Human Salivary Amylase: Genetics of Electrophoretic Variants

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INTRODUCTION

Human α-amylase is an endoamylase which hydrolyzes the α-1,4-glycosidic linkages of polysaccharides and is produced primarily in the pancreas and salivary glands. It appears not only in the saliva and duodenal secretions but in serum and urine as well. With differing electrophoretic methods, salivary amylase (SaAmy) has exhibited variable isozyme patterns. Six enzymatically active amylase bands have been seen in an agar gel system [1]. Disc polyacrylamide gels have provided more complex patterns [2]. Vertical sheet acrylamide gels have provided additional resolution with eight amylase bands [3]. This method has made comparisons possible among zymogram patterns of multiple samples studied under the same conditions.

Earlier data have shown heritable electrophoretic variations in mouse SaAmy [4] as well as in the amylases of *Drosophila melanogaster* [5, 6]. Human pancreatic and salivary variations in two-generation data have been reported [3, 7, 8]. These data suggest that SaAmy does exist in multiple isoenzyme forms and that the variations of these forms are inherited.

Using a modified polyacrylamide electrophoretic system, we have screened individual saliva samples for establishment of a normal pattern of amylase isoenzymes and the detection of variants. When individuals with variant patterns were found, family members were studied to define the heritability.

MATERIALS AND METHODS

Parotid and whole saliva samples from normal and variant subjects gave comparable amylase staining patterns. Therefore, whole saliva samples were collected from 700 medical students, personnel, and families referred for twin studies. The population was primarily adult and Caucasian. The samples were centrifuged and the supernatant electrophoresed or stored at $-20°$ C for later analysis.

Discontinuous vertical sheet polyacrylamide electrophoresis was employed, modifying and combining the techniques of Ritchie et al. [9] and Boettcher and de la Lande [10].

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The contents of a 5.6% polyacrylamide gel, *p*H 8.5, are: acrylamide, 28.0 g; N,N'-methylenebisacrylamide, 0.74 g; N,N,N',N'-tetramethylethylenediamine, 0.34 ml; 0.037 M tris(hydroxymethyl) aminomethane-HCl, *p*H 8.6, 480.0 ml; and amonium persulfate, 3.5% in water, 20.0 ml. Ammonium persulfate was added to promote polymerization in a 6-mm standard (12 × 27 cm) eight-slot starch gel mold. The bridge buffer, *p*H 8.3, contained 0.20 M Tris and 1.5 M glycine. Saliva samples were applied 12 cm from the cathodal end of the gel, sealed with liquid petrolatum, and covered with plastic film. Vertical electrophoresis was carried out in the anodal direction at room temperature for about 15 hr, at a constant current of 20 ma. During electrophoresis, the gel voltage rose from approximately 1.3 to 8.5 v/centimeter. The run was terminated when the buffer front reached a distance of 12 cm from the origin. Gel above the origin and below the front was discarded, and the remaining gel (6-mm thick) was sliced into two 3-mm gels. One half was incubated in 0.02 M phosphate buffer (*p*H 6.9), 0.0067 M NaCl, and 1% hydrolyzed starch for 15 min. The gel was water-rinsed and stained with Gram's KI-Iodine solution for a few seconds until resolution of the amylase banding was evident. Photographs were taken immediately after staining, since the pattern becomes less distinct after 10–15 min. When appropriate, the other half of the gel was protein stained in amido black, 0.5% in methanol-water-acetic acid (5:5:1), and destained in 3% acetic acid solution.

RESULTS

The normal pattern seen was that of alternating light and heavy bands with decreasing intensity toward the anode (fig. 1). Six to eight bands were usually

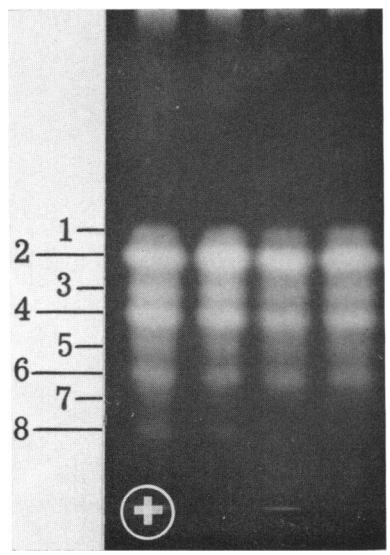

FIG. 1.—Polyacrylamide gel zymograms of normal whole saliva from four individuals. Note the amylase-like activity at the origin and the buffer front.

visualized. In addition, some amylase-like activity was seen at the origin and buffer front. Among the 700 individuals screened, five variant families (four individual probands and two sets of twins serving as a proband set) exhibited a variation from the normal pattern (0.7%). Major electrophoretic variations in

zymograms occurred cathodal to the first normal component. These variations were designated by a surname contraction of the proband. To date, three different cathodal variants have been noted: SaAmy(Tn), SaAmy(Bn), and SaAmy(Al) (fig. 2). Two additional unrelated families also exhibited the Tn variant.

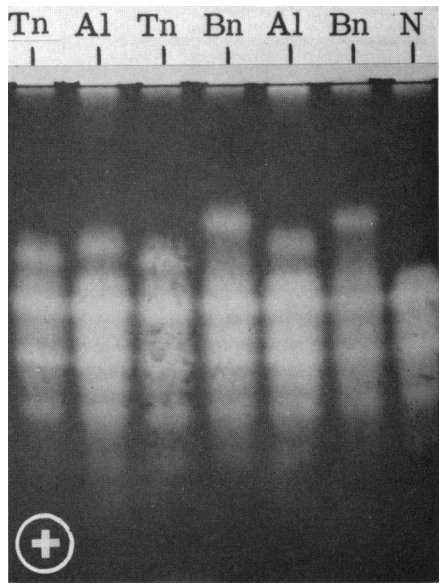

FIG. 2.—Zymograms showing relative migration of the three SaAmy variants: fast—Tn; intermediate—Al; slow—Bn; and normal—N.

The Tn variation is the most anodal of the observed cathodal variants; that is, it migrates nearest to the normal components. The rest of the banding pattern appears normal with respect to presence, relative activity, and electrophoretic mobility. The Bn variant is the most cathodal variant observed, while the Al variation is of intermediate mobility.

Equal volume mixtures of saliva samples of each variant with a normal sample or with those of other variants were subjected to electrophoresis to evaluate possible isozyme interactions. In such gels, all isozymic components were noted in positions identical with those seen in unmixed samples (fig. 3).

The pedigrees summarize the results of family studies (fig. 4). Individuals within each family were either normal or exhibited the SaAmy variant of the proband. Three families exhibited the Tn variation. In two families, X-linkage was excluded. In family 2041, III-8 transmitted the variation to IV-9 and II-8 failed to transmit the trait to his daughter; in family 2209, male-to-male transmission in three generations was noted. After combining all Tn sibships, there was no significant difference in segregation of normal and variant phenotypes ($P > .5$). Also no significant sex difference was apparent among normal and variant individuals ($P > .5$). Dominant inheritance, presumably autosomal, was established for SaAmy(Al) in

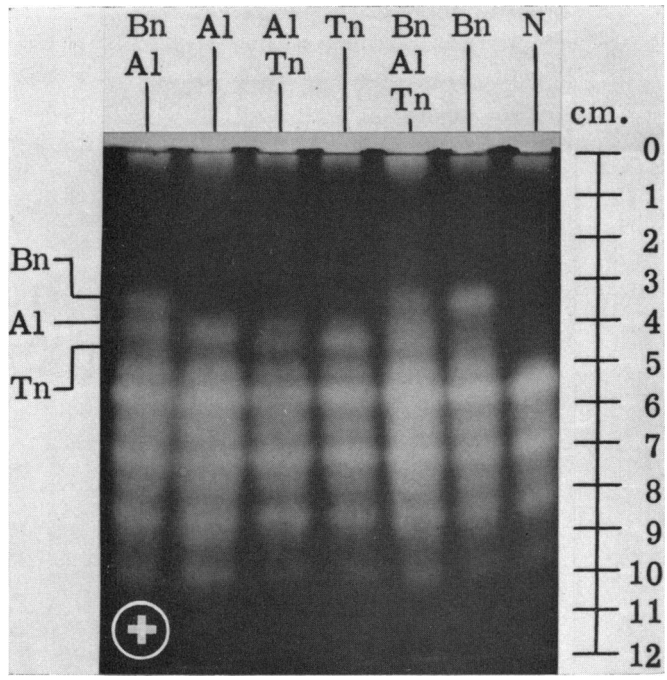

FIG 3.—Zymograms of equal volume mixtures of variant-normal and variant-variant SaAmy. No evidence of interaction is noted.

family 9342, although no affected males have been observed. Dominant inheritance is clearly discernible in family 2244 which manifests SaAmy(Bn).

DISCUSSION

In earlier studies, electrophoresis of human SaAmy in an agar gel system, *p*H 6.8, produced a cathodal isozyme pattern [1]. The normal zymogram showed decreasing enzyme activity, cathode to anode, as seen in our studies. The isozymes were similar to our bands 1, 2, and 4. Components corresponding to bands 3 and 5 in the present investigation were not observed. No significant variations in mobility were reported in the 975 individuals studied. A variation in the amylase activity was studied in two families with no inheritance pattern proposed.

Using a barbital buffer agar system, *p*H 8.4, Kamaryt and Laxova [8] reported normal and variant zymograms of SaAmy from twins and their families. In 111 families, 92.8% had only a single isozyme, 2.7% had two isozymes, and 4.5% had no discernible activity. Autosomal codominant inheritance was proposed from these data.

Wolf and Taylor [2], using an anionic polyacrylamide disc system in barbital buffer, *p*H 8.6, showed banding patterns in parotid amylase similar to those of Ogita [1]. They reported variation with respect to the number of isozymes detected in 11 people, but did not characterize the isozymes further.

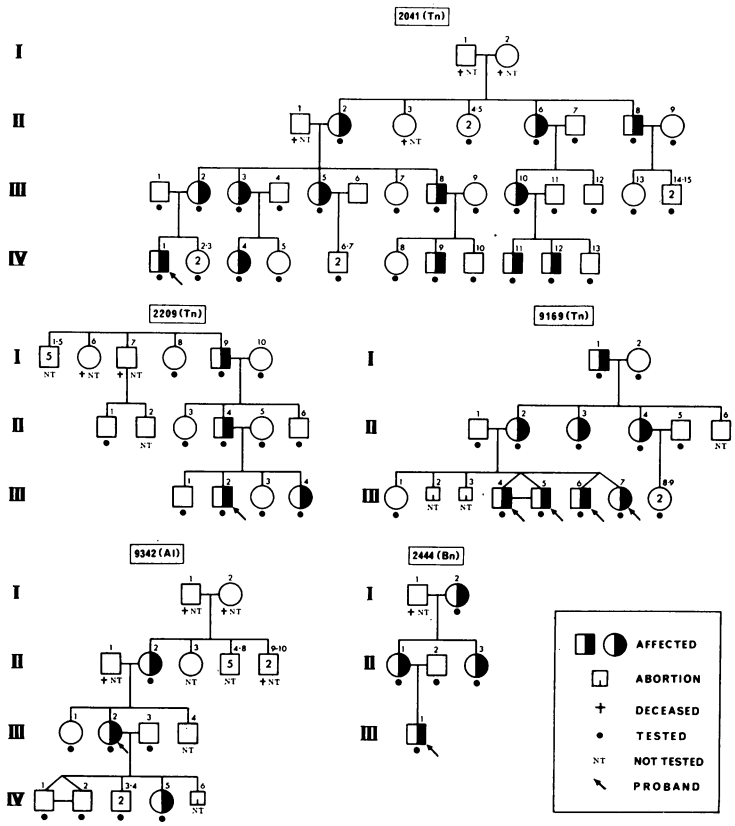

FIG. 4.—Pedigrees of families with SaAmy variants

A more recent study using a polyacrylamide vertical sheet gel has been performed [3]. With a discontinuous system (gel buffer–lithium-borate and tris-citrate, bridge buffer–lithium-borate), variations were seen in two areas of the zymogram. One involved an intensity and mobility alteration of band 1 without variations occurring in the rest of the zymogram. Another variation was observed with individuals manifesting either slow or fast or both slow and fast components of the band-4 region. With these variants, slowing of only band 3 was observed, indicating that bands 3 and 4 had units in common. Thus they postulated that there are at least two loci involved in controlling the structural units of salivary amylase. Autosomal codominant inheritance was proposed.

Our study illustrates the usefulness of a sheet polyacrylamide gel system as a screening procedure for SaAmy variation and as a method to identify the inheritance of such variants. Several interesting differences are noted in comparing the above studies with ours. First, the intensity of the bands in our system shows an alternating pattern; that is, the more intense bands (2, 4, and 6) seem to form one migrating group while bands 1, 3, and 5 form another. These groups also differ in that the rela-

tive distance between the bands is constant and unique for that group. Within each group, the pattern decreases in intensity from the origin toward the anode. Kauffman et al. [11] have presented data from human parotid salivary amylase eluted from columns and subsequently electrophoresed in anionic acrylamide gels that support the existence of two groups of isozymes: group A consisting of their bands 1, 3, and 5; and group B consisting of bands 2, 4, and a *z* band.

When the method of Boettcher and de la Lande [10] was employed in our laboratory, bands which we designate as 3 and 5 were not seen. SaAmy(Bn) was easily identified in both systems, but migrated less anodally in our system. It is possible that our bands 3 and 5 are within the enzymatically active areas of the second and third bands seen with the Boettcher system. Therefore the second and third bands in the Boettcher system would correspond to our more intense bands 2 and 4.

We have observed no obvious variations of band 1, since our slower variants also manifested a normal band 1. Neither have we observed any slowing of migration of any other normal bands. Boettcher and de la Lande [3] imply that single normal bands may be manifested either as mutants with slight changes in electrophoretic mobility or with rather marked variations in the amount of enzymatic protein. They report finding bands in some variants with enzymatic activity that did not necessarily correspond to increased amounts of enzymatic protein. We did not observe these same variations in our protein-stained gels.

The population of individuals tested was rather homogeneous with regard to age, health, and race. Thus, no attempt was made to distinguish differences which might be related to these factors. Although the prevalence of variants was 0.007, each variant type was less frequent: Tn, 0.0043; Bn, 0.0014; and Al, 0.0014. Assuming these variants to be from heterozygotes, the homozygote frequencies should be one in 200,000 or less. Therefore these variants are rare in the population tested. Within each family, the trait seems to be inherited as an autosomal codominant; evidence against X-linkage was observed.

SUMMARY

A 5.6% tris-glycine tris-HCl discontinuous sheet polyacrylamide electrophoresis system was developed to screen 700 individuals for human salivary amylase (SaAmy) variation. The frequency of variants was 0.7%. Three families exhibited the SaAmy (Tn) variant and one each exhibited the SaAmy (Bn) and (Al) variants. The family data are consistent with an autosomal codominant mode of inheritance.

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Symposium on Sickle Cell Disease

Scientists from Africa, the West Indies, and the United States will participate in a Symposium on Sickle Cell Disease at the Commodore Hotel, New York City, November 18–19, 1971. The two-day symposium will cover diagnosis, management, education, and research of sickle cell disease. The meeting is sponsored by the New York Chapter of the National Foundation–March of Dimes and the Foundation for Research and Education in Sickle Cell Disease, and is under the cochairmanship of Drs. Harold Abramson, John F. Bertles, and Doris L. Wethers.

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