

## ORIGINAL ARTICLE

Identification of a 52 kb deletion downstream of the *SOST* gene in patients with van Buchem disease

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Van Buchem disease is an autosomal recessive skeletal dysplasia characterised by generalised bone overgrowth, predominantly in the skull and mandible. Clinical complications including facial nerve palsy, optic atrophy, and impaired hearing occur in most patients. These features are very similar to those of sclerosteosis and the two conditions are only differentiated by the hand malformations and the tall stature appearing in sclerosteosis. Using an extended Dutch inbred van Buchem family and two inbred sclerosteosis families, we mapped both disease genes to the same region on chromosome 17q12-q21, supporting the hypothesis that van Buchem disease and sclerosteosis are caused by mutations in the same gene. In a previous study, we positionally cloned a novel gene, called *SOST*, from the linkage interval and identified three different, homozygous mutations in the *SOST* gene in sclerosteosis patients leading to loss of function of the underlying protein. The present study focuses on the identification of a 52 kb deletion in all patients from the van Buchem family. The deletion, which results from a homologous recombination between *Alu* sequences, starts approximately 35 kb downstream of the *SOST* gene. Since no evidence was found for the presence of a gene within the deleted region, we hypothesise that the presence of the deletion leads to a down regulation of the transcription of the *SOST* gene by a *cis* regulatory action or a position effect.

Van Buchem disease (MIM 239100) is a disorder of the skeleton inherited as an autosomal recessive trait which, according to Beighton,<sup>1</sup> belongs to the group of craniotubular hyperostoses. The condition was first described by van Buchem *et al*<sup>2</sup> in 1955 as “hyperostosis corticalis generalisata familiaris”, but Fosmoe *et al*<sup>3</sup> subsequently introduced the eponym “van Buchem disease”. Radiological examination of patients diagnosed with van Buchem disease shows a generalised hyperostosis mainly involving the skull, mandible, clavicles, ribs, and the diaphysis of the long bones (fig 1A). Clinical complications such as facial nerve palsy, visual disturbances, and hearing loss can occur as a consequence of the entrapment of cranial nerves. The condition is progressive throughout life. In some patients, serum alkaline phosphatase is raised, but in others the level of serum alkaline phosphatase is within the normal range.<sup>4</sup> This may be associated with a greater or lesser activity of the process at a particular moment in life. Van Buchem disease is a very uncommon disorder with no more than 30 published cases. In 1976, Van Buchem *et al*<sup>5</sup> described a total of 15 patients of Dutch origin. Eight patients from this study and five additional van Buchem patients described by Van Hul *et al*<sup>6</sup> in 1998 have been shown to belong to one extended, highly consanguineous family with a common ancestor in the 18th century. Besides these, one family with four affected sibs<sup>6</sup> and a few isolated cases<sup>7–11</sup> have been reported.

The clinical and radiological features of van Buchem disease are reminiscent of those of sclerosteosis. Sclerosteosis (MIM 269500) is a sclerosing bone dysplasia also belonging to the group of craniotubular hyperostoses.<sup>1</sup> The condition is inherited as an autosomal recessive trait and, like van Buchem disease, it is radiologically characterised by an overgrowth of normal bone tissue, mainly involving the skull, mandible, and tubular bones. This disorder was first recognised by Truswell<sup>12</sup> in 1958 as a morphological variant of Albers-Schönberg disease with syndactyly, but later the term “sclerosteosis” was introduced by Hansen.<sup>13</sup> Sclerosteosis can be differentiated

from van Buchem disease by its more severe character and the presence of hand malformations, including syndactyly of the digits, radial deviation of the terminal phalanges, and absent or dysplastic nails. Some patients also present with tall stature, sometimes even gigantism. Raised intracranial pressure can lead to severe headaches and in some cases to sudden death. The incidence of sclerosteosis is very low and most patients, more than 40, live in South Africa.<sup>14</sup> Stein *et al*<sup>15</sup> postulated that sclerosteosis, like van Buchem disease, is primarily the result of an abnormal osteoblastic bone formation. The condition has also been reported in one family from New York,<sup>16</sup> in a tri-racial kindred from Maryland,<sup>15–19</sup> in a consanguineous Brazilian family,<sup>20</sup> and in a few isolated cases.<sup>21–24</sup>

Van Buchem disease and sclerosteosis were previously mapped to the same chromosomal region 17q12-q21,<sup>5,25</sup> supporting the hypothesis that both conditions are allelic variants, which has already been suggested by Beighton *et al*.<sup>14</sup> Recently, we were able to isolate a novel gene, *SOST*, from the linkage interval. This gene harbours different loss of function mutations within *SOST* in two sclerosteosis families and one isolated sclerosteosis patient.<sup>26</sup> These findings are consistent with the identification of another loss of function mutation in *SOST* in sclerosteosis patients from the Afrikaner population in South Africa.<sup>27</sup> Although our data support the hypothesis that mutations in *SOST* result in sclerosteosis, we failed to find *SOST* mutations in the patients from the Dutch van Buchem family used to map the van Buchem disease locus, despite extensive mutation analysis of the *SOST* gene.

Here, we describe the identification of a 51.7 kb deletion located approximately 35 kb downstream of the *SOST* gene, which is homozygously deleted in all patients from the Dutch van Buchem family and in three additional van Buchem patients of Dutch origin.



**Figure 1** (A) X ray of a 68 year old van Buchem patient (patient 1) showing the thickening (hyperostosis) of the skull and mandible. (B) Frontal view of this patient showing an enlarged mandible and facial paresis.

## PATIENTS AND METHODS

### Patients

Patients from the extended Dutch van Buchem family have been described elsewhere.<sup>45</sup> Three other patients are also of Dutch origin. Patient 1 is a 68 year old male. The father of his maternal grandmother and the father of his paternal grandmother were brothers. On examination, macrocephaly and an enlarged mandible (fig 1B), narrow external ear canals, and bilateral facial paresis (right side grade II HB, left side grade V HB<sup>28</sup>) were evident. Audiometry showed a 40-60 dB sensorineural hearing loss in the right ear and 40-100 dB hearing loss in the left ear. Radiological examination of the skull showed a markedly dense skull and mandible (fig 1A). The patient has no history of fractures. Patients 2 and 3 are a sister and brother aged 58 and 65, respectively, with consanguineous parents, second cousins once removed. Around puberty, enlargement of the skull and mandible and thickening of the clavicles and phalanges started to appear in both patients. At the age of 16, the sister showed facial paresis, is now deaf and almost completely blind with exophthalmos and nystagmus, and suffers from disturbances of balance. The brother showed no abnormalities. Radiological examination showed a generalised endosteal hyperostosis from the skull to the lower extremities in both patients.

### Southern blot analysis

Genomic DNA from the van Buchem patients, carriers, and controls was digested with *EcoRI* and *HindIII*. Fragments were separated on a 0.8% agarose gel in  $0.5 \times$  TBE and transferred to a Hybond-N+ nylon membrane (Amersham). Hybridisation was carried out overnight at 65°C with probes derived from PCR products of ESTs and STSs located within the region between D17S1326 and D17S1860. Probes were radioactively

labelled using  $\alpha^{32}\text{P}$ -dCTP and  $\alpha^{32}\text{P}$ -dATP (ICN) and purified on a Sephadex<sup>TM</sup> G50 fine column (Amersham).

### Control samples

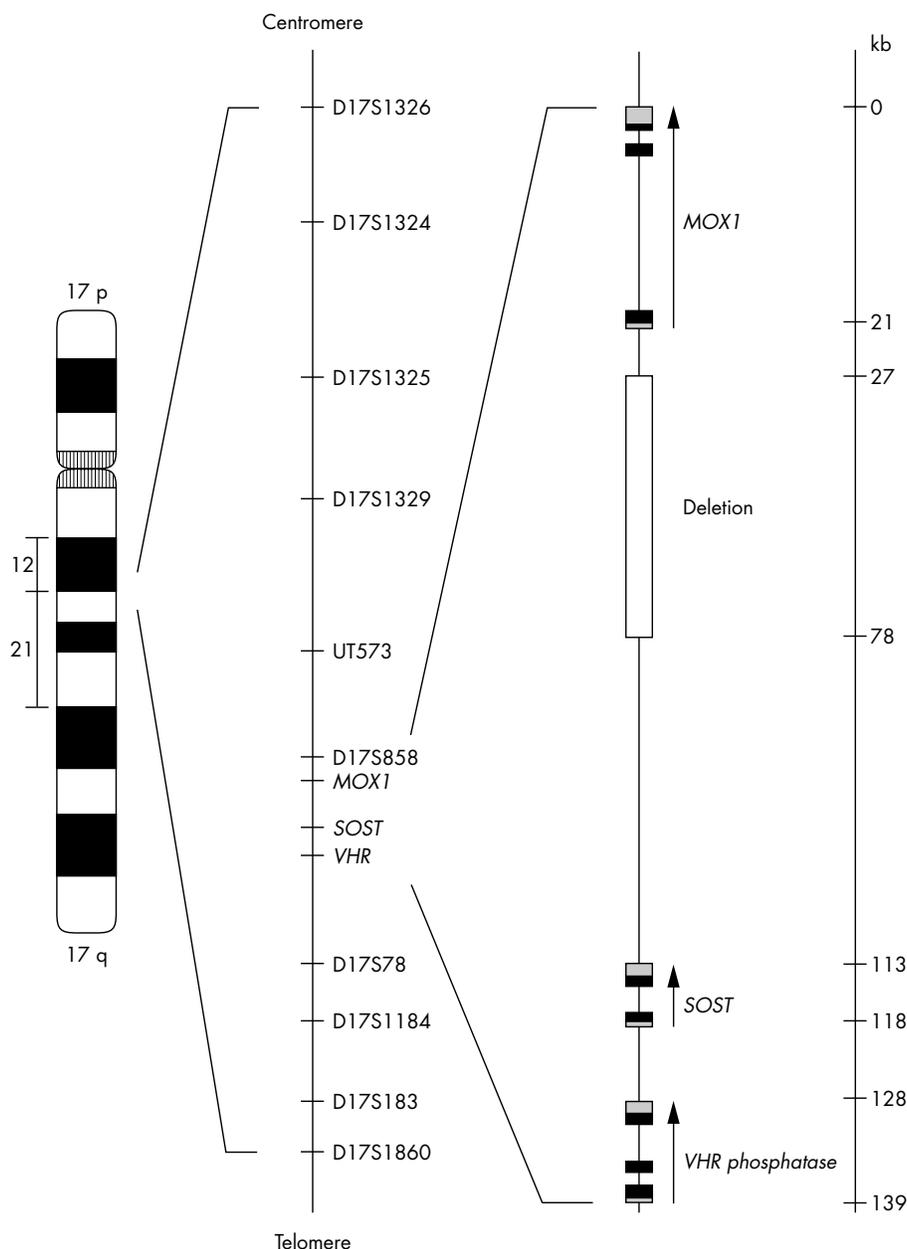
A set of 50 Dutch control and 50 random DNA samples was used to check whether the deletion found in the van Buchem patients exists as a polymorphism in the population. We amplified genomic DNA using three primers: primer 1 on the proximal side outside the deletion (del-F1: 5'-cAgAagAcAggcAgATTTTgg-3'), primer 2 on the proximal side within the deletion (del-R1: 5'-AgAggccATcTcAgcTTgg-3'), and primer 3 on the distal side outside the deletion (del-R2: 5'-AggTgggAAccTATccgTgc-3') using a standard multiplex PCR protocol. PCR products were run on a 1% agarose gel in  $0.5 \times$  TBE. This resulted in a fragment of 0.7 kb when the deletion was present and a fragment of 1.2 kb when the person did not carry the deletion. In heterozygotes for the deletion both the 0.7 kb and 1.2 kb fragment can be seen.

### Identification of transcribed sequences

The complete sequence of the deletion was subjected to the BLAST algorithm<sup>29</sup> and to different exon prediction programs using the NIX program (<http://www.hgmp.mrc.ac.uk/Bioinformatics/>) to identify putative exons and transcribed sequences. Based on the alignment of the results from the different programs, we selected for further investigation those exons which were predicted by more than two programs.

### Tissue expression and library screening

We designed primers located within the predicted exons, MTO sequences, BCC8, and B169 to perform RT-PCR analysis on RNA from 16 different tissues (Human Multiple Tissue cDNA panels I and II, Clontech) and bone tissue. The ICRF human fetal brain



**Figure 2** Map showing the van Buchem linkage interval between D17S1326 and D17S1860, and a detailed map of the region between *MOX1* and *VHR* with the position of the deletion (white box). The axis on the right represents the distances in kb.

and the Stratagene Human Universal cDNA libraries were available for cDNA library screening. Filters were hybridised overnight at 65°C with a radioactively labelled probe.

#### Identification of conserved sequences

The human sequence of the deletion was compared to the homologous mouse sequence located within genomic clone RP23-346P7 (Genbank accession No AC012296) using the “dotter” program by E Sonnhammer.<sup>30</sup> The program creates dot plots for orthologous chromosome regions. Visual inspection allows a straightforward identification of conserved regions.

#### Quantitative TaqMan analysis

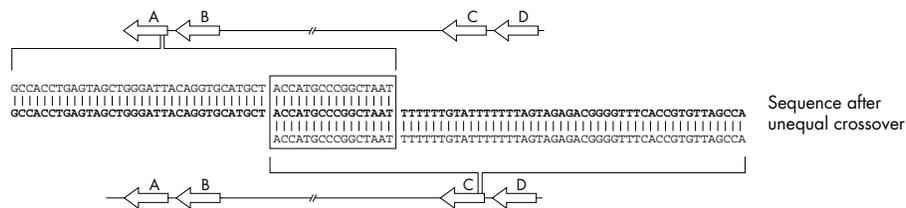
The expression levels of *VHR phosphatase* in van Buchem patients versus controls were determined using a quantitative RT-PCR method (*TaqMan*). Fifty ng of RNA derived from lymphocytes was subjected to reverse transcription using MMLV

reverse transcriptase according to the manufacturer’s protocol (Advantage RT for PCR kit, Clontech). The RT-PCR products were used in a quantitative real time PCR system (ABI PRISM 7700 Sequence Detection System) with the following primer set: *VHRtaq-F* (5′-gccAgcccTgcAAcgA-3′) and *VHRtaq-R* (5′-cgTTcAgcAcATgggTgATg-3′). PCR products were detected using a *VHR* specific *TaqMan* fluorogenic probe (5′-ccTgAcccAcAgAgTTgcccAcgTAGATcc-3′). Experiments were done in duplicate and expression levels were normalised to *GAPDH* expression as an endogenous control.

## RESULTS

### Mutation analysis

We performed sequence analysis to check for sequence variations in *SOST* in patients from the extended Dutch van Buchem family reported previously<sup>45</sup> and the three additional Dutch patients reported here. Sequence comparison between



**Figure 3** Nucleotide sequence flanking and structure of the deletion breakpoints in the Dutch van Buchem patients. The resulting sequence after unequal crossover (bold) has been aligned with the centromeric and telomeric breakpoint sequences. The positions and orientations of the *Alu* repeats are indicated by arrows. The junction region is shown in the box.

van Buchem patients and a control did not result in the identification of any disease causing mutation, although the entire 5 kb of the gene, including the two exons, the complete intron sequence, and the 3'UTR, and approximately 1 kb upstream of the gene was sequenced.

### Analysis of the linkage interval

As no mutation could be found in the *SOST* gene in genomic DNA of van Buchem patients, we searched for other interesting candidate genes in the linkage interval between D17S1326 (proximal) and D17S1860 (distal). About 18 known genes, including *RNU2*, *DDX8*, *MOX1*, *VHR phosphatase*, *dlg3*, *PPY*, *PYY*, and *glucose-6-phosphatase*, map within this region, which is estimated to be approximately 1 Mb. However, based on the function of the encoded proteins, none of these positional candidates could be considered as a strong functional candidate gene for van Buchem disease. We also looked for chromosomal rearrangements in the linkage interval between D17S1326 and D17S1860. Southern blot analysis was carried out on genomic DNA from patients and carriers of the Dutch van Buchem family using probes derived from ESTs and STSs located within this linkage interval. We identified a homozygous deletion in patients with probes derived from MTO-156 (Genbank accession No T27203) and MTO-206 (Genbank accession No T27197).<sup>31</sup> Combined Southern blot and PCR analyses on genomic DNA of van Buchem patients carrying the deletion resulted in the positioning of the centromeric and telomeric breakpoints. The centromeric deletion breakpoint is located just telomeric of the *MOX1* gene (fig 2) within genomic sequence hCIT.501\_O\_10 (Genbank accession No AC004149). The telomeric deletion breakpoint is located within EST sequence B169 (Genbank accession Nos U17906-U17908).<sup>32</sup> Sequence information of the deletion was available from genomic clone hCIT.501\_O\_10 (Genbank accession No AC004149), which includes the centromeric 28 kb of the deletion. For the remaining sequence, a cloning and sequencing effort was performed. The complete deletion spans 51.7 kb and starts approximately 35 kb downstream of the *SOST* gene (fig 2). PCR analysis with primers flanking the proximal and distal breakpoints showed that all patients from the extended Dutch van Buchem family and three additional Dutch van Buchem patients are homozygous for the deletion. Additionally, the parents of the van Buchem patients were shown to be heterozygous. Construction of haplotypes using genetic markers from the chromosome 17 linkage region showed a common haplotype in the three van Buchem patients and the patients from the Dutch van Buchem family, indicating the existence of a common ancestor for all patients (data not shown). We did not find this deletion in DNA samples from 50 random subjects and 50 Dutch controls.

### Mechanism causing the deletion

Analysis of the sequences flanking the deletion breakpoints showed the presence of *Alu* repeats on each side. The *Alu* sequences on the centromeric and telomeric deletion breakpoints are both dimers and are orientated in the same direction. The deletion is most likely the result of an *Alu* mediated, unequal homologous recombination event between two

16 bp repeats within the *Alu* sequences which show 100% identity (fig 3).

### Characterisation of the deletion

In an effort to isolate transcribed sequences from the deletion, we initially performed in silico analyses on the deletion sequence using the BLAST algorithm,<sup>29</sup> the NIX package (<http://www.hgmp.mrc.ac.uk/Bioinformatics>), and the "dotter" program.<sup>30</sup> This resulted in the identification of the cDNA sequences BCC8 and B169, seven MTO sequences already described by Brody *et al*,<sup>31</sup> 10 predicted exons, and two highly conserved regions (fig 4).

The BCC8 cDNA sequence (Genbank accession No U70074) was initially indicated by zoo blot experiments, whereas northern blot analysis showed a transcript of 2.4 kb in heart and skeletal muscle.<sup>32</sup> In addition, the isolation of a cDNA clone from a retina cDNA library has been reported.<sup>32</sup> However, our NIX analysis did not predict this BCC8 sequence to be a putative exon and the dotter program showed no interspecies sequence homology. Moreover, there is no clear open reading frame in the BCC8 sequence and we were not able to amplify BCC8 by RT-PCR from a cDNA panel derived from 16 different tissues. Our data suggest that it is very unlikely that BCC8 is derived from a gene.

B169 (Genbank accession Nos U17906-U17908) is a gene isolated by Friedman *et al*<sup>33</sup> after screening fibroblast and ovarian cDNA libraries. In lymphoblasts, transcripts of 3 and 3.8 kb were detected. Homology searches using the BLAST program showed several EST sequences. We isolated one cDNA clone from the ICRF human fetal brain cDNA library (ICRFp507I01228) using B169 sequence derived probes. However, comparison of the sequences from this cDNA clone and from the ESTs with the genomic sequence of the deletion showed several nucleotide differences. Since the sequence derived from the copy on chromosome 17 did not contain an open reading frame and was not found in any of the EST sequences, it can be concluded that the gene in the deletion is a pseudogene.

BLAST searches on the deletion sequence also showed the presence of seven MTO sequences previously isolated by exon amplification experiments by Brody *et al*.<sup>31</sup> Four MTO sequences (MTO-154, MTO-144, MTO-155, MTO-143) were too small to use for further experiments. BLAST searches did not result in other ESTs derived from these sequences and the dotter program did not show any sequence conservation between man and mouse. RT-PCR analysis using RNA from multiple tissues on the other MTO sequences (MTO-156, MTO-206, MTO-167) did not provide evidence for the expression of any of these. Negative results were also obtained from BLAST searches and the dotter program. These results suggest that the MTO sequences within the deletion do not represent coding sequences.

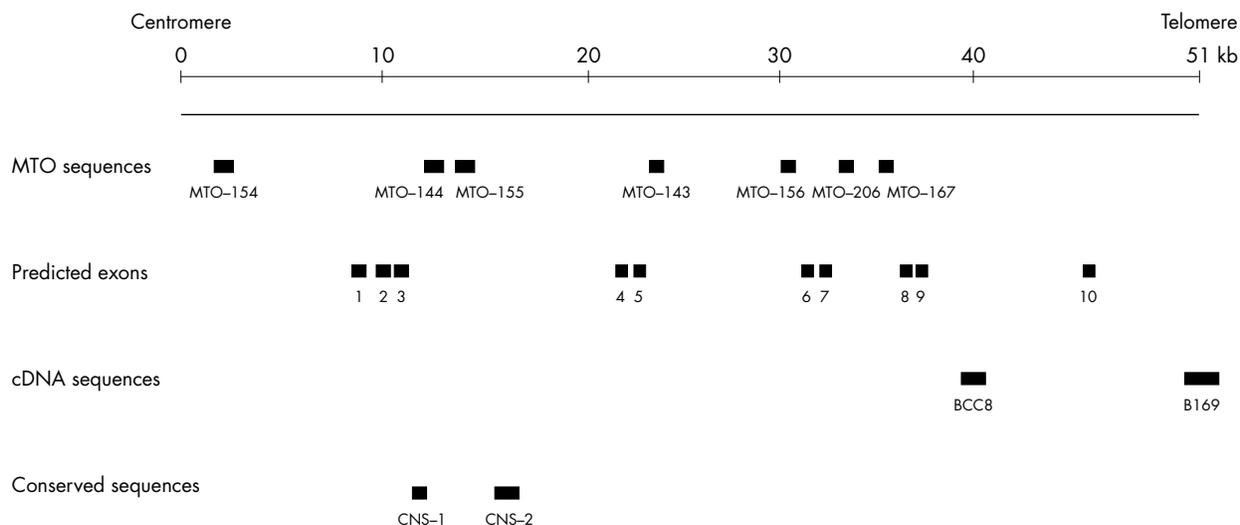
Ten exons were predicted within the deletion by several exon prediction programs using the NIX package. These sequences were subjected to RT-PCR analysis and BLAST homology searches but negative results were obtained. Therefore, we concluded that the sequences do not represent novel genes. The only putative exon which was positive in RT-PCR

A

Position	Name	Accession No	Method	Program	Score	Length (bp)	RT-PCR	dbest	Conserved sequence
2245-2265	MTO-154	T27205	A	-	-	20	-	-	-
9844-10012	EXPR-1	-	NIX	Fex, Hexon, Genemark	M	168	-	-	-
10574-10808	EXPR-2	-	NIX	Fex, Hexon, Genemark, Fgene	M	234	-	-	-
12182-12285	EXPR-3	-	NIX	Fex, Hexon, Fgene	M	103	-	-	-
12577-12734	CNS-1	-	Dotter	-	-	157	-	-	+
12928-12948	MTO-144	T27204	A, NIX	Fex, Hexon, Fgenes	G	20	-	-	-
13671-13692	MTO-155	T27202	A, NIX	Fex, Hexon	M	21	-	-	-
16262-16742	CNS-2	-	Dotter	-	-	480	-	-	+
23220-23658	EXPR-4	-	NIX	Fex, Hexon, Fgenes	M	438	-	-	-
23817-24162	EXPR-5	-	NIX	Fex, Hexon, Fgene	M	345	-	-	-
24331-24349	MTO-143	T27200	A	Fex, Hexon	M	18	-	-	-
30790-30969	MTO-156	T27203	A, NIX	Fex, Hexon	M	179	-	-	-
31247-31542	EXPR-6	-	NIX	Fex, Hexon	M	295	-	-	-
33624-33750	EXPR-7	-	NIX	Fex, Hexon, Fgene	M	126	+	+	-
34097-34234	MTO-206	T27197	A	-	-	137	-	-	-
36205-36318	MTO-167	T27199	A	-	-	113	-	-	-
37132-37311	EXPR-8	-	NIX	Fex, Hexon, Fgenes	M	179	-	-	-
37614-38104	EXPR-9	-	NIX	Fex, Hexon, Fgene	M	490	-	-	-
38525-39600	BCC8	U70074	A, Z	-	-	1075	-	-	-
46289-46460	EXPR-10	-	NIX, dotter	Genscan, Fgenes, GRAIL	G	171	-	-	+
51532-53119	B169	U17906-8	C	-	-	1587	-	+	-

A = exon amplification; C = screening cDNA libraries; Z = zoo blot; M = marginal; G = good.

B



**Figure 4** (A) Summary of the different cDNA sequences, MTO sequences, predicted exons, and conserved sequences with characteristics and analyses performed. (B) Positions of the sequences mentioned above within the deletion.

analysis and shows homology with ESTs is EXPR-7. However, further analysis of this sequence showed the presence of an *Alu* repeat sequence which accounts for the amplicon observed by RT-PCR and also the homology with ESTs. Only one predicted exon (EXPR-10) showed significant conservation between human and mouse, 51% identity over 57 amino acids. However, no homology with any EST was found and screening of the ICRF human fetal cDNA library and the Stratagene Human Universal cDNA library did not result in the isolation

of a cDNA clone from EXPR-10, nor was it positive in our RT-PCR experiments.

Finally, we checked the complete sequence of the deletion for the presence of conserved sequences by performing a human-mouse cross comparison of the genomic sequences using the dotter program developed by E Sonnhammer. This resulted in the identification of two highly conserved regions (fig 4). The first region (Conserved Non-coding Sequence) CNS-1 is 157 bp and shows 84% identity with the mouse

sequence. The second region (CNS-2) is 480 bp and has a degree of conservation between man and mouse of 91%. However, sequence analysis of these regions did not show an open reading frame, thereby making it unlikely that these regions are coding.

### Expression levels of *VHR phosphatase* in van Buchem patients

To look for a possible influence of the deletion on the expression of neighbouring genes, quantitative *TaqMan* analysis was set up. Because of the lack of expression of the *SOST* gene in readily available tissues, this analysis was performed for the *VHR phosphatase* gene, located approximately 10 kb upstream of the *SOST* gene. In lymphoblastoid cell lines from van Buchem patients carrying the deletion and from control samples, the expression levels of the *VHR phosphatase* gene were determined. We were not able to detect an altered level of *VHR* expression in patients as compared to controls (data not shown) since statistical analysis using Student's *t* test did not show a significant difference between the two groups ( $p=0.16$ ).

### DISCUSSION

We previously mapped van Buchem disease and sclerosteosis to the same chromosomal region in 17q12-q21,<sup>5,25</sup> supporting the hypothesis of Beighton *et al*<sup>14</sup> that both conditions could be caused by mutations in the same gene. Recently, we refined the linkage interval using additional microsatellite markers to a region of approximately 1 Mb between D17S1326 and D17S1860. Subsequently, we and others were able to positionally clone a new gene, *SOST*, from this region and we identified three different *SOST* loss of function mutations in sclerosteosis patients.<sup>26,27</sup>

Extensive sequence analyses in patients from the Dutch van Buchem family used to localise the van Buchem disease gene did not result in the identification of a disease causing mutation in the *SOST* gene. This suggested that van Buchem disease in this family might be caused by a regulatory mutation influencing the expression of *SOST*, or by a mutation in another (related) gene located within the linkage interval. Since no interesting functional candidate gene for van Buchem disease was identified in this region, we analysed the complete linkage region between D17S1326 and D17S1860 for chromosomal rearrangements. Southern blot experiments on genomic DNA from patients from the Dutch van Buchem family showed the presence of a 52 kb deletion starting approximately 35 kb downstream of the *SOST* gene. Three other Dutch patients with the typical clinical features of van Buchem disease are also homozygous for the deletion. The absence of this deletion in a set of Dutch and random control DNA samples provided evidence that the deletion was not a polymorphism, but a disease causing mutation.

The deletion is caused by an unequal homologous recombination event between *Alu* sequences, which are located at the deletion breakpoints. A 16 bp sequence at the junction of the deletion is 100% identical at the proximal and distal sides, further substantiating our hypothesis of *Alu* mediated recombination. *Alu* repetitive sequences are frequently involved in homologous and non-homologous recombinations leading to disease.<sup>24,35</sup> In silico analysis of the 52 kb deleted sequence by BLAST searches and exon prediction programs showed 19 putative coding sequences. However, we were unable to confirm experimentally that any of these represent coding sequences, even after extensive analysis. We therefore concluded that no genes are located within the deleted sequence. The absence of genes within the deleted region was verified and confirmed by comparison of the human genomic sequence of the deletion with the homologous mouse sequence.

The absence of a putative coding sequence within the deletion raises the possibility that the deletion has an effect on the transcription of the *SOST* gene in the van Buchem patients.

Two different mechanisms could account for altered transcription: an alteration in the chromatin structure because of the deletion<sup>36</sup> or the presence of a *cis* regulatory element within the deleted region. Mutations in *cis* regulatory elements located more than 10 kb from the disease gene have previously been shown to be responsible for other monogenic conditions, such as aniridia (*PAX6*), campomelic dysplasia (*SOX9*), and X linked deafness (*POU3F4*).<sup>37-39</sup> Interestingly, using man-mouse sequence comparison, we identified two highly conserved non-coding regions, CNS-1 and CNS-2. Despite the fact that these sequences did not show any similarity with known regulatory elements, it has already been shown that conserved non-coding sequences influence the expression of genes. Transgenic mice carrying a deletion of a CNS located between the interleukin-4 (*IL-4*) and interleukin-13 (*IL-13*) genes showed a two- to threefold reduction of the expression of *IL-4*, *IL-13*, and *IL-5*.<sup>40,41</sup> Since *SOST* is not expressed in lymphocytes or other readily available tissue, we were not able to test its transcription in the van Buchem patients. We therefore measured the expression level of *VHR phosphatase*, a gene located approximately 10 kb distally from *SOST* (fig 2). No significant difference was found between deletion patients and controls, indicating that if the deletion alters the *SOST* transcription, it does so without involving the *VHR phosphatase* gene. In light of the findings reported by Loots *et al*,<sup>40</sup> that deleting a CNS did not alter the expression of *RAD50*, a gene located between *IL-13* and *IL-5*, it would not be surprising that the CNS elements within our chromosome 17 deletion regulate only a subset of genes in the region.

In summary, we identified a deletion downstream of the *SOST* gene in Dutch van Buchem patients caused by homologous recombination between *Alu* sequences. In this deletion, no coding sequences could be identified. We propose a regulatory effect of the deletion on the *SOST* gene as the mechanism underlying van Buchem disease in these patients.

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### REFERENCES

- 1 Beighton P. Sclerosteosis. *J Med Genet* 1988;**25**:200-3.
- 2 Van Buchem FSP, Hadders HN, Hansen JF, Wolrding MG. An uncommon familial systemic disease of the skeleton: hyperostosis corticalis generalisata familiaris. *Acta Radiol* 1955;**44**:109-20.
- 3 Fosmoe RJ, Holm RS, Roscoe CM. Van Buchem's disease (hyperostosis corticalis generalisata familiaris): a case report. *Radiology* 1968;**90**:771-4.
- 4 Van Buchem FSP, Prick JJG, Jaspar HHJ. *Hyperostosis corticalis generalisata familiaris (van Buchem's disease)*. Amsterdam: Excerpta Medica, 1976.

- 5 **Van Hul W**, Balemans W, Van Hul E, Dikkers FG, Obee H, Stokroos RJ, Hildering P, Vanhoenacker F, Van Camp G, Willems PJ. Van Buchem disease (hyperostosis corticalis generalisata) maps to chromosome 17q12-q21. *Am J Hum Genet* 1998;**62**:391-9.
- 6 **Dixon JM**, Cull RE, Gamble P. Two cases of van Buchem's disease. *J Neurol Neurosurg Psychiatry* 1982;**45**:913-18.
- 7 **Lopez AG**, Pinero ML, Varo FM. Hiperostosis cortical generalizada (van Buchem). *Rev Clin Esp* 1985;**177**:293-4.
- 8 **Miguez AM**, Esteban BM, Ramallo VG, Quinones JM, Hernandez JA, Lafuente J, Albarran AJ. Partial empty sella turcica in van Buchem's disease. *Med Clin (Barc)* 1986;**87**:719-21.
- 9 **Fryns JP**, Van den Bergh H. Facial paralysis at the age of 2 months as a first clinical sign of van Buchem disease (endosteal hyperostosis). *Eur J Pediatr* 1988;**147**:9-10.
- 10 **Cook JV**, Phelps PD, Chandy J. Van Buchem's disease with classical radiological features and appearances on cranial computed tomography. *Br J Radiol* 1989;**62**:74-7.
- 11 **Bettini R**, Sessa V, Mingardi R, Molinari A, Anzani P, Vezzetti V. Endosteal hyperostosis with recessive transmission (Van Buchem's disease). A case report. *Recenti Prog Med* 1991;**82**:24-8.
- 12 **Truswell AS**. Osteopetrosis with syndactyly, a morphologic variant of Albers-Schönberg disease. *J Bone Jt Surg Br* 1958;**40**:208-18.
- 13 **Hansen HG**. Sklerosteose. *Handbuch Kinderheilkd* 1967;**6**:351-5.
- 14 **Beighton P**, Barnard A, Hamersma H, van der Wouden A. The syndromic status of sclerosteosis and van Buchem disease. *Clin Genet* 1984;**25**:175-81.
- 15 **Stein SA**, Witkop C, Hill S, Fallon MD, Viernstein L, Gucer G, McKeever P, Long D, Altman J, Miller NR. Sclerosteosis: neurogenetic and pathophysiologic analysis of an American kinship. *Neurology* 1983;**33**:267-77.
- 16 **Higinbotham NL**, Alexander SF. Osteopetrosis: four cases in one family. *Am J Surg* 1941;**53**:444-54.
- 17 **Kelly CH**, Lawlah JW. Albers-Schönberg disease: a family survey. *Radiology* 1946;**47**:507-13.
- 18 **Witkop CJ**. Studies of intrinsic disease in isolates with observations on penetrance and expressivity of certain anatomical traits. In: Pruzansky J, ed. *Congenital anomalies of the face and associated structures*. Springfield, IL: C C Thomas 1961:291-308.
- 19 **Witkop CJ**. Genetic diseases of the oral cavity. In: Tiecke RW, ed. *Oral pathology*. New York: McGraw-Hill 1965:834-43.
- 20 **Paes-Alves AF**, Rubin JLC, Cardoso L, Rabelo MM. Sclerosteosis: a marker of Dutch ancestry? *Rev Bras Genet* 1982;**4**:825-34.
- 21 **Pietruscka G**. Weitere Mitteilungen über die Marmorknochenkrankheit (Albers-Schönbergsche Krankheit) nebst Bemerkungen zur Differentialdiagnose. *Klin Monatsbl Augenheilkd* 1958;**132**:509-25.
- 22 **Sugiura Y**, Yasuhara T. Sclerosteosis. A case report. *J Bone Joint Surg Am* 1975;**57**:273-7.
- 23 **Bueno M**, Olivian G, Jimenez A, Garagorri JM, Sarría A, Bueno AL, Ramos FJ. Sclerosteosis in a Spanish male: first report in a person of Mediterranean origin. *J Med Genet* 1994;**31**:976-7.
- 24 **Tacconi P**, Ferrigno P, Cocco L, Cannas A, Tamburini G, Bergonzi P, Giagheddu M. Sclerosteosis: report of a case in a black African man. *Clin Genet* 1998;**53**:497-501.
- 25 **Balemans W**, Van Den Ende J, Paes-Alves AF, Dikkers FG, Willems PJ, Vanhoenacker F, de Almeida-Melo N, Freire Alves C, Stratakis CA, Hill SC, Van Hul W. Localization of the gene for sclerosteosis to the van Buchem disease-gene region on chromosome 17q12-q21. *Am J Hum Genet* 1999;**64**:1661-9.
- 26 **Balemans W**, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuys W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foerzler D, Van Hul W. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). *Hum Mol Genet* 2001;**10**:537-43.
- 27 **Brunkow ME**, Gardner JC, Van Ness J, Paepfer BW, Kovacevich BR, Prohl S, Skonier JE, Zhao L, Sabo PJ, Fu YH, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan JT. Bone dysplasia sclerosteosis results from loss of the *SOST* gene product, a novel cystine knot-containing protein. *Am J Hum Genet* 2001;**68**:577-89.
- 28 **House JW**. Facial nerve grading systems. *Laryngoscope* 1983;**93**:1056-69.
- 29 **Altschul SF**, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990;**215**:403-10.
- 30 **Sonnhammer EL**, Durbin R. A dot-matrix program with dynamic threshold control suited for genomic DNA and protein sequence analysis. *Gene* 1995;**29**:GC1-10.
- 31 **Brody LC**, Abel KJ, Castilla LH, Couch FJ, McKinley DR, Yin GY, Ho PP, Merajver S, Chandrasekharappa SC, Xu J, Cole JL, Struwing JP, Valdes JM, Collins FS, Weber BL. Construction of a transcription map surrounding the *BRCA1* locus of human chromosome 17. *Genomics* 1995;**25**:238-47.
- 32 **Jones KA**, Black DM, Brown MA, Griffiths BL, Nicolai HM, Chambers JA, Bonjardim M, Xu CF, Boyd M, McFarlane R, Korn B, Poutska A, North MA, Schalkwyk L, Lehrach H, Solomon E. The detailed characterization of a 400 kb cosmid walk in the *BRCA1* region: identification and localisation of 10 genes including a dual-specificity phosphatase. *Hum Mol Genet* 1994;**3**:1927-34.
- 33 **Friedman LS**, Ostermeyer EA, Lynch ED, Welch P, Szabo CI, Meza JE, Anderson LA, Dowd P, Lee MK, Rowell SE, Ellison J, Boyd J, King MC. 22 genes from chromosome 17q21: cloning, sequencing, and characterization of mutations in breast cancer families and tumors. *Genomics* 1995;**25**:256-63.
- 34 **Harteveld KL**, Losekoot M, Fodde R, Giordano PC, Bernini LF. The involvement of Alu repeats in recombination events at the  $\alpha$ -globin gene cluster: characterization of two  $\alpha^0$ -thalassaemia deletion breakpoints. *Hum Genet* 1997;**99**:528-34.
- 35 **Tvrdik T**, Marcus S, Hou SM, Fält S, Noori P, Podlutska N, Hanefeld F, Strømme P, Lambert B. Molecular characterization of two deletion events involving *Alu*-sequences, one novel base substitution and two tentative hotspot mutations in the hypoxanthine phosphoribosyltransferase (*HPRT*) gene in five patients with Lesch-Nyhan syndrome. *Hum Genet* 1998;**103**:311-18.
- 36 **Kleinjan DJ**, van Heyningen V. Position effect in human genetic disease. *Hum Mol Genet* 1998;**7**:1611-18.
- 37 **Lauderdale JD**, Wilensky JS, Oliver ER, Walton DS, Glaser T. 3' deletions cause aniridia by preventing *PAX6* expression. *Proc Natl Acad Sci USA* 2000;**97**:13755-9.
- 38 **Wunderle VM**, Crichton R, Hastie N, Goodfellow PN, Schedl A. Deletion of long-range regulatory elements upstream of *SOX9* causes campomelic dysplasia. *Dev Biol* 1998;**95**:10649-54.
- 39 **de Kok YJ**, Vossenaar ER, Cremers CW, Dahl N, Laporte J, Hu LJ, Lacombe D, Fischel-Ghodsian N, Friedman RA, Parnes LS, Thorpe P, Bitner-Glindzic M, Pander HJ, Heilbronner H, Graveline J, den Dunnen JT, Brunner HG, Ropers HH, Cremers FP. Identification of a hot spot for microdeletions in patients with X-linked deafness type 3 (DFN3) 900 kb proximal to the *DFN3* gene *POU3F4*. *Hum Mol Genet* 1996;**5**:1229-35.
- 40 **Loos GG**, Locksley RM, Blankespoor CM, Wang ZE, Miller W, Rubin EM, Frazer KA. Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons. *Science* 2000;**288**:136-40.
- 41 **Hardison RC**. Conserved noncoding sequences are reliable guides to regulatory elements. *TIG* 2000;**16**:369-72.

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## Identification of a 52 kb deletion downstream of the *SOST* gene in patients with van Buchem disease

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