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Mammalian Acatalasemia: The Perspectives of Bioinformatics and Genetic Toxicology

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Abstract

The molecular defects in the catalase gene, levels of m-RNA and properties of the residual catalase studied by scientists are reviewed in human (Japanese, Swiss and Hungarian) and non-human (mouse and beagle dog) acatalasemia with reference to the bioinformatics. Japanese acatalasemia-I, the G to A transition at the fifth position of intron 4 of the catalase gene, limited the correct splicing of the mRNA and synthesized trace catalase with normal properties. Hungarian acatalasemia type C showed a splicing mutation. In the Japanese acatalasemia II and the type A and B of Hungarian acatalasemia, the deletion or insertion of nucleotides was observed in the coding regions, and the frame shift altered downstream amino acid sequences and formed truncated proteins. In the Hungarian acatalasemia D, the substitution of a nucleotide in the exon was found. In mouse and beagle dog acatalasemia, the substitution of nucleotides in the coding regions was also observed. Studies of residual catalase in Swiss, mouse and beagle dog acatalasemia showed that aberrant catalase protein degrades more quickly than normal catalase in cells. The experimental research in genetic toxicology concerning the effect of oxidative stressors (nitrogen monoxide, nitrogen dioxide and so on) on Japanese acatalasemic blood and acatalasemic mice is described. The clinical features of Japanese and Hungarian acatalasemic subjects are also described.

KEYWORDS: acatalasemia, catalase, novel mutation, bioinformatics, genetic toxicology

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Review

Mammalian Acatalasemia: The Perspectives of Bioinformatics and Genetic Toxicology

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The molecular defects in the catalase gene, levels of m-RNA and properties of the residual catalase studied by scientists are reviewed in human (Japanese, Swiss and Hungarian) and non-human (mouse and beagle dog) acatalasemia with reference to the bioinformatics. Japanese acatalasemia-I, the G to A transition at the fifth position of intron 4 of the catalase gene, limited the correct splicing of the mRNA and synthesized trace catalase with normal properties. Hungarian acatalasemia type C showed a splicing mutation. In the Japanese acatalasemia II and the type A and B of Hungarian acatalasemia, the deletion or insertion of nucleotides was observed in the coding regions, and the frame shift altered downstream amino acid sequences and formed truncated proteins. In the Hungarian acatalasemia D, the substitution of a nucleotide in the exon was found. In mouse and beagle dog acatalasemia, the substitution of nucleotides in the coding regions was also observed. Studies of residual catalase in Swiss, mouse and beagle dog acatalasemia showed that aberrant catalase protein degrades more quickly than normal catalase in cells. The experimental research in genetic toxicology concerning the effect of oxidative stressors (nitrogen monoxide, nitrogen dioxide and so on) on Japanese acatalasemic blood and acatalasemic mice is described. The clinical features of Japanese and Hungarian acatalasemic subjects are also described.

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Catalase Enzyme

In almost all aerobic organisms, hydrogen peroxide is produced by normal metabolic process, mostly from superoxide by superoxide dismutase, and then is broken down by the catalase. The peroxidative reaction is remarkable at low hydrogen peroxide concentrations, but the catalatic reaction is predominant at higher hydrogen peroxide concentrations. Human catalase with a molecular mass of 24,000 is a tetramer comprised of 4 identical subunits, each of which consists of 526 amino acid residues [1].

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History and Research Process in Human and Non-human Acatalasemia

1. Human acatalasemia.

1) Japanese acatalasemia: Acatalasemia was first described by the Shigeo Takahara in Japan in 1947 [2, 3]. Takahara described that an 11-year-old pale looking girl came to the Department of Ear, Nose, and Throat Outpatient Clinic in Okayama University Medical School with high fever and complained of severe oral ulcer in 1946. After a radical operation,

Takahara poured hydrogen peroxide on the wound and found that the blood immediately turned a brownish-black and the usual bubbles did not appear. Four out of 7 siblings had mouth gangrene, and all of their blood reacted with hydrogen peroxide in the same manner, indicating a genetic abnormality [4]. This oral gangrene due to acatalasemia was called Takahara's disease. Takahara also found that the heterozygote of acatalasemia had catalase activity in the blood, though its amounts were one half catalase activities of normal person and designated as "hypocatalasemia" [5]. He carried out a large-scale survey of hypocatalasemia in Japanese, Korean and Chinese individuals, and the difference in the frequency of acatalasemic genes among the races of the Far East area was reported [5, 6]. The properties of residual catalase were also described [4].

2) Swiss acatalasemia: Acatalasemia was described by Hugo Aebi [7–10]. Aebi reported that it was impossible to distinguish normal and heterozygous individuals based on blood catalase activity [10].

3) Hungarian acatalasemia: Two sisters of a Hungarian family were found to be the type A of acatalasemic subjects by Góth in 1992 [11, 12]. The blood catalase activities of the acatalasemic A subjects were 4.0 and 6.7% of normal activity [12], and the activity of hypocatalasemias in the family was 45.8% of normal. The higher incidence of diabetes mellitus in Hungarian catalase deficient family members was reported by Góth [13].

4) Peruvian and German acatalasemia: Propositus of acatalasemia was found via a progressive oral gangrene called Takahara's disease and via a hydrogen peroxide test of the blood in 1979 in Peru [14] and in 1999 in Germany [15].

5) Jewish acatalasemia: Iranian born Jewish origin with acatalasemia (7～9% of normal) and with glucose-6-dehydregenase deficiency were found by Szeinberg [16].

2. Non-human acatalasemia.

1) Mouse acatalasemia: Acatalasemic mice (Cs^b) and homologous hypocatalasemic mice (Cs^c) were produced by Feinstein in 1969 by hybridizing mice with low catalase activity [17], which were induced by X-ray irradiation, and the Cs^b and Cs^c genes were transferred into the genes of C3H strain mice. The C3H/AnL Cs^b (male), C3H/Anl Cs^b (female) mice, C3H/AnL Cs^c and C3H/Anl Cs^c mice along with wild-type mice were donated from Argonne National Laboratory to Ogata for collaborative research by courtesy of Feinstein (1979).

2) Beagle dog acatalasemia: Spontaneous occurrence of acatalasemic beagle dog occasionally expressing signs of Takahara's disease was reported by Fukuda in 1982 [18].

Review: A review describing a comparison among Japanese and Swiss acatalasemia in humans and acatalasemic mice was described by Ogata in 1991 [19]. The characterization and comparison of Hungarian acatalasemia to the Japanese and Swiss acatalasemia were performed by Góth in 2001 [20] and 2004 [13].

Characterization of Acatalasemia and Hypocatalasemia

1. Acatalasemia. Takahara defined acatalasemia as a new term meaning "a deficiency of catalase enzyme." The Japanese type of acatalasemia is a rare genetic abnormality defined by an apparent lack of catalase enzyme. About half of acatalasemia originates from ulcerating oral gangrenes; this type is called Takahara's disease [4, 6]. Japanese acatalasemia is inherited as an incomplete recessive trait, and the heterozygote shows activity that is about half of normal activity. Recently, Japanese-type acatalasemia has been characterized by Takahara [4], Ogata [19], Kishimoto [21] and Hirano [22]. In these reports, the Japanese type of acatalasemia is defined as a hereditary or genetic metabolic disorder characterized by an almost total loss of catalase activity in erythrocytes and is inherited as an autosomal incomplete recessive trait, and is often associated with progressive ulcerating oral gangrene. In the characterization of both Japanese and Swiss acatalasemia, an autosomal recessive trait can be used instead of an autosomal incomplete recessive trait, in due consideration of the fact that heterozygote of Swiss acatalasemia was at the level of normal catalase activity in blood. Dr. H. Aebi also pointed out that acatalasemia cannot be considered a disease, since all of acatalasemc subjects show a high level of activities; thus it is suitable to consider it an anomaly (abnormality) [7]. Residual catalase presents in Japanese and Swiss acatalasemias, and the catalase activity is 0.08～0.67% (0.34±0.21%) of normal in Japanese acatalasemia,

0.5～2.0%(0.18±0.1%) of normal in Swiss acatalasemia, and 4.0% and 6.7% of normal in Hungarian acatalasemia A. Góth described Hungarian acatalasemia as an inherited pattern for acatalasemia, in which less than 10% of normal erythrocyte catalase activity was observed, and inherited autosomal recessive trait. Acatalasemia is in a homozygote state and is distributed heterozygous condition [13]. Definition of human acatalasemia also adopted in mouse [23] and beagle dog acatalasemias considering genetic abnormality. The residual blood catalase activity of mouse Cs^b was 1.8% of normal activity and that in the beagle dog was 0.7% as determined by UV spectrophotometry [18].

2. Hypocatalasemia. Some of the family members of acatalasemia, including acatalasemia I confirmed by genetic study [21], were found to have about half of the normal catalase activity and were diagnosed as hypocatalasemia. Family hypocatalasemia is heterozygous and in a carrier state of acatalasemia [4]. Kishimoto *et al.* first reported that the DNA of a hypocatalasemic patient had a normal fragment and a mutant fragment of the catalase gene [21]. Hypocatalasemia (heterozygote) was reported in the family of Hungarian acatalasemia A [12, 13] and the proband of Hungarian B, C and D was found by genetic study. The hypocatalasemia was also found in 13 of 22 subjects in 2 Peruvian acatalasemic subjects in a family [14] and 15 of 23 subjects in an Iranian-born Jewish acatalasemic family [16]. The frequency of hypocatalasemia in far-eastern populations is as follows: In a Japanese population it was 0.25%, in a Ryukyuan population it was 0.007%, among Koreans in Japan it was 1.29% (11/922) and among Chinese in Taiwan it was 0.33% [6]. The gene flow was estimated by the frequency of hypocatalasemia. Heterozygote hypocatalasemias were also detected in mice and beagle dogs. The frequency of heterozygotes was estimated to be 0.17% in Japan [24] as calculated by Kimura's formula [26], which nearly coincides with the value (0.23%) obtained by the screening. From 1947 to 1977, 90 cases of acatalasemia in 45 Japanese families were reported [6]. In the following description of mammalian (human and nonhuman) acatalasemia, acatalasemia is classified into the order referred to each constituent part of the bioinformatics [27]. The data obtained are summarized in Tables 1, 2 and 3. The data of human acatalasemia are summarized in Table 1 and 2 and those of nonhuman acatalasemia are Table 3.

Table 1 Bioinformatics of human acatalasemia; mutation of catalase gene.

Bio informatics	Japanese acatalasemia		Hungarian acatalasemia				Swiss acatal.
	Type I	Type II	Type A	Type B	Type C	Type D	
Genome ①Mutation of nucleotide	splicing mutation (G to A substitut.)	frame shift mutation (T deletion)	frame shift mutation (GA insertion)	frame shift mutation (G insertion)	splicing mutation (G to T substitut.)	coding region mutation (G to A substitut.)	nd
②Position	pos. 5 of intron 4	358(n) in exon 4 TGA 43(+n)*	138(n) in exon 2 TGA134(a)	79(n) in exon 2 TGA 58(a)	pos. 5 of intron 7	pos. 5 of exon 9	nd
③Predicted change in peptide chain	no change	termination introduced at 133 Aa#	termination introduced at 133 Aa	termination introduced at 57 Aa	no change	Arg^{354} to His**	nd
④Pred. change in protein	no change (normal)	truncated protein	truncated protein	truncated protein	decreased pr. in hyp.	aberrant protein	aberrant protein***

Note. acatal., acatalasemia; *Genome*: ① nd, not determined; A, adenine; G, guanine; T, thymine; Substi or substitut., substitution; ② pos., position; (n), nucleotide position; (a), amino acid position; TGA, TGA stop codon; Arg, arginine; His, histidine; ③ Aa, amino acid; Aa#., frameshift 119–133 Aa is estimated [22]. ④ Pred., predicted; pr., protein; hyp., hypocatalasemia; (+n)*., Nucleotide number in downstream from T delition; His**., His is described in the reference [13]; however, the substitution from arginine to cysteine is also described in the reference [32]. aberrant protein***., aberrant protein predicted from data of Table 2.

Table 2 Bioinformatics of human acatalasemia; effect of mutation on elements of bioinformatics.

Bio-informatics	Japanese acatalasemia		Hungarian acatalasemia				Swiss a.
	Type I	Type II	Type A	Type B	Type C	Type D	
①*Tran-scriptome* (mRNA)	udc	nd	nd	nd	nd	nd	nor. level
②*Proteome (Blood)* Cat. act (A/N)	0.6%	<0.05%	4.0 and 6.7%	52.2% (hypocatala-semics)	60.6%* (hypocatala-semics)	53.7% (hypocatala-semics)	0.1～1.3%
Heat stability	stable	nd	unstable	nd	nd	nd	unstable
p*I*	normal	nd	normal	nd	nd	nd	nd
Subunit size	ca 60 kDa of normal	nd	nd	nd	nd	nd	nd
③*Xeno-metabolome* H_2O_2 applicat.	nd	H_2O_2 appli. increased MetHb	nd	nd	nd	nd	nd
④*Phenome***	history of Alv. Pyorr.	history of laryngial cancer	DM. in acatala-semics	DM*** increased in cat. def. families		type 2 DM. at 35 Y (proband)	not describ.

Note. Swiss a., Swiss acatalasemia.; ① udc, under detectable concentration.; nd, not determined; nor. level, normal level; ② Cat. act. (A/N), catalase activity (acatalasemic activity/normal activity); p*I*., isoelectric point; ③ appli., application; MetHb., methemoglobin; ④ Alv. Pyorr., alveolar pyorrhea (chronic marginal peridentis); DM., diabetes mellitus; def., deficient., Y, years old.
The 60.6%*, decreased level of catalase protein is also examined by Western blot analysis. Phenome**, the patients with WAGR including Wilms tumor usually have accompanying hypocatalasemia [19].
DM***, an increased frequency of diabetes (12.7%) is detected in the Hungarian acatalasemic and hypocatalasemic family members than in the normocatalasemic ones (0.0%) [13, 30, 32].
Sections of Bioinformatics. *Genome* in Table 1. and ①*Transcriptome* ②*Proteome* ③*Metabolome* and ④*Phenome* in Table 2.
Reference: Jap. Ⅰ[19, 21, 28, 36, 41, 42], Jap. Ⅱ [22], Swiss [7, 37], Hungarian [11—13, 20, 29—32] by Góth.

Mutation of Catalase Gene (genome)

1. Japanese acatalasemia. Two types of mutation were found in the acatalasemia.

1) **Japanese acatalasemia I: (1) Casual or novel** mutation site of catalase gene: Japanese acatalasemia I: Molecular defect in Japanese acatalasemia was first reported in 1990 [28] by the Japanese acatalasemia group (Wen, Osumi and Hashimoto in Shinshu Univ. School of Med. and Ogata in Okayama Univ. Med. School). The guanine to adenine substitution at the fifth position of intron 4 inhibited the correct splicing of the RNA product (one kind of the splicing mutation), which is considered responsible for the defective catalase synthesis in acatalasemic individuals. This report is the first report of causal gene mutation in human acatalasemia.

(2) Conformation of splicing site: The mutant catalase gene from a typical Japanese acatalasemia was cloned. The nucleotide sequence of the catalase gene was determined and compared with the sequence from a normal catalase gene. Seven base differences were found between the normal and acatalasemic genes. Among them the guanine to adenine substitution at the fifth position of intron 4 seemed most likely to be responsible for defective catalase synthesis in Japanese acatalasemia. To confirm this, a chimeric gene was constructed in which a segment of the normal or acatalasemic catalase gene, encompassing the 3' part of exon 4, the entire intron 4 and the 5' portion of exon 5, was contained within the third exon of human α-globin gene. When this chimeric gene construct was introduced into simian virus-40-transformed simian cells (COS-7), the normal cata-

Table 3 Bioinformatics of non-human acatalasemia

Animals	MouseA (Cs^b)	MouseH (Cs^c)	Beagle dog
①*Genome* Mutation of nucleotide	substitution G→T (CAG→CAT)	substitution A→G (AAT→AGT)	substitution G→A (979#) (GCT→ACT)
Predicted change in Aa	Aa 11 Gln→His	Aa 439 Asn→Ser	Aa 327. Ala→Thr
②*Transcriptome* (m-RNA)	nd	nd	normal level in reticulocytes
③*Proteome* (residual catalase)	reticulocyte activity> erythrocyte activity	nd	reticulocyte activity> erythrocyte activity
Activity, (A/N)%	♂ (2.4%), ♀ (1.1%)	♂ (20%), ♀ (16%)	0.7%
Heat stability*	nd	unstable	unstable
p*I*, value	p*I*: 6.3–7.2 (Nor. 5.0–6.0)	nd	nd
Subunit size	nd	nd	63kDa of monomer**
Life span of catalase; reticulocyte→erythrocyte	shorter	nd	shorter
④*Metabolome* and -Xeno-*metabolome* including prevention of clatinogenic promotion	increased conc. MetHb NO exp. Hb→MetHb. Hg^0exp.→accumulation in central nerve methanol→low met. r.	nd	nd
⑤*Phenome (cartinogenese etc)*	higher incidence of breast cancer is inhibited by vitamin E	nd	occasional exhibiting oral gangrene similar to Takahara's Disease

Note: nd., not determined; A., acatalasemia; H., hypocatalasemia. ① 979# showing nucleotide position; Aa., Amino acid; Gln., glutamine; Hist., histidine; Asn., asparagines; Ala., alanine; Ser., serine; Thr., threonine; ③ p*I*., isoelectric point; ④ exp., exposure; low met. r., low metabolic rate; (① Genome, acatalasemic animals indicated a single nucleotide substitution of base at the position within the catalase-encoding region).
Heat stability*., stability of aberrant catalase in acatalasemic mice and beagle dog might be derived from substitution of a nucleotide in exon. monomer**: subunit size of catalase indicates 63kDa as the monomer subunit [80], Increased concent MetHb***: concentration ratio of MetHb to Hb were 1.6±0.2% in Cs^b and 0.8±0.3% in Cs^a mice.
Refernce: Mouse A [23, 33]. Mouse H [23, 34], Beagle dog [18, 35, 80].

lase/α-globin chimeric gene was spliced correctly as revealed on Northern blotting and RNA mapping. In contrast, the splicing of the acatalasemic chimeric pre-mRNA suggested that one entire exon sequence was skipped. Thus the guanine-to-adenine substitution at the fifth position of intron 4 of the catalase gene prevents the correct splicing of the RNA product. The same splicing mutation was found in the genomic DNA of another case of acatalasemia from an unrelated family. Acatalasemic subjects showing the same splicing mutation as Wen *et al.* [28] reported subsequently with the same female acatalasemic subject described by Kishimoto *et al.* in 1992 [21] using the single-strand conformation polymorphism analysis of polymerase chain reaction (PCR-SSCP analysis). The acatalasemic subjects used to their study were the previously examined acatalasemic female (Okayama Prefecture) and her brother who exhibits hypocatalasemia and unrelated cases of acatalasemia from two other unrelated acatalasemic subjects (Hiroshima Prefecture and Kagawa Prefecture) who lived in the western part of Japan. Results studied show the same splicing mutation. They suggested a single mutated allele has spread in the Japanese acatalasemic population. These acatalasemic cases caused by the mutation are classified as Japanese type I acatalasemia, pre-

sumably.

The presence of residual catalase suggests that the splicing mutation of the catalase gene is of the leaky type.

2) Japanese acatalasemia Ⅱ: An acatalasemia having another type of novel mutation was reported in 1995 by Hirano *et al.* [22], in which a deletion of the 358th thymine in exon 4 of the catalase gene was found in the acatalasemia. The frame shift caused by the nucleotide deletion altered the downstream of the amino acid sequence and generated a new terminal codon TGA 43 bp 3' to the mutation. A truncated peptide chain consisting of 133 amino acid residues might be translated in patient tissue. A truncated protein is expected to be extremely unstable and has almost no catalase activity. This type of acatalasemia is presumably called Japanese acatalasemia Ⅱ of which 1 acatalasemia and 4 hypocatalasemia have been reported.

2. Swiss acatalasemia. No study on mutation of the catalase gene has been reported.

3. Hungarian acatalasemia. Novel gene mutation and change in the structure of catalase protein are described as follows. All data (the type A, B, C and D of Hungarian acatalasemia) are described by Góth L *et al.* [13, 29–32].

1) Type A: A GA insertion at nucleotide position 138 (exon 2) increasing the repeat number from 4 to 5. This GA insertion caused a frame shift mutation with the amino acid sequence from position 68 to 133 and generated a TGA at 134. The trans-located protein lacked the essential amino acid of histidine 74 [29], which is required for binding hydrogen peroxide substrate.

The blood catalase activities of 2 acatalasemic sisters were 4.0% (M. K.) and 6.7% (J. K.) and 45.8% for the 23 hypocatalasemics which were compared with 26 normocatalasemic family members [13].

2) Type B: Nucleotide sequence analysis showed a G insertion at position 79 in exon 2 [30]. This mutation caused a frame shift in the amino acid sequence from 49 to 57, and a TGA stop codon was generated at position 58. This truncated protein is not able to enzymatic function of catalase.

The mean value of the blood catalase activities of 3 hypocatalasemic family members was 52.2% of the mean activity in 4 normocatalasemic family members [13, 30] or 60.1% of reference value of blood catalase activity [30]. The frequency of diabetes (12.7%) is higher level in the catalase-deficient family members than the normocatalasemic ones [13, 30].

3) Type C: The G to T substitution at position 5 of intron 7, indicating a splice site mutation [13, 31]. The effect of this mutation was confirmed by Western blot analysis demonstrating a decreased level of catalase protein.

The mean blood catalase activities of 7 hypocatalasemia was 60.6% [(58.8 MU/L)/(96.9 MU/L)] of normocatalasemic family members [13].

4) Type D: The G to A mutation in position 5 of exon 9 changes the essential amino acid arginine^{354} into histidine [13] or cysteine and may be responsible for the decreased catalase activity [13, 32]. The mean blood catalase activity of hypocatalasemia was 53.7% of normocatalasemic family members. All analytical methods were based on the PCR-SSCP [13].

4. Mouse acatalasemia (Cs^b) and homologous hypocatalasemia (Cs^c). Shaffer *et al.* [33] described that catalase cDNA clone was isolated from normal mice (Cs^a) and Cs^b acatalasemic mouse straines, respectively. Sequence analysis of these cDNAs revealed that nucleotide transversion (G→T) occurs in the third position of amino acid 11 (from glutamine to histidine) of the acatalasemic mouse catalase gene. This was suggested to be the catalase deficiency of the acatalasemic mouse. Glutamine is located within a region that forms the first major α-helix in the amino-terminal arm. Wang *et al.* at [34] reported that the mutant catalase cDNA from acatalasemic (Cs^b), homologous hypocatalasemic (Cs^c) and normal (Cs^a) mice were cloned and expressed in bacteria, A novel missense mutation in homologous hypocatalasemic mouse catalase cDNA leads to amino acid substitution of asparagine (AAT) to serine (AGT) at amino acid position 439 of hypocatalasemic catalase. The substitution at this position may affect the formation of the substrate channel leading to the heme group in the tetrametric catalase molecule, resulting in the low catalase activity in the (Cs^c) mouse.

5. Beagle dog acatalasemia. Nakamura *et al.* [35] isolated full-length cDNA clones of acatalasemic dogs. Comparison of the catalase nucleotide sequences of catalase cDNA between the normal and acatalasemic beagle dogs indicated a single nucleotide substitution of G and A at position 979 within the catalase encoding region causing an amino acid substitution of

alanine (GCT) at amino acid 327 to threonine (ACT).

6. Classification of acatalasemia. These results are summarized as follows; acatalasemia is presumably classified into ① splicing mutation (substitution of a nucleotide) of a nucleotide in the intron observed in Japanese acatalasemia I [28] and Hungarian acatalasemia type C [31]. ② point mutation (substitution of a nucleotide *etc.*) and frame shift mutation in the coding region. Mutation of the nucleotide in the coding region of catalase mainly a frame shift caused by nucleotide deletion or insertion induced alternation of the amino acid sequence [22] which was observed in Japanese acatalasemia II [22] and Hungarian acatalasemia A [29] and B [30], and point mutation (substitution of a nucleotide) of an exon was observed in Hungarian acatalasemia D [32]. Mouse acatalasemia [33] or hypocatalasemia [34] and beagle dog acatalasemia [35] are caused by point mutation within the catalase encoding regions.

Catalase Messenger RNA (transcriptome)

1. Japanese acatalasemia I. The RNA was extracted from the cultured acatalasemic fibroblasts and analyzed by Northern blotting. Acatalasemic extract gave only an extremely faint band, indicating severe reduction of catalase mRNA as reported by Wen *et al*, in 1988 [36]. The results indicate the defective synthesis of catalase protein caused by the serious decrease in catalase mRNA.

2. Swiss acatalasemia. The fibroblasts strain obtained from Swiss acatalasemia containing normal amounts of catalase mRNA was conformed by Northern-blot analysis. Crawforld *et al.* [37] suggested that a structural mutation in the catalase gene is probably responsible for the inactivation of the enzyme in Swiss acatalasemia, as estimated from the results of m-RNA in Northern blotting.

3. Mouse acatalasemia. The results suggested nearly normal levels of mouse catalase m-RNA in acatalasemic mouse (Cs^b) [33].

4. Beagle dog acatalasemia. Total RNA samples of the reticulocyte fraction from normal and acatalasemic dogs served as materials. The expressed level of catalase mRNA and its size, in acatalasemic reticulocytes, were not changed when compared with those in the normal beagle dog reticulocytes [35].

Physicochemical Nature of Residual Catalase in Acatalasemia (related to proteome)

1. Isolation of a minimal amount of residual catalase in Japanese acatalasemic blood.

1) Japanese acatalasemia: Separation of residual catalase was carried out by Sephdex G-100 column chromatography from hemolysates of 4 acatalasemic subjects [43] by the manometric method. The fraction comprising the first peak of the catalase activity was residual catalase, and the catalase like activity of MetHb-H_2O_2 compound from hemoglobin in the second peak was hemoglobin. Residual catalase activity was observed in 4 acatalasemic subjects including a subject with acatalasemia I. Crude catalase fractions of hemolysates obtained from acatalasemic subjects were also fractionated by DEAE column chromatography. The Hb fraction was eluted with 1 mM sodium phosphate buffer (pH6.8), and A and B fractions were obtained at a buffer concentration of 10 mM and C fraction was at a buffer concentration of 100 mM by Thorup [38]. Oxygen evolution from hydrogen peroxide by catalase was determined by oxygen electrode and manometer [39, 40].

2) Swiss acatalasemia: A complete separation of residual catalase and hemoglobin could be achieved by Sephadex G100 column chromatography as reported by Aebi [7].

3) Beagle dog acatalasemia: The residual catalase in the liver was isolated by a procedure including a Fracto gel EMD−DEAE column chromatography.

2. Properties of residual catalase.

1) Immunodiffusion and immunoelectrophoresis.

(1) Japanese acatalasemia: Residual catalase protein in the erythrocytes from Japanese acatalasemia I was confirmed by a double immunodiffusion test used human catalase antibody [40]. Immunoelectrophoresis was also carried out on the crude catalase from the erythrocytes in Japanese acatalasemia against antihuman erythrocyte catalase rabbit serum. The presence of catalase protein was observed [40].

(2) Swiss acatalasemia: Residual catalase protein was also confirmed by double immunodiffusion [7].

2) Electrophoretic mobility of residual protein.

(1) Japanese acatalasemia: After the separation of catalase in acetone extract of acatalasemic erythrocytes by electrophoresis in polyacrylamide gel containing starch, the band showing catalase protein had the

same mobility as in normal one [39].

(2) Swiss acatalasemia: The results of polyacrylamide-starch-agar mixed gel-electrophoresis showed that the erythrocyte catalase of normal fractions was faster than that of acatalasemic fractions [9]. However, catalase in acatalasemic fibroblasts has one weak band but possesses the same electrophoretic mobility as catalase in normal fibroblasts on 12.5% polyacrylamide gel under different conditions of electrophoresis by Crawford in 1988 [37].

(3) Hungarian acatalasemia: Electrophoretic mobility of catalase in the type A of acatalasemic M.K. case was indistinguishable from the normal enzyme [11].

3) Isoelectric point (p*I*) of residual catalase.

(1) Japanese acatalasemia I:

Hemolysate was separated into fractions A, B, and C by using the DEAE column of Thorup [38]. The catalase in the C fraction was subjected to isoelectric focusing (IEF) in agarose gel followed by electroblotting and catalase activity staining [41] or by electroblotting onto polyvinylidene dichloride membrane and immuno-enzymic detection and p*I* value was examined [42]. The p*I*. of acatalasemic catalase in the C fraction is identical to the p*I* of normal catalase in the C fraction. The analyses of p*I* values in the A and B fractions of acatalasemic hemolysates gave results similar to those seen in the C fraction [41]. The p*I* values of residual catalase in the sonicated homogenates in cultured skin fibroblasts in Japanese acatalasemia with history of alveolar pyorrhea also showed the same p*I* value of normal catalase by a similar analytical method [41].

(2) Hungarian acatalasemia: The isoelectric point (p*I*) of catalase in tissues from acatalasemia M. K. was indistinguishable from that of the normal enzyme [11].

(3) Mouse acatalasemia: The p*I* values of catalase molecules in the A, B, and C fractions separated by DEAE column increased in the following order: normal <hypocatalasemic< acatalasemic mouse blood in each fraction [41].

4) Subunit size:

(1) Japanese acatalasemia I: The subunit size of residual catalase in the C fraction prepared from the hemolysates of acatalasemia was also found to be identical with that of the normal fraction, corresponding to a molecular weight of nearly 60 KDa on SDS-polyacrylamide gel followed by electroblotting and immunoenzymatic amplification [42] in Table 2.

(2) Beagle dog acatalasemia: The SDS-PAGE of purified liver catalase from normal and acatalasemic dogs gave a band at molecular weight of 63 KDa as also shown in Table 3.

5) Heat (Thermal) stability:

(1) Japanese acatalasemia: The heat stability of the erythrocyte catalase C fraction from DEAE column chromatography was tested before and after incubation at 55℃ for 60 min, and the remaining activity was tested. As a result, the heat stability of residual catalase in the Japanese acatalasemia tested showed no distinct difference from normal catalase [40, 43, 44], suggesting that presence of trace amounts of m-RNA of normal catalase trace m-RNA of normal catalase.

(2) Swiss acatalasemia: In the C fraction of Swiss acatalasemia, the remaining activity was 25% of the original activity at 55℃ for 60 min, indicating less heat stability than in the Japanese case [9, 10, 43, 44].

(3) Hungarian acatalasemia: During incubation at 25℃ for a 6-h study on the hemolysates of the type A of acatalasemia (M.K.), catalase activity in hemolysates showed higher instability than normal [11].

(4) Mouse hypocatalasemia: In the residual catalase in hemolysates of mouse hypocatalasemia Cs^c, thermal stability was less stable than in normal mice Cs^a [23].

(5) Beagle dog acatalasemia: Recombinant catalase molecules in COS-1 cells of normal and acatalasemic dogs were tested. Thermal stability of catalase in COS cells was examined in recombinant catalase of normal and acatalasemic beagle dogs in COS-1 cells at 45℃ and it was found that over expressed acatalasemic catalase in COS cells decreased rapidly when compared with that of normal dog catalase, showing the lower heat stability of the acatalasemic catalase than normal catalase [35]. Heat stability of aberrant catalase protein in acatalasemic mice and beagle dog might be originated in substitution of a nucleotide in coding regions of catalase.

6) Affinity toward the substrate of residual catalase (Michaelis constant).

Japanese acatalasemia: Normal and residual catalase molecules were separated by Sephadex G-100 in the hemolysates and the Michaelis constants (Km) were

found to be 0.35 and 0.37 mM, respectively. The results measured were suggested that residual catalase activity in acatalasemia due to splice site mutation might not change at the region of the amino acid sequence in the active centers or ion pockets of catalase molecules by the Michaelis constant [43].

7) Immunotitration: Specific catalase activity of Japanese acatalasemia I as determined by immunotitration will be the same as normal catalase described by Ogata and Fujii [45].

Level and Distribution of Residual Catalase in the Blood and Solid Organs

1. Level of catalase activity in blood of acatalasemia and hypocatalasemia.

In the splicing mutation of the leaky type, minute amounts of residual catalase having properties of normal catalase might be biosynthesized. In the mutation of the cording region of catalase, the frame shift mainly caused by the nucleotide deletion or insertion should alter the downstream amino acids sequence and a truncated peptide chain might be translated into aberrant acatalasemic protein [22]. Structural change of residual catalase might be caused by one point mutation of an exon like Hungarian acatalasemia D. Mouse acatalasemia also originated in one point mutation.

1) Japanese acatalasemia: The catalase activities in the blood of acatalasemic subjects in 5 families and the ratio of this activity to normal activity were as follows: ①Gio Family: 19.20 Pu/gHb, (0.60% of normal); ②Min Family: 17.01 (0.53%); ③Sus Family: 4.75 (0.25%); ④Miy Family: Mean, 5.4 (0.17%); and ⑤NAK Family 8.94 (0.28%). The mean of the 5 families measured by perborate methods was 11.02±6.72 Pu/gHb (0.34±0.21%) expressed as (m±SD), and the range of measured values was 4.57 ~21.52 Pu/gHb (0.14~0.70%) [39]. Members of 3 families (Sus, Miy and Nak) suffered from typical Takahara's disease, while a member of one family having acatalasemia I has a history of alveolar pyorrhea. The residual catalase activity of an individual with acatalasemia I was 0.602% of normal (3190 Pu/gHb) [39]. Heterozygote hypocatalasemia showed activity that was 44.9±12.1% of normal. There was almost no overlapping of catalase activity between the normal group (5.04±0.68, Kcat) and the hypocatalasemia group (2.24±0.41, Kcat) by the permanganate titration method. The critical point was calculated to be 3.29 Kcat [43].

2) Swiss acatalasemia: The catalase activity in the blood was 0.10~1.3% of the activity of normal subjects [7] as determined by the perborate method [17]. The average catalase activity of hypocatalasemia amounted to 2.46±0.51 (m±SD) mg catalase/gHb, and the normal activity was 2.50±0.62 mg catalase/gHb. Distinguishing between the normal subjects and heterozygote hypocatalasemia by catalase activity is not possible [10].

3) Hungarian acatalasemia: ①In type A (GA insertion in exon 2) acatalasemias, catalase activity was 4.0 and 6.7% of the normocatalasemic families as measured in the substrate of hydrogen peroxide by the spectrophotometric method and reported by Góth [11, 29]. Truncated protein caused by the results of GA insertion lakes a histidine at position 74 in active center resulted in low catalase activities. Furthermore, ②In type B, 3 hypocatalasemic family members showed activity that was 60.1±5.2% of normal activity 113.3±16.5, MU/l) or 52.2% of the activity in a normocatalasemic family members (68.1 MU/L/130.4 MU/L) ×100. Truncated protein with its 58 amino acid is not able to maintain the enzyme function of catalase [30]. ③In type C, hypocatalasemia (60.6 ±11.9) % of the activity of normocatalsemic family members (58.5 MU/L)/(96.9 MU/L) ×100 was observed A G to C substitution was detected at position 5 of intron 7 Decreased catalase protein caused by the effect of splice site mutation was confirmed by Western blot analysis [31], and ④In type D, 53.7± 7.3% of normocatalasemic (103.6 MU/l) family members was observed due to a G to A substitution at position of exon 9. The type 2 diabetes mellitus was observed at a relatively early age in the 35-year-old proband as reported by Góth [32].

4) Other human hypocatalasemia: Hypocatalasemia [Heterozygote] was recognized in Korean [6], Peruvian [14], German [15] and Jewish [16] populations. Hypocatalasemia can be found with a simple determination of the catalase activity in the blood without using gene analysis. The catalase activity in the blood of Korean and Peruvian hypocatalasemia is similar to that of Japanese hypocatalasemia.

5) Mouse acatalasemia: Residual catalase activity was found to be 2.0% of normal by the perborate

method at 37℃ for 5 minutes and 4.4% of normal at 20℃ by Tottori [52]. The residual catalase activity of acatalasemia (Cs^b) was 1.8% and activity of hypocatalasemia was 18.0% (mean of activity of male and female mice), which was calculated from the report of Feinstein [46] by perborate method (37℃, 5min.).

6) Beagle dog: Residual catalase activity was found to be 0.7% of the normal value using the spectrophotometric method for measuring hydrogen peroxide of the substrate [18].

Cellular Distribution of Residual Catalase Activity in Mature and Immature Red Cells

The results obtained were related to the life span of residual catalase in blood.

1. The residual catalase in erythrocytes and reticulocytes.

1) Human acatalasemia: In the blood of acatalasemic subjects, the reticulocyte rich and poor fractions were separated by 20% albumin density gradient centrifugation, and the number of reticulocytes and the catalase activity in the fractions were measured by Aebi [9].

(1) Japanese acatalasemia: The residual catalase activity in the blood was nearly equally distributed between reticulocytes and erythrocytes in 3 acatalasemia cases in one family and in one acatalasemia case in another family [43].

(2) Swiss acatalasemia: In 3 Swiss acatalasemic subjects, residual catalase activity in the reticulocytes and erythrocytes may differ by a ratio of up to about 300: 1, suggesting that residual catalase has a shorter life span than normal catalase [44].

2) Non-human acatalasemia: Animals made anemic condition by injection with phenyl hydrazine hydrochloride, and the reticulocyte rich fractions were obtained.

(1) Mouse acatalasemia: Blood catalase: The change in the catalase activity as assessed by the perborate method [46] during the maturation of reticulocytes was measured both in normal conditions and in anemic condition induced by phenyl hydrazine and the catalase activity and reticulocyte counts were measured. The results indicated that the catalase activity per reticulocyte in the blood of acatalasemic mice was 55% of that of normal mice. The degradation rate, expressed as (catalase activity in reticulocytes−catalase activity in erythrocytes)/catalase activity in reticulocytes, was 88% in the acatalasemic mice and 34% in the normal mice, respectively. Thus the catalase activity in the erythrocytes of acatalasemic subjects was 10% of the activity in the erythrocytes of normal mice [47, 48].

(2) Beagle dog acatalasemia: Dogs were made anemic by injection of phenyl hydrazine hydrochloride, and a reticulocyte-rich fraction was prepared. Pulse labeling using ^{35}S methionine and immunoprecipitation examination indicated that catalase synthesized in the acatalasemic dog was almost the same as that in the reticulocytes, but the synthesized mutant catalase in the acatalasemia was degraded more rapidly than normal catalase. The degradation was almost completely inhibited by lactcystin, a proteasome specific inhibitor. Nakamura suggested that degradation by the proteasome was most likely involved [35]. These reports suggested that the degradation of mutant catalase with structural change was faster than that of normal catalase in Swiss acatalasemia and mouse and Beagle dog acatalasemias.

Distribution and Turnover of Residual Catalase in Acatalasemic Solid Organs

1. Distribution of catalase activity in solid organs of acatalasemia.

In order to determine the lifespan of residual catalase and the ability to protect against oxygen stress in the solid organs, the distribution of catalase in organs was studied.

1) Japanese acatalasemia.

(1) Case 1: catalase activities in the liver, muscle and bone marrow blood were not recognized under detectable sensitivity (USD) by Euler's titration method [49] in one case of acatalasemia (2 of 4 siblings in the family are acatalasemic subjects) [50].

(2) Case 2: The ratio of residual catalase activity to normal catalase activity in organs was 0.4% in blood as determined by the permanganate method. Activity in the vermiform appendix was found to be 2.7% of normal, Activity in the abdominal mussel could not be measured by Euler's method [49]. Blood catalase activity was under the detectable level in Euler's method. In this acatalasemic family, 4 subjects with acatalasemia, 1 with hypocatalasemia and 1 normal subject were found among 9

siblings. The above-mentioned acatalasemic individuals of one case and other 3 cases of acatalasemia might have similar organ distributions of catalase activity [51].

(3) Case 3: The ratio of residual catalase activity to normal catalase activity in organs was 37.9% in the liver, 31.0% in the small intestine, 30.2% in the large intestine and 4% in the blood [4]. In members of her family who were tested, minute catalase activity was not found, and hypocatalasemia was found in one of her nephews by the manometric method. She has an atypical family tree. She suffered from maxillary cancer [4].

2) Swiss acatalasemia: Catalase activity in the blood of acatalasemic subjects was 0.1～1.3% of normal catalase activity in the liver was less than 0.1 units per mg protein, indicating almost no catalase activity [7]. Activity in the liver of hypocatalasemic subjects was between normal activity and that in acatalasemia. Residual catalase activities in the limphoepithel tissue and in the fibroblasts of acatalasemia were 3% and 2～3% of normal, respectively (PU/mg. pr.) [7].

3) Hungarian acatalasemia. The ratios of the specific catalase activity of each organ in acatalasemic subjects to that of each corresponding organ in normal subjects were 15.2% for the liver, 12.4% for the pancreas 13.0% for the kidney, 13.5% for the lung 15.4% for the spleen and 5.0% for the blood, indicating less activity in blood than in solid organs. These results were calculated from the report as described by Góth [12] in Hungarian acatalasemia-A.

4) Mouse acatalasemia: Distribution in organs: Feinstein recognized higher activities in acatalasemic solid organs than in blood [46]. Tottori reported the activities in the solid organs measured by perborate assay at 20℃ for 5 minutes to avoid heat inactivation at 37℃ [52]. The ratios of catalase activity (PU/mg. pr.) in the organs of acatalasemic subjects to activity in the organs of normal subjects were 65.9% of normal in the liver, 15.6% in the kidney, 69.9% in the lung, 33.7% in the stomach, 30% in the brain, 31.8% in the heart and about 4.3% in the blood. Similar results were described using a different assay method by Wang *et al.* [53].

5) Beagle dog acatalasemia: The ratios of catalase activity in the organs of acatalasemic beagle dogs to the activity in normal dogs were 71% in the liver, 41% in the kidney, 83% in the intestine, 90% in the heart, 56% in the lung, 76% in the pancreas, 35% in the spleen, 100% in muscle and 52% in the brain [35]. The catalase activities of the solid organs of normal and acatalasemic mice were compared activites of solid organs of normal and acatalasemic dog. Relatively lower activity in the brain was found in the solid organs of normal and acatalasemic mice and also normal and acatalasemic beagles.

2. Synthesis and degradation of residual catalase in the acatalasemic mouse liver. The recovery of catalase activity after inhibition with aminotriazole in acatalasemic mice was determined [54]. The kinetics of catalase synthesis and degradation were determined in acatalasemic and normal liver catalase during the recovery of catalase activity after the activity was inhibited with aminotriazole in acatalasemic and normal mice singly. The liver catalase turned over with a half-life of 10.0h in acatalasemic mice and a half-life of 19.2h in normal mice. The results indicated that acatalasemic liver catalase was found to have a shorter life span. The rate of catalase (Ks) was 0.168 (Pu/mg protein/hr) in acatalasemic mice and 0.271 in normal mice. On the other hand, the rate of catalase degradation (Kd) was 0.069 in acatalasemic mouse liver and 0.036 in normal mouse liver. It seems that the rate of catalase synthesis was lower and the rate of degradation was higher in acatalasemic mouse liver than in normal mouse liver, thus giving rise to lower catalase activity in the livers of acatalasemic mice than in the livers of normal mice [54]. The synthesizing activity of catalase in the mouse liver was measured by ^{14}C- leucine incorporation. The results suggested that the incorporation activity of ^{14}C- leucine into catalase protein in acatalasemic mouse liver was lower than the incorporation activity into catalase protein in normal mouse liver [55].

Erythrocyte Metabolism against Oxidation and Related Enzyme (related to metabolome)

1. Superoxide dismutase and glutathione peroxidase. Hydrogen peroxide is generated by oxygen reduction of various oxidases. Superoxide anion generated in cells may be converted to hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide is decomposed by residual catalase and glutathione peroxidase [6] in the acatalasemic blood.

However, when the amounts of hydrogen peroxide generated by microorganisms and/or environmental chemicals exceeds the ability to decompose hydrogen peroxide, cell damage will be produced [3, 6]. The levels of residual catalase were examined in 5 Japanese acatalasemic families having 0.14～0.70% of normal catalase activity in the blood [6, 54]. The leukocyte catalase activity was extremely low (0.13%) in acatalasemia [56]. Assays of SOD activities were carried out by Beauchamp and Fridcvich [6, 57], and the activities of glutathione peroxidase were determined in the blood of 5 Japanese acatalasemic families [6, 58]. The SOD activities of normal controls were 49.1 ± 7.8 U/mgHb, and those of 5 acatalasemic individuals fell in the range of 61.5 ± 7.1 U/mgHb. The acatalasemic blood showed significantly higher activity of superoxide dismutase than did the blood of normal subjects ($p < 0.05$). Glutathione peroxidase activity in acatalasemic blood was shown to be slightly elevated compared with that of normal blood but within normal limits [6, 58].

The superoxide anion is considered to produce singlet oxygen and a hydroxyl radical in the presence of hydrogen peroxide by Harber and Weiss's reaction:

$$O_2^- + H_2O_2 \rightarrow OH^- + OH\cdot + {}^1O_2.$$

It is supposed that the above reaction also occurs in acatalasemia. However, the superoxide anion in this reaction may be catalyzed by superoxide dismutase into hydrogen peroxide, $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$, which is then decomposed with minimal catalase and glutathione peroxidase [6]. The activity of glucose-6-dehydrogenase (G6PD) in erythrocytes in the subjects of 3 acatalasemic family was not significantly different between normal, hypocatalasemia and acatalasemia in 3 families of acatalasemia but in one family lower activity than normal was found in acatalasemia [56]. The mean value of alkaline phosphatase activities in the plasma in an acatalasemic family was within the normal range [56]. One subject of acatalasemia I was found to show activitiies of lactate dehydrogenase in plasma and also AST and ALT in serum were within the normal range respectively. The activity of catalase in the leucocytes was half of normal in hypocatalasemia and extremely low in acatalasemia [56]. The activity of residual catalase in the blood of subjects with acatalasemia I was very low and the activity of G6PD was within the normal range [58].

2. Methemoglobin. Methemoglobin (MetHb) concentration in Japanese acatalasemia is considered to have a tendency to be higher than in normal Japanese subjects, but the difference between normal and acatalasemic subjects tested was small and could not be determined with because the MetHb level fluctuated every day. MetHb concentration for Hb concentration in the blood of wild-type and mutant mice, as in the order of female acatalasemic mice, male acatalasemic mice, female homozygous hypocatalasemic mice and normal mice [59], which is considered to be related to endogenous metabolism.

Effect of Environmental Chemicals on Japanese Acatalasemia and Mouse Acatalasemia (belong genetic toxicology and also belonging Xeno-metabolome)

1. Genetic toxicology. The term genetic toxicology or toxicogenetics (related to the field of pharmacogenetics) was first used by Ogata in the textbook of Biological Monitoring of Exposure to Industrial Chemicals [60]. The investigation of the effect of environmental chemicals on a deficient metabolic enzyme system, known as genetic toxicology or toxicogenetics, can lead to an understanding of the enzymatic reaction involving hazardous chemicals and of the experimental effect of toxic environmental chemicals on genetic diseases. In one example of such investigation, MetHb reductase deficiency or acatalasemia was found to increase in concentration after exposure to MetHb inducer [60].

The effect of environmental chemicals on acatalasemic subjects is described as follows:

1) Exposure of acatalasemic human blood and acatalasemic mice to nitrogen monoxide (NO) or nitrogen dioxide (NO_2): Methemoglobin (MetHb) formed in the blood of human and mouse acatalasemic subjects exposed to NO or NO_2 according to the following equation.

$$2NO_2 + H_2O = 2H^+NO_2^- + NO_3^- \quad (1)$$

$$Hb(O_2)_4 + 2NO_2^- + 6H^+ \rightarrow$$
$$MetHb + 2NO_3^- + H_2O_2 + 2H_2O + O_2 \quad (2) \cdots [61]$$

The reaction of hemoglobin and hydrogen peroxide produces methemoglobin [62].

A peroxidase action of catalase converts NO_2^- to NO_3^-.

Thus, NO_2^- and H_2O_2 decrease.

$$2NO_2^- + H_2O_2 \rightarrow 2H^+ + 2NO_3^- \quad (3) \cdots\cdots [61]$$

As for NO, it reacts with hemoglobin to form nitrosyl hemoglobin and nitrite as follows:

$Hb(O_2)_4 + 4NO \rightarrow Hb(NO)_4 + 4O_2$ (4) and $Hb(NO)_4 + 4O_2 \rightarrow MetHb + 4NO_3^-$ (5) and then $Hb(NO)_4 + 4NO_3^- \rightarrow MetHb + 8NO_2^-$ (6). Thus nitrite ion and MetHb are produced. The equation suggested that hydrogen peroxide participate these chain reactions.

(1) Erythrocyte exposure of Japanese and mouse acatalasemic blood to NO or NO_2 (*in vitro* experiment): Catalase activity in the blood of Japanese acatalasemia I was 0.6% of normal activity [39]. No significant difference in NADH MetHb reductase activity was found between Japanese acatalasemic blood and normal blood [63]. In normal and Japanese acatalasemic erythrocytes exposed to NO or NO_2, the concentrations of MetHb were larger in acatalasemic erythrocytes than normal erythrocytes [60, 63]. The ratios (%) of MetHb to hemoglobin concentration in the acatalasemic erythrocytes exposed to 200 ppm NO gas for 0, 30, 60 and 90 minutes were 0.72, 6.55 ± 0.41, 11.77 ± 0.95 and 20.17 ± 2.84%, respectively, and in normal erythrocytes for 0, 30, 60 and 90 min they were 1.03, 4.75 ± 0.18, 8.81 ± 0.72, and 12.45 ± 1.11%, respectively. MetHb concentration in Japanese acatalasemic erythrocytes was significantly higher than in normal erythrocytes ($p < 0.05$; determined by *t*- test) for all of the above exposure times. Similar results were obtained with human erythrocytes exposed to NO_2.

The levels of MetHb concentrations of normal and acatalasemic Japanese erythrocytes after exposure to NO were higher than after exposure to NO_2 under the same exposure concentration.

(2) Acatalasemic mouse erythrocytes *in vitro* experiment: When acatalasemic mouse erythrocytes were exposed to 200 ppm of NO, the MetHb concentration in the blood was significantly higher than that in normal mice. The results were similar to human erythrocytes exposed to 200 ppm of NO_2.

(3) Acatalasemic mice exposed to NO or NO_2, *in vivo* experiment: The MetHb concentrations in the blood of acatalasemic mice exposed to 100 ppm of NO for 0, 20, 40 and 60 minutes were 1.52 ± 0.16, 17.47 ± 3.48, 25.49 ± 5.06 and 31.58 ± 2.04% of total hemoglobin, respectively, and in normal mice they were 0.85 ± 026, 10.63 ± 3.75, 18.39 ± 0.31 and 22.21 ± 2.72%, respectively. In the entire range of studied concentrations of NO, the mean concentrations of MetHb in the blood of acatalasemic mice were higher than in the blood of normal mice. Similar results on MetHb formation were obtained after exposing mice to NO_2, although the rate of MetHb formation was lower in the blood of NO-exposed mice [60, 62].

(4) Inhibitory effect of α tocopherol on MetHb formation: MetHb formation in both normal and acatalasemic mouse hemolysates exposed to 180 ppm of nitrogen oxide was significantly inhibited by the addition of α-tocopherol [64]. The formation of MetHb in acatalasemic mouse hemolysates exposed to nitrogen oxide was greater than that in normal hemolysates with or without the addition of α tocophenol [64].

(5) MetHb formation in Japanese acatalasemia by hydrogen peroxide: In a study by Hamada *et al.* [65], the oral cavity and larynx of an acatalasemic patient were disinfected with 300 ml of a 1: 2 dilution of 3% hydrogen peroxide, then 11% MetHb concentrations were recognized. The patient was given ascorbic acid intravenously after medical treatment, and the MetHb concentration decreased to 7.5%. The MetHb concentration on postoperative day 3 then became normal (0.5%).

2. Hemolysates and mouse exposure to metallic mercury. 1) Japanese acatalasemic hemolysates: Normal and acatalasemic hemolysates from Japanese subjects were exposed to metallic mercury. The amount of mercuric ions taken up by human acatalasemic erythrocytes was less than that taken up by normal erythrocytes with or without hydrogen peroxide. The data indicated that erythrocyte catalase oxidizes metallic mercury to mercuric ions through its peroxidative action [66]. Deisseroth *et al.* [67] considered that the oxidation of metallic mercury by catalase might be a chain reaction in which ferric ions react with hydrogen peroxide to form $Fe^{3+}OOH$, which oxidizes metallic mercury to mercuric oxide. $Fe^{3+}OH + H_2O_2 \rightarrow Fe^{3+}OOH + H_2O$ and $Fe^{3+}OOH + Hg^0 \rightarrow Fe^{3+}OH + HgO$

2) Mice exposed to metallic mercury vapor: Mercury distribution in mouse organs was studied after normal and acatalasemic mice underwent mer-

cury vapor exposure. The concentration of metallic mercury in the arterial blood of acatalasemic mice is higher than in that of normal mice. The mercury concentration and the organ/blood rate of mercury concentration in the brain, liver, placenta and fetuses in acatalasemic mice are higher than those in normal mice. The rate of the mercury level in the brain to that in the blood of acatalasemic mice was about 2 times higher than that of the normal mice. These observations suggest that lipid soluble metallic mercury passes the blood-brain barrier and the blood-placenta barrier more easily in acatalasemic mice than in normal mice [68–70].

3. Acatalasemic mice injected with methanol. The amount of [^{14}C] carbon dioxide exhaled by acatalasemic and normal mice (Cs^b) injected with [^{14}C] methanol or [^{14}C] formic acid is less than that exhaled by acatalasemic mice, suggesting that catalase plays a major role in murine metabolism for methanol [71].

4. Carbon tetrachloride. After a single intraperitoneal injection of carbon tetrachloride to acatalasemic mice (Cs^b), centrilobular necrosis in the livers of Cs^b mice was historically more severe than that in normal mice. The liver malondialdehyde level in acatalasemic mice was nearly two-fold higher than that in normal mice at 18h after CCl_4 treatment [72]. The carbon-tetrachloride-induced liver injury in acatalasemic mice is inhibited by hepatic iron deprivation [73], indicating that the formation of active oxygen as in hydrogen peroxide or hydroxyl radicals formed from hydrogen peroxide might be related to CCl_4-induced hepatotoxicity.

5. Prevention of carcinogenesis promotion.

1) Mammary tumor: C3H females, both Cs^a and Cs^b, carrying the MuMTV eventually showed essentially a 100% incidence of mammary tumor [74]. Female acatalasemic and hypocatalasemic mice had an increased incidence of spontaneous mammary tumors. The cumulative incidence of spontaneous mammary tumor in vitamin E-supplemented acatalasemic mice (47%) was significantly lower than in vitamin-E-deprived mice (82%). Thus, vitamin E might be useful for tumor prevention [75].

2) Liver tumor: ①Administration of aminotriazole (AT) C3H mice are also a strain with a high natural incidence of liver tumors [74]. The effect of prolonged mouse diet on liver tumor appearance in normal and acatalasemic C3H mice was tested. A large proportion (21/87) of the acatalasemic group developed liver tumors than did the normal catalase mice, and they were detected earlier (beginning at 35 weeks) [74]. ②Administration of diethylnitrosamine (DEN): Acatalasemic mice (C3H/AnLCs^b) were more susceptible to DEN, leading to enhanced hepatocartinogemesis in comparison with normal mice [76].

Escherichia coli Incorporated Mouse Acatalasemic Gene

In order to clarify whether the reactive oxygen species are involved in hydroquinone-induced cytotoxicity, *E. coli* strains that express murine catalase derived from catalase (Cs^b and Cs^c) and wild-type Cs^a in catalase-deficient *E. coli* UM255. Hydroquinone toxicity was examined using a zone-inhibited test, and UM255 showed the largest inhibition zone, followed by Cs^b, Cs^c and Cs^a. The results suggest that hydrogen peroxide is involved in hydroquinone-induced cytotoxicity in a catalase-deficient *E. coli* mutant [77].

Catalase Gene Mutation and Disease Reported (included in the phenome)

1. Takahara's disease. (1) Occurrence of Takahara's disease: In human acatalasemia, about 41% of Japanese actatalasemia, a Peruvian acatalasemia (1979) and a German acatalasemia (1999) have been associated with the oral progressive gangrene known as Takahara's disease. The pathogenesis of the oral gangrene can be explained as follows. Some germs, pneumococcus and β-streptcoccus, exist in crevices of teeth produce hydrogen peroxide. In Japanese acatalasemia, hydrogen peroxide is not decomposed and will destroy hemoglobin in the blood, resulting in local malnutrition and the development of the gangrenous disease [4]. Peruvian acatalasemia (n =2) and German acatalasemia with Takahara's disease were reported by Delgado WA *et al.* [14] and Pemer H *et al.* [15], respectively. Swiss acatalasemia without Takahara's disease was reported by Aebi *et al.* in [7]. Hungarian acatalasemia without Takahara's disease and higher prevalence rates of diabetes mellitus was reported by Góth L in 1992 [11].

(2) Trend of occurrence of Takahara's disease: The incidence rate of Takahara's disease in Japanese acatalasemic individuals from 1945 to 1955 was 0.68

(17/25), and from 1956 to 1970, it was 0.27 (13/49). The difference between the rates is significant ($p < 0.001$) [78]. A yearly decrease in incidence through the improvement of nutritional conditions and oral hygiene and also the use of antibiotics were considered to be a possible cause for the difference. The means by which acatalasemia was found included surgical operation (23.3%), Takahara's disease (24.4%) and survey of hypocatalasemia (52.3%) [79]. Acatalasemic beagle dogs occasionally exhibit an oral gangrenous disease very similar to Takahara's disease [80].

2. Diabetes mellitus. In the report by Góth [32], a proband of Hungarian acatalasemia of type D developed type 2 diabetes mellitus at a relatively early age (35 years). The frequency of diabetes in the catalase deficient family members was (12.7%) which was higher than normocatalasemic family members (0.0%) [13, 30] Góth described that increased hydrogen peroxide levels may damage oxidative-sensitive β cells in the pancreas and decrease insulin production. The exact mechanisms by which hydrogen peroxide affects pancreatic function/insulin production are still unknown [13].

3. Wilms tumor- anirida, genitourinary abnormalities and mental retardation triad. The patients with this condition (WAGR) caused by chromosomal deletion in 11p 13 usually accompany with hypocatalasemia [19].

Bioinformatics of Japanese Acatalasemia I

Studies of acatalasemia I demonstrated the following; ① splicing mutation (leaky type) [28], ② pre-mRNA [28] (appropriate amounts) and mRNA (under the detectable concentration) [36], ③ residual catalase (0.60% of normal activity [39], with p*I* and subunit size at the normal level) [41, 42] and ④ history of alveolar pyorrhea [81].

The sections describing acatalasemia I in this manuscript concerned the confirmed cases. The Japanese acatalasemic subjects examined almost lived in the Okayama acatalasemia I.

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