See related article on pg 1421

COMMENTARY

Autosomal Recessive Congenital Ichthyosis

Judith Fischer[1]

Recent progress in the genetics of autosomal recessive congenital ichthyosis (ARCI) has illustrated the power of genetic strategies for the investigation of newly recognized metabolic pathways and for the mechanisms of barrier function in normal skin. Parallel biochemical studies have elucidated important functional aspects of these findings (Brash *et al*., 2007), and it is now time to determine how the genetic and biochemical features correlate with the clinical phenotypes of patients. The story of ARCI provides an instructive example of synergy among geneticists, biochemists, and clinicians.

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Autosomal recessive congenital ichthyosis and its genes

ARCI is a heterogeneous group of disorders of keratinization characterized mainly by abnormal skin scaling over the whole body. These disorders are mostly nonsyndromic and limited to skin; a total of 60–70% of patients present with severe symptoms, including a collodion membrane at birth. The main skin phenotypes are lamellar ichthyosis and congenital ichthyosiform erythroderma, although phenotypic overlap in the same patient or in patients of the same family can occur. Some patients have nonlamellar, nonerythrodermic ichthyosis. Since 1995, genetic analyses have implicated six genes. Mutations in transglutaminase 1 (*TGM1*), identified simultaneously by two groups in 1995 (Huber *et al*., 1995; Russell *et al*., 1995), are the most common cause of ARCI. Between 2002 and 2006, five other ARCI genes were identified via homozygosity mapping in consanguineous families (Jobard *et al*., 2002; Lefèvre *et al*., 2003, 2004, 2006). These findings provided evidence for the existence of an epidermis-specific hepoxilin pathway, based on the metabolism of arachidonic acid. These genes include two lipoxygenases (*ALOX12B*, *ALOXE3*), an ATP-binding cassette transporter (*ABCA12*), a potential receptor (*ichthyin*), and a gene coding for a protein of the cytochrome P450 family (*CYP4F22*).

Mutational spectrum in ARCI

In the study reported in this issue, Eckl *et al*. (2009) present mutation screening of *ALOX12B* and *ALOXE3* genes in 250 ARCI patients. Consanguineous families were first tested for homozygous regions before sequencing of the corresponding genes. Previous analysis of the *TGM1* gene had revealed mutations in 38% of patients (data not presented), whereas *ALOX12B* and *ALOXE3* mutations were found in 17 patients each—6.8% for *ALOX12B* and 6.8% for *ALOXE3*, including 13 patients from a previous publication (Eckl *et al*., 2005). Eight of the mutations in *ALOX12B* and three mutations in *ALOXE3* were novel. Analysis of the literature and the results of this paper confirmed the presence of two mutational hotspots in *ALOXE3* (p.Arg234X and p.Pro630Leu), which were first reported by Jobard *et al*. (2002) and Eckl *et al*. (2005), respectively, whereas no recurrent mutations were observed in *ALOX12B*. It is unclear whether all 250 patients were sequenced for the three genes (*TGM1* and the two lipoxygenases) that are responsible for more than half of the mutations (51.6%). Based on Eckl's estimate that between 30 and 40% of patients have no mutations in the six known ARCI genes (Eckl *et al*., 2009), mutations in the other three genes—*ichthyin*, *CYP4F22*, and *ABCA12*—should account for 8–18% of the remaining cases.

In our cohort of 520 independent families, however, we were able to identify mutations in all but 22% of the patients, and we observed differences in the distribution of mutations in populations of different origin. By direct sequencing of the six ARCI genes, we found that 32% of the patients harbored mutations in *TGM1*, 12% in *ALOX12B*, and 5% in *ALOXE3* (49% of the total, similar to the 51.6% reported by Eckl *et al*.). Moreover, 29% of the mutations were situated in the three remaining ARCI genes (16% in *ichthyin*, 8% in *CYP4F22*, and 5% in *ABCA12*) (Figure 1). For a better estimation of the percentages, especially that of patients without mutations in the six known genes, it will be necessary to sequence all ARCI genes in patient collections, including Eckl's cohort.

> Large cohorts are essential to understanding ARCI.

Causative mutations and polymorphisms

Another issue is the genetic classification of patients in whom only one mutation has been detected, which Eckl *et al*. (2009) reported in 3 of 34 patients (9%). It is well known that larger deletions may play a role in these cases, but very few molecular researchers have systematically tried to identify such large deletions.

Unfortunately, the molecular diagnosis remains uncertain in these cases. They should not be included in studies of genotype–phenotype correlations, even when the first mutation is a previously detected missense mutation or a mutation leading to a truncated protein.

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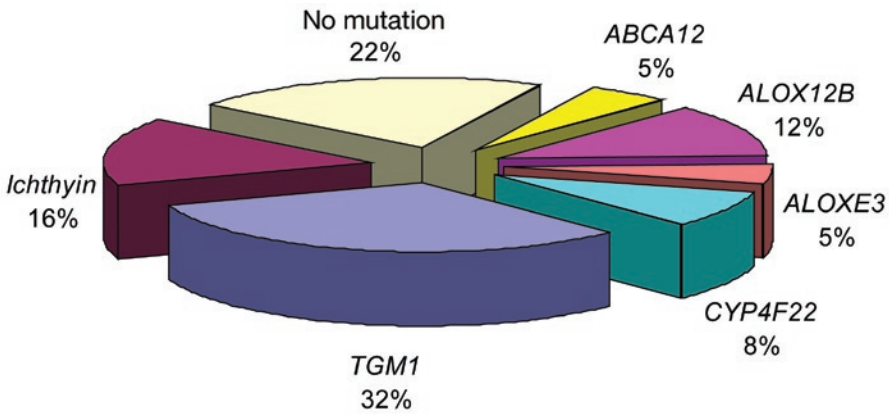

Figure 1. Distribution of mutations in a cohort of 520 ARCI families. The results of direct sequencing of 520 patients from 520 independent families; 32% of the patients present mutations in *TGM1*, 16% in *ichthyin*, 12% in *ALOX12B*, 8% in *CYP4F22*, and 5% each in *ALOXE3* and *ABCA12* (only exons 28–32 were sequenced in *ABCA12* for the majority of patients). In 22% of the patients, no mutations could be detected.

If a mutation is a missense mutation, which leads to a replacement of one amino acid in the peptide sequence, it is usual to provide the results of screening in 120 healthy population-matched controls. However, there is no guarantee that the mutation is causative when it is absent from the general population. In the case of autosomal recessive disorders, there are four to five times more healthy carriers than affected individuals in the general population, so the detection of carriers in such population-matched controls is insufficient proof to classify a sequence variation as a noncausative polymorphism. The strategy used by Eckl *et al*. (2009) to test for enzymatic activity was especially helpful in determining whether the missense mutations in the *ALOXE3* and *ALOX12B* genes were causative.

The authors have provided a didactic example of the difficulties of molecular diagnosis with patient ISA, from the Indian subcontinent, who was previously reported to harbor a homozygous p.Leu237Met mutation in *ALOXE3* but in whom this mutation was shown to have normal enzymatic activity using a construct created by site-directed mutagenesis (Eckl *et al*., 2005). This patient, in whom no consanguinity was initially known, was described as presenting with a remarkably severe phenotype, including erythroderma; large thick, dark scales over his entire body; and facial involvement, symptoms that are more characteristic of *TGM1* mutations, as noted by the authors. In the current article, the mystery about patient ISA continues: a second homozygous mutation is now reported (p.Arg145His) in exon 3 of *ALOXE3*; it was also described as enzymatically active but was later found to correspond functionally to a splice site mutation leading to a skipped exon (p.Arg119GlyfsX12). Phenotypic information is absent from the current article.

Genotype–phenotype correlation

Because the ARCI group of disorders is characterized by both genetic and clinical heterogeneity, the genetic forms cannot be clearly distinguished based on the phenotype. Our attempts to correlate the skin phenotype of patients from 520 families with a specific genotype were successful (manuscript in preparation) in only about one-third of the cases. However, we have observed that clinical criteria such as hyperlinearity and general or partial hyperkeratosis of the palms and soles are often more genotype specific than are the details about skin phenotype. Therefore, it is to be expected that a clear palmoplantar description would provide a more precise "prediction score" for the genotype–phenotype correlation.

As reported in this issue, Eckl *et al*. describe a significant phenotypic variation in ARCI resulting from *ALOX12B* and *ALOXE3* mutations, based on the presence or absence of keratoderma, respectively. Other clinical features, such as alopecia and hypohydrosis, did not exhibit significant differences, but it is possible that these differences are more striking in patient groups in which the causative genes do not exhibit a close functional relationship, as is the case with *ALOXE3* and *ALOX12B*. It is also possible that examination of more than 34 patients will be necessary to detect statistical differences.

The immense importance of large collections of patients and families for the complete study of these heterogeneous disorders has become evident. These collections allow the identification of genes that are causative in only a very small group of patients, and they can help to identify genotype–phenotype correlations.

A complete list or database of sequence variations found in ARCI patients, including information about causative mutations and noncausative polymorphisms and corresponding clinical data, would be useful for diagnosis and for future projects, including clinical trials.

In several cases, sequence variations reported as causative mutations (cf. p.P127S in *ALOX12B*; Eckl *et al*., 2005) have turned out to be neutral polymorphisms (Lesueur *et al*., 2007). Clear and precise clinical information such as that reported by Eckl *et al*. in this issue (see the supplementary information for that article) will be very useful in this type of study.

A more precise clinical classification that takes into account key elements of ARCI (such as palmoplantar features) would certainly be useful for correlations with genotypes. However, most ARCI patients have no access to mutation screening because of the high screening costs and the large number of genes that must be analyzed in the absence of clear phenotypic correlation with specific gene mutations. Therefore, the development of a diagnostic chip that could provide less expensive and more complete diagnosis should be a priority for both patients and clinicians. The future will show whether the lessons learned in the genetic, biochemical, and clinical analyses of ARCI will finally lead to a revolution in treatment.

CONFLICT OF INTEREST
The author states no conflict of interest.

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See related article on pg 1471

Shedding Light on Proteolytic Cleavage of CD44: The Responsible Sheddase and Functional Significance of Shedding

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CD44 is the major cell-surface receptor for hyaluronan, which is implicated in cell–cell and cell–matrix adhesion, cell migration, and signaling. Studies have shown that CD44-dependent migration requires CD44 to be shed from the cell surface and that matrix metalloproteinase–mediated cleavage may provide an underlying mechanism. However, the full spectrum of proteases that may participate in CD44 shedding has yet to be defined. In this issue, Anderegg *et al.* demonstrate that ADAM10, but not ADAM17 or MMP14, mediates constitutive shedding of CD44 in human melanoma cells and that knockdown of ADAM10 blocks the antiproliferative activity of the soluble proteolytic cleavage product of CD44.

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Roles of CD44 and ADAM10 in melanoma progression

Studies have suggested an important role for CD44 in melanoma growth and progression. Thus, increased expression of CD44 and CD44–hyaluronan (HA) interaction correlate, respectively, with melanoma progression and metastatic proclivity of melanoma cells (De Wit *et al.*, 1996). CD44 mediates HA-induced melanoma cell proliferation (Ahrens *et al.*, 2001a), and CD44–HA interaction is required for melanoma development in mouse models (Bartolazzi *et al.*, 1994). Functional blocking anti-CD44 antibody inhibits growth and metastasis of human melanoma cells (Guo *et al.*, 1994), and soluble CD44 inhibits melanoma tumor growth by blocking HA binding to cell-surface CD44 (Ahrens *et al.*, 2001b). In addition, hepatocyte growth factor engagement of its receptor, c-Met, upregulates expression of CD44v6 in murine melanoma cells (Recio and Merlino, 2003), and HA-mediated interaction between CD44 and epidermal growth factor receptor promotes melanoma cell motility by activating protein kinase C signaling (Kim *et al.*, 2008).

ADAM (a disintegrin and metalloproteinase) is a family of cell-surface proteases that are related to matrix metalloproteinases (MMPs) and contain several defined functional domains, including metalloproteinase, disintegrin, cysteine-rich, and transmembrane domains, as well as a cytoplasmic tail. ADAMs serve as the major class of sheddases for many important cell-surface receptors, and they play an essential role in regulating interactions between tumor cells and their microenvironment and in initiating the activation of key signaling pathways. Expression levels of many ADAM family members are elevated in human cancers, and studies have suggested important roles for ADAMs in cancer growth and progression (reviewed by Mochizuki and Okada, 2007). Currently, little is known about the role of ADAM10 and the other ADAM family members in

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