Genetic Testing for Familial Cancer Syndromes...CON

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Department of Surgery
Grand Rounds
1/10/2011
Disclosures

- Terri Chin........................................New Jersey
- Dr. Kounalakis...............................New Jersey
- Travis Walker.................................NOT from NJ
Discovery of DNA

FRIEDRICH MIESCHER, MD
(1844-1895)

- 1869 isolated “nuclein” (Nucleic acid) from leukocytes.
- Collected bandages with pus
- Isolated cells in sodium sulfate solution
- Alkaline extraction followed by acidification forming precipitate
- Published in 1871
MOLECULAR STRUCTURE OF NUCLEIC ACIDS
A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey 1. They kindly made their manuscript available to us in advance of publication. Their model consists of three triple-twisted chains, with the phosphates near the fiber axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together; especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear too small.

Another triple-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each of which contains the same number of nucleotide units; they have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joined by deoxyribose residues with 3,5' linkages. The two chains (but not the bases) are related by a dyad perpendicular to the fiber axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the bases in the two chains run in opposite directions.

Each chain loosely resembles Furberg's model. It is, the bases on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's standard configuration, the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 A. in the direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fiber axis is 10 A. As the phosphates are on the outside, the atoms have easy access to them.

The structure is an open one, and as water content is rather high. At lower water contents we would expect the bases to fill so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fiber axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a base from the other chain, so that the two lie side by side with identical 2,3-orientations. One of the purine must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 6; purine position 6 to pyrimidine position 1.

It is assumed that the bases only occur in the structure in the most plausible tautomeric forms (i.e., with the keto rather than the enol conformations). It is found that only specific pairs of bases can bond together. These pairs are adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain, does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally 2-7 that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atoms would make too close a van der Waals contact. The previously published X-ray data 8 on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented here when we devised our structure, which rests mainly upon not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of coordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. J. E. Donohue for constant advice and criticism, especially on stereochemical distances. We have also been stimulated by a knowledge of the general results of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. H. R. Franklin and co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge, April 2.

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Human Genome

Nature

Science
Human Genome

- 46 chromosomes
- Estimated 25,000 genes
University of Colorado Adult Medical Genetics

► Adult Medical Genetics clinic began seeing patients in August 2002

► Clinic appointments every Monday (Matthew Taylor, MD)
University of Colorado Adult Medical  
Genetics

► Benefits:
  ▪ Plan for the future  
  ▪ Preventive actions...drugs, lifestyle, surgery

► Risks:
  ▪ inherited mutation can be missed  
  ▪ does not tell us how or when the disease will develop  
  ▪ Family relationships may be affected by this information  
  ▪ genetic test results are uninformative or ambiguous  
  ▪ may affect your ability to be insured (health, life and disability).
In 1994, the following Colorado state law was passed. This can be found specifically in Title 10, Art. 3, Part II: §10-3-1104.7

- Prohibits the utilization of information derived from genetic testing from being used to deny access to health care insurance.
- Provides for privacy protection of genetic information.
- Genetic test: Any laboratory test of human DNA, RNA, or chromosomes that is used to identify the presence or absence of alterations in genetic material which are associated with disease or illness.
University of Colorado Adult Medical Genetics

► COSTS

- BRCA 1/2: $2400 for the initial test

- APC gene sequencing: $1500

- HNPCC (Lynch syndrome) MLH1, MSH2, MSH6 sequencing $2700
Breast Cancer

- **Lifetime Risk: 1 in 8**
  
  (12.5%)
BRCA 1 & 2

- **BRCA 1**
  - First discovered in early 90’s; cloned in 1994
  - Located on Chromosome 17
  - Tumor suppressor, DNA repair
  - Autosomal Dominant
  - Increased risk breast cancer

- **BRCA 2**
  - Cloned in 1995
  - Chromosome 13
  - Increased risk of breast and ovarian cancer
  - Male Breast Cancer
Genetic Screening Guidelines

- Individual from a family with a known BRCA1/BRCA2 mutation
- Personal history of breast cancer\(^1\) plus one or more of the following:
  - diagnosed at age \(\leq 45\) years
  - diagnosed at age \(\leq 50\) years, with one or more close blood relative with breast cancer at \(\leq 50\) years and/or one or more close blood relative with epithelial ovarian/fallopian tube/primary peritoneal cancer
  - two breast primaries\(^2\) when first breast cancer diagnosis occurred prior to age 50
  - diagnosed at any age, with two or more close blood relatives with breast and/or epithelial ovarian/ fallopian tube/primary peritoneal cancer at any age
  - close male blood relative with breast cancer
  - personal history of epithelial ovarian\(^3\) /fallopian tube/primary peritoneal cancers
  - for an individual of ethnicity associated with higher mutation frequency (e.g., founder populations of Ashkenazi Jewish, Icelandic, Swedish, Hungarian or Dutch), no additional family history may be required\(^4\)
- Personal history of epithelial ovarian/fallopian tube/primary peritoneal cancers
- Personal history of male breast cancer
- Family history only with one of the following:
  - First- or second-degree blood relative meeting any of the above criteria
  - Third-degree blood relative with \(\geq 2\) close relatives with breast and/or ovarian cancer (at least one close blood relative with breast cancer \(\leq 50\) years)
Majority of patients overestimate perceived risk of breast cancer

Overestimate family history

Correlation between perceived risk and desire for gene testing
Prevalence and penetrance of *BRCA1* and *BRCA2* mutations in a population-based series of breast cancer cases

► Screened over 2000 women diagnosed with breast cancer over 5 year period
► Diagnosis before age of 55
► Used PCR to evaluate for BRCA 1 & 2

### Table 2  Prevalence of *BRCA1* and *BRCA2* mutations in breast cancer cases by age

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number</th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>Total</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>n</td>
<td>% a</td>
<td>n</td>
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<tr>
<td>&lt;35</td>
<td>57</td>
<td>2</td>
<td>4.1 (0.5, 14.1)</td>
<td>4</td>
</tr>
<tr>
<td>35–44</td>
<td>341</td>
<td>3</td>
<td>1.0 (0.2, 3.0)</td>
<td>4</td>
</tr>
<tr>
<td>45–54</td>
<td>917</td>
<td>3</td>
<td>0.3 (0.1, 1.1)</td>
<td>8</td>
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<tr>
<td>Total</td>
<td>1435</td>
<td>8</td>
<td>0.7 (0.3, 1.3)</td>
<td>16</td>
</tr>
</tbody>
</table>

* a Adjusted for 15% failure rate in DNA amplification by PCR for mutation analysis
Screening the General Population

**WHAT GENE TESTS CAN TELL YOU**

THERE are genetic tests for 1,400 diseases. These involve taking a swab from inside the mouth or a blood test.

Genetic testing is only predictive — it can reveal if you have a high risk of developing a disease such as breast cancer or whether you carry a genetic mutation for a disease that you can pass on to your children.

But this doesn’t mean that you, or your children, will become ill.

However, there are very few conditions where a positive result for a faulty gene means you will develop it.

These include Huntington’s disease, a degenerative disease that affects the muscles and leads to dementia.
Figure 3. Yield of testing for BRCA mutations in a hypothetical population based on assumptions in Table 6.

- **Women** (n = 100,000)
  - **Average risk** (n = 92,707)
    - NNS to prevent 1 case of cancer
      - Breast: (n = 11,049)
      - Ovarian: (n = 7,072)
    - Cases of cancer prevented
      - Breast: (n = 8)
      - Ovarian: (n = 13)
  - **Moderate risk** (n = 6,862)
    - NNS to prevent 1 case of cancer
      - Breast: (n = 12,222)
      - Ovarian: (n = 436)
    - Cases of cancer prevented
      - Breast: (n = 6)
      - Ovarian: (n = 16)
  - **High risk** (n = 431)
    - NNS to prevent 1 case of cancer
      - Breast: (n = 182)
      - Ovarian: (n = 189)
    - Cases of cancer prevented
      - Breast: (n = 2)
      - Ovarian: (n = 2)

Total cases of cancer prevented
- Breast: (n = 16)
- Ovarian: (n = 31)

NNS = number needed to screen. *Based on estimates for mastectomy.
†Based on estimates for oophorectomy.
Surveillance of BRCA1 and BRCA2 Mutation Carriers With Magnetic Resonance Imaging, Ultrasound, Mammography, and Clinical Breast Examination

- 236 patients with BRCA 1 / 2 followed for 3 years
- Combined Mammogram, MRI, US, and CBE
- 22 cancers detected

- Sensitivity and Specificity:
  - MRI: 77% and 95%
  - Mammogram: 36% and 99%

- Conclusions: “MRI likely to become screening modality for BRCA 1 & 2 mutation carriers”
Retrospective review of 358 high-risk patients undergoing prophylactic mastectomy

236 patients BRCA 1/2 mutation carriers

<table>
<thead>
<tr>
<th>History of BC</th>
<th>No (Unaffected)</th>
<th>Yes (Affected)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>177 (%)</td>
<td>181 (%)</td>
<td></td>
</tr>
<tr>
<td>Breast reconstruction (BR)</td>
<td>9 (5)</td>
<td>68 (37)</td>
<td>&lt;.001</td>
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<td>Yes</td>
<td>166 (94)</td>
<td>110 (61)</td>
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<tr>
<td>Unknown</td>
<td>2 (1)</td>
<td>3 (2)</td>
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<tr>
<td>Type of BR</td>
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<td>95 (54)</td>
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<td>Silicone prosthesis</td>
<td>6 (3)</td>
<td>14 (8)</td>
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<td>Autologous tissue</td>
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<td>No</td>
<td>82 (49)</td>
<td>55 (30)</td>
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<tr>
<td>Total number of complications after BR</td>
<td>127</td>
<td>88</td>
<td>.74</td>
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<tr>
<td>Early (&lt;6 weeks after BR)</td>
<td>42 (33)</td>
<td>31 (26)</td>
<td></td>
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<tr>
<td>Late (6 weeks after BR)</td>
<td>85 (67)</td>
<td>57 (45)</td>
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<td>58 (30)</td>
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<td>.67</td>
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<td>64 (73)</td>
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<tr>
<td>Early complications</td>
<td>27 (66)</td>
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<td>14 (45)</td>
<td></td>
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<tr>
<td>No</td>
<td>8 (19)</td>
<td>14 (45)</td>
<td>.09</td>
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<td>4 (13)</td>
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<tr>
<td>Infection</td>
<td>20 (48)</td>
<td>12 (39)</td>
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<tr>
<td>Necrosis</td>
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<tr>
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<td>1 (2)</td>
<td>2 (0)</td>
<td></td>
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<tr>
<td>Late complications</td>
<td>11 (13)</td>
<td>7 (12)</td>
<td>.91</td>
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<tr>
<td>Surgery due to late complication</td>
<td>74 (87)</td>
<td>50 (68)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4 (4)</td>
<td>0 (0)</td>
<td>.33</td>
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<td>5 (1)</td>
<td>3 (5)</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>31 (27)</td>
<td>20 (35)</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>2 (2)</td>
<td>3 (5)</td>
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</tr>
<tr>
<td>Prosthesis rupture</td>
<td>31 (27)</td>
<td>19 (34)</td>
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<td>Dog ear</td>
<td>16 (19)</td>
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# Early Complications

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<th>Women with complications after BR</th>
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<th>55</th>
<th>(50)</th>
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<tr>
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<td>82</td>
<td>(49)</td>
<td>55</td>
<td>(50)</td>
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<tr>
<td>Total number of complications after BR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>127</td>
<td>88</td>
<td></td>
<td></td>
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<tr>
<td>Early (&lt;6 weeks after BR)</td>
<td>42</td>
<td>(33)</td>
<td>31</td>
<td>(35)</td>
<td>.74</td>
</tr>
<tr>
<td>Late (&gt;6 weeks after BR)</td>
<td>85</td>
<td>(67)</td>
<td>57</td>
<td>(65)</td>
<td></td>
</tr>
<tr>
<td>Surgery due to late complication</td>
<td>No</td>
<td>38</td>
<td>(30)</td>
<td>24</td>
<td>(27)</td>
</tr>
<tr>
<td>Yes</td>
<td>89</td>
<td>(70)</td>
<td>64</td>
<td>(73)</td>
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<tr>
<td>Early complications</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Surgery due to early complication</td>
<td>No</td>
<td>27</td>
<td>(64)</td>
<td>17</td>
<td>(55)</td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>(36)</td>
<td>14</td>
<td>(45)</td>
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<td>Type of early complication</td>
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<td>Necrosis</td>
<td>11</td>
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<td>(48)</td>
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## Late Complications

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<td>No</td>
<td>11</td>
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<tr>
<td>Yes</td>
<td>74</td>
<td>(87)</td>
<td>50</td>
<td>(88)</td>
<td></td>
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<tr>
<td>Type of late complication</td>
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<td></td>
<td></td>
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<tr>
<td>Infection</td>
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<td>(4)</td>
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<td>(0)</td>
<td>.33</td>
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<tr>
<td>Necrosis</td>
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<td>(1)</td>
<td>3</td>
<td>(5)</td>
<td></td>
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<tr>
<td>Capsular formation</td>
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<td>(37)</td>
<td>20</td>
<td>(35)</td>
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<td>(2)</td>
<td>3</td>
<td>(5)</td>
<td></td>
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<td>Poor cosmetic appearance $^d$</td>
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<td>(37)</td>
<td>19</td>
<td>(34)</td>
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<td>Dog ear</td>
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<td>(19)</td>
<td>12</td>
<td>(21)</td>
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Conclusions

- Breast cancer is very common
- BRCA 1 and 2 increases lifetime risk for breast cancer
- Genetic screening is important but...
  - Genetic tests are expensive
  - Patients often overestimate their risk of cancer and family history
  - Prevalence of BRCA 1 and 2 is low
  - No proven benefit for genetic screening the general population for BRCA 1 and 2
  - Screening would increase the use of MRI
  - Prophylactic mastectomies in BRCA 1 and 2 carriers have both early and late complications
Townsend: Sabiston Textbook of Surgery 18th edition, Chapter 34 “Diseases of the breast.”

Email Correspondence with Matthew Taylor, MD. Director of University of Colorado Adult Medical Genetics.


