Van Buchem Disease (Hyperostosis Corticalis Generalisata) Maps to Chromosome 17q12-q21

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Summary

Van Buchem disease (hyperostosis corticalis generalisata; OMIM 239100 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispimim?239100]) is an autosomal recessive disorder characterized by hyperostosis of the skull, mandible, clavicles, ribs, and diaphyseal cortices of the long bones. The most striking clinical features are the enlargement of the jaw and the thickness of the skull, which may lead to facial nerve palsy, hearing loss, and optic atrophy. Increased formation, by osteoblasts, of qualitatively normal bone has been proposed as the underlying pathological mechanism, but the molecular defect is unknown. We studied 11 van Buchem patients and their highly inbred family, who live in The Netherlands in a small ethnic isolate, that had a common ancestor ∼9 generations ago. A genomewide search with highly polymorphic microsatellite markers showed linkage to marker D17S1299 on chromosome 17q12-21 (maximum LOD score of 8.82 at a recombination fraction [v] of .01). Analysis of additional markers from that region delineated a candidate region of 1 cM, between markers D17S1787 and D17S934. Interestingly, the only marker not showing recombination with the disease locus was an intragenic marker of the thyroid-hormone receptor ?1 (THRA1) gene, which generated a LOD score of 12.84 at . Since thyroid hormones are known to stimulate bone resorption, the THRA1 gene might be involved in the etiology and pathogenesis of van Buchem disease. Unraveling the underlying mechanism for this disorder could contribute to the understanding of the regulatory processes conditioning bone density and the underlying pathological processes.

Introduction

Van Buchem disease is an autosomal recessive sclerosing bone dysplasia that van Buchem et al. first described in 1955. They called this condition “hyperostosis corticalis generalisata familiaris,” but Fosmoe et al. (1968) initiated use of the eponym “van Buchem disease,” which is widely accepted. The most characteristic feature is endosteal hyperostosis of the mandible, skull (both the calvarium and the cranial base), ribs, clavicles, and diaphyses of the long bones (van Buchem et al. 1962). Van Buchem disease therefore has been classified as a cranio-tubular hyperostosis (Beighton 1988). The characteristics of this group of sclerosing bone diseases is that, in addition to increased bone density, there is a disruption of bone contours. Many subperiosteal osteophytes (exostoses) can form on these altered bones, resulting in a very rough bone surface. The principal clinical features are macrocephaly and an impressively enlarged mandible, which can be very broad and prognathic. The weight of the skull and mandible is grossly increased, in some patients to more than three times the normal weight. In most cases, the bone anomalies are symmetric and progressive, starting in the 1st decade of life. Interestingly, van Buchem patients hardly ever have fractures. Owing to encroachment on the cranial foramina, by the hyperostotic bone, the clinical complications include cranial nerve paralysis (5th, 7th, 8th, and 10th cranial nerves), neuralgic pain, sensorineural hearing loss, and visual problems—such as optic atrophy—that sometimes lead to blindness (van Buchem 1971). Increased intracranial pressure also has been described (van Buchem et al. 1955). Surgical treatment for both decompression of cranial nerves and recontouring of the mandible has been performed (Schendel 1988).

The prevalence of van Buchem disease is very low, with <30 cases reported. Van Buchem et al. (1976) described a total of 15 patients, who were all of Dutch origin. One family with four affected siblings (Dixon et al. 1982) and a few isolated cases (Lopez et al. 1985; Miguez et al. 1986; Fryns and Vandenberghe 1988;
Cook et al. 1989; Bettini et al. 1991) also have been reported. In addition, as already suggested by Eastman and Bixler (1977), several case reports have been published concerning subjects who probably did not have van Buchem disease (Dyson 1972; Owen 1976; Ruckert et al. 1985; Schendel 1988; Rodriguez et al. 1995); rather, these subjects probably had the more benign, autosomal dominant endosteal hyperostosis, first described by Worth and Wollin (1966).

On the basis of normal bone structure, the normal mineral content of bone, and increased alkaline phosphatase in serum, van Buchem et al. (1976) have suggested that the disease is due to increased formation of normal bone. However, the primary defect of van Buchem disease is unknown.

In order to localize the gene for van Buchem disease, we performed a genetic linkage study in a small village located in The Netherlands. This village is an ethnic isolate with a very high degree of consanguinity. Eight of the 15 Dutch patients described by van Buchem (1971) lived in this village and were known to originate from common ancestors. We restudied this family and found 11 patients. Six are survivors from the last study by van Buchem et al. (1976), whereas five new patients were diagnosed by us.

**Subjects and Methods**

**Patients and Pedigree**

The van Buchem–disease pedigree described by van Buchem et al. (1976) has been extended and now contains 13 affected individuals, of whom 2 (patients 12 and 13) had died by the time of our study. The remaining 11 patients, from seven sibships, were investigated in this study. Information obtained from an extended genealogical study, which covered the entire population of the small village and dated back to the beginning of the seventeenth century, enabled us to extend the pedigree described by van Buchem et al. (1976). Family relationships for all 13 patients were documented, and a couple who were married in 1751 was identified as the common ancestors for 14 of the 18 parents of the 13 patients (fig. 1). The remaining 4 parents (the mother of patient 1, the mother of patients 6–8, the mother of patient 12, and the father of patient 13) could not be linked to these common ancestors. Six of the patients (patients 3–8) have been described in detail (van Buchem 1971; van Buchem et al. 1976), whereas five patients (patients 1, 2, and 9–11) were diagnosed in this study (fig. 2). The diagnosis of van Buchem disease was based mainly on...
Figure 2 Clinical pictures of seven patients showing characteristic features of van Buchem disease. Frontal (A) and lateral (B) views of patient 3. This patient, at age 65 years, was the oldest patient studied. C–G, Pictures of the five new van Buchem patients (patients 1, 2, and 9–11, respectively). All patients showed the characteristic features of protruding chin, high forehead, and facial nerve paralysis, as illustrated in panel C.
the phenotypic abnormalities of the skull and mandible, facial nerve involvement, and radiological pictures of the skeleton. Facial nerve grading (House 1983) proved to be a good diagnostic criterion, since severe dysfunction (grade V) on at least one side was seen in all 11 patients examined, with the exception of patient 8, who showed a moderately severe dysfunction (grade IV) on both sides. The most prominent features were found in patient 3 (fig. 2A and B), who is the oldest (age 65 years) among the patients. This suggests that, in van Buchem patients, the skull and mandible grow continuously throughout life, which was confirmed by comparison with pictures of patients at a younger age. Radiological examination of the van Buchem patients revealed increased bone mass of the long bones (fig. 3A) and hyperostosis of the skull and mandible (fig. 3B and C; fig. 4). In four of the five new patients, minimal sensorineural hearing loss was found. This may be due to compression of the 8th cranial nerve.

Genotyping

Genomic DNA was obtained from peripheral blood leukocytes, by standard techniques. Microsatellite markers were analyzed by use of an automated DNA-sequencing apparatus (Applied Biosystems [model 373]), using fluorescently labeled primers. For allele identification, GENESCAN and GENOTYPER software was used in combination with the Linkage Designer program (Van Camp et al. 1997). The Cooperative Human Linkage Center Human Screening Set (Weber version 6)—which contains 391 markers, covering the entire human genome, with an average spacing of 10 cM—was used in a genomewide linkage analysis. The markers are mainly tri- and tetranucleotides and have an average heterozygosity of 76%. PCR reactions were performed by use of a Hybaid OmniGene thermal cycler (Biozym) containing 80 ng genomic DNA, 200 μM each dNTP, 1 × PCR buffer, 2.5 mM MgCl₂, 0.5 units AmpliTaq Gold DNA polymerase, and either 24 ng each primer, for blue (FAM-labeled) and green (TET-labeled) markers, or 48 ng each primer, for yellow (HEX-labeled) markers, in a total volume of 20 μl. After initial denaturation at 94°C for 5 min, the samples underwent 30 cycles of amplification (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min), with a final extension at 72°C for 30 min. Two multiplex reactions were performed, one containing all blue and green markers and the other containing yellow markers. The two separate multiplex reactions were brought together by pooling of 5 μl blue and green markers with 10 μl yellow markers. Loading samples were prepared by addition of 4 μl deionized formamide and 0.7 μl TAMRA-2500 internal size standard (Perkin Elmer–Applied Biosystems) to 1.5 μl of the pooled reaction. After denaturation at 94°C for 5 min, 2.5 μl of each sample was loaded on a polyacrylamide gel and was electrophoresed in accordance with the manufacturer’s instructions.

Radioactive PCR amplification (Hughes 1993) of polymorphic markers was performed by use of 80 ng DNA template and 4 pmol each primer, in a total volume of 20 μl. Sequences for primer pairs are listed in the Genome Database (http://gdbwww.gdb.org/). Prior to

Figure 3  X-rays showing generalized sclerosis in van Buchem patients. A, Lateral view of the elbow, showing diffuse diaphyseal sclerosis. The cortex is thickened, and there is narrowing of the medullary canal. B and C, Anteroposterior and lateral views of the skull, showing extensive sclerosis of the calvarium and the skull base, enlargement of the mandible, and obliteration of the paranasal and mastoid air spaces (Van der Wouden 1971).
Figure 4  Skull and mandible of one of the patients described originally by van Buchem et al. (1955). This skull of a 52-year-old female case weighs 2,357 g. Frontal (A) and lateral (B) views show the markedly enlarged and thickened hyperostotic mandible with many bone excrescences. The entire skull, including the zygomatic ouch nose, is severely thickened and has a rough surface covered with flat excrescences or exostoses. Sutures have disappeared, owing to new bone formation. The orbital, foramen opticum, and nasal openings are narrowed. The skull shows impressive thickening (C), and the mandible has a blown-up appearance (D and E), owing to massive enlargement of the entire mandible, which is covered with bony excrescences.
amplification, one primer was end-labeled with $^{32}$P with polynucleotide kinase (Pharmacia). Radioactive PCR conditions were similar to the conditions used for the fluorescently labeled markers, with the exception that the number of PCR cycles was 26. Primers used for analysis of an intragenic marker of the thyroid-hormone receptor $a_1$ (THRA1) gene have been described elsewhere (Futreal et al. 1992).

**Genome Search**

First, we conducted a genome search with DNA from the 11 patients only, including one sibship of 3 patients, two sibships of 2 patients, and four affected distant relatives. For markers suggestive of linkage, 22 additional individuals were analyzed. These included the 11 available siblings of the patients, the parents of patients 2 and 6–11, and, finally, the father and 2 siblings of the mother of patient 2 (fig. 1). Informed consent was obtained from all individuals.

**Linkage Analysis**

Linkage analysis was performed by use of the FASTLINK programs (Cottingham et al. 1993; Schäffer et al. 1994). MLINK two-point linkage analysis between the microsatellite markers and the disease was performed under the assumption that van Buchem disease is an autosomal recessive disease with a disease frequency of 1/100,000. Allele frequencies of the microsatellite markers were set at 1/$n$, where $n$ is the published number of alleles for each marker. Linkage analysis was performed with consideration of the known consanguineous relationships shown in the pedigree in figure 1.

**Results**

**Genome Search**

After testing 391 polymorphic tri- and tetranucleotide markers, which covered the entire genome, we analyzed the allele distribution of the 11 patients. Within the three sibships, only 15 markers were free of recombinations. D17S1299 was most suggestive of linkage, since it was homozygous for one allele in eight patients and heterozygous in the remaining three patients (fig. 1). Moreover, the heterozygosity in the three patients could be explained by a single recombinational event that had occurred several generations back. The suggestion that van Buchem disease is linked to the chromosomal region around D17S1299 was confirmed by linkage analysis. After inclusion of the 22 additional individuals, a LOD score of 8.82 at a recombination fraction ($\theta$) of .01 was obtained.

**Delineation of the Candidate Region**

To delineate the candidate region more precisely, we analyzed eight markers from the highly saturated Généthon microsatellite map (Dib et al. 1996). D17S1299 is not precisely mapped on the Généthon map but was assigned to a 1.5-cM interval between D17S1814 and D17S932 (see http://www.CHLC.org/ABI/ABIRefMaps .html). The two-point LOD scores provided evidence for linkage of van Buchem disease to this chromosomal region (table 1). Haplotype analysis of all affected chromosomes in the 11 patients enabled us to delineate the candidate region (table 2). A shared recombined haplotype in patients 9–11 excludes the region proximal to D17S1787 as the region harboring the disease gene. A recombination in patient 1 makes D17S934 the flanking marker on the distal side of the candidate region. Therefore, the 0.7-cM region between D17S1787 (proximal side) and D17S934 (distal side) contains the van Buchem–disease gene (fig. 5 and table 2). Since these markers are cytogenetically close to the border of chromosomal bands 17q12 and 17q21, the van Buchem–disease gene must be located in the chromosomal region 17q12-q21.

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Note.—Underlined alleles indicate those that are divergent from the shared haplotype.
Figure 5  Delineation of the genetic candidate region for the van Buchem gene, on chromosome 17, by analysis of key recombinants. The blackened bars represent the regions containing the disease gene, whereas the unblackened bars represent excluded regions. The vertical lines represent regions that were uninformative. A recombination event in patients 9–11 excludes the region proximal to D17S1787; whereas a recombination with D17S934 in patient 1 excludes the region distal to D17S934. The remaining candidate region, between D17S1787 and D17S934, spans 0.7 cM on the genetic map.

Analysis of an Intragenic Marker of the THRA1 Gene

Since the THRA1 gene has been assigned to 17q11.2-12 (Fain et al. 1991) and since thyroid hormones are known to stimulate bone resorption (Mundy et al. 1976), we analyzed an intragenic marker for the THRA1 gene (Futreal et al. 1992). No recombinations between the disease and this marker were detected (data not shown), and a two-point LOD score of 12.84 at $\theta = .00$ was obtained.

Discussion

Bone is a dynamic tissue that is constantly remodeled throughout life by two processes, bone formation and bone resorption. The balance of these processes is under the control of both systemic factors (such as parathyroid hormone and vitamin D) and local factors (such as transforming growth-factor $\beta$ and fibroblast growth factor) (Canalis 1983; Canalis et al. 1993). Disruption of this balance can cause a diverse spectrum of pathological conditions. At one end of this spectrum are conditions that cause decreased bone density, such as osteoporosis. At the other end of this spectrum are diseases that cause increased bone mass or density, caused by increased bone formation or reduced bone resorption (Whyte 1997). Not all of these diseases are determined genetically, but >20 distinct genetic entities have been described (Whyte 1996). The disorders causing high bone mass are called “osteoscleroses” if trabecular bone is increased and “hyperostoses” if cortical bone is increased (Beighton 1988). They include conditions such as osteopetrosis, endosteal hyperostosis, and pycnodysostosis, as well as many others. Thus far, the molecular bases of only two conditions causing increased bone mass have been unraveled (Whyte 1997). First, an autosomal recessive form of osteopetrosis with renal tubular acidosis and cerebral calcification has been shown to be caused by inactivating mutations within the carbonic anhydrase II gene (Sly et al. 1983). Second, in 1996, the cathepsin K gene was identified as the disease-causing gene in pycnodysostosis (Gelb et al. 1996), a rare autosomal recessive condition that might have affected the French impressionist painter Henry Toulouse-Lautrec. Two more gene locations causing increased bone mass have been reported recently, one for an autosomal dominant form of osteopetrosis (Albers-Schönberg disease), at chromosome 1p (Van Hul et al. 1997), and one for a gene causing high bone mass, at chromosome 11q (Johnson et al. 1997). In general, however, positional cloning strategies have only started to unravel the molecular genetics of sclerosing bone disorders.

In this study, we were able to find a locus for another type of sclerosing bone disorder, van Buchem disease, or endosteal hyperostosis. Bone tissue in van Buchem disease has a normal structure, with little modeling defect. The underlying mechanism has been suggested, by van Buchem et al. (1976), to be due to increased osteoblast activity, but little pathological or biochemical evidence has been provided to support this hypothesis.

Van Buchem disease is rare, with <30 patients described. Most patients (a total of 15) have been reported by van Buchem et al. (1976) and were of Dutch descent. Eight patients from this group and five new patients, from this study, are all living in a small village. This village was an island until 1941, when part of the surrounding Zuyderzee was reclaimed and the village became a port. The current population is ~15,000 individuals, almost all of whom are descendents from only 151 inhabitants that survived the plague in 1637. The community was and still is isolated geographically, religiously, and professionally from the rest of The Netherlands.

This inbred van Buchem–disease pedigree now includes 13 patients with a common ancestor. As would be expected, when the structure of the pedigree is considered, high LOD scores for linkage to the disorder were found, and a very small candidate region of 0.7 cM, between markers D17S1787 and D17S934, was delineated (fig. 5). No additional markers between these two flanking markers are currently available, so that, for now, the candidate region cannot be narrowed further. Many genes, such as EDH17B, ERBB2, GCSF, RARA, and THRA1, are assigned to the chromosomal region 17q12-q21 (Black et al. 1993). However, detailed physical mapping of these genes, relative to the small can-
South Africa, with van Buchem disease, are also found. Most of the sclerosteosis, two symptoms that have not yet been reported for some patients with sclerosteosis, gigantism and syndactyly (Beighton et al. 1977). In adults resulting in cranial nerve compression and in an autosomal recessive condition is also characterized by a high intracranial pressure. The clinical symptoms of van Buchem disease also resemble those of sclerosteosis. This sometimes confusing. The clinical symptoms of van Buchem disease belong to the group of endosteal hyperostoses. The correct diagnosis of these hyperostoses is often hard to make, and the nomenclature is sometimes confusing. The clinical symptoms of van Buchem disease also resemble those of sclerosteosis. This autosomal recessive condition is also characterized by a massive overgrowth of the mandible and sclerosis of the skull resulting in cranial nerve compression and increased intracranial pressure (Beighton et al. 1977). In some patients with sclerosteosis, gigantism and syndactyly, two symptoms that have not yet been reported for van Buchem disease, are also found. Most of the sclerosteosis cases are found in the Afrikaner population of South Africa, with >40 patients described (Beighton et al. 1984). On the basis of (1) the close clinical resemblance between van Buchem disease and sclerosteosis and (2) the Dutch origin of the majority of patients with these diseases, Beighton et al. (1984) postulated that these conditions should be lumped together. This is corroborated by the report of a sclerosteosis family in a region of Brazil that was occupied by the Dutch in the seventeenth century (Alves et al. 1982). The localization of the van Buchem–disease gene will allow testing of the hypothesis that dominant endosteal hyperostosis and/or sclerosteosis are allelic to van Buchem disease.

Because van Buchem disease has an autosomal recessive mode of inheritance, it is likely that the disease-causing mutation(s) leads to loss of function of the van Buchem gene. This would imply that the physiological function of the gene is to inhibit bone formation or to stimulate bone resorption. The clinical observation that the hyperostosis observed in van Buchem disease progresses with age suggests that the van Buchem gene is not a developmental gene but a gene that is very actively involved in bone metabolism at an older age. Antisense treatment of the van Buchem gene therefore might become a form of gene therapy for fractures and diseases with low bone density.

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