result further confirmed that aCGH as an ideal tool for diagnosing and characterising complex multigene disorder.

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