Ascorbate or vitamin C (VC) is an essential reducing agent and antioxidant that participates in a variety of metabolic processes. Unlike rodents and other animals, humans depend on dietary intake of VC to meet their daily requirement. VC intracellular accumulation is mediated through two distinct pathways. In one pathway, VC is transported as such by high affinity sodium dependent carriers. In a second pathway, oxidised vitamin C is transported as such by high affinity sodium dependent VC transporters types 1 and 2, hSVCT1 and hSVCT2, respectively, and based on homology, human dependent VC transporters types 1 and 2, hSVCT1 and hSVCT2, were identified as anonymous expression sequence tags (ESTs). Subsequently, and based on homology, human SLC23A1 and YSPL3 genes, expression profile, and function were then identified. Both genes were first identified as anonymous expression sequence tags (ESTs). Subsequently, and based on homology, human SLC23A1 and SLC23A2 genes were identified as nucleobase transporters YSPL3 and YSPL2, respectively. Their full length coding sequences, expression profile, and function were then identified. The EST location of YSPL3 and the physical location of the SLC23A2 gene on chromosome 5 were also recently confirmed.

The known enzymatic roles of VC in collagen hydroxylation, carnitine biosynthesis, formation of the catecholamine norepinephrine, and as an inhibitor of oxidation make both SLC23A2 and SLC23A1 candidate genes for a variety of human disorders. These may include monogenic diseases affecting the skeleton, fat metabolism, and the endocrine glands, as well as polygenic conditions, such as osteoporosis, obesity, hypertension, and aging. Thus, the knowledge of the precise genetic and physical location of these two genes is essential for the investigation of their possible contribution to these states.

Two bacterial artificial chromosome (BAC) clones, 261-J-23 which contains SLC23A2 (coding for hSVCT1) and 361-O-11 which contains SLC23A1 (coding for hSVCT2), were obtained from a commercially available library (Research Genetics, Huntsville, AL) after screening by polymerase chain reaction (PCR). DNA was extracted from these BACs as previously described. The primers for the identification of 261-J-23 were 737F (5'-GACACCATGTAGTGACCCCTG-3') and 738R (5'-CCTTACACCTTCATATTTG-3'), and for the identification of 361-O-11 777F (5'-CAGGATCGATC AGGTGTGT-3') and 778R (5'-TCTTCTGGA GTACCTGGATG-3'). The PCR conditions for both sets included 30 cycles, an annealing temperature of 57°C, and a final extension step of five minutes at 72°C. Both BACs contained the full length of the two genes (data not shown).

The 10000 rad SHGC G3 radiation hybrid (RH) panel was used to map the location of the SLC23A2 and SLC23A1 genes with regards to sequence tagged sites (STS) mapped to the human genome. PCR were performed with the above two sets of primers. The products of the reactions were run in agarose gels and scored. The RH mapping data were analysed as previously described and the most closely linked STSs were identified. The chromosomal and genetic locations of these STSs are available on line at http://gdbwww.gdb.org/ and http://www-genome.wi.mit.edu.

Since the location of SLC23A2 on 5q23-qtel was recently published, we did not perform fluorescence in situ hybridisation (FISH) for this gene; its location relative to other genes on 5q was further refined by RH (fig 1). An amplicon from the SLC23A2 gene was mapped by RH in close proximity to STS SHGC1784 (also known as AFM240x9) with logarithm of odds (lod) score 12.5 and distance 9 centiRays (cR) or 252 kilobases (kb) on chromosome 5 (for which 28 kb equals 1 cR). Two markers centromeric to SHGC1784, SHGC11945, and SHGC11406 also linked to SLC23A2 with lower lod scores and distances.

**Figure 1** RH mapping of the SLC23A1 and SLC23A2 genes, nearby RH markers, and respective lod scores and distances.
scores, 10.8 and 9.9, respectively, at distances 15 cR (420 kb) and 19 cR (532 kb). SHGC1784 is located between polymorphic markers D5S1995 and D5S436, which have also been localised to the distal 5q region.15 Thus, the most likely order of the above markers on 5q is cen-D5S1995-SHGC11406-SHGC11945-SHGC1784-D5S436-tel (figs 1 and 2).

FISH analysis using previously described protocols11 12 16 17 with BAC 361-O-11 as a probe showed that SLC23A1 maps to 20p12.2-12.3 (fig 3). An amplicon from SLC23A1 was mapped by RH in proximity to STS SHGC31723 with lod score 4.4 and distance 47 cR or 1128 kb on chromosome 20 (for which 24 kb equals 1 cR).15 One more marker, SHGC34960 that is telomeric to SHGC31723, was linked to SLC23A1 with lod score 4.4 at distance 1152 kb. Marker SHGC31723 is located between polymorphic markers D20S97 and D20S779; the most likely order of the markers on 20p is tel-D20S97-SHGC34960-SHGC31723-D20S779-cen.

Plasma VC concentrations in humans range from 10 to 100 µmol/l; in contrast, millimolar concentrations of VC are found in pituitary, adrenal cortex and medulla, pancreas, ovary, and testes.23 Other cells and tissues also contain millimolar ascorbate concentrations, including neutrophils, lymphocytes, platelets, monocytes, liver, heart, fibroblasts, and neurones. VC is accumulated against its concentration gradient in endocrine and other tissues.31 8 The Na + dependent VC transporters are predicted to play a pivotal role in ascorbate accumulation.18 Once inside cells, VC serves as an electron donor for enzy-

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Figure 2 Positioning of the SLC23A2 gene coding for hSVCT1 on chromosomal region 5q relative to other known loci in this area.

Figure 3 FISH analysis of normal female peripheral lymphocyte metaphase spreads using the BAC 361-O-11 which contains the SLC23A1 gene coding for hSVCT2. The centromere of chromosome 20 is identified by the red signal (α satellite probe), whereas the green signal is from the BAC 361-O-11 (hSVCT2) which maps to 20p12.2-p12.3.
matic and chemical reactions.\(^1\) VC is a specific electron donor for eight isolated enzymes and has been shown to be an electron donor for several of these enzymes in cells and tissues. VC is also a reducing agent, or antioxidant, in chemical reactions both inside and outside cells. For example, VC will quench reactive oxygen species generated from superoxide and hydrochloric acid. For VC to fulfill these roles, it must be transported against its concentration gradient. Therefore, normal SVCT1 and SVCT2 activities are essential for subsequent ascorbate action. Because ascorbate is involved in many distinct metabolic processes, aberrant transport could have implications for a variety of disorders.\(^1\)

The genetic locations of the two main VC transporters provide information for their possible linkage with human diseases that have also been mapped to the respective areas. The gene for hSVCT1 (SLC23A4) maps to 5q23-3q11. This area harbours the loci for an autosomal recessive form of Charcot-Marie-Tooth disease (Mendelian inheritance in man (MIM) 601596)\(^2\) and limb-girdle muscular dystrophy type 1A (MIM 159000)\(^3\) (fig 2) and corresponds to mouse chromosome 18.\(^4\) The gene for hSVCT2 (SLC23A1) maps to 20p12-p11. Interestingly, this area contains the locus for another neurological disorder, Hallervorden-Spatz disease (MIM 234200),\(^5\) which is characterised by progressive dementia and brain iron accumulation.\(^6\) The human chromosomal region 2p12 corresponds to the mouse distal chromosome 2.\(^1\) Neither the human nor the corresponding mouse loci of the VC transporter genes have been linked to osteoporosis\(^7\) or other bone defects, although the markers proximal to these genes have not been used in the reported studies. On the other hand, both human chromosomes 5 and 20 have been reported to be involved extensively in tumour genetic changes; trisomy 20 often characterises fibrosar lesions arising in both soft tissue and bone.\(^8\)

In summary, the present study reported the genetic and physical location of the human VC transporter genes on chromosomes 5 and 20, respectively. These data, along with the new available DNA sequences of SLC23A1 and SLC23A2, will facilitate the investigation of the possible involvement of these two genes in disease.