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Probing the Gene eXpression Database for candidate genes

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We report on a strategy for the identification of candidate genes for multiple malformation syndromes using expression data available in public databases. The basis for this pilot study was the assumption that, for a multiple malformation syndrome, the expression pattern of the causative gene should at least cover the organs or tissues affected by the syndrome. Twenty malformation syndromes were selected from the OMIM and defined by three to five main symptoms. These key symptoms were translated into anatomical terms that were used to query the Gene eXpression Database (GXD). The searches covered 65% of the database and yielded an average of 16 candidate genes per syndrome. Of these, 23% were ubiquitously expressed or housekeeping genes. Further database evaluation of these potential candidate genes was based on positional information and on information from mouse knockouts. In a first experiment, the correct gene was identified as a candidate in four of seven syndromes for which the causative gene is already known. In addition, this strategy identified new candidate genes for disorders for which the genetic basis is unknown. We identified candidate genes for the Walker-Warburg, DOOR, C, scalp–ear–nipple and oculocerebral hypopigmentation syndromes. Our results suggest that it may ultimately be feasible to identify disease genes by probing gene expression databases with simple syndrome descriptions.

Keywords: candidate gene; gene expression; database; syndrome; Internet

Introduction

Four main strategies for identifying human disease genes are often distinguished, namely functional cloning, positional cloning, positional candidate, and position-independent candidate strategies. In functional cloning, information about the presumed cellular function is used to identify the unknown disease gene. The gene product is already known and the gene coding for it can quickly be identified. This approach has been

useful for those diseases in which well-known metabolic pathways or structural proteins are affected, such as phenylketonuria, alkaptonuria and haemophilia.^{1–3} In positional cloning, the gene is isolated on the basis of its chromosomal localisation, without prior information about its function. To achieve this, first the localisation of the disease gene must become known. This is done by means of linkage analysis using extended families. Next, genetic and physical maps of the chromosome area are constructed and potential genes in the region identified. Finding a disease-associated mutation is required to prove which of the genes in the region is the disease gene.

Positional cloning is gradually being superseded by the positional candidate approach. Thanks to the increasing volume of gene sequences, either partial or

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complete, deposited in public databases, candidate genes can often be found by database searches, targeting the area where a disease gene has been localised. In this way, the efforts involved in cloning and analysing large pieces of chromosomal DNA can often be avoided. When a disease gene is mapped to a specific chromosomal segment, additional criteria are useful to prioritise the different positional candidates. Among these criteria is the expression pattern of the positional candidate genes.⁴ A different approach from the positional cloning and positional candidate strategies is that of the position-independent candidate. For example, genes that are expressed in a single tissue can be considered as candidate genes for those inherited disorders in which only that tissue is affected. Examples are the collagen genes in the chondrodysplasias,⁵ retina-specific genes in inherited blindness⁶ and cochlea-specific genes in deafness.⁷ Natural or induced mutations in model organisms such as the mouse or similar syndromes in humans for which the gene is already known^{8,9} may further suggest a gene for a particular disorder. Human multiple malformation syndromes are defined by specific recurring patterns of abnormalities in tissues of different embryonic origin. In analogy to the candidate approach for single-tissue disorders, genes that have an expression pattern matching the syndromal pattern of abnormalities can be considered as candidates. To our knowledge, a generalised approach that compares gene expression data with syndrome-specific patterns of organ or tissue involvement has not been attempted. Here we report on our experience with such a strategy for gene identification based on matching gene-expression patterns with syndrome-specific patterns of organ involvement.

Methods

Syndrome Definition

In order to search databases for candidate genes, a syndrome must first be translated into an expression pattern. To define those areas in which gene expression is critical for the development of the syndrome, its defining features should be separated from rare or nonspecific signs. This is a central problem in syndromology for which many solutions have been proposed.¹⁰ These solutions, however, have not been incorporated into clinical syndromology practice nor in the available syndrome databases. Ideally, one would use a database that attaches weights to signs and symptoms based on their sensitivity and specificity relative to other syndromes. The London Dysmorphology Database¹¹ (LDDb) does define a syndrome as a pattern, but does not distinguish essential from non-essential symptoms. The (P)OSSUM databases^{12,13} can allocate a weight to symptoms, but as this

must be done by the user, this does not obviate the problem. We chose to circumvent this problem by asking two experienced clinical geneticists to define a set of 20 syndromes by three to five main symptoms. The symptoms were chosen from the listings in the LDDb, as this is the most comprehensive database to date. A third clinical geneticist decided where the first two disagreed on a particular core symptom.

Definition of Search Terms

To search gene expression databases, the phenotypic abnormalities of the syndrome must be translated into search terms. At the time this work was undertaken, the expression databases that were available (such as the Genome Database (GDB) and the Gene eXpression Database (GXD)) were based entirely on literature abstracts. Therefore we defined the most commonly used developmental terms in the literature and used these in our searches. It should be noted that the newer GXD versions do have standardised expression terms.

To define the most commonly used terms, the 1997 issues of three major developmental biology journals (*Genes and Development*, *Mechanisms of Development*, *Development*) were examined for possible search terms. Both abstracts and the body of text of approximately 540 papers were evaluated. This resulted in a list of 1775 terms. Several of these were synonymous terms (for example, genito-urinary and urogenital). The 1775 terms were checked for occurrence in 3000 MedLine abstracts which had been selected using 'embryo' and 'development' as keywords. A simple shell script was written to count the terms and run on a PC running RedHat Linux 5.0–5.2, kernel versions 2.0.34–2.0.36. Terms that occurred at a frequency greater than 15 per 1000 articles were selected for use as search terms. A total of 153 terms was thus selected, less than 1% of the original list. These terms were arranged in a hierarchical look-up table which closely follows the branching structure of the LDDb feature list. Most of the headings of the LDDb feature list are matched by at least one term in the thesaurus. The search terms are grouped under organ systems headings as syndrome features in the LDDb. Syndrome features selected by the expert panel could thus be translated into developmental search terms by selecting the appropriate organ system heading and choosing the terms describing the affected tissue.

Selection of a Suitable Database

The starting point for the candidate gene selection procedure described here is the hypothesis that the gene which causes a multiple congenital malformation syndrome is expressed in the tissues affected in that particular syndrome. To find candidate genes based on this assumption, a database must contain searchable expression information for human genes. No such databases are at present available. We therefore used mouse data as a second-best approach. There are many databases available on the mouse as a model organism,¹⁴ but only the Gene eXpression Database (GXD), maintained by the Jackson Laboratories (www.informatics.jax.org), contains systematically ordered expression data that can be searched using anatomical or functional terms. This database was selected to examine the feasibility of candidate identification based on expression patterns.

Candidate Gene Selection

To find candidate genes for a specific syndrome, between two and four search terms were used to query the Gene

eXpression Database. Compound searches, wildcarding and the use of regular expressions (a pattern that is matched against a string) are not allowed in this database. Each new search term requires a new query. The result of a query is a list of hyperlinked genes and their associated literature references, encoded in HTML (HyperText Markup Language). The genes in the listings are expressed in the tissue described by the search term. Candidate genes for the syndrome are those that are expressed in all tissues affected in the syndrome, ie are present in all listings. A shell-script was written to compare the downloaded files and select those genes that were present in each of the listings downloaded for that syndrome.¹⁵

The Pilot Study

In order to evaluate the strategy outlined above, a pilot study was conducted. Twenty malformation syndromes were selected from the OMIM (Online Mendelian Inheritance in Man) database¹⁶ by querying it with 'syndrome' and picking OMIM numbers from the list at random. Syndromes for which the gene is already known, chromosome abnormalities and metabolic diseases were excluded. Syndromes were selected if the component abnormalities involved two or more anatomically distinct organ systems. The selected syndrome features were translated into search terms by use of the look-up table. A potential problem is that genes may be indexed under synonymous terms. Using only one term from the list of possible synonyms would give rise to many potential candidate genes being missed. To evaluate the magnitude of this problem, searches were made either using 'blanket terms', ie terms describing the organ system in the broadest sense, or by a list of synonyms, for example, 'limb' vs 'hand + digit + ulna + radius'. Separate sets were compiled either of 'blanket terms' or of sets of synonyms. These terms were then entered in the GXD. The resulting HTML-encoded

listings were downloaded and saved for later evaluation. Candidate gene listings were prepared by using the shell script that identifies genes which arise in all searches. In order to evaluate the potential for this approach to uncover disease genes, a set of seven syndromes for which the molecular basis is already known was also examined.

Results

Characteristics of the Search Terms

The 29 blanket search terms (Table 1) used to describe the total of 20 syndromes in the initial pilot study together recovered 1596 (65%) of 2467 genes in the GXD (number as determined on 15 June 1998). One third of the database, 886 genes, was not accessed by this set of terms. This could be due to our limited selection of terms, but other causes such as missing expression data also seem possible. To clarify this, the abstracts of 40 genes not among those retrieved by the 29 blanket terms were evaluated (genes listed in Table 2). It turned out that 18 out of 40 genes either had no abstract or an abstract without anatomical search terms. Of another 18 genes, the abstracts contained terms which are not among the 29 terms selected in the initial study, but are in the main list of 153 terms. Of the remaining four genes, one had a term not present in either list and one entry had been removed from the database. A small number (two out of 40) genes was associated with abstracts which described a different

Table 1 Syndromes selected at random from OMIM and their description in expression patterns

<i>Syndrome (No. of genes returned)</i>	<i>MIM number</i>	<i>Translation/search terms</i>
C-syndrome (20)	211750	Cranium, digit, brain
Cerebro-costo-mandibular syndrome (13)	117650	Rib, palate
FG syndrome (3)	305450	Cranium, ear, brain, hindgut
Wolcott-Rallison syndrome (1)	226980	Vertebra, limb, pancreas
Weill-Marchesani syndrome (8)	277600	Eye, tooth, digit
Branchio-oculo-facial syndrome (15)	113620	Branchial, eye, skeleton
Cerebro-oculo-facial-skeletal syndrome (5)	214150	Brain, skull, eye, digit
Costello syndrome (61)	218040	Heart, brain, skin
Oculocerebral-hypopigmentation syndrome (12)	257800	Brain, eye, pigment
DOOR syndrome (5)	220500	Ear, kidney, digit
Johnson-McMillin neuro-ectodermal syndrome (32)	147770	Brain, skin, tooth
Walker-Warburg syndrome (56)	236670	Muscle, eye, brain
Megalocornea-mental retardation syndrome (18)	249310	Cornea, brain
Nance-Horan syndrome (50)	302350	Eye, tooth
OFD type 1 (1)	311200	Brain, palate, tongue, digit
OFD type 3 (1)	258850	Tongue, digit, brain
Opitz-Frias syndrome (7)	145410	(O)esophagus ^a , genital
Scalp-ear-nipple syndrome (4)	181270	Skin, ear, mammary
Senior-Loken syndrome (35)	266900	Retina, kidney
Varadi-Papp syndrome (4)	277170	Cerebellum, oral, digit

^aboth 'oesophagus' and 'esophagus' were used as search terms and the results concatenated since both terms return different sets of genes.

Table 2 Properties of genes not found using 29 blanket search terms

<i>Gene</i>	<i>Abstract</i>	<i>Gene</i>	<i>Abstract</i>
Hp1a	no terms	Canp3	no abstract
Hsd17b4	terms in list	Cd3e	terms in list
Iap1	terms in list	Cea10	terms in list
Lamp1	terms in list	Cebpa-rsl	no terms
Uty	no terms	Cmkbrll2	terms in list
Abl	terms in list	Dabl	no terms
Ar	terms in list	Dmahp	no terms
Cebpd1	terms in list	Epsl5-rs	no terms
Fgdl	no terms	Err2	about other gene
Lbx1	terms in list	Gnal2	no terms
Mif	terms in list	Gnao	terms in list
Rnu6	no terms	Gnaz	terms in list
Clk4	no terms	H2-T17	no terms
Rab6kifl	no terms	Has3	no terms
Tbx14	none	Hbb-y	about other gene
Scal	terms in list	Il3rb2	terms in list
Etv6	terms in list	Mage-rsl	no terms
Etv5	no terms	Semak	removed from db
Fgfr3-ps	no terms	Tcra-C	terms in list
Finl3	no terms	Zfx	terms in list

'Terms in list': terms found in abstracts can also be found in extended list of 153 search terms.

gene from the one indexed. Assuming that our sample of 40 genes analysed is representative of the 886 genes that were not retrieved, the following calculations can be made. Of all the genes currently in the GXD database, some 65% are accessible by a set of 29 simple blanket-type search terms and this increases to 81% if an extensive list of 153 (including several synonymous terms) is used. Of the remaining 19%, 17% are not accessible by anatomical search terms. Approximately 2% of the searches will retrieve false positives, that is genes which are not expressed in the tissue being queried. Based on these results, the core set of 29 terms was used in a pilot study to evaluate further the feasibility of the candidate gene selection procedure.

Database Search by Syndrome Patterns

In order to test the feasibility of using expression patterns for the selection of candidate genes, we first examined seven syndromes for which the causative gene is known. In four of these, the disease gene was

identified as one of 19 to 45 candidate genes (Table 3). In the other three cases, the gene was not found by using the selected combination of search terms. The *TBX5* gene for Holt-Oram syndrome was retrieved by using 'limb' as a search term, but not by 'heart'. Alternative search terms were used. Queries using 'cardiac', 'ventricle' and 'atrium' were raised, all with negative results. Similarly, the *Jag2* gene was not identified for Alagille syndrome, since only the 'heart' query was positive, but not 'eye' or 'liver'. The gene for Townes-Brocks syndrome (*HSAL1* or *SSAL*) did not feature at all in the GXD, presumably because the mouse homologue has not been identified. Thus, our analysis of seven multiple congenital anomaly syndromes identified the causative gene in the majority, but also showed up some of the weaknesses of this system using current databases such as GXD.

We examined a larger series of syndromes for which the genes were not yet known in order to gain a better view of whether candidate gene lists are specific for

Table 3 Result of search with syndromes of known genesis

<i>Disorder/MIM number</i>	<i>Search terms</i>	<i>Causative gene identified?</i>	<i>Selectivity</i>
Tricho-Dento-Osseous	Ectoderm Skeleton Tooth	yes; <i>DLX3</i>	1/19
Ulnar Mammary	Limb Mammary	yes; <i>TBX3</i>	1/22
Townes-Brocks	Limb Ear	no; <i>HASL1</i>	NA
Holt-Oram	Limb Heart	no; <i>TBX5</i>	NA
Hand-Foot-genital	Limb Genital	yes; <i>HOXA13</i>	1/45
Renal-Coloboma	Renal Eye	yes; <i>PAX2</i>	1/22
Alagille	Liver Eye Heart	no; <i>Jag2</i>	NA

NA = no applicable.

each syndrome description, and whether candidates on these lists are in fact biologically plausible, ie real candidates. Since only 29 terms from the list were used to describe 20 syndromes, the possibility of overlap between syndrome descriptions exists, perhaps causing different syndromes to be described by an identical set of terms. As can be seen in Table 1, this was not the case in the pilot. Thus, a relatively limited number of terms still conferred a unique syndrome 'pattern' in this pilot experiment. The syndrome signatures were used to query the Gene eXpression Database. The gene listings resulting from each query were downloaded and combined to generate a list of candidate genes specific to each syndrome. As expected, different numbers of terms yielded different numbers of candidate genes. For four syndromes, two terms were used to define the characteristic pattern of organ involvement. These searches yielded on average 25 genes per syndrome. For twelve syndromes, three terms were used. The average yield of these searches was 16 genes per syndrome. Four syndromes were described with four terms each. The yield in these searches was four genes per syndrome. Based on these results the pilot was continued with the 12 syndromes that were described using a combination of three blanket terms. A total of 20 different terms was used to describe these 12 syndromes. The total number of candidate genes retrieved from the GXD for the 12 syndromes combined was 115. This is 7.4% of the total number of 1552 genes that could have been retrieved by the 20 search terms separately. The number of genes picked up by the searches varied from one (OFD type 3; terms 'tongue', 'digit', 'brain') to 61 (Costello syndrome; terms 'heart', 'brain', 'skin'). For the 115 genes that were retrieved for the 12 syndromes, 57 genes (49.6%) were picked up only once and 58 genes (50.4%) between two and eight times. An example of a syndrome query is given in Table 4. Here 56 genes were retrieved for the Walker-Warburg syndrome (WWS) and evaluated for their potential as candidate genes. Gene function was assessed using the Mouse Genome Database.¹⁷ Mouse knockout models were searched in TBase.¹⁸ The search terms used in individual searches were 'muscle', 'eye' and 'brain'. As shown in Table 3, 15 genes were not considered further as syndrome-specific candidate genes on the basis of their known role as housekeepers or their ubiquitous expression. Another 20 genes are associated with human or mouse phenotypes which do not resemble WWS. Therefore the likelihood of these genes causing WWS is reduced. Among these were 10 mouse knock-

out models, illustrating the value of knockouts for the evaluation of candidate genes. This proportion can be expected to increase in future, as more developmentally important genes are targeted in the mouse. Another 11 genes were not likely candidates because their products are known to function in single biochemical pathways that are not likely to explain the syndromic pattern of organ involvement. For example, *Isl1* is a LIM-homeodomain gene required for the development of pancreatic islet cells and motor neurons.¹⁹ The first analysis left 10 genes which merit further evaluation. Of these, eight are associated with human or mouse phenotypes featuring brain, eye and/or muscle abnormalities. The combination of data on expression patterns and function suggested that the remaining two genes may be candidates for the disorder (Table 3). To prioritise between these 11 candidate genes, positional information was sought.

Recently, the muscle-eye-brain disease (*MEBD*) gene was mapped to human chromosome 1p32.²⁰ Whether *MEBD* is allelic to WWS is not known, but the syndromes have a similar pattern of organ involvement. No positional candidate genes could be identified in this region, so it was of interest to check whether any of the candidate genes identified above maps near this region or is in a syntenic mouse chromosomal region. None of the genes in our list has been localised to the 1p32 region in humans. However, *Cdh4*, which has not yet been mapped in humans, may be in the relevant area, as it is in a region on mouse chromosome 2 which shows synteny with human 1p32-33 and therefore is an interesting candidate. *Cdh4* belongs to the cadherin family of cellular adhesion molecules. It is expressed in muscle, retina and brain; inhibition of cadherin action in the brain leads to neuronal migration defects.^{21,22} In view of its expression pattern, *Cdh4* is an attractive candidate gene for this disorder. Thus, the search for Walker-Warburg syndrome uncovered 11 candidate genes, one of which is also a positional candidate for *MEBD*. Similar results were obtained for the other 11 syndromes in this limited pilot experiment. On average, 23% of the genes were ubiquitously expressed or housekeeping genes. Of the remainder, six genes (or their associated pathways) were considered potential candidates for one of the syndromes in the pilot study, namely *Bmp4*, *Fgf4*, *Dct*, *Mitf*, *Pdgfra* and *Lef-1*. For these genes, mouse mutants either occur naturally or have been generated, which facilitates their evaluation (Table 5). These are discussed below. A search for DOOR syndrome using 'ear', 'kidney' and 'digit' as

Table 4 Evaluation of WWS candidate genes

Strong candidates

Similar mouse / human phenotypes

Hnf3beta	-/- mouse has head and brain abnormalities
Lama2	<i>dystrophia muscularis</i> mouse mutant
Ncam	-/- mouse has mild brain phenotype/lethal
Otx2	-/- mouse has no rostral structures, +/- NTD
Pax3	Human: WS1, -/- mouse has exencephaly
Pax7	-/- mouse has head and brain abnormalities
Pdgfra	<i>Patch</i> mouse, may have NTD
Shh	Human: HPE, -/- mouse has severe defects of brain, limb, midline structures, foregut
Six3	Human: HPE, -/- mouse has eye/brain phenotype ⁴⁰

Expression and/or function

Cdh4	expression in retina, brain, muscle; cell adhesion
Utrn	part of dystrophin complex, expressed in neural progenitors

Less likely candidates

Dissimilar human/mouse phenotype

Bmp2	-/- mouse post-implantation lethal
Bmpr	-/- mouse lacks mesoderm
Cd44	-/- mouse has immune disturbance
Cdh2	-/- mouse has inflammatory bowel disease
Col10a1	Human: Schmidt metaphyseal dysplasia
Col2a1	Human: SED type skeletal dysplasias
Col1a1	Human: osteogenesis imperfecta
Dlx1	-/- mouse has abnormal 1st/2nd branchial arch
Dmd	Human: m. Duchenne
Egfr	<i>waved-2</i> mouse mutant
Fgf4	-/- mouse failure of embryogenesis
Flk1	-/- mouse failure of vascular development
Gja1	Human: Implicated in situs inversus
Lamb1-1	<i>neonatal cutis laxa</i> mouse mutant
Msx-1	Human: missing molars
Nfl	-/- mouse has ALS-like phenotype
Pax1	<i>undulated</i> mouse mutant
Tgfb1	-/- mouse has severe inflammatory disease
Tnc	-/- mouse is normal
Vim	-/- mouse is normal

Known housekeepers/ubiquitously expressed

Actb	actin beta, cytoskeletal element
Crya2	lens protein
Ccnd2	cyclin D2
Cdkn1c	p57 tumor suppressor
Dag1	part of Ca ²⁺ signalling pathways
Ecm2	extracellular matrix protein
Pkca	protein kinase c, 'a' subunit
Pkcb	Protein kinase c, 'b' subunit
Slc2a1	glucose transporter
Slc2a2	idem
Slc2a4	idem
Sparc	extraembryonic matrix glycoprotein
Tubb	component of microtubules
Tubb3	idem
Vwf	Von Willebrand factor

Functional data negative/equivocal

Cdh1	required for compaction of embryos
Col16a1	connective tissue component
Col19a1	connective tissue component
Gfap	glial cytoskeleton
Isl1	LIM homeodomain protein
Lama1	mainly expressed in genitals
Lamb1-2	pseudogene
Nfm	neurofilament medium chain
Sim2	may be involved in Down's syndrome
Syp	component of synaptic vesicles

NTD: neural tube defect; HPE: holoprosencephaly; WS1: Waardenburg syndrome type 1; SED: spondyloepiphyseal dysplasia; ALS: amyotrophic lateral sclerosis.

Table 5 Potential candidate genes found for some of the syndromes described by three terms

Syndrome	Candidate genes	Arguments for gene
C-syndrome terms: cranium, digit, brain	<i>Fgf4</i>	Overexpression in mouse gives cranial and limb abnormalities
Oculocerebral hypopigmentation syndrome terms: brain, eye, pigment	<i>Dct</i> <i>Mitf</i> <i>Pdgfra</i>	Slaty mouse has similar pigment disturbances; <i>Dct</i> expressed in brain. Involved in pigmentation and eye development, Waardenburg syndrome type II in humans. <i>Patched</i> mouse has CNS and pigment anomalies
DOOR syndrome terms: ear, kidney, digit	<i>Bmp4</i>	−/+ mouse has polydactyly, cystic kidneys and inner ear defects
Scalp–ear–nipple syndrome terms: skin, ear, mammary	<i>Lef1</i>	−/− mouse lacks mammary tissue, hair and teeth

terms uncovered five candidate genes, of which *BMP4* may be especially convincing. The heterozygous *Bmp4* knockout mouse has pre-axial polydactyly, cystic kidney malformations and in a number of cases shows circling behaviour consistent with an inner ear defect. Shortening of the nasal and frontal bones, giving the appearance of a boxer's nose, were also noted.²³ DOOR syndrome is a disorder characterised by onychodystrophy, triphalangeal thumbs, mental retardation, seizures and sensorineural deafness. Cystic malformations of kidneys and urinary tract have also been described.²⁴ The face is characterised by a short, broad nose. There is no mention of dystrophic claws or seizures in the knockout mice, but the mouse phenotype seems to resemble the human DOOR phenotype sufficiently to warrant consideration of *Bmp4* as a serious candidate gene for this disorder.

One search also resulted in *Fgf4* as a candidate gene for C syndrome (trigonocephaly and polydactyly). An insertional mutation in the mouse upregulating the expression of *Fgf3* and *Fgf4* in the mouse causes craniofacial malformations.²⁵ The mutant is called *Bulgy eye* (*Bey*). *Bey* mice are viable and fertile but show facial shortening with increased interorbital distance and craniosynostosis. According to Carlton *et al*, who described the mutant, the *Bey* mutation resembles Crouzon syndrome.²⁵ Although two *Fgfs* are upregulated here, this study suggests that *Fgf4* may be involved in the regulation of suture closure. *Fgf4* is involved in limb patterning as well. In the Strong's luxoid mouse mutant, ectopic expression of *Fgf4* causes polydactyly.²⁶ *Fgf4* is normally secreted by the ZPA and is one of the regulators of antero-posterior polarity in the limb.²⁷ These data suggest that either *Fgf4* or a gene regulating its expression in the developing skull and limbs may be involved in C syndrome, which is characterised by trigonocephaly and polydactyly.²⁸ For three other syndromes in the list, potential candidate

genes were also obtained. Various mutations in dopa-chrome tautomerase, *Dct*, have been found in the *Slaty* mouse mutant, which has patchy to diffuse hypopigmentation.²⁹ This phenotype models the pigmentation phenotype of oculocerebral hypopigmentation syndrome. Moreover, *Dct* is also expressed in the brain although its role in that organ is as yet unclear.²⁹ Mutations in the *Mitf* gene cause microphthalmia in the mouse and are responsible in humans for about 15% of Waardenburg syndrome type II cases.³⁰ In the mouse, a number of *Mitf* alleles exist, all associating disturbances of pigmentation with various other abnormalities.³¹ This suggests that *Mitf* may be relevant for a number of different pigmentation phenotypes in humans. As such this gene is a candidate for oculocerebral hypopigmentation syndrome in which microphthalmia has been reported to occur.³² *Pdgfra* is mutated in the *Patch* mouse, that shows patchy depigmentation³³ and, depending on its genetic background, other anomalies varying from cardiovascular malformations to neural tube defects.³⁴ Although neural tube defects are not a feature of oculocerebral hypopigmentation syndrome, the mouse appears to be more prone to developing neural tube defects upon perturbation of developmental genes and it is conceivable that a similar mutation in humans could give rise to a more subtle neural tube phenotype. Finally, the search for the scalp–ear–nipple syndrome produced *Lef-1* as a candidate. Scalp–ear–nipple syndrome is characterised by hairless nodules on the scalp, malformed external ears, hypo- to aplasia of nipples and breast tissue and widely spaced or missing secondary teeth.³⁵ Lymphoid enhancer factor-1 is an HMG-domain DNA-binding protein expressed in the neural crest, mesencephalon, tooth germs and other sites during embryogenesis.³⁶ It is a component of the *Wnt* pathway, and probably modulates the actions of β -catenin.³⁷ Homozygous deficiency of this gene causes postnatal lethality in mice. Mutant mice lack teeth,

mammary glands, whiskers and hair. All anlagen of these tissues are reduced in number and are arrested in their development. Although the phenotype is lethal in mice and nodules of the skin were not described, the lack of hair, missing teeth and aplasia of breast tissue suggest that *Lef-1* may be a candidate gene for scalp-ear-nipple syndrome.

Discussion

If it is to be used for the purpose of generating position-independent candidate genes, a gene expression database should ideally have the following features:

- include all human genes;
- be updated regularly;
- all genes in the database should be examined for expression in a large number of tissues, so that both presence and absence of expression are listed;
- contain data in the form of gene expression patterns for defined embryonal stages, determined by comparable, standardised methods;
- the database should allow searching with uniform search terms for genes, tissues, and stages of development.

It appears that the GXD at present fails to fulfil several of these requirements.

At the time our study was undertaken, the GXD contained less than 3% of the total number of genes expected to be present in the mouse. Often, only a few tissues have been examined for gene expression patterns, so there is no way of knowing whether the data presented accurately reflect the expression pattern of a gene. At the time of our study no standard terminology had been implemented in the GXD to describe expression patterns, which often necessitates multiple searches with synonyms for a single tissue. In addition, a limited number of errors are probably inevitable in the construction of this and similar large databases. On average, 2% of all query results will not be associated with verifiable expression data and hence constitute a false positive result.

Although it is possible to search for stage-specific gene expression patterns, stage grouping in the GXD database was not very precise. Embryonic stages are described as days post coitum (dpc) instead of embryonic Theiler stages. Since dpc sometimes cover several

Theiler stages and embryonic tissues sometimes appear over the course of one Theiler stage, this is a source of potential error. Lastly, gene expression patterns listed in the GXD had been determined by a number of different methods. Comparing expression data for various candidate genes and assessing the reliability of these data is not yet optimal. It may be argued that there are examples of mutations in genes that are ubiquitously expressed, but cause a very specific pattern of organ involvement. Examples are eye diseases such as RP3 and gyrate atrophy which are due to mutations in ubiquitously expressed genes. This does not in itself invalidate the approach outlined here. In fact, a general feature of all candidate gene strategies is that they represent educated guesses and hence should be regarded as hypothesis-generating rather than as problem-solving.

It is of interest to note that out of seven syndromes for which the gene is known, a search based on organ involvement returned a list of genes which included the real disease gene in four of seven cases (trichodonto-osseous, hand-foot-genital, unlar-mammary, and renal-coloboma syndromes, Table 3). In addition, our limited pilot experiment of 20 syndromes of unknown genesis yielded credible candidate genes for several of these (Table 5). Therefore, even though many problems still have to be solved, it would appear to be possible to use simple search terms that describe organs or anatomical regions, by which one can convert human malformation syndromes into a syndrome-specific developmental gene expression pattern. Using only 29 simple terms at present covers 65% of the GXD and a manageable list of 153 terms covers 84%. Thus, the number of terms that is required to translate a syndrome to an expression pattern is not large.

As the number of genes in expression databases increases, so will the numbers retrieved in this type of search. Therefore, secondary criteria for the ranking of candidates will have to be developed. For this purpose, genetic localisation would be a useful possibility. Also, the number of genes retrieved will be smaller if more search terms, or specific periods for embryonic gene expression are used to define the syndromes.

In conclusion, our data suggest that it may ultimately be possible to probe gene expression databases for candidate genes for multiple congenital malformation syndromes using simple syndrome descriptions. The potential value of this is illustrated by recent data.³⁸ For instance, the genes for Townes-Brocks syndrome (*SALI*) was initially identified on the basis of positional

information. Upon examination of the expression pattern of its mouse homolog (*Msall*) it became clear that it matched the pattern of abnormalities observed in the syndrome. This established the *SALI* gene as an excellent candidate for Townes-Brocks syndrome, a hypothesis which was borne out by subsequent mutation analysis. It is also encouraging that in our analysis of seven syndromes due to known gene mutations, we could identify the gene for four of these using expression data as the only clues. If this method is to be used on a larger scale, the expression databases will need to meet as many of the requirements listed above as possible. Projects such as GENEX, an internet-accessible database that integrates mouse anatomy data with gene expression data,³⁹ may ameliorate the situation in the near future. In the GENEX database, expression patterns are listed according to Theiler stages. The terms used to describe various tissues and organs were standardised from the beginning. As the authors of the database are also responsible for the expression studies, uniform methodology is assured. When this and other similar databases for human developmental data are fully operational, most of the above requirements will be met. With the expected accumulation of genomics/proteomics data, these and other databases can be used to identify position-independent candidate genes for human congenital malformation syndromes. Such candidate genes can be the starting point for further investigations. In most cases these will be in the form of targeted linkage analyses in small families. In cases where such family material is unavailable, direct mutation analysis may be the strategy of choice.

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