

Type 2 Gaucher disease: the collodion baby phenotype revisited

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Abstract

The association of Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase (EC 3.2.1.45), and congenital ichthyosis was first noted a decade ago. Subsequently, a null allele type 2 Gaucher mouse was generated that also exhibited ichthyotic skin, confirming that the skin disorder and enzyme deficiency were directly related. This paper details the clinical and molecular characterisation of 6 cases of type 2 Gaucher disease presenting with the collodion baby phenotype. The identified mutant glucocerebrosidase alleles include two novel mutations (S196P and R131L) and two rare point mutations (R120W and R257Q), as well as alleles resulting from recombination with the nearby glucocerebrosidase pseudogene. There is significant genotypic heterogeneity in this rare subset of patients with type 2 Gaucher disease. Gaucher disease should be considered in the differential diagnosis of congenital ichthyosis in the newborn period.

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Ten years ago two Lebanese siblings were described who were born in Australia with the collodion baby phenotype.¹ Subsequently, it was discovered that they had type 2 (acute neuronopathic) Gaucher disease, which results from the inherited deficiency of the lysosomal enzyme glucocerebrosidase (EC 3.2.1.45). Type 2 Gaucher disease culminates in early death as a result of devastating neurological disease.^{2,3} The term "collodion baby" does not refer to a specific disease, but rather to the presentation of a newborn infant encased in a cellophane like skin wrapping. This skin condition can later develop into a classic form of ichthyosis. It was speculated that the occurrence of these two distinct conditions, the first a lysosomal storage disorder, and the second a congenital skin abnormality, might be the result of a contiguous gene effect, coincidence, or multiple effects of glucocerebrosidase deficiency.¹ Two years later a third, unrelated infant was born in Australia with collodion skin and Gaucher disease, lending support to the hypothesis that the occurrence of these two conditions together was not coincidental.⁴

In 1992, the first mouse knockout model of Gaucher disease was generated by homologous recombination with a null glucocerebrosidase

allele.⁵ Mice homozygous for this null mutation have no detectable glucocerebrosidase activity and survive for only a few hours after birth. These severely affected mice were noted to have dry, ichthyotic skin. Further histological and ultrastructural analyses of the skin from the affected mice revealed hyperkeratosis and disruption of the lamellar structure of the stratum corneum.^{6,7} We postulated that the deficiency of glucocerebrosidase in the skin of the null allele mice accounted for the observed skin abnormalities, because glucocerebrosidase is abundant in mammalian epidermis and the content and ratios of ceramides to glucosylceramides is essential for the maintenance of the epidermal permeability barrier.⁷ This hypothesis could also explain the congenital ichthyosis observed in some infants with type 2 Gaucher disease. Ultrastructural analyses performed on infants with type 2 Gaucher disease confirmed that the structure of the stratum corneum was disrupted in a manner similar to that seen in the affected mice. These microscopic skin changes were present in all of the patients with type 2 Gaucher disease studied, regardless of whether the infants had obvious skin involvement.⁸

During the 10 years since the first cases of Gaucher disease with the collodion phenotype were published, many additional cases of Gaucher disease with congenital ichthyosis have been described, including a fourth case from Australia.⁹ We have performed mutational analyses on this new patient and on several previously described patients with Gaucher disease and collodion skin, including the three patients described originally. We have summarised the results of the mutation analyses of all cases of type 2 Gaucher disease with congenital ichthyosis that have been described to date and demonstrate that there is considerable genotypic diversity in this clinically homogeneous subgroup of patients with Gaucher disease.

Case 1

This male infant was the second child of consanguineous (first cousin) Lebanese parents with no other relevant family history.⁹ An ultrasound study at 18 weeks of gestation was reportedly normal. An ultrasound performed at 26 weeks of gestation because of maternal abdominal pain was suggestive of fetal hydrops. Additional imaging studies over the following months showed hepatomegaly, decreased fetal movement, and hyperextension of the neck. Amniocentesis and karyotyping were performed, revealing a 46,XY male fetus. He was born at 34 weeks of gestation and died shortly

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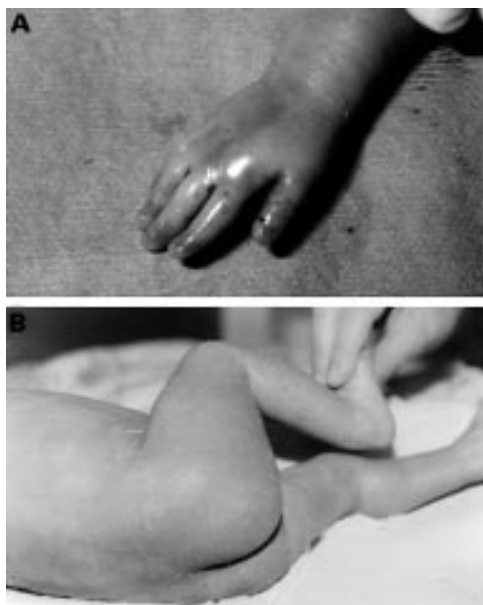


Figure 1 (A) Postmortem photograph of the hand (case 1) showing tight, shiny skin. (B) Body (case 6) showing peeling and scaling of the skin.

after birth. Postmortem examination revealed a thick, collodion like skin, ectropia of the eyes, and multiple dysmorphic features, including malformed and low set ears, a flattened nasal bridge, and a highly arched palate. He had pronounced hepatosplenomegaly, contractures of the knees and interphalangeal joints, and tight shiny fingers (fig 1A). Histological examination of multiple organs revealed Gaucher cells. The diagnosis of Gaucher disease was confirmed enzymatically, with a leucocyte glucocerebrosidase activity of 9.5 pmol/min/mg (normal, 600–3200).

Case 2

This child was one of two siblings who were the first described cases of Gaucher disease with collodion skin.¹ He was the first born child of non-consanguineous Lebanese parents. At delivery at term he weighed 3500 g and was noted to have a pronounced hepatosplenomegaly and thick collodion skin, which peeled off over the first 5 days, leaving residual erythroderma. Laboratory studies showed a pronounced thrombocytopenia. Seizures and laryngospasm developed during the 1st week of life. A leucocyte enzyme assay for β -glucocerebrosidase activity confirmed the diagnosis of Gaucher disease. He died at 3 months of age after respiratory arrest.

Case 3

This child, a sibling of case 2, was a full term, 3400 g male infant with collodion skin, generalised joint contractures, and gross hepatosplenomegaly. The skin on the soles of his feet and forearms was desquamating. He was apneic at birth, had refractory seizures and opisthotonos, and required assisted ventilation until his death at 11 days of age. His white blood cell β -glucocerebrosidase activity was 35 pmol/min/mg. At postmortem examination, Gaucher cells were identified in multiple tissues.

A cousin of cases 2 and 3 was born to consanguineous first cousin parents and was subsequently diagnosed with type 2 Gaucher disease. No mention was made of collodion skin in the limited medical records available, but this child also died in infancy after requiring ventilatory support.

Case 4

This 2480 g female infant was born at 34 weeks of gestation to non-consanguineous Australian parents.⁴ She had collodion skin with ectropia, hepatosplenomegaly, thrombocytopenia, and apnoea. Leucocyte β -glucocerebrosidase activity was 53 pmol/min/mg. She died at 3 weeks of age. Liver histopathology confirmed the diagnosis of type 2 Gaucher disease.

Case 5

This 3150 g boy was born at term to Mexican parents who were not known to be consanguineous.¹⁰ At birth his skin was described as collodion-like, but the lesions cleared within 2 weeks. He subsequently developed feeding difficulties and had regression of motor milestones. On examination at 6 months of age, he had hypotonia, spasticity, abnormal eye movements, an opisthotonic posture, and hepatosplenomegaly. Fibroblast β -glucocerebrosidase values were 3% of control values. He died at 7 months of age.

Case 6

This child was the sister of case 5. Amniocentesis established that the fetus was glucocerebrosidase deficient. The parents elected to continue the pregnancy, and she was born at 37 weeks' gestation, weighing 2560 g, with collodion skin and hepatosplenomegaly (fig 1B). The skin condition resolved during the 1st month of life, but she developed neurological abnormalities and died at age 9 months.

Methods

MUTATION DETECTION

Genomic DNA was extracted from cultured fibroblasts from the probands (cases 1, 3, 4, 5, and the cousin of case 3). The DNA was initially screened for the presence of common Gaucher mutations as described previously.¹¹ The complete glucocerebrosidase gene was amplified by long template polymerase chain reaction (PCR) to look for large deletions and fusion products.¹² The 11 exonic regions, including flanking intronic sequences and a 1 kb segment at the 5' untranslated promoter region, were then selectively amplified using primers complementary to the functional gene but not the pseudogene.¹³ Several exons were amplified as a single PCR product, which was sequenced with primers specific for each exon. The PCR reactions were prepared in 100 μ l volumes containing 400–1000 ng of genomic DNA, 1 μ l of each 20 μ M primer, 10 μ l of 10 \times PCR buffer, 8 μ l of 2.5 mM dNTPs, and 2.5 U Ex Taq polymerase (TaKaRa Biomedicals, Otsu, Japan). DNA sequencing using the fluorescent dideoxy termination method was performed on both strands using the 373A DNA sequencer (Applied Biosystems, Foster City,

California, USA). The identified mutations were subsequently confirmed where possible by restriction digestions using the restriction enzymes BsmAI, Tsp45I, NciI, and AciI.

SOUTHERN BLOTS

The restriction enzymes SstII and SspI were used to digest genomic DNA from cases 1, 3, 4, 5, and a normal control. The digestions were electrophoresed on a 0.6% I.D.NA agarose gel (FMC, Rockland, Maine, USA), transferred to supported nitrocellulose membranes (Schleicher and Schuell, Keene, New Hampshire, USA) and hybridised with a glucocerebrosidase cDNA probe as described previously.^{13 14}

Results

MUTATION IDENTIFICATION

Case 1 was found to be homozygous for a recombinant allele including point mutations, L444P, A456P, and V460V, two base changes in intron 9, and one base change in intron 11, all alterations present in sequence from the glucocerebrosidase pseudogene.

Case 3 was found to carry a rare mutation, R120W,¹⁵ and a previously undescribed mutation, S196P. The former mutation obliterates an NciI site in exon 5, and the latter mutation destroys a Tsp45I site in exon 6. Restriction digests using these enzymes on DNA from the cousin of this patient revealed that she was homozygous for mutation S196P.

Mutational analysis of the glucocerebrosidase gene in case 4 revealed a 55 bp deletion in exon 9 of one allele. This 55 bp segment is ordinarily absent in the pseudogene. An uncommon mutation, R257Q,¹⁶ was identified on the second allele. This mutation obliterates a BsmAI site in exon 7, and its presence was confirmed by restriction digestion.

Case 6 was found to be homozygous for a previously undescribed mutation, R131L, in exon 5. Restriction digestion confirmed the presence of this mutation, because it obliterates an AciI site.

SOUTHERN BLOT ANALYSES

Southern blot analyses were performed on SspI and SstII digested DNA from the four probands and control samples. All samples had identical DNA fragment patterns. Bands known to be characteristic of a fusion gene were not seen.¹³

Discussion

In the decade since the association between congenital ichthyosis and Gaucher disease was first noted, both murine and human studies

have confirmed that this phenotype is part of the spectrum of clinical presentations of type 2 disease. However, it is still very difficult to ascertain the frequency of this form of Gaucher disease. We are aware of at least 14 published cases of neonatal Gaucher disease with ichthyosis.^{1 4 6 8-10 17-20} Greater awareness of this association by neonatologists, dermatologists, and geneticists might lead to an increased recognition of similar cases. Clearly, Gaucher disease belongs in the differential diagnosis of congenital ichthyosis.

Although the deficiency of epidermal glucocerebrosidase must contribute to the ichthyosis observed, it is not clear why only some infants with type 2 Gaucher disease display the collodion baby phenotype. Histological and ultrastructural studies of skin from patients with type 2 Gaucher disease without overt skin manifestations reveal that these fetuses and infants also have extracellular lamellar bilayer abnormalities of the epidermis.⁸ In fact, skin analyses might provide a means of distinguishing patients with type 2 Gaucher disease from those with type 1 or type 3 disease before the development of severe neurological signs. It has been speculated that the altered cutaneous desquamation seen in patients with congenital ichthyosis could be a result of abnormal intercellular cohesion and/or the induction of an abnormal skin permeability barrier leading to hyperproliferation.⁸

In this report, we provide the genotypic analyses of four previously described families with Gaucher disease and the collodion baby phenotype. In two of these families (cases 1, 5, and 6) there were homozygous genotypes presumably because of consanguinity, although the parents of cases 5 and 6 were not aware that they had common ancestors. We did not have parental DNA from cases 5 and 6 to confirm their mutations, but long PCR amplification of the complete glucocerebrosidase gene did not demonstrate any intergenic deletions, and sequencing at six known polymorphic sites in the glucocerebrosidase gene did not reveal any heterozygosity. Case 1 was found to have a known recombinant allele, sometimes referred to as RecNciI,² which includes pseudogene sequence beginning in intron 9. This genotype has also been seen in an infant of Afghani background with tight and shiny skin,¹⁴ as well as in two hydropic fetuses of Macedonian/Ashkenazi Jewish extraction.²¹ The mutation found in case 5, R131L, had not been described before, although an R131C mutation was found in a child with type 2 Gaucher disease.²²

The other two infants described were compound heterozygotes. Case 4 carried a 55 bp deletion in exon 9, a mutation that has been described previously in patients with type 1 and type 2 Gaucher disease.^{23 24} Although this 55 bp segment is absent from the glucocerebrosidase pseudogene, this patient did not have any other mutations deriving from pseudogene sequence. The second mutation in this patient, R257Q, has been described previously in a patient with type 1 Gaucher disease carrying a mild mutation, N370S, on the second allele.

Table 1 Genotypes observed in collodion babies with Gaucher disease

Case	Ethnic background	Consanguinity	Genotype
1	Lebanese	Yes	Recombinant allele (RecNciI)/recombinant allele (RecNciI)
2, 3	Lebanese	No	R120W/S196P
4	Australian	No	R257Q/55bp del
5, 6	Mexican	?	R131L/R131L
7	American ¹⁹	No	L444P and E326K/E233X
8	Dutch/Surinamese ²⁰	No	R359X/V398F
9	Afghani ¹⁴	Yes	Recombinant allele (RecNciI)/recombinant allele (RecNciI)

*DNA was only available from one parent.

Case 2 was found to have a previously undescribed mutation, S196P, on one allele and a known mutation, R120W, on the second. Mutation R120W has been described both in patients with type 1 and type 2 Gaucher disease. The observation that a cousin who was homozygous for mutation S196P also had type 2 Gaucher disease, confirms that this mutation is associated with early lethality.

Table 1 summarises the genotypes of these patients, together with those of other cases from the literature. No specific genotypes are associated with this phenotype. However, several of the patients have mutant alleles that result from recombination with the glucocerebrosidase pseudogene.

As the spectrum of phenotypes associated with metabolic disorders continues to expand, lysosomal storage diseases are increasingly being recognised in the newborn period. Gaucher disease is a disorder that must be considered in the neonatal nursery when confronted with a child with congenital ichthyosis.

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