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Gene copy number variations in Asian patients with congenital bilateral absence of the vas deferens

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BACKGROUND: Congenital bilateral absence of the vas deferens (CBAVD) is a distinct clinical entity accounting for ~25% of obstructive azoospermia in infertile men. The association between CBAVD and mutated *CFTR* (cystic fibrosis transmembrane conductance regulator) alleles is well demonstrated in Caucasians, but the identity of CBAVD-susceptibility genes remains elusive in Asians. We investigate genomic copy number variations (CNVs) in a patient cohort of Taiwan.

METHODS AND RESULTS: Genome-wide screening for genetic CNVs was conducted on eight individuals with CBAVD using array-based comparative genomic hybridization. One recurrent CNV was detected on 3q26.1 in five patients, and another was detected on a reproduction-related gene PANK2 in two patients. For the former, we further characterized the breakpoints in CBAVD and assessed the incidence in healthy individuals by tiling path arrays. The deletion in each patient was confirmed, and seven out of the eight controls were also affected. Examination of the homozygous loss of *PANK2* by PCR in a larger cohort showed a homozygous deletion in only one of the 26 CBAVD males, and not in any of the 20 azoospermic patients without CBAVD, nor in any of the 16 control subjects.

CONCLUSIONS: Our results suggest that 3q26.1 may not be a critical region for CBAVD. Additionally no strong association was found for *PANK2* in this reproduction disorder. Other reproduction-related genes, such as *PBX1*, *BRD3*, *COL18A1* and *HMOX1*, identified by this initial study may inspire further investigation.

Key words: array-based comparative genomic hybridization / copy number variations / congenital bilateral absence of the vas deferens / 3q26.1 microdeletion / tiling path array.

Introduction

Congenital bilateral absence of the vas deferens (CBAVD) is a distinct clinical entity affecting 1–2% of infertile men (Holsclaw *et al*., 1971) and is the most common cause of obstructive azoospermia in patients who have not undergone elective sterilization (Jarow *et al*., 1989). This reproductive tract disorder also occurs in a mild form of cystic fibrosis (CF) (Holsclaw *et al*., 1971; de la Taille *et al*., 1998). CF, a progressive lung disease that is characterized by mutations of the CF transmembrane conductance regulator (*CFTR*) gene (Riordan *et al*., 1989), accounts for the most common autosomal recessive disorder in Caucasians. In the last decade, the linkage between the phenotypic and genotypic abnormalities of CBAVD has been investigated using various mutation detection methods. The results showed that the *CFTR* gene carries genetic variations in 50–74% of the alleles tested (Patrizio and Zielenski, 1996; de Meeus *et al*., 1998; Claustres *et al*., 2000; Josserand *et al*., 2001; Wang *et al*., 2002; Dayangaç *et al*., 2004). However, the involvement of the *CFTR* gene in this infertility disorder is not observed in Asian populations (Welsh *et al*., 2001; Wong *et al*., 2003). A recent survey of the mutation spectrum of the *CFTR* gene among 36 Taiwanese infertile men with CBAVD was performed using temporal temperature gradient gel electrophoresis mutational analysis. The results showed that the number of mutations was limited that the most common mutation IVS8-5T accounted for 81% of the mutations identified, and that the most mutant alleles (64%) remained unknown (Wu *et al*., 2005), leaving the causes for the majority of affected Taiwanese individuals unknown. Based on these previous reports, we postulated that the underlying cause of Taiwanese CBAVD may be distinct from those of Europeans.

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Microarray-based comparative genomic hybridization (array-CGH) is a high-throughput genomic analysis technology that enables efficient screening for copy number variations (CNVs). This technology is well documented in cancer research to identify amplicons and genome instability related to carcinogenesis (Yao *et al*., 2006; Lai *et al*., 2007). Additionally, array-CGH is also suitable for the analysis of DNA number aberrations that cause human genetic disorders. Several studies using array-CGH have detected subtle genomic CNVs for 15q21 syndrome (Pramparo *et al*., 2005), X-linked hypopituitarism (Solomon *et al*., 2004), X-linked mental retardation (Bauters *et al*., 2005) and language disorder (Murthy *et al*., 2007). In the present study, we employed a commercially available oligonucleotide array-based CGH (Brennan *et al*., 2004) for genome-wide mapping of CNVs in Taiwanese men with CBAVD, followed by PCR analysis. All the patients had been diagnosed with CBAVD based on physical examination of the scrotal contents, followed by semen analysis and transrectal ultrasound for providing further evidence for confirmation. In addition, impaired sperm production and testis failure were ruled out as causes of infertility by examining spermatogenesis markers (serum FSH, LH, Testosterone and Prolactin levels) prior to sperm retrieval.

Materials and Methods

Control individuals

To obtain genomic DNA for use as a reference, healthy Taiwanese men were recruited to contribute DNA samples. Since several hereditary diseases do not affect carriers until adulthood, we set the age criteria of the normal controls to 30 to rule out subjects who look 'healthy' but have genetic defects. We also excluded individuals with diabetes mellitus, major systemic disorders, psychiatric disorders or neurological disorders (such as epilepsy), mental retardation, facial dysmorphysm and clinical evidence of brain, trunk or limb anomalies, from the control recruitment since people affected by these disorders may carry genetic defects.

After giving informed consent, 10 participants underwent screening interviews followed by the withdrawal of blood samples for DNA extraction and analyses. The reference DNA pool was obtained by pooling equal amounts of chromosomal DNA extracted from the lymphocytes of the 10 healthy men. Reference samples for array-CGH analysis were taken from this normal DNA pool.

Assessment and recruitment of CBAVD patients

Men with infertility were referred to us for diagnosis at Taipei Medical University Hospital in Taipei, Taiwan. The diagnosis of CBAVD was based on physical examination of the scrotal contents; men with the absence of palpable vas deferens on both sides, but with normal testes size (long axis >2 cm) were selected for further screening. Clinical examinations for CF symptoms did not identify any classic CF symptoms in any of the patients. Every patient provided detailed clinical and family histories. In addition to routine semen analysis, special examination for semen pH and fructose content was performed to confirm the CBAVD diagnosis. A total of 26 patients, V1–V26, underwent transrectal ultrasonography for the evaluation of morphology and size of the seminal vesicles, prostate and ejaculatory ducts. We also performed renal ultrasonography to assess the existence and outlines of both kidneys and hormonal assays and chromosomal analyses to rule out testicular azoospermia.

After informed consent was obtained from each participant, blood samples were withdrawn and genomic DNA was extracted for analysis.

Genomic DNA extraction

Genomic DNA was prepared from peripheral blood lymphocytes using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Array-based CGH analysis

A microarray containing over 44 000 oligonucleotides probes designed to cover coding, non-coding and intergenic sequences in the human genome (human genome CGH microarray 44B, Agilent Technologies, Palo Alto, CA, USA) was used for array-CGH analysis. Genomic DNA fragmentation, labelling and array hybridization were performed according to the standard array-CGH protocol (version 3) provided by Agilent Technologies as previously described (Lee *et al*., 2008). To rule out probable normal copy number polymorphisms (CNPs) (Sebat *et al*., 2004), which are more likely related to ethnicity, array hybridization experiment was performed with DNA pools from the healthy Taiwanese men and from healthy Caucasian men (Promega, Madison, WI, USA).

The hybridized arrays were scanned using a G2565B DNA microarray scanner (Agilent Technologies), and the microarray images were analyzed using FEATURE EXTRACTION software (version 8.1.1, Agilent Technologies) as previously described (Lee *et al*., 2008).

Tiling path CGH arrays analysis

We performed tiling path array analysis using an Agilent system custom oligonucleotide Chromosome 3 array (HG18; NCBI build 36) covering 0.068948–199.324720 Mb designed under the following specifications: isothermal probe (45–60 bp) with target T_m of 85 and average interval spacing of 14.17 kb. Reference and test sample genomic DNA (250 ng each) was fluorescently labelled using the Agilent ULS Genomic DNA Labelling Kit (Agilent Technologies). For this study, each control DNA sample and CBAVD patient DNA sample was used as an independent test sample against a commercial reference DNA pool of Caucasian men (Promega). All hybridization and array scanning were performed as previously described (Lee *et al*., 2008).

Quantitative real-time PCR

The fold change of *PANK2* gene copy numbers were determined by real-time PCR using the LightCycler FastStart DNA Master^{PLUS} SYBR Green I kit (Roche Molecular Systems, Inc., Branchburg, NJ, USA) and the LightCycler Instrument 1.5 (Roche Molecular Systems). Quantification was performed by comparing the target locus to the reference *ACTB* (encoding beta-actin). The relative *PANK2* copy number level was normalized to normal control genomic DNA as calibrator. The change in copy number of the *PANK2* gene relative to the *ACTB* and the calibrator were determined by using the formula $(PANK2_{CBAVD}/ACTB_{CBAVD})/(PANK2_{control}/ACTB_{control})$ as described previously (Sasaki *et al*., 2007), where $PANK2_{CBAVD}$ and $ACTB_{CBAVD}$ are the quantity from the CBAVD sample. The cycling conditions were as follows: pre-incubation at 95°C for 10 min, 40 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 10 s. The specific oligonucleotide primer pairs were selected from the Universal Probe Library (Roche Molecular Systems), and the specificity of each primer pair was verified. The primers were

PANK2, 5′-agtgtggtcctgcgacttctaag-3′, 5′-gctcaaatttgtacgctccac-3′.

ACTB, 5′-acttctcgagtctccacactgtc-3′, 5′-cctgggcttgagaggtagagtgt-3′.

The mean threshold cycle number (C_t) for each gene in each sample was obtained from triplicate experiments.

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Assay for homologous loss of PANK2

The presence of *PANK2* allele was determined using PCR followed by electrophoresis. Heterozygous and homozygous losses of *PANK2* were readily distinguishable by the presence of 215 bp amplified fragment. PCR was carried out using 10 ng genomic DNA as a template in a 50 μl reaction mixture containing 1.25 U of Tag DNA polymerase (Roche Molecular Systems), 250 μM dNTPs, 10 pmol of each sense (5′-gagaccctgtctccg ttttt-3′) and antisense (5′-gtgcctgaagataccccaaa-3′) primer and PCR buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl pH 8.3). Details of PCR conditions were as follows: initial denaturation at 95°C for 10 min was followed by 35 cycles of 94°C for 45 s, 58°C for 45 s and 72°C for 45 s and ended by an extension at 72°C for 7 min. The *GAPDH* gene used for internal control was amplified under the same PCR conditions with primers (sense: 5′-cctgccaaatattgatgacatcaag-3′; antisense: 5′-accctgttgctgtagccaaa-3′). Amplified products were electrophoresed on a 3% agarose gel. The absence of a specific amplified fragment was interpreted as homozygous loss of *PANK2*.

Results

From a total of eight Taiwanese men affected with CBAVD, genomic DNA derived from peripheral lymphocytes was extracted and subjected to genome-wide array-CGH analysis using a commercially available oligonucleotide array that offers an average resolution of approximately of 35 kb. A total of 66 CNVs with high aberrant scores (<-1 or >1) were found: 36 CNVs were assigned to known genes, 13 CNVs were assigned to intergenic regions and 17 were assigned to anonymous genes (function unknown). To filter out of the CNVs that are more likely to be related to ethnicity, the five CNPs were identified from the reference array which was performed with a normal male DNA pool in our laboratory (as described in Materials and Methods). Thus, *PXT1* (6p21.31, STEPP), *RAB11FIP3* (16p13.3, KIAA0665, Rab11-FIP3, eferin), *SOLH* (16p13.3, CAPN15), *CRYAA* (21q22.3, HSPB4) and *C21orf57* (21q22.3) were excluded from subsequent data analysis. Additionally, the CNVs that have been reported among healthy controls on the Database of Genomic Variants (http://projects.tcag.ca/variation) website (Iafrate *et al*., 2004; Redon *et al*., 2006; Wong *et al*., 2007) were also excluded. Finally, five out the 14 remaining CNVs for known genes were referred to as reproduction-related, according to an online search for the relevant literature on PubMed (http://www.ncbi.nlm.nih.gov) using the keyword 'reproduction'. The five reproduction-related genes identified were *PBX1* (Schnabel *et al*., 2003), *BRD3* (Boyer *et al*., 2004; Shang *et al*., 2004), *PANK2* (Kuo *et al*., 2005, 2007), *COL18A1* (Nasu *et al*., 2003) and *HMOX1* (Ozawa *et al*., 2002; Watanabe *et al*., 2004; Shiraishi *et al*., 2005), which mapped to 1q23.3, 9q34.2, 20p13, 21q22.3 and 22q12.3, respectively (Table I). *PANK2* was the only reproduction-related gene deleted in two CBAVD patients (V3 and V8) of this small study. There was no reproduction-related gene affected in Patients V1, V4 and V7. The detailed information on CNVs of reproduction-irrelevant or anonymous genes in these eight patients is provided in Table II. In Table III, we also summarized the intergenic sequences that showed CNVs. We noted that the genomic imbalance involving intergenic sequences located on chromosome 3 around 163.997 Mb (HG18) is prevalent (five of eight) in the CBAVD patients analyzed. The representative array results for *PANK2* deletion and 3q26.1 microdeletion are shown in Fig. 1A and B, respectively.

All of the five reproduction-related genes were selected for qPCR analysis, but only the copy numbers of *PANK2* were cross-validated in all samples (V1–V8), as shown in the Fig. 2. Thus, we choose this reproduction-related gene for further investigation. To evaluate the association of copy number loss in *PANK2* with CBAVD, we recruited another 11 subjects with CBAVD, Patients V9–V19, for quantitative PCR assessment. According to the interpretation of normalized gene dosage ratios described in Fig. 2, *PANK2* seemed to be affected by homozygous loss in three patients V3, V8 and V16, while variations were not observed in 10 healthy controls analysed.

To examine whether the losses of *PANK2* are homozygous or heterozygous in affected individuals and to ascertain the association with CBAVD, we performed PCR followed by electrophoresis in 16 normal controls and 26 CBAVD subjects (V1–V26). In addition, we also recruited 20 azoospermia patients without CBAVD with an intent to examine whether *PANK2* is involved in spermatogenesis or in the development of the vas deferens. Homozygous deletion of PANK2 was detected in only one of the 26 (3.85%) CBAVD patients, whereas no deletions were detected in any cases of control individuals or azoospermic patients tested (Fig. 3).

To better map the deletion breakpoints of 3q26.1 in CBAVD and assess the frequency of this microdeletion among unaffected individuals, we investigate seven of the original CBAVD subjects (V2–V8) and eight control subjects using a chromosome 3 tiling path microarray comprised of ~15 000 oligonucleotide probes. All patients with this microdeletion, V3, V4, V6, V7 and V8 were validated. However, we

Table I Summary of the affected reproduction-related genes among CBAVD males in Taiwan

The CNVs of reproduction-related genes in CBAVD									
Cytogenetic location	**Gene symbol**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
1q23.3	PBX1					−2.67			
9q34.2	BRD3		−3.15						
20p13	PANK2			−2.22					−3.5
21q22.3	COL18A1			−1.59					
22q12.3	HMOX1						2.59		

The number indicates the score of CNVs for the eight CBAVD individuals (V1–V8): no change is defined by $n = 0$, high score loss is defined by $n < -1$ and high score gain is defined by $n > 1$. *PBX1* (pre-B-cell leukaemia transcription factor 1), *BRD3* (bromodomain containing 3), *PANK2* (pantothenate kinase 2), *COL18A1* (collagen, type XVIII, alpha 1) and *HMOX1* (heme oxygenase 1).

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Table II Summary of the affected reproduction-irrelevant genes among CBAVD males in Taiwan

The CNVs of reproduction-irrelevant or anonymous genes in CBAVD									
Cytogenetic location		**Gene symbol**	**V1**	**V3**	**V2**	**V4**	**V5**	**V6**	**V7**	**V8**
Chr2	p21	TACSTD1			−2.63					
	q11.2	LIPT1							−3.16	
Chr5	q22.2	C5orf18							−3.13	
Chr6	p21.2	KCNK16							−2.84	
		C6orf102							−2.84	
	q23.2	VNN3			−3.15					
Chr7	p22.3	MGC9712		−1.42						
		MGC10911		−1.42						
		KIAA1908		−1.42						
chr8	q21.11	FLJ11011							−3.36	
chr9	q34.3	MGC20 262				1.57		2.78		
		ENST00000361063				1.57				
chr11	p15.5	THC2056818			−2.47					
chr13	q14.2	CHC1L			−2.44					
chr14	q13.3	NKX2-8		−3.21						
	q32.33	SIVA		−1.23						
		THC2199436		−1.23						
		C14orf175		−1.23						
		C14orf79		−1.23						
chr16	p11.2	KIAA0339		−2.06						
	q24.2	ZNF469			−2.35					
	q24.3	LOC348180		−1.01						
		FLJ40 448		−1.01						
chrX	p21.3	BC024027	−3.17							
	p11.3	ZNF157					−3.5			
	p11.21	MAGED2					−3.34			

Their score of copy number alteration in the eight individuals (V) is displayed by a numbered grade of alteration (*n*) as described for Table I. The affected genes of known function are: TACSTD1 (tumour-associated calcium signal transducer 1), LIPT1 (lipoyltransferase 1), KCNK16 (potassium channel, subfamily K, member 16), VNN3 (vanin 3), THC2056818 (HTH-type transcriptional regulator budR), NKX2-8 (NK2 transcription factor related, locus 8), SIVA (CD27-binding (Siva) protein), ZNF157 (zinc finger protein 157), MAGED2 (melanoma antigen, family D, 2).

Table III Summary of affected intergenic sequences among CBAVD males in Taiwan

The CNVs of Intergenic sequences in CBAVD										
Cytogenetic location (Mb)			**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
Chr 1	q25.2	176.938320–176.938379			−2.36					
Chr 3	p36.12	24.564676–24.564735								−4.55
	q26.1	163.997228–163.997287			−4.32	−5.75		−5.61	−4.88	−3.98
	Qter	196.270291–196.270350	2.9		−3.24			−2.33	−2.29	
Chr 5	q14.3–21.1	96.406507–96.406566								−2.86
Chr 11	q13.2	66.988388–66.988447		−2.43						
Chr 12	Pter	1.919511–1.919570						−3.3		
Chr 13	q14.12	44.338530–44.338589								−2.84
Chr 15	q12	23.860478–23.860537								−3.02
Chr 16	p13.2–13.12	11.499205–11.499264								−2.79
Chr 19	Pter	0.737550–0.737609						−3.15		
Chr 20	q13.12–13.2	47.532907–47.532953		−2.34						
Chr Y	q11.223	23.283689–23.283748						−2.33		

Their score for copy number alteration in the eight individuals is displayed by a number as described in Table I. [a]The chromosomal positions (HG18; NCBI build 36) of probes.

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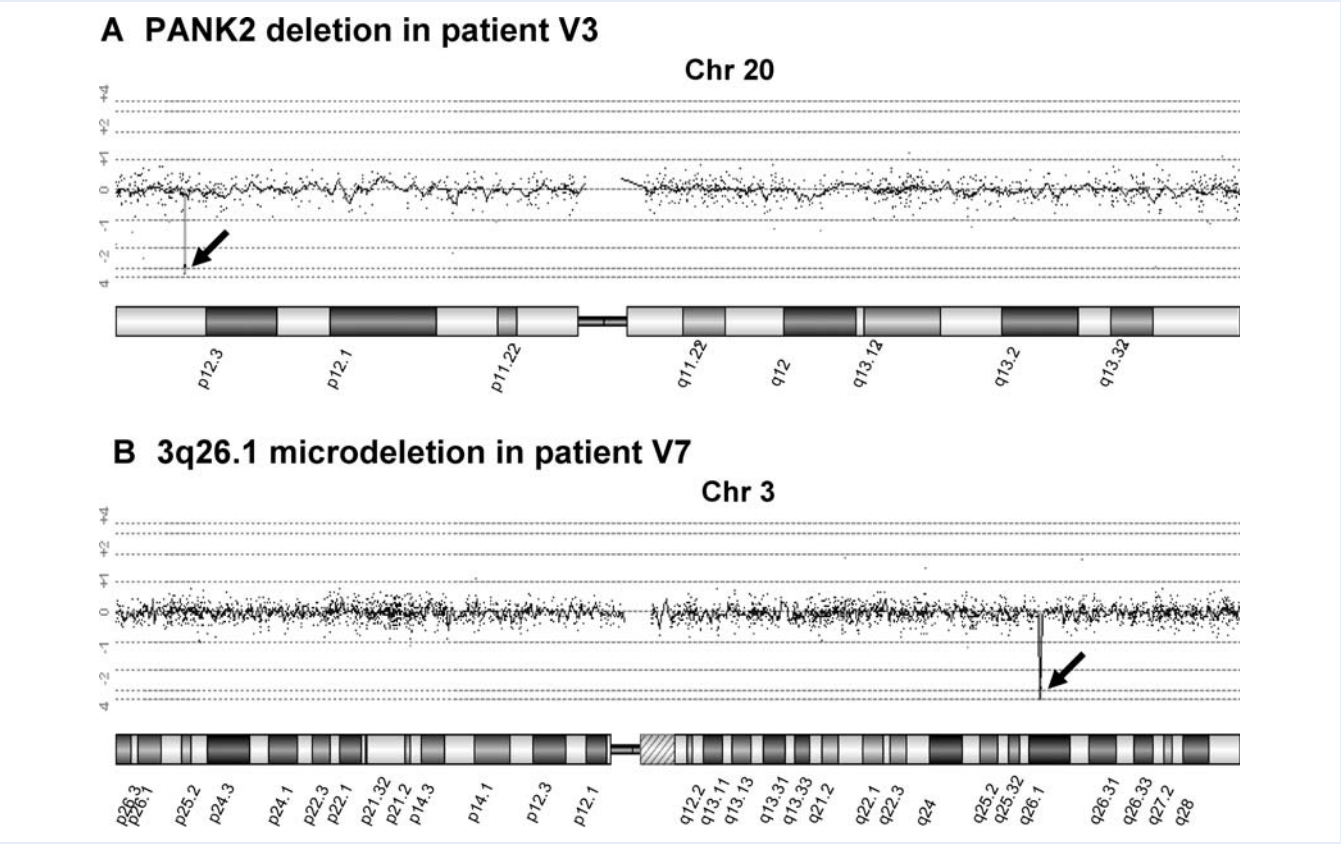

Figure 1 Genomic copy number variations in CBAVD.
(**A**) PANK2 deletion in CBAVD. The array-CGH profile is shown adjacent to an ideogram of chromosome 20. (**B**) 3q26.1 microdeletion in CBAVD. The array-CGH profile is shown adjacent to an ideogram of chromosome 3. The array results show the log2 ratio of the reference versus patient DNA on the Y-axis. The X-axis shows the position of each probe along the chromosome. Arrows in (A) and (B) indicate the aberrant loci mapping to PANK2 and 3q26.1, respectively. Images were produced using CGH Analytics version 3.4 software.

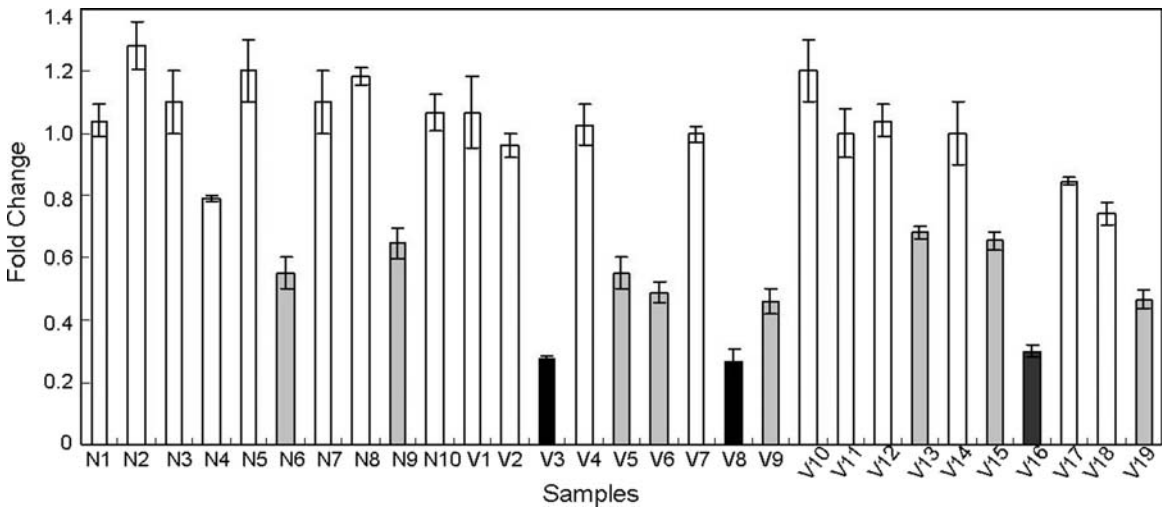

Figure 2 Quantitative real-time PCR (qPCR) results for PANK2.
The fold change in gene copy number for the target gene (PANK2) relative to the endogenous reference gene (ACTB) was compared for the CBAVD samples (V1–V19) and the controls (N1–N10) as described in Materials and Methods. The fold change for PANK2 and ACTB of the control sample is set at 1. The normalized fold changes were interpreted as follows: no change (0.7–1.4, white bar), homozygous loss (<0.3, black bar), over representation (>1.4, black bar) and ambiguous (0.3–0.7, gray bar).

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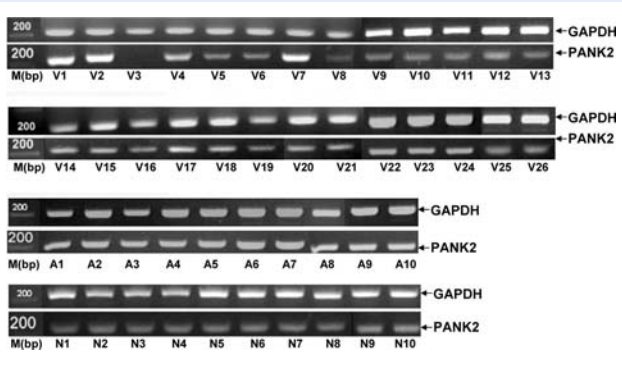

Figure 3 Homozygous deletions of PANK2 in CBAVD.
Agarose gel electrophoresis picture of the PCR products of PANK2 and GAPDH were shown for all cases of CBAVD (V1–V26), representative cases of azoospermia patients without CBAVD (A1–A10) and healthy controls (N1–N10). M, DNA marker. The absence of the specific amplified fragment of 215 bp was interpreted as homozygous loss of PANK2.

also identified seven controls deleted for 3q26.1, giving a frequency of 87.5% (Fig. 4), which suggests that the 3q26.1 microdeletion may not be pathogenic. In this analysis, we characterized this microdeletion to ~104 kb ranging from 163.997227 to 164.101835 Mb on chromosome 3, and containing sequences coding for the hypothetical protein, LOC647107.

Discussion

In this study, we have performed a genome-wide examination of genetic copy number aberrations in eight CBAVD patients. Array-CGH analysis identified five reproduction-related genes: *PBX*, *BRD3*, *PANK2*, *COL18A1* and *HMOX1*. All showed a single incidence, except *PANK2* which was affected in two patients. Subsequent qPCR analysis for the five reproduction-related genes showed only deletion of *PANK2* was cross-validated in all cases and was thus selected for further study. We employed performed deletion analysis by PCR in a larger cohort, including 26 CBAVD patients, 20 azoospermic patients without CBAVD and 16 healthy control men. Homozygous loss of *PANK2* was detected in only one CBAVD patient (3.8%), while no loss was found in azoospermic and control subjects. Thus, the *PANK2* gene has the potential to explain only a small percentage of this disorder, suggesting that other genetic factors must play a role. Furthermore, this study showed that the submicroscopic region located on 3q26.1 which was frequently deleted in CBAVD patients, was also deleted in control subjects, suggesting that this microdeletion is a CNP among Asian men and is not disease-causing.

The *PANK2* gene encodes pantothenate kinase 2 (Pank). Pantothenate kinase is the first enzyme in the biosynthetic pathway of co-enzyme A (CoA), catalyzing the ATP-dependent phosphorylation of pantothenate. As a ubiquitous metabolic substrate, CoA plays a critical role in the tricarboxylic acid cycle, in the synthesis of some amino acids and in the fatty acid metabolism. One isoform of Pank in mammals, Pank2 is probably uniquely located on mitochondria (Hörtnagel *et al*., 2003;

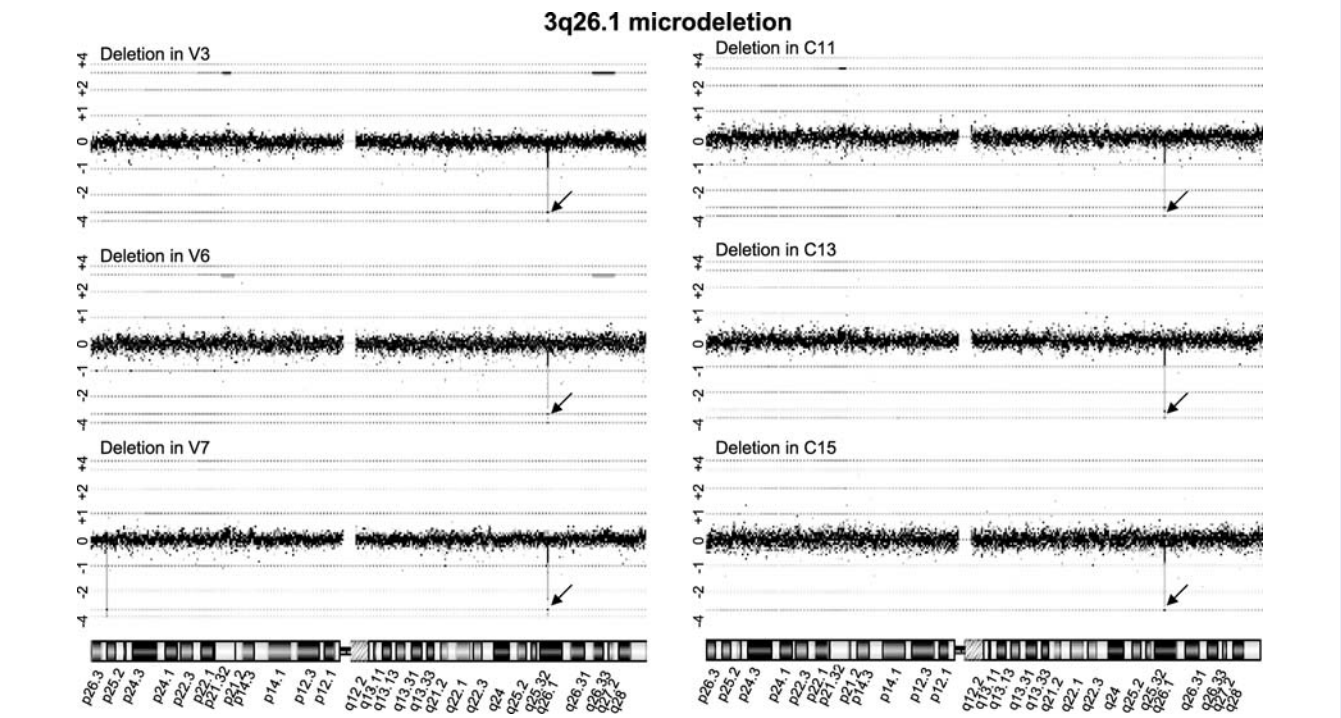

Figure 4 Recurrent 3q26.1 microdeletions in CBAVD patients and controls.
Tiling path array demonstrates the presence of the 3q26.1 deletion with high frequency in both CBAVD and control subjects. The array-CGH results show the log 2 ratio of the reference versus patients DNA on the vertical axis. The horizontal axis shows the position along the chromosome. Black arrows highlight the deletions.

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Johnson et al., 2004), strongly implying its involvement in energy metabolism. Previous studies of human Pank2 (hPank2) have been mostly limited to the central nervous system, because a defect in this gene can give rise to a devastating neurodegenerative disorder named pantothenate kinase-associated neurodegeneration (PKAN, also known as Hallervorden-Spatz syndrome), which is mainly caused by a deficiency of CoA (Zhou et al., 2001; Hayflick et al., 2003a,b). The novel role of Pank2 in reproduction was discovered in knock-out mice with homozygous loss of the orthologous murine gene, mPank2. The homozygous male mutants are azoospermic due to an arrest in spermiogenesis, which leads to complete absence of elongated and mature spermatids (Kuo et al., 2005, 2007). Contrary to the conditions observed in the infertile knock-out mice, all cases of CBAVD patients we analyzed were diagnosed without defects in spermiogenesis. Similarly, azoospermia had not been reported for men with PKAN. Here, rise two interesting questions: (i) Is the discrepancy due to a difference in spermiogenesis between human and mouse? (ii) Does the gene product of *PANK2* play a different pathological role in human and mouse? A recent study carried out by Leonardi et al. (2007) supported the above speculations by showing that although the enzymatic properties of hPank2 and mPank2 are indistinguishable, there are obvious differences in gene structure, protein localization and relative abundance of protein in human and murine tissues. Interestingly, they found that PANK2 transcripts were low in mouse brain and were extremely high in mouse testis, whereas hPank2 were highest in the human brain but were considerably less abundant in human testis. What is the role of *PANK2* in human reproduction and how might the homozygous deletion lead to CBAVD? To assist in addressing these questions, a study of the association between *PANK2* and CBAVD with a higher sample size would be required, and detailed analysis of the expression patterns of *PANK2* during wolffian duct development (Hannema et al., 2007) would also be signified.

In conclusion, we have attempted to contribute to the discovery of causative CNVs, which are important to understand the pathogenesis of CBAVD in Asian populations. However, the incidence for potentially susceptible loci mapping to known genes was rare in our patient cohort, and the highly recurrent microdeletion on 3q26.1 seems not be pathogenic. Our exploratory data provide information on CNVs for CBAVD in Asian populations and suggest that other genetic events, including inversion, translocation, promoter mutations and even submicroscopic CNVs may be implicated in this form of male infertility. Several follow-up analyses are needed to assess the reproduction-related gene disrupted by CNV identified by this pilot study.

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Author roles

C.H.L. and H.S.C. designed research.
C.H.L. and Y.N.W. performed research.
C.H.L. and C.C.W. wrote the paper.
C.H.L. and H.S.C. revised the paper.

References

Bauters M, Van Esch H, Marynen P, Froyen G. X chromosome array-CGH for the identification of novel X-linked mental retardation genes. *Eur J Med Genet* 2005;**48**:263–275.

Boyer A, Lussier JG, Sinclair AH, McClive PJ, Silversides DW. Pre-sertoli specific gene expression profiling reveals differential expression of Ppt1 and Brd3 genes within the mouse genital ridge at the time of sex determination. *Biol Reprod* 2004;**71**:820–827.

Brennan C, Zhang Y, Leo C, Feng B, Cauwels C, Aguirre AJ, Kim M, Protopopov A, Chin L. High-resolution global profiling of genomic alterations with long oligonucleotide microarray. *Cancer Res* 2004; **64**:4744–4748.

Claustres M, Guittard C, Bozon D, Chevalier F, Verlingue C, Ferec C, Girodon E, Cazeneuve C, Bienvenu T, Lalau G *et al*. Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. *Hum Mutat* 2000;**16**:143–156.

Dayangaç D, Erdem H, Yilmaz E, Sahin A, Sohn C, Ozgüç M, Dörk T. Mutations of the CFTR gene in Turkish patients with congenital bilateral absence of the vas deferens. *Hum Reprod* 2004;**19**:1094–1100.

de la Taille A, Rigot JM, Mahe P, Gervais R, Dumur V, Lemaitre L, Mazeman E. Correlation between genitourinary anomalies, semen analysis and CFTR genotype in patients with congenital bilateral absence of the vas deferens. *Br J Urol* 1998;**81**:614–619.

de Meeus A, Guittard C, Desgeorges M, Carles S, Demaille J, Claustres M. Genetic findings in congenital bilateral aplasia of vas deferens patients and identification of six novel mutations. *Hum Mutat* 1998;**11**:480.

Hannema SE, Hughes IA. Regulation of Wolffian duct development. *Horm Res* 2007;**67**:142–151.

Hayflick SJ. Unraveling the Hallervorden-Spatz syndrome: pantothenate kinase-associated neurodegeneration is the name. *Curr Opin Pediatr* 2003a;**15**:572–577.

Hayflick SJ, Westaway SK, Levinson B, Zhou B, Johnson MA, Ching KH, Gitschier J. Genetic, clinical, and radiographic delineation of Hallervorden-Spatz syndrome. *N Engl J Med* 2003b;**348**:33–40.

Holsclaw DS, Perlmutter AD, Jockin H, Shwachman H. Genital abnormalities in male patients with cystic fibrosis. *J Urol* 1971; **106**:568–574.

Hörtnagel K, Prokisch H, Meitinger T. An isoform of hPANK2, deficient in pantothenate kinase-associated neurodegeneration, localizes to mitochondria. *Hum Mol Genet* 2003;**12**:321–327.

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. Detection of large-scale variation in the human genome. *Nat Genet* 2004;**36**:949–951.

Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. *J Urol* 1989;**142**:62–65.

Johnson MA, Kuo YM, Westaway SK, Parker SM, Ching KH, Gitschier J, Hayflick SJ. Mitochondrial localization of human PANK2 and hypotheses of secondary iron accumulation in pantothenate kinase-associated neurodegeneration. *Ann N Y Acad Sci* 2004; **1012**:282–298.

Josserand RN, Bey-Omar F, Rollet J, Lejeune H, Boggio D, Durand DV, Durieu I. Cystic fibrosis phenotype evaluation and paternity outcome in 50 males with congenital bilateral absence of vas deferens. *Hum Reprod* 2001;**16**:2093–2097.

Kuo YM, Duncan JL, Westaway SK, Yang H, Nune G, Xu EY, Hayflick SJ, Gitschier J. Deficiency of pantothenate kinase 2 (Pank2) in mice leads to retinal degeneration and azoospermia. *Hum Mol Genet* 2005;**14**:49–57.

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Kuo YM, Hayflick SJ, Gitschier J. Deprivation of pantothenic acid elicits a movement disorder and azoospermia in a mouse model of pantothenate kinase-associated neurodegeneration. *J Inherit Metab Dis* 2007;**30**:310–317.

Lai LA, Paulson TG, Li X, Sanchez CA, Maley C, Odze RD, Reid BJ, Rabinovitch PS. Increasing genomic instability during premalignant neoplastic progression revealed through high resolution array-CGH. *Genes Chromosomes Cancer* 2007;**46**:532–542.

Lee CH, Fang CY, Sheu JJ, Chang Y, Takada K, Chen JY. Amplicons on chromosome 3 contain oncogenes induced by recurrent exposure to 12-O-tetradecanoylphorbol-13-acetate and sodium n-butyrate and Epstein-Barr virus reactivation in a nasopharyngeal carcinoma cell line. *Cancer Genet Cytogenet* 2008;**185**:1–10.

Leonardi R, Zhang YM, Lykidis A, Rock CO, Jackowski S. Localization and regulation of mouse pantothenate kinase 2. *FEBS Lett* 2007; **581**:4639–4644.

Murthy SK, Nygren AO, El Shakankiry HM, Schouten JP, Al Khayat AI, Ridha A, Al Ali MT. Detection of a novel familial deletion of four genes between BP1 and BP2 of the Prader-Willi/Angelman syndrome critical region by oligo-array CGH in a child with neurological disorder and speech impairment. *Cytogenet Genome Res* 2007;**116**:135–140.

Nasu K, Fujisawa K, Nishida Y, Kai S, Sugano T, Miyakawa I, Tateishi Y. Expression of collagen XVIII mRNA and protein in human umbilical vein and placenta. *Reprod Fertil* 2003;**15**:107–114.

Ozawa N, Goda N, Makino N, Yamaguchi T, Yoshimura Y, Suematsu M. Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress. *J Clin Invest* 2002;**109**:457–467.

Patrizio P, Zielenski J. Congenital absence of the vas deferens: a mild form of cystic fibrosis. *Mol Med Today* 1996;**2**:24–31.

Pramparo T, Mattina T, Gimelli S, Liehr T, Zuffardi O. Narrowing the deleted region associated with the 15q21 syndrome. *Eur J Med Genet* 2005;**48**:346–352.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W *et al.* Global variation in copy number in the human genome. *Nature* 2006;**444**:444–454.

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL *et al.* Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989;**245**:1073–1080.

Sasaki H, Kawano O, Endo K, Yukiue H, Yano M, Fujii Y. EGFRvIII mutation in lung cancer correlates with increased EGFR copy number. *Oncol Rep* 2007;**17**:319–323.

Schnabel CA, Selleri L, Cleary ML. Pbx1 is essential for adrenal development and urogenital differentiation. *Genesis* 2003;**37**:123–130.

Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J *et al.* Large-scale copy number polymorphism in the human genome. *Science* 2004; **305**:525–528.

Shang E, Salazar G, Crowley TE, Wang X, Lopez RA, Wang X, Wolgemuth DJ. Identification of unique, differentiation stage-specific patterns of expression of the bromodomain-containing genes Brd2, Brd3, Brd4, and Brdt in the mouse testis. *Gene Expr Patterns* 2004; **4**:513–519.

Shiraishi K, Naito K. Increased expression of Leydig cell haem oxygenase-1 preserves spermatogenesis in varicocele. *Hum Reprod* 2005; **20**:2608–2613.

Solomon NM, Ross SA, Morgan T, Belsky JL, Hol FA, Karnes PS, Hopwood NJ, Myers SE, Tan AS, Warne GL *et al.* Array comparative genomic hybridisation analysis of boys with X linked hypopituitarism identifies a 3.9 Mb duplicated critical region at Xq27 containing SOX3. *J Med Genet* 2004;**41**:669–678.

Wang Z, Milunsky J, Yamin M, Maher T, Oates R, Milunsky A. Analysis by mass spectrometry of 100 cystic fibrosis gene mutations in 92 patients with congenital bilateral absence of the vas deferens. *Hum Reprod* 2002; **17**:2066–2072.

Watanabe S, Akagi R, Mori M, Tsuchiya T, Sassa S. Marked developmental changes in heme oxygenase-1 (HO-1) expression in the mouse placenta: correlation between HO-1 expression and placental development. *Placenta* 2004;**25**:387–395.

Welsh MJ, Romsey BW, Accurso F, Cutting GR. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). *Metabolic and Molecular Bases of Ingerited Disease*. New York: McGraw-Hill, 2001, 5121–5188.

Wong LJ, Alper OM, Wang BT, Lee MH, Lo SY. Two novel null mutations in a Taiwanese cystic fibrosis patient and a survey of East Asian CFTR mutations. *Am J Med Genet A* 2003;**120**:296–298.

Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE, MacAulay C, Ng RT, Brown CJ, Eichler EE *et al.* A comprehensive analysis of common copy-number variations in the human genome. *Am J Hum Genet* 2007;**80**:91–104.

Wu CC, Alper OM, Lu JF, Wang SP, Guo L, Chiang HS, Wong LJ. Mutation spectrum of the CFTR gene in Taiwanese patients with congenital bilateral absence of the vas deferens. *Hum Reprod* 2005; **20**:2470–2474.

Yao J, Weremowicz S, Feng B, Gentleman RC, Marks JR, Gelman R, Brennan C, Polyak K. Combined cDNA array comparative genomic hybridization and serial analysis of gene expression analysis of breast tumor progression. *Cancer Res* 2006;**66**:4065–4078.

Zhou B, Westaway SK, Levinson B, Johnson MA, Gitschier J, Hayflick SJ. A novel pantothenate kinase gene (PANK2) is defective in Hallervorden-Spatz syndrome. *Nat Genet* 2001;**28**:345–349.

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