Assignment of the Human Transglutaminase 2 (TGM2) and Transglutaminase 3 (TGM3) Genes to Chromosome 20q11.2

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Transglutaminases (TGases) catalyze the formation of an $N^{\epsilon}(\gamma$-glutamyl)lysine isodipeptide crosslink in proteins between the γ-amide of a donor glutamine and the ϵ-NH_2 of an acceptor lysine, resulting in the formation of an insoluble macromolecular structure (2, 5). There are five known and partially characterized TGase genes in the human haploid genome: the gene of the membrane-associated TGase1 of 92 kDa (TGM1) maps to chromosome position 14q11.2 (9, 12, 13); the gene encoding the a subunit of the blood clotting factor XIII (F13A1) of 80 kDa maps to 6p25–p24 (1); the gene for the subplasma membrane inactive TGase-like protein constituent band 4.2 of 75 kDa (EPB42) maps to 15q15 (11); but the locations of the genes for the ubiquitous tissue or soluble TGase2 of 80 kDa (4) and for the proenzyme soluble TGase3 of 77 kDa (7) are unknown. The TGase2 enzyme has been implicated in many cellular processes (5) including apoptosis (6). Interestingly, the three TGase1, TGase2, and TGase3 enzymes are expressed during terminal differentiation in the epidermis and certain other stratified squamous epithelia. Determination of the chromosomal locations of the TGM2 and TGM3 genes could facilitate linkage study of their roles, if any, in a large variety of genetic diseases of the skin and related tissues. We have now mapped these two genes using both analysis of hybrid cell panels and chromosomal fluorescence *in situ* hybridization.

The two genes were chromosomally localized by Southern blot hybridization [TGM3, using a 3′-noncoding cDNA probe (7)] or species-specific PCR amplification [TGM2, using primers that amplified a portion of the 3′-noncoding region of the gene (4, 7, 8)] of DNAs isolated from a panel of human/rodent somatic cell hybrids (9, 10). TGM3 was detected as a 9.2-kb hybridizing band in *Eco*RI digests of human and somatic cell hybrid DNAs, and this band was easily resolved from weakly cross-hybridizing 12- and 13-kb bands in mouse and Chinese hamster DNAs, respectively. The human–hamster hybrids (9) consisted of 29 primary hybrids and 13 subclones (24 positive of 42 total), and the human–mouse hybrids represented 18 primary hybrids and 34 subclones (16 positive of 62 total). The same series of somatic cell hybrid DNAs was used as DNA templates for PCR amplification of 783- and 863-bp segments of human TGM2 (7). There was complete concordance for the retention of both genes in the panel of somatic cell hybrids, indicating that both genes were located on the same human chromosome. Both genes segregated concordantly with human chromosome 20 and discordantly (>16%) with all other human chromosomes (data not shown).

Using established methods (9), they were then regionally localized by *in situ* hybridization of metaphase spreads with biotinylated genomic probes (13-kb gTGM2-7 encoding exons I-IV, and 14.2-kb gTGM3-2 encoding exons II–IV) (8). These two probes do not cross-hybridize on Southern blots. Fluorescent G banding of the metaphase spreads was also performed to permit alignment of hybridization signals with specific bands. Location of the FITC signal in typical metaphase spreads is shown in Fig. 1. In the case of both the TGM3 (Figs. 1A and 1B) and the TGM2 (reviewed but data not shown) genes, the center of the signal was localized predominantly in band 20q11.2, with overlap into band 20q12 (Fig. 1C). The position of the signals were also determined as a fraction of the total length of chromosome 20 and were 0.609

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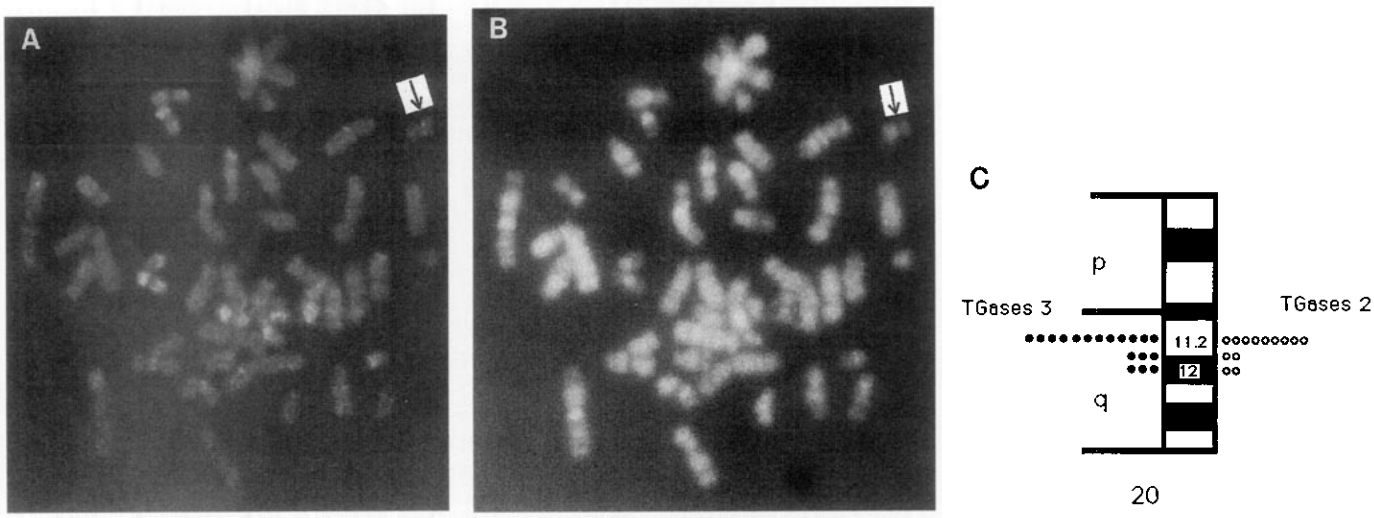

FIG. 1. Localization of the TGM2 and TGM3 genes by fluorescence *in situ* hybridization. (**A**) Typical metaphase spread hybridized with the TGM3 genomic probe showing the fluorescent hybridization signal on chromosome 20. (**B**) Photograph of the same metaphase spread showing the fluorescent G banding pattern. (**C**) Idiogram of chromosome 20 showing distribution of grains with TGM2 (**left**) and TGM3 (**right**) probes projected onto the banded chromosome pattern.

± 0.009 (18 chromosomes measured) for the TGM2 gene and 0.629 ± 0.009 (13 chromosomes measured) for the TGM3 gene.

Our data indicate that these two TGase genes are located close together. Further work involving linkage analysis and/or the use of YACs will now be necessary to determine precisely how close the TGM2 and TGM3 genes are to one another. Interestingly, however, the other three human TGase genes have been scattered throughout the human genome. Analyses of gene structures (8) and amino acid sequence homology scores (4, 7, 9) for the TGase2 and TGase3 systems reveal that they are more closely related to each other than the other TGase family members. Together with the present new information on chromosomal locations, these data predict that these two members of the family have most recently duplicated. Further work is in progress to assess their different functional and expression properties and roles in diseases.

Following submission of the manuscript, a report localizing the TGM2 gene to human chromosome 20 appeared (3).

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