

## ARTICLE

# Expansion of a 12-kb VNTR containing the *REXO1L1* gene cluster underlies the microscopically visible euchromatic variant of 8q21.2

Christine Tyson<sup>1</sup>, Andrew J Sharp<sup>2</sup>, Monica Hrynychak<sup>1</sup>, Siu L Yong<sup>3</sup>, Edward J Hollox<sup>4</sup>, Peter Warburton<sup>2</sup> and John CK Barber<sup>\*5</sup>

Copy number variants visible with the light microscope have been described as euchromatic variants (EVs) and EVs with extra G-light material at 8q21.2 have been reported only once before. We report four further patients with EVs of 8q21.2 ascertained for clinical (3) or reproductive reasons (1). Enhanced signal strength from two overlapping bacterial artificial chromosomes (BACs) and microarray analysis mapped the EV to a 284-kb interval in the reference genome. This interval consists of a sequence gap flanked by segmental duplications that contain the 12-kb components of one of the largest Variable Number Tandem Repeat arrays in the human genome. Using digital NanoString technology with a custom probe for the RNA exonuclease 1 homologue (*S. cerevisiae*)-like 1 (*REXO1L1*) gene within each 12-kb repeat, significantly enhanced diploid copy numbers of 270 and 265 were found in an EV family and a median diploid copy number of 166 copies in 216 controls. These 8q21.2 EVs are not thought to have clinical consequences as the phenotypes of the probands were inconsistent, those referred for reproductive reasons were otherwise phenotypically normal and the *REXO1L1* gene has no known disease association. This EV was found in 4/3078 (1 in 770) consecutive referrals for chromosome analysis and needs to be distinguished from pathogenic imbalances of medial 8q. The *REXO1L1* gene product is a marker of hepatitis C virus (HCV) infection and a possible association between *REXO1L1* copy number and susceptibility to HCV infection, progression or response to treatment has not yet been excluded.

*European Journal of Human Genetics* (2014) 22, 458–463; doi:10.1038/ejhg.2013.185; published online 18 September 2013

**Keywords:** 8q21.2; copy number variant; euchromatic variant; DNA array; NanoString technology; *REXO1L1*; hepatitis C

## INTRODUCTION

At least 1% of individuals have a copy number variation (CNV) larger than 1 Mb<sup>1</sup> and, when copy number is high enough, CNVs become visible with the light microscope. These have been described as euchromatic variants (EVs)<sup>2</sup> (Chromosome Anomaly Collection) and involve (1) constitutional cytogenetic amplification of known CNV regions or (2) duplication, triplication or deletion of large segmentally duplicated (SD) euchromatic tracts which may include heterochromatin and accompany pseudo-dicentric variants (Table 1).<sup>3–25</sup> Most of the EVs are clinically innocuous but the 8p23.1 EV has been associated with a number of traits<sup>5–8</sup> and the 4p16.1 EV co-segregated with microtia.<sup>4</sup> EVs need to be distinguished from pathological imbalances with which they may be similar or even identical under the light microscope.<sup>5,19,21,24,25</sup>

Recently, Manvelyan *et al*<sup>9</sup> reported three patients of Middle European and Japanese descent with a microscopically visible CNV of 8q21.2. This CNV was characterised by a 60% increase in the signal strength from a single bacterial artificial chromosome (BAC) but an extra copy of a single BAC of ~123 kb would not normally account for the microscopic visibility of this 8q21.2 EV. Here, we have

confirmed the existence of this 8q21.2 EV in four new patients from Canada (Table 2) and set out to refine the location and extent of this EV. We have used digital NanoString technology to quantify the copy number of the variable number tandem repeat (VNTR) array within the EV and find that expansion of this 8q21.2 VNTR is common and most likely to account for the microscopic visibility of the 8q21.2 EV. We estimate the prevalence of the 8q21.2 EV among postnatal referrals for chromosome analysis and suggest that CNV of the RNA exonuclease 1 homologue (*S. cerevisiae*)-like 1 (*REXO1L1*) gene cluster within the VNTR may be related to susceptibility to hepatitis C infection.

## METHODS

Chromosomes were prepared and analysed using standard procedures on a total of 3078 consecutive clinical referrals for peripheral blood analysis over a 21 month period. Single- or dual-colour fluorescence *in situ* hybridisation (FISH) was performed with BACs from 8q21.2, a centromere probe for chromosome 8 (D8Z2) and, for Patient 4, a centromere probe for chromosomes 9 (D9Z3) with a centromere probe for chromosome 11 as a control (Cytocell, Cambridge, UK). DNA was extracted from peripheral blood using

<sup>1</sup>Cytogenetics, Department of Pathology, Royal Columbian Hospital, New Westminster, British Columbia, Canada; <sup>2</sup>The Mindich Child Health and Development Institute, Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, Hess Center for Science and Medicine, New York, NY, USA; <sup>3</sup>Department of Medical Genetics, UBC, Vancouver, British Columbia, Canada; <sup>4</sup>Department of Genetics, University of Leicester, Leicester, UK; <sup>5</sup>Department of Human Genetics and Genomic Medicine, Faculty of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK

\*Correspondence: Dr JCK Barber, Department of Human Genetics and Genomic Medicine, Faculty of Medicine, Southampton University School of Medicine, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK. Tel: +44 2380 796586; Fax: +44 2380 794760; E-mail: john.barber@oton.ac.uk  
Received 30 January 2013; revised 4 July 2013; accepted 24 July 2013; published online 18 September 2013

**Table 1** Summary of euchromatic variants

	EV	Repeats	Amplicon size	Control		Gene content	Trait	Similar imbalance	Reference(s)
				copy number <sup>a</sup>	EV copy number <sup>b</sup>				
1	amp 4 p16.1	OR/DEF	750 kb	2 (200)	7 (0/200)	<i>DEFB131; DUB3; DUB4; OR; DRD5</i>	Microtia	dup(4)(p16.1p16.1)	3
2	dup 8 p23.2	—	2.5 Mb	2 (50)	3 (3/50)	<i>Part CSMD1</i>	—	dup(8)(p23.1p23.3)	4
3	amp 8 p23.1	OR/DEF	>260 kb	2–9 (576)	8–12	<i>DEFB4; OR; SPAG11; HE2; FAM90A; DUB</i>	Psoriasis Psoriasis HIV load ?Influenza infection	dup(8)(p23.1p23.1)	5 6 7 8
4	amp 8 q21.2	REPP and REPD	12 kb	97–277 (216)	265–270 (4/3078)	<i>REXO1L1</i> gene cluster	?HCV infection		9 Present paper
5	amp 9 p12	SDs	~1 Mb	1–3 (100)	7–12	<i>CNTNAP3; ANKRD20A</i>	None		10
6–7	del or dup 9 p11.2–p13.1	SDs	~5 Mb	4	3–5	Multiple genes, gene fragments, pseudogenes and CNVs inc.	None		11–12
8–10	dup or trp 9 ins qh/q12	SDs	~5 Mb	4	5–6	<i>PGM5; CNTNAP3;</i>	None		12–14
11–13	del, dup/trp, or amp 9 q13–q21.1	SDs	~5 Mb	4	3–8	<i>ZNF658; ANKRD20A; CBWD; FOXD4L; FAM75A</i> and <i>FAM27A</i>	None	ins(9) (q13;q32q34.3)	12 14–16
14	dup dic 9 p12–q22 or p13.1–q21.11	SDs	~40 Mb	2	3		None		12 17–18
15	amp 15 q11.2	SDs	~1 Mb	<i>IGVH</i> 1–3; <i>NF1</i> 1–4	<i>IGVH</i> 4–9; <i>NF1</i> 5–10	<i>IGVH(D1&amp;2); BCL8A; NF1; GABRA5</i>	None	dup(15)(q11.2q13)	19–21
16	amp 16 p11.2	SDs	692–945 kb	3–8, median 4 (46)	8–10	<i>IGVH; HERC2P4; TP53TG3; SLC6A10</i>	None	dup(16)(p11.2p21.2)	22–24
17	dup dic 16 p11.2–q12.1	SDs	940 kb of ~12.3 Mb	2	3	<i>CCNYL3</i>	None	dup(16)(q11.2q13)	25

Abbreviations: amp, amplification; dic, dicentric; dup, duplication; EV, euchromatic variant; inc, including; ins, insertion; kb, kilobase pairs; Mb, megabase pairs; OR, olfactory receptor; SDs, segmental duplications (stretches of DNA that are at least 1000 bases in length and share a sequence identity of at least 90%); trp, triplication.

<sup>a</sup>Figures in parentheses are the number of individuals in which control copy number was established.

<sup>b</sup>Figures in parentheses are the number of individuals with EVs among control or study populations.

? Denotes possible association with influenza infection raised by reference 8.

Puregene DNA Isolation Kits (Gentra, Minneapolis, MN, USA) and used for microarray analysis with Affymetrix Cytogenetics 2.7M (Patient 1) or Affymetrix Cytoscan HD (Patient 4) microarrays according to the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA). The Affymetrix CEL data files were converted into CYCHP files using the annotation files na30.2 (Patient 1; 2.7M array) or na32.1 (Patient 4; Cytoscan HD array) and the microarray data were analysed using the Affymetrix Chromosome Analysis Suite (ChAS) Software v1.1 (Patient 1; 2.7M array) or v1.2 (Patient 4; Cytoscan HD array) (Affymetrix). The reference model file was provided by Affymetrix. Quality control parameters were SNPQC  $\geq 1.1$  and MAPD  $\leq 0.27$  (Patient 1; 2.7M array) or SNPQC  $\geq 15$  and MAPD  $\leq 0.25$  (Patient 4; Cytoscan HD array). Base pair coordinates correspond or have been converted to release GRCh37 (hg19). DNA for NanoString analysis was extracted using standard methods and used for genomic copy number analysis with a custom NanoString code set comprising 10 normalisation probes targeting invariant autosomal loci, and 27 custom probes targeting highly CNV sites including one targeting the *REXO1L1* gene cluster (probe sequence GTTCCCCTAC AAGCGCTCCCCTCAGGAATCTCGCGGCCGACTACCTGGCACAGATCATCC AGGACAGCCA). Patient DNA samples and 216 anonymous controls of self-reported European ancestry were processed using the NanoString nCounter Analysis System (NanoString Technologies, Seattle, WA, USA)<sup>26</sup> according to the manufacturer's instructions. The ethnicity of patients and/or parents was based on their self-reported national origins.

## Clinical details

Patient 1 was a woman of 33 referred for recurrent miscarriages, Patient 2 a woman of 26 with secondary amenorrhoea referred to exclude a possible diagnosis of Turner's syndrome, Patient 3 a boy of 3 with autism and Patient 4 a newborn girl with a large ventricular septal defect and dysmorphic features (mild hypotelorism, abnormal palpebral fissures and a smooth, thin upper lip). Her father was phenotypically normal and healthy.

## RESULTS

Our results and those of Manvelyan *et al*<sup>9</sup> are summarised in Table 2.

### Karyotyping

Additional G-light material was found at band 8q21.2 in all four index patients (Figures 1a–d) among 3078 consecutive clinical referrals for peripheral blood analysis over a 21-month period. The additional material could have been the result of duplication, insertion, paracentric inversion or conformational alteration. However, the banding pattern was not consistent with an inversion and the extra material appeared to be cytogenetically identical to the microscopically visible CNVs described by Manvelyan *et al*.<sup>9</sup> The same variant was found in the father of Patient 4 (Figure 1e) and, following microarray analysis of Patient 4 (see below), mosaic trisomy 9 was

**Table 2 Summary of results**

Patient <sup>a</sup>	Sex	Ethnicity	Indication	Conventional karyotype	FISH <sup>b</sup>	Array		EV copy number	Reference
						mapping	Amplicon		
1	F	Middle European	FH ID and CF	46,XX,var(8)(q21.2q21.2)	96G1 enh	—	—	—	9
2	M	Middle European	Fertility problems	46,XY,var(8)(q21.2q21.2)	96G1 enh	—	?123 kb	?3	
3	M	Japanese	Azoospermia	46,XY,var(8)(q21.2q21.2)	96G1 enh	—	—	—	
<b>1</b>	F	Palestinian	Recurrent miscarriages	46,XY,var(8)(q21.2q21.2)	96G1 enh, 90G23 enh	292 kb	—	—	
<b>2</b>	F	Caucasian	Secondary amenorrhoea	46,XX,var(8)(q21.2q21.2)	96G1 enh, 90G23 enh	—	—	—	
<b>3</b>	M	Canadian	Autism	46,XY,var(8)(q21.2q21.2)	96G1 enh, 90G23 enh	—	—	—	Present paper
<b>4</b>	F	Middle European	MCA	mos 46,XX/47,XX,+9,var(8)(q21.2q21.2)	96G1 enh, 90G23 enh	284 kb	12 kb	265	
<b>Father of 4</b>	M	Czech	Daughter	46,XY,var(8)(q21.2q21.2)	96G1 enh, 90G23 enh	—	12 kb	270	

Abbreviations: CF, cystic fibrosis; enh, enhanced; F, female; FH, family history; ID, intellectual difficulties; kb, kilobase pairs; M, male; Mb, Megabase pairs; MCA, multiple congenital anomalies; var, variant.

<sup>a</sup>Patients from present paper are in bold.

<sup>b</sup>All BACs from the RP11-library. ? used as an amplicon of 123 kb and EV copy number of 3 was implied but not proven in reference 9.

confirmed in 2/200 G-banded metaphases (data not shown). Other parental samples were requested but not received.

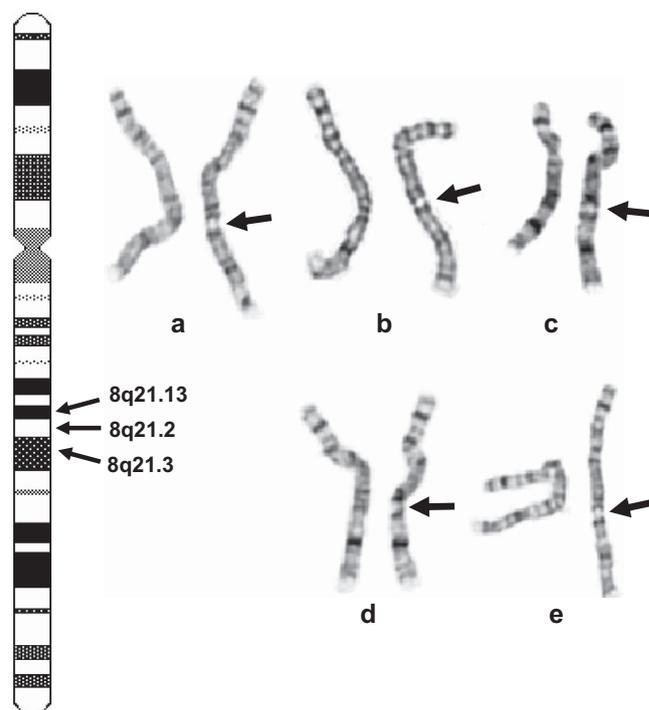
### FISH

Using a panel of five BACs from medial 8q, Manvelyan *et al*<sup>9</sup> found that only BAC RP11-96G1 gave signals that were 60% larger on the EV chromosome than on the normal chromosome. We noted that RP11-96G1 overlaps with RP11-90G23 and that RP11-90G23 contains segmental duplications (SDs) of 8q21.2 that might mediate amplification of the sequence between them, represented as gap in the human genome assembly (hg19). Enhanced signals on one chromosome 8 were consistently found using single- or dual-colour FISH with both BACs in all four new patients (Figures 2a–d) but the increase in size was more consistent with duplication than with amplification (Figures 2a–d). However, these larger signals could not be separated in prometaphase (Figure 2c) or interphase cells (Figure 2a). Following microarray analysis (see below), mosaic trisomy 9 was found in Patient 4 and confirmed in 14/200 interphase nuclei (data not shown).

### Microarray

Affymetrix Cytogenetics Whole-Genome 2.7M Array analysis in Patient 1 and Cytoscan HD array analysis in Patient 4 were normal except that evidence of low-level trisomy 9 mosaicism was detected in Patient 4 and subsequently confirmed using FISH and karyotyping (see above) (data not shown). DNA for microarray testing was requested from Patient 3 but not received, and not thought to be warranted in Patient 2 on clinical grounds. The normal array results exclude a duplication of the great majority of chromosome 8 covered by the arrays or an unbalanced insertion into 8q from elsewhere. The array results also localise the variant region to a 284-kb interval in distal 8q21.2 (Figure 2e) between base pairs 86 553 129 and 86 836 909 (hg19) in Patient 4 (Figure 2f). The corresponding 174 kb interval between base pairs 86 738 256 and 86 912 598 (hg18) in Patient 1 converts to an almost identical 292 kb between base pairs 86 551 004 and 86 843 310 in the current build of the human genome (hg19). This common region in Patients 1 and 4 contains the sequence gap and flanking SDs REPP and REPD in the reference genome (Figure 2g).

The karyotype of the index Patients 2 and 3 was 46,XX or XY,var(8)(q21.2q21.2).ish var(8)(RP11-90G23enh,RP11-96G1enh), the karyotype of index Patient 1 was 46,XX,var(8)(q21.2q21.2).ish var(8)(RP11-90G23enh,RP11-96G1enh).arr(1-22)x2 and that of Patient 4 was mos 46,XX,var(8)(q21.2q21.2)[198]/47,XX,var(8)(q21.2q21.2),+9[2].ish var(8)(q21.2q21.2)(RP11-90G23enh,

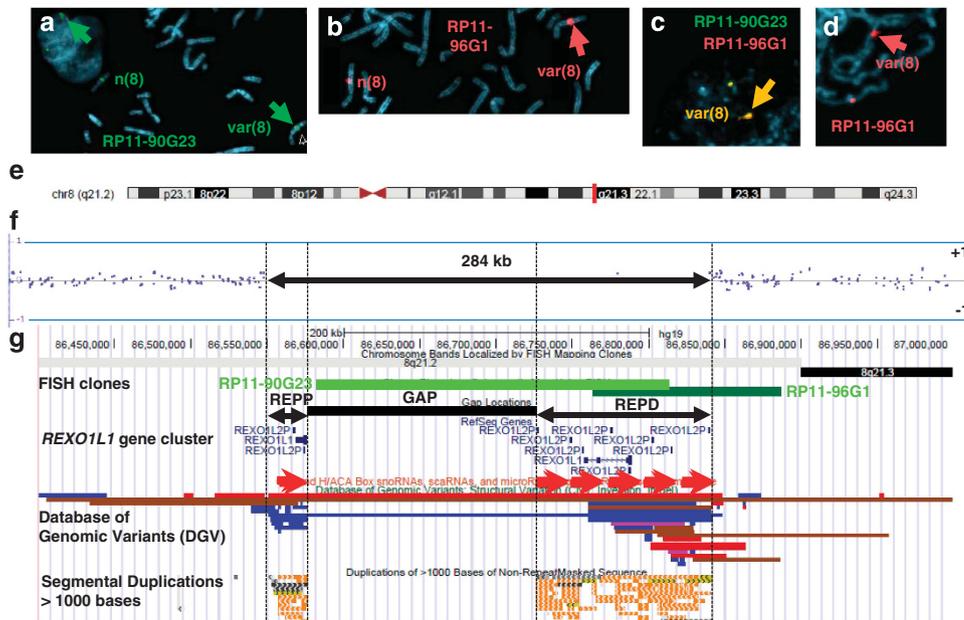


**Figure 1** Idiogram of a normal chromosome 8 and partial G-banded karyotypes of the EV chromosomes 8 with the variant chromosome on the right in each case and the additional G-light material indicated by the black arrow: (a) Patient 1; (b) Patient 2; (c) Patient 3; (d) Patient 4 and (e) the father of Patient 4.

RP11-96G1enh).nuc ish(D9Z3 × 3,D11Z1 × 2)[14/200].arr(9)(1-141 213 431)x2~3.

### Molecular genetics

Pulse-field electrophoresis (PFGE) had previously demonstrated that the 8q21.2 SDs contain elements of a tandem VNTR array consisting of 50–150 copies of a 12-kb repeat that spanned 0.6–1.7 Mb in two pedigrees.<sup>27</sup> We used digital NanoString technology with a custom probe for the *REXO11* gene cluster to determine total (diploid) copy number of the 12 kb repeat. There was a close linear correlation between total copy number measured using PFGE Southern blot and the NanoString nCounter Analysis System ( $R^2 = 0.98$ ) (Figure 3a). Total copy numbers of 265 and 270 were found in Patient 4 and her



**Figure 2** (a–e) FISH and microarray results: (a, b) single-colour FISH with BACs RP11-90G23 (green) and BAC RP11-96G1 (red) in metaphases and an interphase nucleus from Patient 1 with the variant chromosome indicated by the large arrows; (c) dual-colour FISH with BACs RP11-90G23 (green) and RP11-96G1 (red) in an interphase nucleus from Patient 1 showing the colocalisation of the combined signals (yellow); (d) FISH with BAC RP11-96G1 (red) in a prometaphase from Patient 1 showing the lack of separation of the signals on the variant chromosome; (e) idiogram of chromosome 8 showing the location of the CNV region in distal 8q21.2; (f) Affymetrix Cytoscan HD array analysis of base pairs 86 460 508–87 259 456 in Patient 4 using the Affymetrix ChAS Software. The normal copy number results cluster along the horizontal weighted  $\log_2$  ratio=0 line and flank the variant 284 kb region between base pairs 86 553 129 and 86 836 909 (hg19). The horizontal lines above and below represent  $\log_2$  ratios of +1 and -1, respectively. (g) A Portable Document Format (PDF) screen shot of the variant and flanking regions of 8q21.2 from the UCSC browser (GRCh37/hg19). The FISH clones are in green with RP11-90G23 manually annotated from BAC end sequence data (base pairs 86 570 255–86 817 480). The flanking SDs are represented by black double-headed arrows between black vertical lines and have been labelled REPP (for REPEAT Proximal) and REPD (for REPEAT Distal). The sequence gap between the SDs is represented by the black bar labelled GAP. The RefSeq genes are in blue with the tandem *REXO1L1* gene array highlighted by the red arrows underneath. The region contains no non-coding sequences. The DGV tracks show gains (blue), losses (red) and inversions (brown). The UCSC SD track is mostly in terracotta.

father, respectively, compared with a median of 166 and an average of 173 in 216 normal controls (Figure 3b). Copy numbers ranged from 97 to 277 in the controls with a positive skew of 0.7 (Figure 3b) and, by comparison, the copy numbers in the EV family were greater than the 99th centile and significantly higher using the Mann–Whitney Rank Sum Test ( $P=0.018$ ).

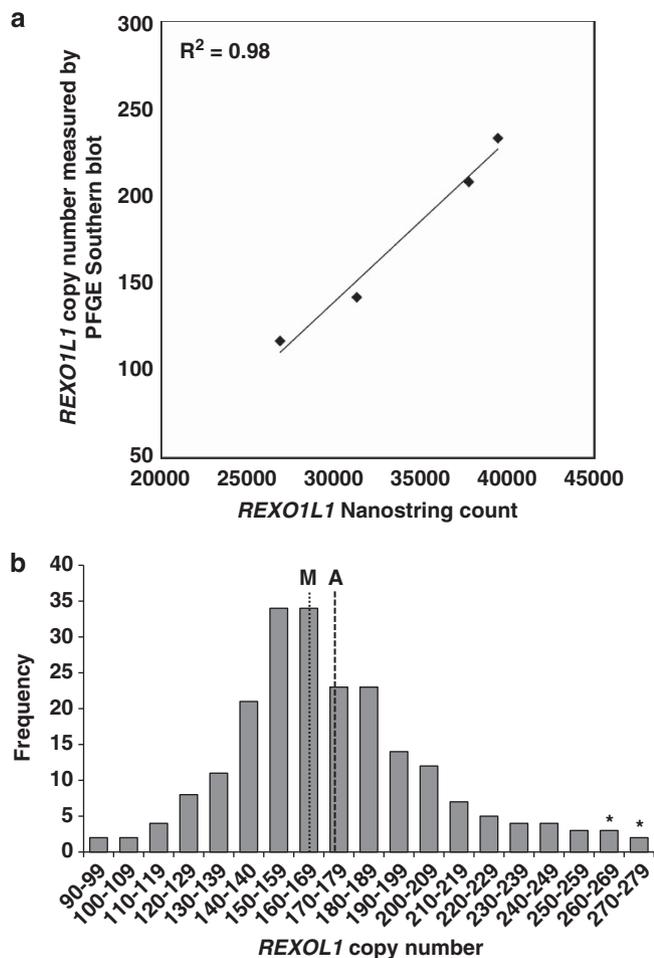
## DISCUSSION

Our results map the 8q21.2 EVs to the sequence gap and flanking SDs in distal 8q21.2 that are clearly larger than 284 kb in the reference genome (Figures 2e and g) and most likely to underlie this EV. Compared with the other 16 EVs in Table 1, the 8q21.2 EVs have by far the smallest amplicon, the highest copy number and, in common with the 4p16.1 and 8p23.1 EVs, are flanked by SDs of the kind that underlie the majority of other EVs.

The NanoString analysis indicates that an average of 173 diploid copies of the *REXO1L1* gene cluster and 12 kb repeat are present within the 8q21.2 VNTR in our 216 controls. This implies that an average chromosome 8 contains 87 copies spanning over 1 Mb or almost half of band 8q21.2 which is a predicted 2.3 Mb in the UCSC browser<sup>28</sup> or an estimated 2.1 Mb (assuming that 8q21.2 is 2% of 8q<sup>29</sup> and that 8q is 105 Mb<sup>30</sup>). If accompanied by an average chromosome 8, then the 265 and 270 copies in Patient 4 and her father imply EV chromosomes with ~180 copies spanning over 2.1 Mb. Thus, given the ~2:1 ratio of the enhanced FISH signals (Figures 2a–d),<sup>9</sup> the simplest interpretation of our results is that an average VNTR array

of ~90 copies is duplicated to give an EV chromosome of ~180 copies, a diploid total of 270 copies and a differential length of at least 1 Mb (Figure 2g). However, the EV FISH signals could not be resolved into separate blocks (Figures 2a and c) and there is a relatively even distribution of copy numbers from a minimum of 97 to a maximum of 277 in the 216 controls (Figure 3b). In addition, the copy numbers in EV carriers are significantly higher than the great majority of controls and above the 99th centile of the control range. We therefore propose that expansion of the VNTR array is most likely to underlie the 8q21.2 EVs, and that their microscopic visibility reflects the combination of an expanded VNTR array on the EV chromosome with an average or smaller than average VNTR array on the homologous chromosome 8. An analogous situation was found for the beta defensin repeat underlying the 8p23.1 EV: this was detected in rare instances of high diploid copy numbers unequally split between homologous chromosomes 8;<sup>31</sup> only subsequently did the frequency of similar diploid copy numbers in control populations imply the presence of individuals with above average copy numbers on both homologous chromosomes.<sup>5</sup> Interphase FISH has been used to determine relative haploid copy numbers but becomes impractical when amplicon size is small and copy number very high.<sup>32</sup> Further investigations will be needed of EV and non-EV carriers in larger families using quantitative allele-specific copy number techniques<sup>33</sup> to confirm or exclude our proposal.

In the meantime, the microscopically visible CNV of 8q21.2 is not believed to be population specific now that it has been described in



**Figure 3** (a) Comparison of the total (diploid) copy number of the *REXO1L1* gene cluster measured by PFGE Southern blot and the NanoString nCounter Analysis System showing the close linear correlation; (b) distribution of the frequency of *REXO1L1* copy numbers (y axis) binned into intervals of 10 copies from 90 to 280 (x axis) in 216 controls. The median copy number of 166 is indicated by the dotted line labelled M, the mean value of 173 by the dashed line labelled A and the copy numbers of 265 and 270 in Patient 4 and her father represented by the black stars.

seven index patients of Middle European (3), Japanese (1), Palestinian (1), Caucasian (1) and unspecified Canadian (1) descent (Table 2). These seven were ascertained with congenital anomalies, autism, secondary amenorrhoea, infertility, miscarriages and a family history of intellectual difficulties and cystic fibrosis (Table 2). However, this EV is unlikely to be a cause of congenital anomalies or autism because: (1) there was no consistency in the indications in Patients 2, 3 and 4, and the congenital anomalies in Patient 4 were most likely explained by her trisomy 9 mosaicism; (2) the three patients referred for reproductive reasons were otherwise normal (Patient 1; Manvelyan *et al*,<sup>9</sup> Patients 1 and 2); (3) the 12-kb VNTR repeat array contains only the *REXO1L1* gene cluster that has no known pathology to date; (4) the variant region overlaps RP11-96G1 which is a common benign CNV<sup>34</sup> and corresponds with locus 4603a in the Database of Genome Variants (DGVs).<sup>35</sup> A possible association with infertility and/or miscarriage<sup>9</sup> would need testing in much larger cohorts and is more likely to reflect bias of ascertainment.<sup>36</sup>

This EV was found in 4/3078 (1 in 770) consecutive referrals over a 21-month period which suggests that this cytogenetically visible

variant is not uncommon and may be misinterpreted as a pathogenic imbalance. This frequency could be an underestimate as this EV is usually discernible only in high quality chromosome preparations of at least 550 band level resolution and may have gone undetected in lower resolution chromosomes or where band 8q21.2 is unclear. In addition, Whitby *et al*<sup>34</sup> found CNVs of RP11-96G1 in 142/1275 (1 in 9) clinically affected patients, which suggests that ~1% (9/770) of these CNVs may be microscopically visible. The DGV also indicates that the background frequency of CNVs across the 8q21.2 EV region is high with gains and losses found in 10/49 patients and controls<sup>35</sup> and 9/90 Yoruban individuals.<sup>37</sup> Gains were also found in 26/30 HapMap control samples from four populations, including Yorubans, European-Americans, Japanese and Chinese individuals.<sup>38</sup> In addition, inversions between the 8q21.2 SDs have been detected in individuals of Yoruban and non-African descent.<sup>39</sup> Interestingly, a diploid *REXO1L2P* median copy number of 172, almost identical to the 173 in our controls, was found using sequencing in 159 ethnically diverse subjects from the 1000 Genomes Project,<sup>32</sup> and the Database of Genomic Structural Variation records 272 copies, within the EV range, of a 7-kb segment including the *REXO1L1* gene in a Southern Kalahari hunter-gatherer (KB1).<sup>40</sup>

RP11-96G1 at 8q21.2 is the third most common benign CNV identified by Whitby *et al*<sup>34</sup> using BAC array CGH and, like the second most common (RP11-122N11 at 8p23.1), becomes microscopically visible at high copy number,<sup>31</sup> contains tandemly repeated DNA families,<sup>27</sup> predisposes to polymorphic inversions<sup>41</sup> and has been associated with neocentromere formation.<sup>42</sup> The 8q21.2 VNTR is one of the nine autosomal tandem arrays >2 kb that contain a single gene and show CNV.<sup>27</sup> Three of these nine have been associated with one or more traits including (1) higher copy number of the salivary amylase gene (*AMY1*) at 1q21.1 with high starch diets;<sup>43</sup> (2) increasing copy number of the 8p23.1 beta defensin genes with higher predisposition to psoriasis,<sup>5,6</sup> higher human immunodeficiency virus (HIV) load<sup>7</sup> and, possibly, resistance to influenza virus infection;<sup>8</sup> and (3) lower copy number of the *CCL3L1* gene at 17q21.1 with weak protection against malarial anaemia<sup>44</sup> and susceptibility to chronic hepatitis C virus (HCV) infection.<sup>45</sup> It is therefore interesting that immunological reaction against the human *REXO1L1* gene product (GOR) is a marker of HCV infection including occult HCV in which antibody assays against the HCV are negative.<sup>46</sup> This may simply be a reflection of the partial homology between the GOR epitopes and the HCV-encoded core protein sequence,<sup>46</sup> but it is also conceivable that *REXO1L1* copy number affects susceptibility to HCV infection, progression or response to treatment.<sup>47</sup> A critical region including *REXO1L1* and a number of candidate genes is amplified in a variety of cancers<sup>48</sup> but overexpression of *REXO1L1* in cancer has apparently been recorded only once.<sup>49</sup>

In conclusion, we have confirmed the existence of the 8q21.2 EV and mapped the variation to a region containing the 8q21.2 VNTR arrays with multiple copies of a 12-kb repeat and the *REXO1L1* gene cluster. We propose that expansion of this VNTR explains the microscopic visibility of the 8q21.2 EVs, which need to be distinguished from pathogenic imbalances especially at prenatal diagnosis. Light microscopically visible imbalances with normal array results may be regarded as a general indicator of the possibility of novel EVs in other parts of the genome.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

This work was supported by NIH grant UL1RR029887 to Mount Sinai School of Medicine, NIH grants 1R01DA033660 and 1R01HG006696 to AJS, and grant 2012ALZNIRG69983 from the Alzheimer's Association to AJS. We would like to thank all the families concerned, Monika Sharma of the University Health Network in Toronto, Canada, for her technical expertise and Viv K Maloney for the idiogram of chromosome 8.

## WEB RESOURCES

The URLs are Chromosome Anomaly Collection: <http://www.ngrl.org.uk/Wessex/collection/>; Database of Genomic Variants: <http://dgv.tcag.ca/dgv/app/home>; Database of Genomic Structural Variation: <http://www.ncbi.nlm.nih.gov/dbvar>; and UCSC web browser: <http://genome.ucsc.edu/>.

- 1 Itsara A, Cooper GM, Baker C *et al*: Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet* 2009; **84**: 148–161.
- 2 Barber JCK: Directly transmitted unbalanced chromosome abnormalities and euchromatic variants. *J Med Genet* 2005; **42**: 609–629.
- 3 Balikova I, Martens K, Melotte C *et al*: Autosomal-dominant microtia linked to five tandem copies of a copy-number-variable region at chromosome 4p16. *Am J Hum Genet* 2008; **82**: 181–187.
- 4 Harada N, Takano J, Kondoh T *et al*: Duplication of 8p23.2: a benign cytogenetic variant? *Am J Med Genet* 2002; **111**: 285–288.
- 5 Hollox EJ, Huffmeier U, Zeeuwen PL *et al*: Psoriasis is associated with increased beta-defensin genomic copy number. *Nat Genet* 2008; **40**: 23–25.
- 6 Stuart PE, Huffmeier U, Nair RP *et al*: Association of  $\beta$ -defensin copy number and psoriasis in three cohorts of European origin. *J Invest Dermatol* 2012; **132**: 2407–2413.
- 7 Hardwick RJ, Amogne W, Mugusi S *et al*:  $\beta$ -defensin genomic copy number is associated with HIV load and immune reconstitution in sub-saharan Africans. *J Infect Dis* 2012; **206**: 1012–1019.
- 8 Hardwick RJ, Machado LR, Zuccherato LW *et al*: A worldwide analysis of beta-defensin copy number variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. *Hum Mutat* 2011; **32**: 743–750.
- 9 Manvelyan M, Cremer FW, Lance J *et al*: New cytogenetically visible copy number variant in region 8q21.2. *Mol Cytogenet* 2011; **4**: 1.
- 10 Lecce R, Murdolo M, Gelli G *et al*: The euchromatic 9p+ polymorphism is a locus-specific amplification caused by repeated copies of a small DNA segment mapping within 9p12. *Hum Genet* 2006; **118**: 760–766.
- 11 Di Giacomo MC, Cesarano C, Bukvic N *et al*: Duplication of 9p11.2-p13.1: a benign cytogenetic variant. *Prenat Diagn* 2004; **24**: 619–622.
- 12 Kosyakova N, Grigorian A, Liehr T *et al*: Heteromorphic variants of chromosome 9. *Mol Cytogenet* 2013; **6**: 14.
- 13 Wojcicki SA, Rhodes CH, Brodhurst CA *et al*: The G positive band of the rare euchromatic 9q variant is derived from 9p12. *Appl Cytogenet* 1997; **23**: 125–129.
- 14 Willatt LR, Barber JCK, Clarkson A *et al*: Novel deletion variants of 9q13-q21.12 and classical euchromatic variants of 9q12/qh involve deletion, duplication and triplication of large tracts of segmentally duplicated pericentromeric euchromatin. *Eur J Hum Genet* 2007; **15**: 45–52.
- 15 Joseph-George AM, He Y, Marshall CR *et al*: Euchromatic 9q13-q21 duplication variants are tandem segmental amplifications of sequence reciprocal to 9q13-q21 deletions. *J Med Genet* 2011; **48**: 317–322.
- 16 Barber JC, Rodrigues R, Maloney VK *et al*: Another family with a euchromatic duplication variant of 9q13-q21.1 derived from segmentally duplicated pericentromeric euchromatin. *Cytogenet Genome Res* 2013; **141**: 64–69.
- 17 Starke H, Seidel J, Henn W *et al*: Homologous sequences at human chromosome 9 bands p12 and q13-21.1 are involved in different patterns of pericentric rearrangements. *Eur J Hum Genet* 2002; **10**: 790–800.
- 18 Goumy C, Mihaescu M, Tchirkov A *et al*: An unusual familial chromosome 9 'variant' with variable phenotype: characterization by CGH analysis. *Morphologie* 2005; **89**: 71–75.
- 19 Barber JC, Cross IE, Douglas F *et al*: Neurofibromatosis pseudogene amplification underlies euchromatic cytogenetic duplications and triplications of proximal 15q. *Hum Genet* 1998; **103**: 600–607.
- 20 Fantes JA, Mewborn SK, Lese CM *et al*: Organisation of the pericentromeric region of chromosome 15: at least four partial gene copies are amplified in patients with a proximal duplication of 15q. *J Med Genet* 2002; **39**: 170–177.
- 21 Carelle-Calmels N, Girard-Lemaire F, Guérin E *et al*: Proximal 15q familial euchromatic variant and PWS/AS critical region duplication in the same patient: a cytogenetic pitfall. *Eur J Med Genet* 2008; **51**: 547–557.
- 22 Barber JC, Reed CJ, Dahoun SP, Joyce CA: Amplification of a pseudogene cassette underlies euchromatic variation of 16p at the cytogenetic level. *Hum Genet* 1999; **104**: 211–218.
- 23 López Pajares I, Villa O, Salido M *et al*: Euchromatic variant 16p+. Implications in prenatal diagnosis. *Prenat Diagn* 2006; **26**: 535–538.
- 24 Barber JCK, Hall V, Maloney VK *et al*: 16p11.2-p21.2 duplication syndrome; a genomic condition differentiated from euchromatic variation of 16p11.2. *Eur J Hum Genet* 2013; **21**: 182–189.
- 25 Barber JCK, Brasch-Andersen C, Maloney VK *et al*: A novel pseudo-dicentric variant of 16p11.2-q11.2 contains euchromatin from 16p11.2-p11.1 and resembles pathogenic duplications of proximal 16q. *Cytogenet Genome Res* 2013; **39**: 59–64.
- 26 Geiss GK, Bumgarner RE, Birditt B *et al*: Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* 2008; **26**: 317–325.
- 27 Warburton PE, Hasson D, Guillem F *et al*: Analysis of the largest tandemly repeated DNA families in the human genome. *BMC Genomics* 2008; **9**: 533.
- 28 Furey TS, Haussler D: Integration of the cytogenetic map with the draft human genome sequence. *Hum Mol Genet* 2003; **12**: 1037–1044.
- 29 Francke U: Digitized and differentially shaded human chromosome ideograms for genomic applications. *Cytogenet Cell Genet* 1994; **65**: 206–218.
- 30 Morton NE: Parameters of the human genome. *Proc Natl Acad Sci USA* 1991; **88**: 7474–7476.
- 31 Hollox EJ, Armour JA, Barber JC: Extensive normal copy number variation of a beta-defensin antimicrobial-gene cluster. *Am J Hum Genet* 2003; **73**: 591–600.
- 32 Sudmant PH, Kitzman JO, Antonacci F *et al*: Diversity of human copy number variation and multicopy genes. *Science* 2010; **330**: 641–646.
- 33 Alkan C, Kidd JM, Marques-Bonet T *et al*: Personalized copy number and segmental duplication maps using next-generation sequencing. *Nat Genet* 2009; **41**: 1061–1067.
- 34 Whitby H, Tsalenko A, Aston E *et al*: Benign copy number changes in clinical cytogenetic diagnostics by array CGH. *Cytogenet Genome Res* 2008; **123**: 94–101.
- 35 lafrate AJ, Feuk L, Rivera MN *et al*: Detection of large-scale variation in the human genome. *Nat Genet* 2004; **36**: 949–951.
- 36 Barber JCK: Reply to Dundar *et al*: Why a causal association between classical euchromatic variants of chromosome 9 and multiple miscarriages is unlikely. *Genet Couns* 2010; **21**: 469–471.
- 37 Matsuzaki H, Wang PH, Hu J *et al*: High resolution discovery and confirmation of copy number variants in 90 Yoruba Nigerians. *Genome Biol* 2009; **10**: R125.
- 38 Perry GH, Ben-Dor A, Tsalenko A *et al*: The fine-scale and complex architecture of human copy-number variation. *Am J Hum Genet* 2008; **82**: 685–695.
- 39 Kidd JM, Cooper GM, Donahue WF *et al*: Mapping and sequencing of structural variation from eight human genomes. *Nature* 2008; **453**: 56–64.
- 40 Schuster SC, Miller W, Ratan A *et al*: Complete Khoisan and Bantu genomes from southern Africa. *Nature* 2010; **463**: 943–947.
- 41 Giglio S, Broman KW, Matsumoto N *et al*: Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. *Am J Hum Genet* 2001; **68**: 874–883.
- 42 Hasson D, Alonso A, Cheung F *et al*: Formation of novel CENP-A domains on tandem repetitive DNA and across chromosome breakpoints on human chromosome 8q21 neocentromeres. *Chromosoma* 2011; **120**: 621–632.
- 43 Perry GH, Dominy NJ, Claw KG *et al*: Diet and the evolution of human amylase gene copy number variation. *Nat Genet* 2007; **39**: 1256–1260.
- 44 Carpenter D, Färnert A, Rooth I *et al*: CCL3L1 copy number and susceptibility to malaria. *Infect Genet Evol* 2012; **12**: 1147–1154.
- 45 Grünhage F, Nattermann J, Gressner OA *et al*: Lower copy numbers of the chemokine CCL3L1 gene in patients with chronic hepatitis C. *J Hepatol* 2010; **52**: 153–159.
- 46 Quiroga JA, Castillo I, Bartolome J, Carren V: Serum immunoglobulin G antibodies to the GOR autoepitope are present in patients with occult hepatitis C virus (HCV) infection despite lack of HCV-specific antibodies. *Clin Vaccine Immunol* 2007; **14**: 1302–1306.
- 47 Schaefer EA, Chung RT: The impact of human gene polymorphisms on HCV infection and disease outcome. *Semin Liver Dis* 2011; **31**: 375–386.
- 48 Byrne JA, Chen Y, Martin La Rotta N, Peters GB: Challenges in identifying candidate amplification targets in human cancers: chromosome 8q21 as a case study. *Genes Cancer* 2012; **3**: 87–101.
- 49 Ramakrishna M, Williams LH, Boyle SE *et al*: Identification of candidate growth promoting genes in ovarian cancer through integrated copy number and expression analysis. *PLoS One* 2010; **5**: e9983.